# Sociodemographic factors and STIs associated with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection in Zambian female sex workers and single mothers

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## Abstract

Sexually transmitted infections (STIs) in women caused by *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (NG) are epidemiologically distinct. In this study, associations with sociodemographic and clinical risk factors are explored separately for CT and NG. Multivariate logistic regression (MLR) models quantify associations between potential CT and/or NG risk factors within a cross-sectional study of high-risk women in two Zambian cities, Lusaka and Ndola. CT was associated with living in Lusaka, younger age, and literacy. Long-acting reversible contraception (LARC) was predictive of CT in Ndola, but protective in Lusaka. In Lusaka only, CT was associated with lower education and reported unprotected sex. NG was associated with younger age, lower education, concurrent *Trichomonas vaginalis*, bacterial vaginosis, and incident syphilis infection. Signs and symptoms were rare and not associated with either infection. CT was more prevalent, nearly 11%, compared to NG, 6.8%. The higher prevalence of CT could explain the lack of association with other STIs. The associations observed with NG could be the result of high-risk sexual networks or lack of protective immunity. Risk factors for CT and NG are distinct and may differ geographically, which should be considered when developing diagnostic tools or guiding presumptive treatment in specific populations.

## Keywords

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## Introduction

*Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) are bacteria that result in sexually transmitted infections (STIs) worldwide. In women, these infections are frequently asymptomatic and can cause pelvic inflammatory disease, pregnancy complications, and infertility, as well as increase the risk for HIV acquisition[1–3]. Both infections are curable with antibiotics, however the rise of antibiotic resistant NG strains are an increasing threat to treatment[4].

Longitudinal and cross-sectional studies have suggested partial protective immunity to CT. In a study of female sex workers (FSW) in Nairobi, younger age and fewer years working as a FSW were associated with increased incidence of CT, suggesting that despite continued exposure, protective immunity prevented recurrent infections in older and more experienced FSWs[5]. Similarly, among FSWs in Benin, longer duration of sex work was associated with protection against CT/NG infection[6]. Animal studies have shown that the immunity to CT is mediated by CD4^{+} T cells[7, 8] and the cytokines INF-y and IL-13 have been associated with CT/NG protection in humans[5]. No protective immunity has been described in NG, but rather an array of host-evasion strategies precludes the establishment of an effective immune response[9].

Although the mode of transmission for CT and NG is the same, each pathogen has unique characteristics. Due to similar symptomology and limited resources, symptomatic women may be treated for both infections under syndromic STI management[10]. However, with potential protective immunity to CT and the mounting risk of antibiotic resistance by NG[4], it is important to investigate how the risk profiles for infection by each of these unique bacteria differ within the same study population.

## Materials and Methods

### Study Population:

An established prospective cohort of HIV-uninfected high-risk women (HRW) in Lusaka and Ndola, Zambia was examined[11–13]. The women included were either FSW or single mothers with children under the age of five (SM) referred to the Zambia-Emory HIV Research Project (ZEHRP) from local post-natal clinics or invited from community outreach at sex work hot spots.

Data from a 2016 cross-sectional sub-study on intra-vaginal practices (IVP) in this cohort was the source of STI test results and clinical observations[14]. The IVP study included a survey of sociodemographic factors, IVP, symptoms, and sexual behaviors. A clinical exam was performed where venous blood and endocervical swabs were collected by a nurse. All participants were STI tested, regardless of reported symptoms. Samples were tested for HIV by rapid test (Determine HIV-1/2 Ag/Ab Combo, Uni-Gold confirmatory) and GeneXpert; syphilis by rapid plasma reagin (RPR); *Trichomonas vaginalis* (TV), candida, and sperm by wet mount microscopy; and bacterial vaginosis (BV) by KOH whiff test and clue cell identification. Women with bacterial STIs received free treatment onsite and partners were invited for treatment. Women testing HIV positive were referred to government clinics for

antiretroviral therapy. A total of 825 unique clinic visits where CT/NG GeneXpert testing was performed were available for analysis. There were several instances where the same individual was tested at two separate clinic visits, however these entries were not excluded from the analysis because GeneXpert testing and survey responses were available for both time points.

**Variables:**

All samples and data were gathered between September 2016 and January 2019. The two outcome variables for this study were CT (including CT/NG co-infected), and NG (including CT/NG co-infected). The reference group for both outcomes was the CT and NG uninfected population. In the models where CT was the outcome of interest, those infected with NG only were omitted from the analysis; the reverse is true for the models where NG was the outcome of interest.

Clinical signs were any of the following on either the external or internal genitalia during pelvic examination: inguinal adenopathy, inflammation, ulceration, condyloma or warts, cervicitis, cervical discharge or pus, vaginal discharge, erosion or friability of the cervix or vagina, non-menstrual bleeding, or adnexal tenderness or mass. Symptoms were recorded if the participant spontaneously reported the symptom or if they reported it after being specifically prompted, and whether the symptom was currently present or previously treated elsewhere and was now resolved. The following symptoms were evaluated: cystitis, dysuria, vaginal itching, vaginal discharge, dyspareunia, lower abdominal pain, or acute/chronic/recurrent genital ulcer.

Laboratory tests assessed the presence of TV, HIV, sperm on a vaginal swab, candida, BV, and syphilis. BV was considered present if a woman had both a positive KOH whiff test and clue cells observed on microscopy. The presence of an incident syphilis infection was determined by a proxy, receiving treatment for syphilis, due to the high prevalence of serofast RPR results in Lusaka[15, 16].

Age groups (18–24 and 25+) were tabulated based on birth year and age in 2016. Literacy was determined based on whether the participant could read English or the predominant local dialect (Nyanja, the lingua franca in Lusaka or Bemba, the predominant language of communication in Ndola) without difficulty. Education was based on the level of schooling completed and grouped by either none/primary or secondary/college.

Long-acting reversible contraception (LARC) was considered to be either the hormonal implant or the intrauterine device (IUD); injectable contraception was not categorized as LARC. Other variables considered were city of residence, reported sex without a condom at least once in the past one-three months, reported history of transactional sex, and self-reported pregnancy.

**Logistic Regression Modeling:**

Overall frequencies of each covariate, as well as frequencies stratified by city, were first compared via chi-square or Fisher's exact test for each outcome to the uninfected group. Instances where the stratified chi-square or Fisher's exact p-value was less than 0.05 in

one city but not the other, or significant in both cities but not overall, were included as an interaction term by city in the logistic regression model.

The probability of a positive outcome follows a binomial distribution. Each covariate and the interaction terms were initially considered in bivariate logistic regression models. Variables that resulted in a significant association at the bivariate level were included in the full multivariate logistic regression model (MLR). For each of the full models, a data subset was created that excluded missing values for all predictors in the model. The full model then underwent bi-directional stepwise model selection using the R function stepAIC to arrive at the final MLR model.

Collinearity of the full model was assessed by examining the correlations between each of the variables in the model and the variance inflation factors (VIF). All of the correlation coefficients were below 0.5 and all of the VIFs were below 10, so no variables were eliminated due to collinearity.

**Ethics:**

These investigations were approved by the Institutional Review Board of Emory University and the Research Ethics Committee of the University of Zambia. Participants completed written informed consent before survey administration or study specimen collection.

## Results

### Clinical signs and symptoms are not significantly associated with CT/NG status

There were a total of 68 CT-only cases (26 Lusaka, 42 Ndola), 34 NG-only cases (6 Lusaka, 28 Ndola), and 22 CT & NG co-infected cases (4 Lusaka, 18 Ndola). The remaining 701 individuals were both CT and NG uninfected (169 Lusaka, 532 Ndola). The prevalence of CT was higher in Lusaka compared with Ndola (15% vs. 10%, p=0.053), while the prevalence of NG was not different (5% vs. 7%, respectively). Twenty-four women seroconverted to HIV during cohort follow-up and were HIV+ at the time of sample collection for CT/NG testing. HIV was not associated with either CT and/or NG (4/24 HIV+ vs. 119/794 HIV-, p=0.77).

Pelvic exams were performed on 530 participants at the time of CT/NG testing. The majority of women, across all infection categories, had vaginal discharge not associated with CT or NG (Table 1). The number of women with other abnormalities noted on gynecologic exam was small: external ulceration in 2 women and internal ulcers in 1; cervicitis in 2; and pus in the cervix in 5. None of these signs were associated with CT and/or NG (not shown). The only clinical sign that was statistically significantly related with CT or NG was internal genitalia adnexal tenderness (Fisher's exact p=0.03, n=2); both women who presented with this sign were infected with CT, whereas there were zero women with this sign in any other infection status group. All other signs and reported symptoms were present at low frequencies and were not statistically associated with either CT or NG.

Symptoms reported by 559 women were also uncommon, with cystitis/dysuria reported by 3, vaginal itching by 6, dyspareunia by 2, lower abdominal pain by 4, and acute genital ulcer by 3. No symptoms were associated with CT and/or NG (not shown).

### STIs and vaginal dysbiosis are associated with prevalent CT/NG infection

Due to the limited number of positive STI observed, many covariates contained only a small sample size, however several statistically significant associations were detected. TV infection was statistically significantly associated with NG overall and within each city (Table 2), as well as with CT overall and within Lusaka. Receiving treatment for an incident syphilis infection and the BV composite variable of positive KOH whiff test and presence of clue cells were statistically significantly associated with NG overall and within Ndola, however were not associated with CT. Visualization of sperm on microscopy was statistically significantly associated with CT overall. Visualization of candida and testing newly positive for HIV by rapid test were not associated with either of the outcomes regardless of stratification.

### Various sociodemographic factors are associated with prevalent CT and/or NG infection

Sociodemographic and risk behaviors varied in their associations to each outcome when stratified by city (Table 3). Age group and education level, which also appeared to interact with city, were associated with both outcomes. Literacy, unprotected sex in the last 1–3 months, and LARC usage were statistically associated with at least one outcome in at least one city. Transactional sex and pregnancy status were not associated with any outcome in either city.

Of the IVP that were included in this study, only washing the external genitalia with detergent was found to be statistically significantly associated with NG (Fisher's exact p=0.05) (Table 1).

### CT infection is associated with younger age, higher literacy, lower education, unprotected sex, and LARC usage in a multivariate logistic regression model

Bivariate associations with CT infection were seen with city, age group, literacy, unprotected sex, LARC, and TV (Table 4). Interaction terms with city for age group, literacy, education, unprotected sex, LARC usage, and TV were included based on statistical significance in Tables 2 and 3. The full MLR model contained all interaction terms and their lower order terms. Stepwise model selection eliminated the interaction terms with TV, literacy, and age group, as well as the main effect for TV. The data subset used for the MLR, which removed missing observations, included 68 cases of CT and 461 CT/NG uninfected individuals.

Women in the 18–24 age group had 2.05 (p=0.02) times higher odds of being infected with CT compared to women 25 years or older, controlling for literacy, education by city, unprotected sex by city, and LARC by city (Table 4). Interestingly, women that could not easily read English, Bemba, or Nyanja had less than half the odds (Adj. OR 0.48, p=0.02) of being infected with CT compared to women that could easily read English, Bemba, or Nyanja, controlling for all other factors in the model.

Significant associations were observed in Lusaka, but not in Ndola, for education and unprotected sex interaction terms. Women who did not attend secondary school in Lusaka had 4.46 (p=0.01) times the odds of being infected with CT than women living in Lusaka who attended secondary school, controlling for all other factors. In Ndola, the odds of not attending secondary school and CT infection was only 1.25 (p=0.53) times higher. Reporting at least one unprotected sex act within the last 1–3 months, compared to none, increased the odds of CT infection by 4.38 (p=0.01) in Lusaka and 1.37 (p=0.38) in Ndola, when controlling for all other factors. Using the implant or IUD had an inverse relationship between cities; controlling for all other covariates in the model, in Lusaka women who used a LARC method had 0.14 (p=0.02) times the odds of being infected with CT, compared to women who were not using a LARC method. The opposite was true in Ndola, where women using a LARC method had 2.22 (p=0.01) times the odds of being infected with CT compared to women not using LARC.

### NG infection is associated with younger age, lower education, TV, BV, and incident syphilis infection in a multivariate logistic regression model

In the initial bivariate analyses of NG infection, age group, education, TV, BV, incident syphilis infection, and washing the external genitalia with detergent were statistically significantly associated (Table 5). Interaction terms by city were included for education, BV, and incident syphilis infection as these variables suggested different effects on NG infection by city, based on chi-square and Fisher's exact test results (Tables 2 & 3). The full MLR model contained all interaction terms, their main effects, and predictors for age group, TV, and external detergent IVP. Stepwise model selection reduced the model to ultimately contain age group, education, TV, BV, and incident syphilis infection. City was no longer represented in the model in interaction terms or as a main effect. The data subset on which the model was tested contained 34 cases of NG and 389 CT/NG uninfected individuals.

Controlling for all other factors in the model, increased odds of NG were observed for age 18–24 (Adj. OR 3.43, p=0.01), not completing secondary school (Adj. OR 3.53, p=0.01), testing positive for TV (Adj. OR 6.84, p<0.01), BV (Adj. OR 2.31, p=0.03), or being treated for syphilis (Adj. OR 9.12, p<0.01) (Table 5).

## Discussion

In this study, no clinical signs or symptoms were associated with CT or NG, which implies limited sensitivity of syndromic management. Opposing associations between CT and LARC usage were observed between cities, with LARC significantly increasing the odds of CT infection in Ndola, and significantly decreasing the odds of CT infection in Lusaka. Interestingly, the prevalence of LARC in both cities is nearly identical, 33.3% in Lusaka and 34.6% in Ndola. We have previously shown that, when sensitive measures of condomless sexual exposure in HIV discordant couples are included, hormonal contraception overall, and LARC in particular, are not associated with HIV transmission[17, 18]. We also found that LARC use was associated with less condomless sex in discordant couples[19]. In the HRW studied here, the measure of condomless sex was imprecise, therefore the interpretation of the LARC associations with CT must be interpreted with caution.

Similar to other studies, we detected associations between NG and BV[20], incident syphilis, and TV. The association between NG and BV may be related to the association observed between IVP and NG in this study due to an underlying relationship between IVP and BV[21], although this connection appears inconsistent[14]. The association between NG and other STIs may indicate that women infected with NG engage in risky behavior within a sexual network with a high prevalence of STIs, or hint at potential immunomodulatory aspects of NG infection which create an immunosuppressive environment advantageous for NG and other vaginal pathogens[9].

The lack of association between CT and other STIs may be an artifact of the higher prevalence of CT in this population overall, nearly 11%, compared to NG at 6.8%. This can be demonstrated by the prevalence of CT/NG co-infections in each group, where only 24% of those infected with CT were also infected with NG, whereas among those infected with NG, 39% were co-infected with CT. Our observations are consistent with CT/NG co-infection rates in comparable populations[22]. In particular, within a similar cohort of HIV-uninfected high-risk South African women, 100% (n=43) of the women infected with NG were also co-infected with another STI, whereas only 84% (n=130) of those infected with CT had an STI co-infection[23].

Our data do not strongly support partial immunity to CT. Compared to women over 25 years, young women had twice the odds of being infected with CT controlling for other variables in the model, whereas they had more than 3 times the odds of being infected with NG, a pathogen which does not establish protective immunity. If protective immunity to CT developed, we would expect the adjusted odds ratio for young age in the CT model to be higher than that in the NG model, rather than less. Furthermore, apart from lacking protective immunity, young women may have additional unmeasured risk factors such as increased risk-taking behavior or physiological risk factors such as an immature cervix or cervical ectopy, which has been shown to be associated with CT infections[24]. Together, our findings support that young women are more likely to be infected with CT or NG than older women, but do not indicate that this may be the result of protective immunity.

Other limitations include the fact that this was a cross-sectional design examining women who were part of an existing prospective cohort and who did not come to the clinic due to CT/NG symptoms, which likely dampened any associations we might have detected with clinical indicators. However, on the other hand, this highlights the prevalence of asymptomatic CT and NG infections. Finally, the data regarding risk behaviors and sexual practices were obtained via self-report, which could be subject to recall and social desirability bias.

Overall, the results presented here describe various demographic, social, and clinical factors that are associated with CT/NG infections among HRW in Zambia. This information is useful for parsing out unique risk factors for CT versus NG and understanding how the epidemiology of these diseases vary within the same population. These data could also be useful for developing risk assessment tools to improve detection strategies in low-resource settings, especially among asymptomatic women. However, detection and treatment should be accompanied by social intervention, such as risk-reduction counseling, voluntary couples

testing[25], and partner services[26]. Without such services reinfection will occur, potentially at an increased rate as it is hypothesized that early treatment disrupts the development of protective immunity to CT[27, 28]. Together with a comprehensive intervention program or effective vaccine, and epidemiologic guidelines, case detection and clinical outcomes can improve.

## Acknowledgements

## References

1. CDC. Gonorrhea - CDC Fact Sheet, https://www.cdc.gov/std/gonorrhea/stdfact-gonorrhea.htm (2017).

2. CDC. Chlamydia - CDC Fact Sheet, https://www.cdc.gov/std/chlamydia/stdfact-chlamydia.htm (2017).

3. Wijgert JHHM, Morrison CS, Brown J, et al. Disentangling contributions of reproductive tract infections to HIV acquisition in African Women. Sexually Transmitted Diseases 2009; 36: 357–364. [PubMed: 19434010]

4. Wi T, Lahra MM, Ndowa F, et al. Antimicrobial resistance in Neisseria gonorrhoeae: Global surveillance and a call for international collaborative action. PLoS Med 2017; 14: e1002344. 2017/07/08. DOI: 10.1371/journal.pmed.1002344. [PubMed: 28686231]

5. Cohen CR, Koochesfahani KM, Meier AS, et al. Immunoepidemiologic profile of Chlamydia trachomatis infection: importance of heat-shock protein 60 and interferon- gamma. J Infect Dis 2005; 192: 591–599. 2005/07/20. DOI: 10.1086/432070. [PubMed: 16028127]

6. Diabate S, Chamberland A, Geraldo N, et al. Gonorrhea, Chlamydia and HIV incidence among female sex workers in Cotonou, Benin: A longitudinal study. PLoS One 2018; 13: e0197251. 2018/05/11. DOI: 10.1371/journal.pone.0197251. [PubMed: 29746535]

7. Su H and Caldwell HD. CD4+ T cells play a significant role in adoptive immunity to Chlamydia trachomatis infection of the mouse genital tract. Infect Immun 1995; 63: 3302–3308. 1995/09/01. [PubMed: 7642259]

8. Perry LL, Feilzer K and Caldwell HD. Immunity to Chlamydia trachomatis is mediated by T helper 1 cells through IFN-gamma-dependent and -independent pathways. J Immunol 1997; 158: 3344–3352. 1997/04/01. [PubMed: 9120292]

9. Liu Y, Feinen B and Russell MW. New concepts in immunity to Neisseria gonorrhoeae: innate responses and suppression of adaptive immunity favor the pathogen, not the host. Front Microbiol 2011; 2: 52. 2011/08/13. DOI: 10.3389/fmicb.2011.00052. [PubMed: 21833308]

10. WHO. Guidelines for the Management of Sexually Transmitted Infections. February 2004. 14 Syndromic management, http://apps.who.int/medicinedocs/en/d/Jh2942e/2.4.html#Jh2942e.2.4 (2004, accessed October 22, 2019).

11. Kilembe W, Inambao M, Sharkey T, et al. Single Mothers and Female Sex Workers in Zambia Have Similar Risk Profiles. AIDS Research and Human Retroviruses 2019; 35: 814–825. 2019/06/18. DOI: 10.1089/AID.2019.0013. [PubMed: 31204869]

12. Malama K, Sagaon-Teyssier L, Parker R, et al. Client-Initiated Violence Against Zambian Female Sex Workers: Prevalence and Associations With Behavior, Environment, and Sexual History. Journal of Interpersonal Violence 2019 2019/07/04. DOI: 10.1177/0886260519860083.

13. Wall KM, Kilembe W, Inambao M, et al. Implementation of an electronic fingerprint-linked data collection system: a feasibility and acceptability study among Zambian female sex workers. Global Health 2015; 11: 27. 2015/06/28. DOI: 10.1186/s12992-015-0114-z. [PubMed: 26115656]

14. Kilembe W Intravaginal practices (IVP) and Genital Tract Infection in High Risk Female Cohort in Lusaka and Ndola, Zambia. London School of Hygiene and Tropical Medicine, 2017.

15. Dionne-Odom J, Karita E, Kilembe W, et al. Syphilis treatment response among HIV-discordant couples in Zambia and Rwanda. Clinical Infectious Diseases 2013; 56: 1829–1837. 2013/03/15. DOI: 10.1093/cid/cit146. [PubMed: 23487377]

16. Dionne-Odom J, Karita E, Kilembe W, et al. Reply to Yang et al. Clinical Infectious Diseases 2013; 57: 1212–1213. 2013/05/28. DOI: 10.1093/cid/cit353.

17. Wall KM, Kilembe W, Vwalika B, et al. Hormonal contraception does not increase women's HIV acquisition risk in Zambian discordant couples, 1994–2012. Contraception 2015; 91: 480–487. 2015/02/25. DOI: 10.1016/j.contraception.2015.02.004. [PubMed: 25708502]

18. Wall KM, Kilembe W, Vwalika B, et al. Hormonal Contraceptive Use Among HIV-Positive Women and HIV Transmission Risk to Male Partners, Zambia, 1994–2012. J Infect Dis 2016; 214: 1063–1071. 2016/07/28. DOI: 10.1093/infdis/jiw322. [PubMed: 27462093]

19. Khu NH, Vwalika B, Karita E, et al. Fertility goal-based counseling increases contraceptive implant and IUD use in HIV-discordant couples in Rwanda and Zambia. Contraception 2013; 88: 74–82. 2012/11/17. DOI: 10.1016/j.contraception.2012.10.004. [PubMed: 23153896]

20. Wiesenfeld HC, Hillier SL, Krohn MA, et al. Bacterial vaginosis is a strong predictor of Neisseria gonorrhoeae and Chlamydia trachomatis infection. Clin Infect Dis 2003; 36: 663–668. 2003/02/21. DOI: 10.1086/367658. [PubMed: 12594649]

21. Low N, Chersich MF, Schmidlin K, et al. Intravaginal practices, bacterial vaginosis, and HIV infection in women: individual participant data meta-analysis. PLoS Med 2011; 8: e1000416. 2011/03/02. DOI: 10.1371/journal.pmed.1000416. [PubMed: 21358808]

22. Torrone EA, Morrison CS, Chen PL, et al. Prevalence of sexually transmitted infections and bacterial vaginosis among women in sub-Saharan Africa: An individual participant data meta-analysis of 18 HIV prevention studies. PLoS Med 2018; 15: e1002511. 2018/02/28. DOI: 10.1371/journal.pmed.1002511. [PubMed: 29485986]

23. Menezes LJ, Pokharel U, Sudenga SL, et al. Patterns of prevalent HPV and STI co-infections and associated factors among HIV-negative young Western Cape, South African women: the EVRI trial. Sex Transm Infect 2018; 94: 55–61. 2017/05/12. DOI: 10.1136/sextrans-2016-053046. [PubMed: 28490581]

24. Lee V, Tobin JM and Foley E. Relationship of cervical ectopy to chlamydia infection in young women. J Fam Plann Reprod Health Care 2006; 32: 104–106. 2006/07/11. DOI: 10.1783/147118906776276440. [PubMed: 16824301]

25. WHO. Guidance on Couples HIV Testing and Counselling Including Antiretroviral Therapy for Treatment and Prevention in Serodiscordant Couples: Recommendations for a Public Health Approach, https://apps.who.int/iris/bitstream/handle/10665/44646/9789241501972_eng.pdf?sequence=1 (2012).

26. Garrett NJ, Osman F, Maharaj B, et al. Beyond syndromic management: Opportunities for diagnosis-based treatment of sexually transmitted infections in low- and middle-income countries. PLoS One 2018; 13: e0196209. 2018/04/25. DOI: 10.1371/journal.pone.0196209. [PubMed: 29689080]

27. Brunham RC, Pourbohloul B, Mak S, et al. The unexpected impact of a Chlamydia trachomatis infection control program on susceptibility to reinfection. J Infect Dis 2005; 192: 1836–1844. 2005/10/20. DOI: 10.1086/497341. [PubMed: 16235186]

28. Brunham RC and Rekart ML. The arrested immunity hypothesis and the epidemiology of chlamydia control. Sex Transm Dis 2008; 35: 53–54. 2007/12/25. DOI: 10.1097/OLQ.0b013e31815e41a3. [PubMed: 18157065]

**Table 1:**

Prevalence of signs and IVP by CT and/or NG status and city, Zambian HRW, 2016

| | Overall | | | | | | | | | | Lusaka | | Ndola | |
| | CT Only (n=68) | | NG Only (n=34) | | CT & NG Co-infected (n=22) | | CT/NG Uninfected (n=701) | | Fisher's p-value CT vs Uninfected | Fisher's p-value NG vs Uninfected | Fisher's p-value CT vs Uninfected | Fisher's p-value NG vs Uninfected | Fisher's p-value CT vs Uninfected | Fisher's p-value NG vs Uninfected |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | (%) | N | (%) | N | (%) | N | (%) | | | | | | |
| **Signs (n=530)[a]** | | | | | | | | | | | | | | |
| Internal genitalia discharge vagina | 29 | (57) | 16 | (67) | 12 | (80) | 304 | (69) | 0.33[b] | 0.71 | 1.00 | 1.00 | 0.71 | 0.41 |
| Internal genitalia adnexal tenderness | 2 | (4) | 0 | (0) | 0 | (0) | 0 | (0) | 0.03 | 1.00 | 0.17 | 1.00 | 0.10 | 1.00 |
| No clinical signs observed | 20 | (39) | 7 | (29) | 3 | (20) | 130 | (30) | ref | ref | ref | ref | ref | ref |
| Pelvic exam not performed (Missing) | 17 | (25) | 10 | (29) | 7 | (32) | 261 | (37) | | | | | | |
| Washes External Genitalia with Water | | | | | | | | | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Yes | 52 | (100) | 23 | (100) | 12 | (100) | 438 | (100) | | | | | | |
| No | 0 | (0) | 0 | (0) | 0 | (0) | 1 | (0) | | | | | | |
| Washes Internal Genitalia with Water | | | | | | | | | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Yes | 45 | (100) | 22 | (100) | 11 | (100) | 385 | (100) | | | | | | |
| No | 0 | (0) | 0 | (0) | 0 | (0) | 1 | (0) | | | | | | |
| Washes External Genitalia with Soap | | | | | | | | | 0.21[b] | 0.14[b] | 0.80[b] | 0.14 | 0.17[b] | 0.34[b] |
| Yes | 19 | (37) | 7 | (30) | 4 | (33) | 194 | (44) | | | | | | |
| No | 33 | (63) | 16 | (70) | 8 | (67) | 244 | (56) | | | | | | |
| Washes Internal Genitalia with Soap | | | | | | | | | 0.06[b] | 0.60 | 0.34 | 1.00 | 0.16[b] | 0.60 |
| Yes | 2 | (4) | 2 | (9) | 1 | (9) | 56 | (15) | | | | | | |

| | Overall | | | | | | | | Lusaka | | | Ndola | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CT Only (n=68) | | NG Only (n=34) | | CT & NG Co-infected (n=22) | | CT/NG Uninfected (n=701) | | Fisher's p-value CT vs Uninfected | Fisher's p-value NG vs Uninfected | Fisher's p-value CT vs Uninfected | Fisher's p-value NG vs Uninfected | Fisher's p-value CT vs Uninfected | Fisher's p-value NG vs Uninfected |
| | N | (%) | N | (%) | N | (%) | N | (%) | | | | | | |
| No | 43 | (96) | 20 | (91) | 10 | (91) | 330 | (85) | | | | | | |
| **Washes External Genitalia with Detergent** | | | | | | | | | | | | | | |
| Yes | 19 | (37) | 10 | (43) | 9 | (75) | 165 | (38) | 0.34[b] | 0.05[b] | 1.00 | 1.00 | 0.07[b] | 0.21[b] |
| No | 33 | (63) | 13 | (57) | 3 | (25) | 274 | (62) | | | | | | |
| **Washes Internal Genitalia with Detergent** | | | | | | | | | | | | | | |
| Yes | 2 | (4) | 2 | (9) | 3 | (27) | 28 | (7) | 0.59 | 0.17 | 1.00 | 1.00 | 0.39 | 0.18 |
| No | 43 | (96) | 20 | (91) | 8 | (73) | 357 | (93) | | | | | | |

CT, *Chlamydia trachomatis*; HRW, high-risk women; IVP, intra-vaginal practices; NG, *Neisseria gonorrhoeae*; ref, reference group.

[a] The following signs were also investigated during physical examination, but none where clinically observed: External genitalia inguinal adenopathy >1cm unilateral, External genitalia inguinal adenopathy >1cm bilateral, External genitalia inflammation, External genitalia condyloma/warts, Internal genitalia inflammation of vagina, Internal genitalia ulcer vagina, Internal genitalia erosion or friability cervix, Internal genitalia erosion or friability vagina, Internal genitalia non-menstrual bleeding cervix, Internal genitalia non-menstrual bleeding vagina, Internal genitalia condyloma/warts cervix, Internal genitalia condyloma/warts vagina, Internal genitalia adnexal mass.

[b] $\chi 2$ p-value.

**Table 2:**

Prevalence of lab results by CT and/or NG status and city, Zambian HRW, 2016

| | CT Only (n=68) | | NG Only (n=34) | | CT & NG Co-infected (n=22) | | CT/NG Uninfected (n=701) | | Overall | | Lusaka | | Ndola | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | (%) | N | (%) | N | (%) | N | (%) | Fisher's p-value CT vs Uninfected | Fisher's p-value NG vs Uninfected | Fisher's p-value CT vs Uninfected | Fisher's p-value NG vs Uninfected | Fisher's p-value CT vs Uninfected | Fisher's p-value NG vs Uninfected |
| *Trichomonas vaginalis* (microscopy) | | | | | | | | | 0.01 | <0.01[a] | 0.03 | 0.01 | 0.08 | 0.03 |
| Positive | 5 | (7) | 4 | (13) | 4 | (19) | 23 | (3) | | | | | | |
| Negative | 62 | (93) | 27 | (87) | 17 | (81) | 651 | (97) | | | | | | |
| Incident syphilis infection (based on RPR) | | | | | | | | | 0.26 | <0.01 | 1.00 | 0.26 | 0.15 | <0.01 |
| Yes | 2 | (4) | 4 | (16) | 2 | (13) | 13 | (3) | | | | | | |
| No | 54 | (96) | 21 | (84) | 13 | (87) | 454 | (97) | | | | | | |
| HIV (rapid test) | | | | | | | | | 0.50 | 0.24 | 1.00 | 1.00 | 0.61 | 0.08 |
| Positive or Discrepant | 1 | (1) | 3 | (9) | 0 | (0) | 20 | (3) | | | | | | |
| Negative | 66 | (99) | 31 | (91) | 22 | (100) | 675 | (97) | | | | | | |
| BV (Both KOH+ & Clue Cells) | | | | | | | | | 0.23[a] | <0.01[a] | 1.00 | 1.00 | 0.08[a] | <0.01[a] |
| Positive | 14 | (27) | 14 | (50) | 9 | (45) | 147 | (26) | | | | | | |
| Negative | 37 | (73) | 14 | (50) | 11 | (55) | 425 | (74) | | | | | | |
| Sperm (microscopy) | | | | | | | | | 0.05[a] | 0.56 | 1.00 | 1.00 | 0.07 | 0.77 |
| Positive | 1 | (2) | 4 | (14) | 0 | (0) | 43 | (7) | | | | | | |
| Negative | 64 | (98) | 25 | (86) | 20 | (100) | 609 | (93) | | | | | | |
| Candida (microscopy) | | | | | | | | | 0.76 | 0.25 | 1.00 | 1.00 | 1.00 | 0.24 |
| Positive | 2 | (4) | 0 | (0) | 0 | (0) | 24 | (4) | | | | | | |
| Negative | 53 | (96) | 27 | (100) | 20 | (100) | 555 | (96) | | | | | | |

BV, bacterial vaginosis; CT, *Chlamydia trachomatis*; HRW, high-risk women; KOH, potassium hydroxide; NG, *Neisseria gonorrhoeae*; RPR, rapid plasma reagin.

[a] $\chi^2$ p-value.

**Table 3:**

Prevalence of demographic factors and risk behaviors by CT and/or NG status and city, Zambian HRW, 2016

| | CT Only (n=68) | | NG Only (n=34) | | CT & NG Co-infected (n=22) | | CT/NG Uninfected (n=701) | | Overall | | | Lusaka | | Ndola | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | (%) | N | (%) | N | (%) | N | (%) | $\chi^2$ p-value CT vs Uninfected | $\chi^2$ p-value NG vs Uninfected | | $\chi^2$ p-value CT vs Uninfected | $\chi^2$ p-value NG vs Uninfected | $\chi^2$ p-value CT vs Uninfected | $\chi^2$ p-value NG vs Uninfected |
| City | | | | | | | | | 0.06 | 0.29 | | | | | |
| Lusaka | 26 | (38) | 6 | (9) | 4 | (18) | 169 | (24) | | | | | | | |
| Ndola | 42 | (62) | 28 | (91) | 18 | (82) | 532 | (76) | | | | | | | |
| HRW Group | | | | | | | | | 0.33 | 0.09 | | 0.25 | 0.68[a] | 0.66 | 0.11 |
| Single Mothers | 38 | (68) | 11 | (46) | 4 | (29) | 250 | (54) | | | | | | | |
| Female Sex Workers | 18 | (32) | 13 | (54) | 10 | (71) | 215 | (46) | | | | | | | |
| Age Group | | | | | | | | | 0.01 | $<0.01$ | | 0.17 | 0.05[a] | 0.02 | $<0.01$ |
| Age 18–24 | 44 | (67) | 26 | (79) | 21 | (95) | 412 | (59) | | | | | | | |
| Age 25+ | 22 | (33) | 7 | (21) | 1 | (5) | 284 | (41) | | | | | | | |
| Literacy | | | | | | | | | 0.05 | 0.96 | | 0.78 | 0.09[a] | 0.02 | 0.35 |
| Reads English, Bemba, or Nyanja | 45 | (66) | 17 | (52) | 12 | (55) | 361 | (52) | | | | | | | |
| Illiterate | 23 | (34) | 16 | (48) | 10 | (45) | 328 | (48) | | | | | | | |
| Education | | | | | | | | | 0.81 | 0.02 | | 0.04 | 0.01[a] | 0.73 | 0.27 |
| No School or Primary School Only | 42 | (62) | 28 | (85) | 15 | (68) | 426 | (62) | | | | | | | |
| Secondary School or Higher | 26 | (38) | 5 | (15) | 7 | (32) | 261 | (38) | | | | | | | |
| Sex in Exchange for Money | | | | | | | | | 0.73 | 0.12 | | 0.95 | 0.50[a] | 0.71 | 0.19 |
| Never | 38 | (56) | 13 | (39) | 8 | (36) | 339 | (49) | | | | | | | |
| Ever | 30 | (44) | 20 | (61) | 14 | (64) | 350 | (51) | | | | | | | |
| Unprotected Sex (Last 1–3 months) | | | | | | | | | 0.03 | 0.22 | | 0.01 | 1.00[a] | 0.23 | 0.34 |
| None | 16 | (31) | 9 | (41) | 4 | (22) | 201 | (42) | | | | | | | |

| | CT Only (n=68) | | NG Only (n=34) | | CT & NG Co-infected (n=22) | | CT/NG Uninfected (n=701) | | Overall | | Lusaka | | Ndola | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | $\chi^2$ p-value CT vs Uninfected | $\chi^2$ p-value NG vs Uninfected | $\chi^2$ p-value CT vs Uninfected | $\chi^2$ p-value NG vs Uninfected | $\chi^2$ p-value CT vs Uninfected | $\chi^2$ p-value NG vs Uninfected |
| | N | (%) | N | (%) | N | (%) | N | (%) | | | | | | |
| At least once | 35 | (69) | 13 | (59) | 14 | (78) | 272 | (58) | | | | | | |
| Pregnancy | | | | | | | | | 0.43[a] | 0.29[a] | 0.52[a] | 1.00[a] | 0.37[a] | 0.25[a] |
| Not Pregnant | 54 | (96) | 23 | (96) | 13 | (93) | 453 | (97) | | | | | | |
| Pregnant | 2 | (4) | 1 | (4) | 1 | (7) | 12 | (3) | | | | | | |
| Uses LARC Method | | | | | | | | | 0.12 | 0.11 | 0.05 | 0.72[a] | <0.01 | 0.13 |
| No LARC | 43 | (63) | 20 | (65) | 9 | (43) | 449 | (67) | | | | | | |
| Uses Implant or IUD | 25 | (37) | 11 | (35) | 12 | (57) | 224 | (33) | | | | | | |

CT, *Chlamydia trachomatis*; HRW, high-risk women; LARC, long-acting reversible contraception; IUD, intrauterine device; NG, *Neisseria gonorrhoeae.*

[a]Fisher's exact p-value.

**Table 4:**

Logistic regression models of factors associated with CT, Zambian HRW, 2016

| Predictor of CT[a] | Bivariate Association | | | | Multivariate Final Model[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | Odds Ratio | Lower 95% CI | Upper 95% CI | p-Value | Adj. Odds Ratio | Lower 95% CI | Upper 95% CI | Adj. p-Value |
| Age Group | | | | | | | | |
| Age 18–24 | 1.76 | 1.08 | 2.85 | 0.02 | 2.05 | 1.13 | 3.72 | 0.02 |
| Age 25+ | ref | - | - | - | ref | - | - | - |
| Literacy | | | | | | | | |
| Reads English, Bemba, or Nyanja | ref | - | - | - | ref | - | - | - |
| Illiterate | 0.64 | 0.40 | 1.00 | 0.05 | 0.48 | 0.26 | 0.89 | 0.02 |
| Education × City | | | | | | | | |
| No School or Primary School Only vs Secondary School or Higher in Lusaka | 2.26 | 1.03 | 4.99 | 0.04 | 4.46 | 1.42 | 13.98 | 0.01 |
| No School or Primary School Only vs Secondary School or Higher in Ndola | 0.90 | 0.51 | 1.61 | 0.73 | 1.25 | 0.62 | 2.54 | 0.53 |
| Unprotected Sex (Last 1–3 months) × City | | | | | | | | |
| At least once vs None in Lusaka | 3.72 | 1.39 | 9.96 | 0.01 | 4.38 | 1.44 | 13.30 | 0.01 |
| At least once vs None in Ndola | 1.51 | 0.77 | 2.96 | 0.23 | 1.37 | 0.68 | 2.76 | 0.38 |
| Uses LARC Method × City | | | | | | | | |
| Uses Implant or IUD vs No LARC in Lusaka | 0.37 | 0.13 | 1.02 | 0.06 | 0.14 | 0.03 | 0.69 | 0.02 |
| Uses Implant or IUD vs No LARC in Ndola | 2.43 | 1.41 | 4.2 | <0.01 | 2.22 | 1.17 | 4.20 | 0.01 |

Adj, adjusted; CI, confidence interval; CT, *Chlamydia trachomatis*; HRW, high-risk women; LARC, long-acting reversible contraception; IUD, intrauterine device; ref, reference group.

[a] The following variables were not significant in bivariate analyses and are not tabled: cohort, sex in exchange for money, pregnancy, HIV rapid test result, sperm (microscopy), candida (microscopy), BV, and new syphilis infection.

[b] Stepwise model selection done in both directions using the stepAIC function in R. The following variables were not significant in initial adjusted analyses and were removed from the final multivariate model via bi-directional stepwise selection and are not tabled: age group × city, literacy × city, TV, and TV × city. The final model contained 529 observations, including 68 with the outcome of interest.

**Table 5:**

Logistic regression models of factors associated with NG, Zambian HRW, 2016

| Predictor of NG[a] | Bivariate Association | | | | Multivariate Final Model[b] | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Odds Ratio | Lower 95% CI | Upper 95% CI | p-Value | Adj. Odds Ratio | Lower 95% CI | Upper 95% CI | Adj. p-Value |
| Age Group | | | | | | | | |
| Age 18–24 | 3.57 | 1.72 | 7.40 | <0.01 | 3.43 | 1.38 | 8.53 | 0.01 |
| Age 25+ | ref | - | - | - | ref | - | - | - |
| Education | | | | | | | | |
| No School or Primary School Only | 2.20 | 1.14 | 4.24 | 0.02 | 3.53 | 1.35 | 9.21 | 0.01 |
| Secondary School or Higher | ref | - | - | - | ref | - | - | - |
| Trichomonas vaginalis (microscopy) | | | | | | | | |
| Positive | 4.63 | 1.99 | 10.77 | <0.01 | 6.84 | 2.20 | 21.28 | <0.01 |
| Negative | ref | - | - | - | ref | - | - | - |
| BV (Both KOH+ & Clue Cells) | | | | | | | | |
| Positive | 2.64 | 1.46 | 4.79 | <0.01 | 2.31 | 1.10 | 4.87 | 0.03 |
| Negative | ref | - | - | - | ref | - | - | - |
| Incident syphilis (based on RPR) | | | | | | | | |
| Yes | 5.98 | 2.18 | 16.42 | <0.01 | 9.12 | 2.71 | 30.66 | <0.01 |
| No | ref | - | - | - | ref | - | - | - |

Adj, adjusted; BV, bacterial vaginosis; CI, confidence interval; HRW, high-risk women; KOH, potassium hydroxide; NG, *Neisseria gonorrhoeae*; ref, reference group; RPR rapid plasma reagin.

[a] The following variables were not significant in bivariate analyses and are not tabled: cohort, literacy, sex in exchange for money, unprotected sex, pregnancy, LARC usage, HIV rapid test result, sperm (microscopy), and candida (microscopy).

[b] Stepwise model selection done in both directions using the stepAIC function in R. The following variables were not significant in initial adjusted analyses and were removed from the final multivariate model via bi-directional stepwise selection and are not tabled: city, education × city, BV × city, new syphilis infection × city, and washes external genitalia with detergent. The final model contained 423 observations, including 34 with the outcome of interest.