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High Prevalences of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections in Anal and Pharyngeal Sites among Men who Have Sex with Men and Transgender Women in Lima, Peru

Short Title: Chlamydia and Gonorrhea in Anorectum and Oropharynx

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Summary

Objectives: The goal of this study was to analyze the prevalence of pharyngeal and anal chlamydial and/or gonococcal infections and their associated risk factors among men who have sex with men (MSM) in Lima, Peru.

Methods: A cross sectional analysis was performed on 718 MSM recruited for a subsequent community-based randomized controlled trial. The presence of *C. trachomatis* and *N. gonorrhoeae* were evaluated using Aptima Combo2 CT/NG in technician-assisted pharyngeal swabs and self-collected anal swabs. Behavioral and epidemiological data were also analyzed to explore correlates of both infections.

Results: Overall prevalence *of C. trachomatis* was 19.0% and 4.8% in anal and pharyngeal sites respectively, while prevalence of *N. gonorrhoeae* was 9.6% and 6.5% in anal and pharyngeal sites, respectively. The prevalence of each infection declined with participant's age.

Conclusions: We found a high prevalence of rectal and pharyngeal chlamydial and gonococcal infections. Efforts must be made to prevent extra-urogenital chlamydial and gonococcal infections in populations at high risk like MSM and transgender women in Peru.

Keywords: Chlamydia trachomatis, Neisseria gonorrhoeae, anogenital conditions, gay men, Latin America

Strengths and limitations of this study:

- First study in Latin America to describe the prevalence of non-genital infections in high-risk MSM and transgender populations
- Comprehensive assessment of anal and pharyngeal CT and NG infections.

- Ethnographic sampling might preclude generalizability of our findings to populations outside of the geographic areas under study.
- Oral sex was accessed in-depth; therefore it is not possible to link specific oral sex practices and pharyngeal infections.

Key Messages:

1. Rectal and pharyngeal testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* increase the detection of asymptomatic infections in men who have sex with men and transwomen.
2. Nucleic Acid Amplification tests (NAAT) are a valuable tool to diagnose extragenital gonococcal and chlamydial infections.
3. Screening of gonococcal and chlamydial infection should be offered as part of the regular medical care to High-risk populations and people living with HIV.

Introduction

The most common curable genital sexually transmitted infections (STI) worldwide are those caused by *Chlamydia* and *Neisseria* species.[1] They remain a major public health challenge because of their high prevalence and incidence, especially among vulnerable populations such as men who have sex with men (MSM) and transgender women (TW).[2,3] Simultaneous testing for both bacteria is highly recommended and many current diagnostic approaches combine the detection of both agents since co-infection is common.[4,5]

With regard to *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) infections in extragenital sites, their transmission from the urogenital tract of one individual to the oropharynx of another and vice versa has been previously reported. [6-8] Common sexual practices among MSM and TW such as fellatio, anilingus and both receptive and insertive condomless anal intercourse can facilitate CT and NG infections in non-commonly exposed anatomical sites. Those facts highlight the importance and complexities of the transmission of these bacteria given the potential of the anorectum and oropharynx to be infected or colonized and later serve as silent reservoirs and new sources of infections. However, many public health programs in middle-income countries such as Peru still do not routinely screen for NG and CT in anorectal and pharyngeal sites. [5,9,10]

The importance and potential impact of screening for NG and CT in non-genital sites, for men and women at high risk, has been shown in several previous studies. CT and NG infections in the oropharynx and/or the anorectum are much more frequent among MSM and TW than in heterosexual men. [6,11-15] The inclusion of CT and NG screening in some STI programs at public STI clinics worldwide has shown a significant increase in their detection especially when testing is targeted and based upon reported sexual practices and STI past history in these high-risk populations.[6,10,16]

The goal of this study was to describe the prevalence of CT and NG infections in both anorectum and oropharynx among MSM and TW in Lima, Peru.

Methods

Study Design, Study Population and Setting: We analyzed the baseline data of the "Comunidades Positivas and Enhanced Partner Therapy Trial" (ClinicalTrials.gov identifier: NCT00670163) collected during 2008-2009. This study was implemented in Lima, Peru between 2007 and 2012 and sponsored by the NIMH (NCT00670163). Using a snowball-like, peer-based referral approach, we recruited a total of 718 participants 18 to 45 years old, born biologically male, living and/or socializing in the study neighborhood reporting anal or oral sex with other men in the previous 12. This sample size was defined *a priori* to meet the aims of the original trial. Of the 718, 701provided self-collected anal swabs and 712 provided technician-assisted pharyngeal swabs. Free treatment was provided to all participants testing positive for CT and/or NG according to the Peruvian STI Management Guidelines (Azithromycin, 1.0 g single dose orally for CT and Cefixime, 400 mg single dose orally for NG).[17]

Data collection: Following voluntary informed consent, participants responded to a questionnaire using a Computer-Assisted Personal Interview (CAPI) system.[18] A subset of questions about HIV status was administered using Audio Computer-Assisted Self-Interviewing (ACASI) to protect confidentiality. The questionnaire consisted of 300 questions and took approximately 60 minutes to complete. Data collected included demographic characteristics, general health and health care seeking behavior, exposure to HIV/STI prevention messages, HIV testing history, sexual risk behaviors, and substance use. After completing the questionnaire, all participants went through pre-test counseling for STIs, including HIV infection, with a trained counselor according to the Peruvian STI Management guidelines.[17]

Laboratory Procedures: We trained all participants to self-collect anal swabs, using pictures and mirrors for guidance. Trained technicians collected pharyngeal swabs. We initially stored all collected samples in Aptima collection tubes and then transported them to our laboratory facilities in Lima where NAAT was performed. The detection of *CT* and/or *NG* infections was performed by using a NAAT test, the Transcription Mediated Amplification (TMA) with the Aptima Combo2 CT/NG test (Gen Probe Incorporated, San Diego, CA) following the manufacturer's directions. NAAT technology is well known for being highly sensitive and specific. [13,16,19,20] The results were given to the participants within two weeks of sample collection, along with post-test counseling and treatment as necessary according to Peruvian STI Management Guidelines.[17] Laboratory quality assurance was performed on a randomly chosen 10% of the samples and conducted by the Laboratory of the San Francisco Department of Public Health in California.

Variables and Statistical Analysis:

Outcomes: The four outcome variables of interest for this analysis were anorectal chlamydiasis, anorectal gonorrhoea, oropharyngeal chlamydiasis and oropharyngeal gonorrhoea. We calculated their prevalences and 95% confidence intervals overall and for each of the three self-reported sexual identity sub-groups (Gay/Homosexual, Male-to-Female transgender women, and Bisexual/Heterosexual). Composite outcomes for concomitant co-infection and presence of either infection at each anatomical site were also calculated.

Predictor variables: The following variables were used to describe the study population and analyzed to explore their associations with the outcome variables and predictive capacity: age; age at sexual debut; and number of sexual partners over the last 6 months (first summarized with median and interquartile range given their distribution departure from normality, and also later re-categorized in tertiles); educational attainment, self-reported sexual identity; preferred position during anal sex (insertive or receptive); condom use during anal intercourse (regardless of position) or oral sex during the previous 6 months; and if had compensated sex ever in life. Self-reported sexual identity was originally inquired by presenting each option by separate as well as an "other" open option for the respondent to type in on his/her own words what was most appropriate. Later collapsing of original single categories and re-distribution of "other" responses were discussed and agreed by the study team and convened by sample size convenience, prior knowledge about the field, as well as reasonably similar or close to similar expected and observed distribution of covariates during the exploratory phase of the analysis. Oral sex and condomless anal intercourse were summary binary variables constructed from specific responses provided by participants when asked for specific sexual behaviors with up to their last 3 most recent sexual partners. Birthplace and current work/study status were also used but only to describe the overall study sample.

Basic hypothesis testing: We tested the associations between categorized variables and each of the four outcomes by using either Chi square's test or Fisher's exact test when needed. Differences in the distribution of numeric variables between subjects with and without infection were assessed using the Wilcoxon's Rank Sum test. Within each sexual identity group, we also assessed the linear trend of the prevalence of each infection across age groups using the Chi square test for linear trends.

Statistical modelling approach: To model the outcomes as a function of the predictor variables we fitted crude and multivariable Log-Binomial Models under the Generalized Linear Models framework using a logarithmic link and binomial family settings. [21] We choose this approach over the traditional logistic regression widely used in the biomedical literature since it provides a quantification of the association in the form of Prevalence Ratios (PR) which are easier to communicate and interpret than Odds Ratios and more appropriate for a cross-sectional study like the present.[22] Moreover, in comparison to other alternatives described such as the Cox or Poisson regression models, the PR obtained are less prone to errors in interval estimation as well as less biased. [22-24]

Selection of variables for modelling: The selection and inclusion of variables into the models relied on a conceptual framework developed following guidelines previously described for epidemiological applications.[25] Conceptually, we considered three hierarchical levels of variables related to the outcome: a distal level (age and education); an intermediate level (number of partners, age at sexual debut and compensated sex); and, a proximal level (sexual identity, sexual role, and either condomless receptive anal intercourse or oral sex-the latter according to the outcome under analysis). These were used to construct a predictive rather than an explanatory model.[26] Using crude models, each given outcome was regressed on each variable from each level and only one variable from each level was selected according to its best fit measured by the lowest reported value of Akaike Information Criterion (AIC) for the distal and intermediate levels, or the Bayesian Information Criterion (BIC) for the proximal level. We adopted this approach given the specific properties of each information criterion: AIC tends to favor the best model among a set of candidate models while BIC tends to favor the "one true model" among a set of candidate models.[27-29] In our case, within the proximal level, we conceptualized the true crude model as the one including the more conceptually relevant exposure variable for the given outcomes under study (oral sex or condomless receptive anal intercourse). Moreover, from the biological transmission of infection point of view, these specific behaviors are the most relevant epidemiological exposures to consider.

Final models construction and diagnostics: A full multivariable model was constructed with the most relevant epidemiological exposures identified and adjusted by other selected variables (confounders) found to be significant at p=0.20 by means of the likelihood ratio test against their corresponding empty models.[30,31] We repeated this process for each of the 4 non-composite and the 2 composite outcomes (either infection). In order to avoid overfitting and instability in the final models we limited the number of variables to include in the models in such a way that there is between 10-16 events in the outcome per each dichotomous or dummy predictor along with sample size and total number of events considerations.[32] We assessed potential collinearity among predictors by measuring uncentered variance inflation factors and taken into account the context of other factors such as sample size that might also influence the variance of regression coefficients.[33] Specification link test and quadratic term adjusted prevalence ratios, along with their 95% confidence intervals computed from the Log-Binomial Models are presented to quantify the strength of the associations given the cross-sectional nature of the data, study design and analysis.

Ethical approval: The study was approved by the Institutional Ethics Committees at the University of California at Los Angeles and the Universidad Peruana Cayetano Heredia.

Results

Participant Characteristics: During 2008 and 2009, we enrolled 718 participants in Lima and surrounding areas. Participant age range was 18 to 45 years with a mean of 29 (SD 7.4 years). Most participants were born in the coastal region of Peru: 53.6% (385/718) in Lima and 32.2% (232/718) in other coastal cities. The remaining 14.1% (101/718) were born in non-coastal regions of Peru. Almost 80% of participants were concurrently working and/or studying (568/718). The mean age of sexual debut was 14 (SD 3.4 years). Most participants self-identified as gay or homosexual (64.8%), and preferring a receptive position during anal intercourse (67.5%). The median number of sexual partners in the last six months was 5 (IQR range, 2 – 15). Additional characteristics of the overall study population, stratified by specific outcome are described in Table 1.

Infections by anatomical site, infectious agent and sexual identity: Anal swabs were self-collected by 97.6% of participants (701/718) and in 99.2% (712/718) staff collected pharyngeal swabs for CT and NG testing. Overall anal infection by *Chlamydia trachomatis* was 19.0% (95% CI: 16.1-22.1) while pharyngeal infection was 9.6% (95% CI: 7.5-12.0). The prevalence of *Neisseria gonorrhoeae* was 4.8% (95% CI; 3.3-6.6) for anal infection and 6.5% (95% CI: 4.8-8.5) for pharyngeal infection. This pattern was evident within each sexual identity subgroup (Gay/homosexual, transgender women and bisexual), see Table 2.

In the anorectum, the prevalence of CT infection was almost twice (19%) as common as for GC (9.6%); this was true for the overall study population and within each sexual identity group. In the oropharynx, the prevalence of GC was slightly higher than for CT in all cases except for the bisexual sub-group. Comparing across sexual identities, the prevalence of each infection was more common in TW participants, followed by Gay/Homosexual and less common among bisexuals (See Table 3).

Overall, the prevalence of each of the four infections decreased with increasing age (Figure 1). The test for trends over age group was significant ($p<0.05$) in the following cases: within gay/homosexual for anal chlamydiasis, within gay/homosexual and transgender women for anal gonorrhea, and within transgender women for pharyngeal gonorrhea. For pharyngeal chlamydiasis a borderline significance ($p=0.08$) was found within TW.

Discussion

Using a snowball-like peer-based referral approach, we were able to reach a high-risk group of MSM and TW and detected high prevalence of CT and NG infections using both staff-collected pharyngeal and self-collected anal swabs.

Gonococcal prevalence in anorectal samples was higher than a previous reported sentinel surveillance data from the Peruvian Ministry of Health, 1996-2002. That report detailed a decrease in anal NG infection from 5.1% in 1996 to 0.2% in 2002,[34] However, their method for gonococcal diagnostics was culture of anal secretion and it is very well known that culture has low sensitivity for the detection of infection.[35] Culturing gonorrhea from samples with multiple other organisms can be very difficult; NAAT and especially TMA offer a reliable method for the rapid and accurate detection of anal and pharyngeal NG infection without the limitations of culture.[36] Chlamydial prevalence also was quite high in this group of MSM, especially in anorectal samples, where we found the highest prevalence of infection. A previous report of people living with HIV infection tested for pharyngeal chlamydia infection did not find any positive cases.[37] Other studies among MSM and heterosexual young men in Peru only detected chlamydia in urine samples. A national survey conducted among young adults found a prevalence of 4.2% in urogenital samples. [38]

Prevalence of CT and NG decreased with age; this can be explained by an increase in the awareness of STIs and decrease in exposure and the number of sex partners with age. Also, even if not treated, CT infection can be cleared in certain groups of infected people.[39] This inverse association between age and chlamydial infection has been also reported in other studies.[40,41]

This is the first study in Peru that used TMA technology for CT and NG infection testing in extragenital sites and our study highlights the potential importance of extragenital screening of CT and NG. Currently, the Peruvian STI Management Guidelines[17] establish that all female sex workers and male sex workers are subject to Periodic Medical Assessment, this monthly strategy includes STI assessment based on a syndromic approach. If urethral discharge is found, gonorrhea culture must be performed and treatment for chlamydia and gonorrhea should be offered. There is no further testing for chlamydial infection, and asymptomatic cases for both CT and NG infections, especially in extra-genital sites, can easily be missed. According to the current US Centers for Disease Control and Prevention recommendations nucleic acid testing should be available for CT and NG diagnosis.[36]

Our data also showed that the MSM in our study had a high number of sex partners. We found an association between having anal NG infection with the number of sex partners in the last six months. The high level of infection can be explained in part because of the high level of other infections (NG pharyngeal, CT anal and pharyngeal). The high burden of infection in the population contributes to higher rates of exposure in persons with multiple sex partners.

There were several limitations in our study. The participants tested were not randomly selected and all belonged to groups at high risk for STI from low-income neighborhoods in the metropolitan area of Lima, thus our results are not generalizable to all MSM and TW. However, given that this is a hard to reach population, ethnographic techniques were used to identify all of the MSM/TW within a neighborhood. Currently there are no FDA-cleared CT/NG tests available for non-urogenital sites but the CT/NG Aptima Combo-2 test has shown excellent performance when used for pharyngeal and anal samples. [13,42,43]

In conclusion, infection by *Chlamydia trachomatis* and *Neisseria gonorrhoeae* extragenital sites (anorectum and oropharynx) are very common among MSM and TW. Their overall prevalence tends to decrease with older age. Screening for extragenital CT and NG at non-health care facilities located in the actual venues where communities at high risk congregate by using temporary mobile teams is feasible through a combination of coordinated work with the community and by using robust laboratory methods like NAAT technology. Those approaches could be used by the Ministry of health in order to enhance the control of STIs among groups at high risk in Peru.

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Licence for Publication Statement

Table 1: Overall study population characteristics and their bivariate associations with anal and pharyngeal infections caused by C trachomatis and N gonorrhoeae among 718 MSM/TW participants in Lima, Peru 2008-2009.

Characteristics		Total enrolled (N=718)		Provided anal swabs (N=701)				Provided throat swabs (N=712)			
				(CT+ = 133)		(GC+ = 67)		(CT+ = 34)		(GC+ = 46)	
		n	%	n	%	n	%	n	%	n	%
Demographic characteristics											
Age											
Median (IQR)		29	(23-35)	**26**	**(22-30)**	**24**	**(21-29)**	**25**	**(22-29)**	**25**	**(23-29)**
Tertile 1: 18-25		261	(36.4)	**61**	**(24.1)**	**37**	**(14.6)**	**18**	**(7.0)**	**24**	**(9.3)**
Tertile 2: 26-33		242	(33.7)	**50**	**(21.2)**	**25**	**(10.6)**	**12**	**(5.0)**	**16**	**(6.7)**
Tertile 3: 34-45		215	(29.9)	**22**	**(10.4)**	**5**	**(2.4)**	**4**	**(1.9)**	**6**	**(2.8)**
Educational attainment											
At least some beyond HS		204	(28.4)	42	(21.1)	19	(9.6)	9	(4.5)	15	(7.5)
Full/partially attended HS		448	(62.4)	83	(19.0)	38	(8.7)	22	(4.9)	24	(5.4)
Elementary or none		66	(9.2)	8	(12.1)	10	(15.2)	3	(4.6)	7	(10.6)
Self-reported sexual identity											
Gay/Homosexual		465	(64.8)	87	(19.2)	39	(8.6)	19	(4.1)	25	(5.4)
Transgender women		208	(29.0)	41	(20.1)	25	(12.3)	14	(6.7)	20	(9.6)
Bisexual/Heterosexual		45	(6.2)	5	(11.4)	3	(6.8)	1	(2.3)	1	(2.3)
Sexual Characteristics											
Sexual role during anal sex											
Pasivo		485	(67.5)	89	(18.8)	45	(9.5)	23	(4.8)	30	(6.2)
Moderno		188	(26.2)	41	(22.2)	20	(10.8)	9	(4.8)	13	(7.0)
Activo		45	(6.3)	3	(7.0)	2	(4.7)	2	(4.4)	3	(6.7)
Sexual debut											
Median (IQR)		14	(12-16)	14	(12-16)	14	(12-15)	13	(12-15)	14	(13-17)
Tertile 1: 13 or younger		294	(41.0)	61	(21.0)	28	(9.7)	18	(6.2)	15	(5.1)
Tertile 2: 14-15		188	(26.2)	38	(20.8)	24	(13.1)	9	(4.8)	14	(7.5)
Tertile 3: 16 or older		236	(32.8)	34	(14.9)	15	(6.6)	7	(3.0)	17	(7.3)
Sexual partners over the last 6 mo*											
Median (IQR)		5	(2-15)	5	(2-15)	**7**	**(4-30)**	**7**	**(3-30)**	6	(2-18)
1 partner		111	(16.1)	14	(12.8)	**5**	**(4.6)**	2	(1.9)	8	(7.4)
2 partners		117	(16.9)	29	(25.7)	**4**	**(3.5)**	5	(4.3)	5	(4.3)
3-10 partners		262	(37.9)	52	(20.4)	**28**	**(11.0)**	10	(3.8)	16	(6.1)
More than 10 partners		201	(29.1)	37	(18.7)	**26**	**(13.1)**	13	(6.5)	14	(7.0)
Sexual behaviors over the last 6 mo											
C-IAI with men	Yes	131	(18.3)	18	(14.2)	**5**	**(3.9)**	4	(3.1)	8	(6.2)
	No	587	(81.7)	115	(20.0)	**62**	**(10.8)**	30	(5.2)	38	(6.5)
C-RAI sex with men	Yes	469	(65.3)	**97**	**(21.2)**	49	(10.7)	23	(5.0)	30	(6.5)
	No	249	(34.7)	**36**	**(14.8)**	18	(7.4)	11	(4.4)	16	(6.5)
Oral sex with men	Yes	472	(65.7)	92	(19.7)	46	(9.9)	25	(5.3)	33	(7.0)

	No	246	(34.3)	41	(17.5)	21	(8.9)	9	(3.7)	13	(5.4)
Compensated sex	Yes	394	(54.9)	73	(19.0)	**47**	**(12.2)**	23	(5.9)	26	(6.6)
	No	324	(45.1)	60	(19.0)	**20**	**(6.3)**	11	(3.4)	20	(6.3)

IQR: Interquartile range ; p<0.05 for bolded values ; p<0.10 for underlined values ; *n=691 for this variable

C-RAI: Condomless receptive anal intercourse C-IAI: Condomless insertive anal intercourse

CT+: Positive result for Chlamydia trachomatis infection GC+: Positive result for Neisseria Gonorrhoeae

Values between parenthesis are percentages in row display except for the following variables: age, age at sexual debut and number of partners

Bivariate associations between categorical variables tested either using chi square or Fisher's exact test according to expected values

Differences in the distribution of age, age at sexual debut and number of partners between subjects with and without each of the infections tested using Wilcoxon's Rank Sum Test

Table 2: Prevalence of anal and pharyngeal infections caused by C trachomatis and/or N gonorrhoeae among 718 MSM/TW participants and sub-groups by self-reported sexual identity

Type of infection	Total enrolled (N=718)		Gay/Homosexual (N=465)		Transgender women (N=208)		Bisexual/Hetero (N=45)		p value
	n/N	%	n/N	%	n/N	%	n/N	%	
Anal									
C trachomatis	133/701	(19.0)	87/453	(19.2)	41/204	(20.1)	5/44	(11.4)	0.40
N gonorrhoeae	67/701	(9.6)	39/453	(8.6)	25/204	(12.3)	3/44	(6.8)	0.28
Either	169/701	(24.1)	105/453	(23.2)	57/204	(27.9)	7/44	(15.9)	0.18
Both concurrently	31/701	(4.4)	21/453	(4.6)	9/204	(4.4)	1/44	(2.3)	0.77
Pharingeal									
C trachomatis	34/712	(4.8)	19/460	(4.1)	14/208	(6.7)	1/44	(2.3)	0.25
N gonorrhoeae	46/712	(6.5)	25/460	(5.4)	20/208	(9.6)	1/44	(2.3)	0.06
Either	73/712	(10.2)	41/460	(8.9)	30/208	(14.4)	2/44	(4.6)	0.04
Both concurrently	7/712	(1.0)	3/460	(0.7)	4/208	(1.9)	0/44	(0.0)	0.24

p value from Chi square or Fisher's exact test to assess the association between infections and sub-groups

Values between parentheses are percentages in column display

Out 718 participants, 701 provided anal swabs and 712 pharyngeal swabs

Table 3: Multivariable models for each specific single or composite either infection by anatomic site among 718 MSM/TW participants in Lima, Peru 2008-2009.

Anatomic site	Variables included and their categories		Infection					
			C Trachomatis		N gonorrhoeae		Either bacteria	
			PR (95%CI)	p	PR (95%CI)	p	PR (95%CI)	p
Anal	C-RAI	Yes	1.4 (1.0-2.0)	0.07	1.6 (0.9-2.7)	0.11	1.4 (1.0-1.8)	0.03
		No	Ref		Ref		Ref	
	Partners	>10	1.4 (0.8-2.5)	0.25	2.6 (1.0-6.5)	0.04	1.5 (1.0-2.5)	0.07
		3-10	1.6 (0.9-2.7)	0.11	2.3 (0.9-5.7)	0.08	1.5 (0.9-2.3)	0.12
		2	2.1 (1.2-3.7)	0.01	0.8 (0.2-2.2)	0.74	1.7 (1.0-2.8)	0.05
		1	Ref		Ref		Ref	
	Age (years)	34-45	0.5 (0.3-0.7)	<0.01	0.2 (0.1-0.5)	<0.01	0.4 (0.2-0.6)	<0.01
		26-33	0.9 (0.7-1.3)	0.52	0.8 (0.5-1.2)	0.28	0.8 (0.6-1.1)	0.18
		18-25	Ref		Ref		Ref	
	Sample size		675		675		675	
	Number of events		132		63		164	
	AIC		654.5		397.1		723.3	
	BIC		686.1		428.7		754.9	

Anatomic site	Variables included and their categories		Infection					
			C Trachomatis		N gonorrhoeae		Either bacteria	
			PR (95%CI)	p	PR (95%CI)	p	PR (95%CI)	p
Pharingeal	Oral sex	Yes	1.4 (0.7-2.9)	0.38	1.3 (0.7-2.4)	0.45	1.2 (0.8-2.0)	0.38
		No	Ref		Ref		Ref	
	Age (years)	34-45	0.3 (0.1-0.8)	0.02	0.3 (0.1-0.7)	<0.01	0.3 (0.2-0.6)	<0.01
		26-33	0.7 (0.3-1.4)	0.32	0.7 (0.4-1.3)	0.26	0.7 (0.5-1.2)	0.22
		18-25	Ref		Ref		Ref	
	Sample size		686		686		686	
	Number of events		30		43		67	
	AIC		272.9		339.4		464.9	
	BIC		291.1		357.7		483.1	

C-RAI: Condomless receptive anal intercourse

Partners: Number of sexual partners reported over the last 6 months

Number of events: Number of subjects with the specific outcome modeled

AIC: Akaike Information Criterion BIC: Bayesian Information Criterion

Oral sex: No distinction in the specific role during oral sex, just engagement over the last 6 months

PR: Adjusted Prevalence Ratios along with 95%-level confidence interval

Ref: Specific sub-category used as baseline reference for the analysis

Contributorship statement

SRL monitored data collection for the whole study, drafted and revised the paper; ERS and KAK wrote the statistical analysis plan, cleaned and analyzed the data, drafted and revised the paper; JAF supervised the laboratory testing; ASS monitored the data collection and revised the paper; JTG monitored data collection and supervised ethical issues of the study; JDK assessed the biological and treatment component; TJC and CFC designed the study and revised the paper.

Competing interests

None declared

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Data sharing statement

No additional data available

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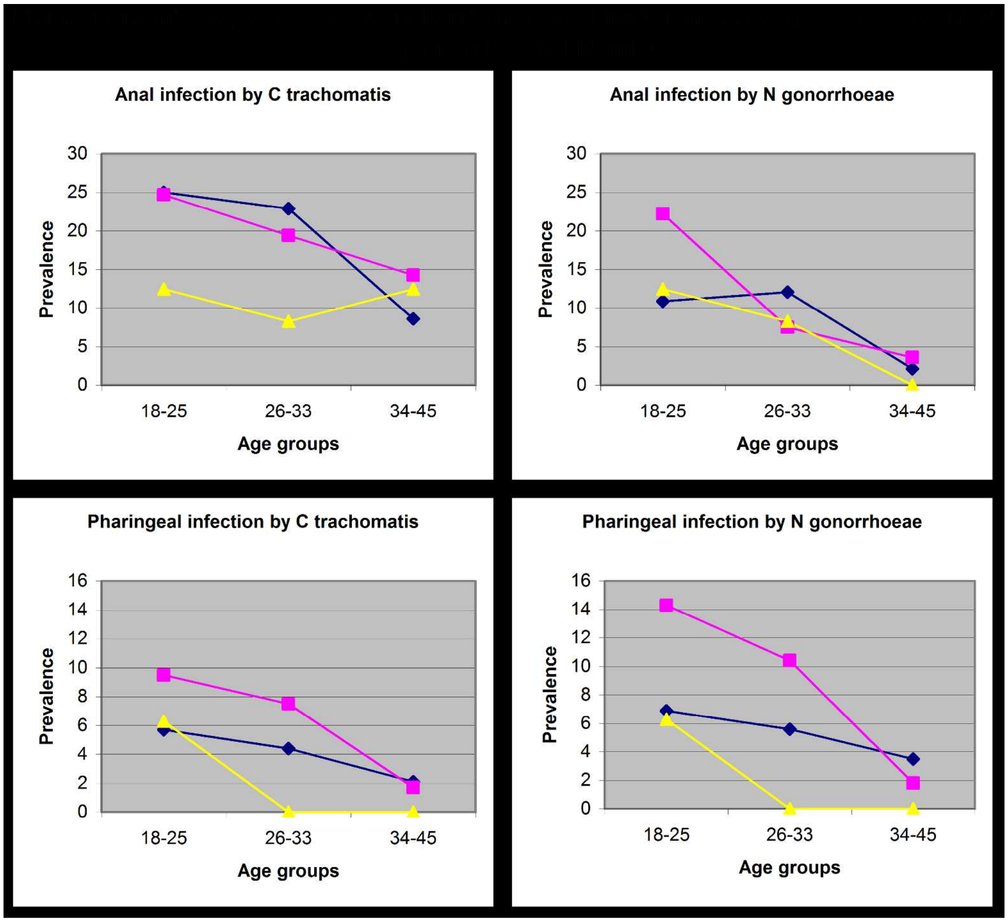

Legend: Pink = Transgender women; Blue = Gay/Homosexual; Yellow = Bisexual
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High Prevalences of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections in Anal and Pharyngeal Sites among a community-based sample of Men who Have Sex with Men and Transgender Women in Lima, Peru

Short Title: Chlamydia and Gonorrhea in Anorectum and Oropharynx

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Summary

This study aimed to characterize the epidemiology of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae (NG)* infections among men who have sex with men (MSM) and transgender women (TW) in Lima, Peru. A cross sectional analysis was performed with 510 MSM and 208 TW recruited for a subsequent community-based randomized controlled trial. The presence of CT and NG were evaluated using Aptima Combo2 in pharyngeal and anal swabs. We also explored correlates of these infections. Overall prevalence of C. trachomatis was 19.0% (95% CI: 16.1-22.1) and 4.8% (95% CI: 3.3-6.6) in anal and pharyngeal sites respectively, while prevalence of N. gonorrhoeae was 9.6% () and 6.5% () in anal and pharyngeal sites, respectively. The prevalence of each infection declined significantly among participant's older than 34 years (p<0.05). Efforts towards prevention and treatment of extra-urogenital chlamydial and gonococcal infections in high risk populations like MSM and TW in Lima, Peru are warranted.

Keywords: Chlamydia, Gonorrhea, anorectal, oropharynx, co-infection, MSM, Peru

Key messages

1. Currently, there is no further testing for chlamydial or gonorrheal infection; and asymptomatic in extra-genital sites, can easily be missed and neglected.
2. Rectal and pharyngeal testing for Chlamydia trachomatis and Neisseria gonorrhoeae using NAAT increase the detection of asymptomatic infections in men who have sex with men and transwomen in Lima, Peru.
3. Screening for gonococcal and chlamydial infection should be offered as part of the regular medical care to high-risk populations and people living with HIV and could include community approach through mobile teams.

Introduction

The most common curable genital sexually transmitted infections (STI) worldwide are those caused by *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG).[1] They remain a major public health challenge because of their high prevalence and incidence, especially among high risk populations such as men who have sex with men (MSM) and transgender women (TW).[2, 3] Simultaneous testing for both bacteria is highly recommended and many current diagnostic approaches combine the detection of both agents since co-infection is common.[4, 5]

With regard to CT and NG infections in extragenital sites, their transmission from the urogenital tract of one individual to the oropharynx of another and vice versa has been previously reported.[6-8] Common sexual practices among MSM and TW such as fellatio, anilingus and both receptive and insertive condomless anal intercourse can facilitate CT and NG infections in exposed anatomical sites. These facts highlight the importance and complexities of the transmission of these bacteria given the potential of the anorectum and oropharynx to be infected or colonized and then to serve as silent reservoirs and new sources of infections. However, many public health programs in low and middle-income countries such as Peru still do not routinely screen for NG and CT in anorectal and pharyngeal sites. [5, 9, 10]

The importance and potential impact of screening for CT and NG in non-genital sites, for high-risk men and women, has been shown in several previous studies.[11, 12] CT and NG infections in the oropharynx and/or the anorectum are much more frequent among MSM and TW than in heterosexual men. [6, 13-17] The inclusion of CT and NG screening in some STI programs at public STI clinics worldwide has showed a significant increase in their detection especially when testing is targeted and based upon reported sexual practices and STI past history in these high-risk populations.[6, 10, 18]

This study aimed to characterize the epidemiology of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae (NG)* infections among men who have sex with men (MSM) and transgender women (TW) in Lima, Peru.

Methods

Study Design, Study Population and Setting:

We analyzed the baseline data of the "Comunidades Positivas and Enhanced Partner Therapy Trial" (ClinicalTrials.gov identifier: NCT00670163) collected during 2008-2009 for a larger clinical trial which was implemented in Lima, Peru until 2012. This timeframe included planning, implementation and data analysis.. We recruited 718 participants who were 18 to 45 years old, born biologically male, who lived and/or socialized in the study neighborhoods, had anal or oral sex with other men in the previous 12 months and expressed sexual preference for men. HIV status was not considered for enrollment purposes. Recruitment used a snowball-like, peer-based referral approach in sites previously identified by ethnographic methods. This sample size was defined *a priori* to meet the aims of the original trial. Among the trial participants, 701 agreed to provide self-collected anal swabs and 712 agreed to provide technician-assisted collection of pharyngeal swabs for screening. We offered free treatment to all participants resulting with a positive test for CT and/or NG according to the Peruvian STI Management Guidelines (Azithromycin, 1.0 g single dose orally for CT and Cefixime, 400 mg single dose orally for NG).[19]

Data collection:

Mobile study teams went to each study neighborhood for 1 to 2 weeks to implement all study procedures using temporary offices. Once the participant volunteered and consented in written to participate in the study, they were asked to respond to a questionnaire using a Computer-Assisted Personal Interview (CAPI) system.[20] The questionnaire collected information on demographic characteristics, general health and health care seeking behavior, exposure to HIV/STI prevention messages, HIV testing history, sexual risk behaviors, and substance use and took approximately 60 minutes to complete. After completing the questionnaire, all participants went through pre-test counseling for STIs, including HIV infection, with a trained counselor according to the Peruvian STI Management guidelines.[19]

Laboratory Procedures:

We trained all participants to self-collect anal swabs, using pictures and mirrors for guidance. Trained technicians collected pharyngeal swabs by swabbing the tonsils. We initially stored all collected samples in Aptima collection tubes and then transported them to our laboratory facilities in Lima where nucleic acid amplification testing (NAAT) was performed. Specifically, the detection of *CT* and/or *NG* infections was performed by means of a Transcription Mediated Amplification (TMA) with the Aptima Combo2 CT/NG test (Gen Probe Incorporated, San Diego, CA) following the manufacturer's directions. NAAT technology is well known for being highly sensitive and specific.[15, 18, 21, 22] The results were given to the participants within two weeks of sample collection, along with post-test counseling and treatment as necessary according to Peruvian STI Management Guidelines.[19] Laboratory quality assurance was performed on a randomly selected 10% of the samples and conducted by the Laboratory of the San Francisco Department of Public Health in California.

Variables and Statistical Analysis:

Outcomes: The four outcome variables of interest for this analysis were anorectal chlamydiasis, anorectal gonorrhoea, oropharyngeal chlamydiasis and oropharyngeal gonorrhoea. We calculated their prevalences and 95% confidence intervals overall and for each of the three self-reported sexual and gender identity sub-groups (Gay/Homosexual, Male-to-Female transgender women, and Bisexual/Heterosexual). Composite outcomes for any infection and concomitant co-infection at each anatomical site were also calculated. The concomitant co-infection outcome was generated in order to gain statistical power for later modeling analysis, especially for the oral infection outcomes given their very low absolute numbers.

Correlates: The following variables were used to describe the study population and analyzed to explore their associations with the outcome variables: age, age at sexual debut, and number of sexual partners over the last 6 months (given their distribution departure from normality, these were re-categorized in tertiles), educational attainment, self-reported sexual/gender identity, preferred role during anal sex, engagement in condomless anal intercourse, engagement in oral sex (without differentiation if giving or receiving) , and ever engaging in compensated sex. Oral sex and condomless anal intercourse were summary binary variables constructed from responses provided by participants when asked for specific sexual behaviors with up to 3 most recent sexual partners in the past 6 months.

Statistical analysis: We tested associations between categorized variables and each of the four outcomes by using either Chi square's test or Fisher's exact test when needed. Differences in the distribution of numeric variables between subjects with and without infection were assessed using the Wilcoxon's Rank Sum test. Within each sexual/gender identity group, we also assessed the linear trend of the prevalence of each infection across age groups using the Chi square test for linear trends. To model the outcomes as a function of the exposure (correlate) variables of a priori interest we fit multivariable Log-Binomial Models under the

Generalized Linear Models framework using a logarithmic link and binomial family settings.[23] This approach estimates Prevalence Ratios (PR)which are more appropriate for a cross-sectional study like the present.[24] Moreover, in comparison to other alternatives, such as the Cox or Poisson regression models, the PR obtained are less prone to errors in interval estimation as well as less biased.

The selection and inclusion of variables into the final multivariate models relied on a conceptual framework developed by the authors following guidelines previously described for epidemiological applications.[25] Conceptually, we considered three hierarchical levels of variables related to the outcome: a distal level (age and education), an intermediate level (sexual identity, sexual role, number of partners and age at sexual debut) and a proximal level (compensated sex and either condomless receptive anal intercourse or oral sex-the latter according to the outcome under analysis). Each anal infection outcome was regressed on each variable at a time and only one variable from each level was selected according to its best fit measured by the lowest reported value of Akaike Information Criterion (AIC) for the distal and intermediate levels, or the Bayesian Information Criterion (BIC) for the proximal level. We adopted this approach given the specific properties of each information criterion: AIC tends to favor the best model among a set of candidate models while BIC tends to favor the "one true model" among a set of candidate models.[26, 27] For the oral infections outcomes we followed the same approach but later further limited the number of correlates in the final model to avoid overfitting and stick to the proximal and distal levels only. In general, In order to avoid overfitting and instability in the final models we limited the number of variables to include in the models in such a way that there is about between 10-16 events in the outcome per each dichotomous or dummy predictor along with sample size and total number of events considerations.[28] We assessed potential collinearity among predictors by measuring uncentered variance inflation factors*Ethical approval*: The study was approved by the IRB of the University of California at Los Angeles and for the Institutional Ethics Committee of the Universidad Peruana Cayetano Heredia.

Results

Participants Characteristics:

During 2008 and 2009, we enrolled 718 participants in Lima and surrounding areas. Participant age range was 18 to 45 years old. Most of them were born in the coastal region of Peru: 53.6% (385/718) in Lima and 32.2% (232/718) in other coastal cities. The remaining 14.1% (101/718) were born in non-coastal regions of Peru. Almost 80% of participants were concurrently working and/or studying (568/718). The mean age of sexual debut was 14 years. Most participants self-reported as gay or homosexual (64.8%), and having preference for a pasivo sexual role (67.5%). The median number of sexual partners in the last six months was 5.. Further details are described in Table 1.

Infections by anatomical site, infectious agent and sexual identity:

Anal swabs were provided by 97.6% of participants (701/718) and 99.2% (712/718) provided pharyngeal swabs for CT and NG testing. Overall, anal infection by *Chlamydia trachomatis* was 19.0% (95% CI: 16.1-22.1) while pharyngeal infection was 4.8% (95% CI: 3.3-6.6). The prevalence of *Neisseria gonorrhoeae* was 9.6% (95% CI: 7.5-12.0) for anal infection and 6.5% (95% CI: 4.8-8.5) for pharyngeal infection. This pattern of higher CT infection in anorectum and higher NG infection in pharinx was evident within each sexual identity subgroup (Gay/homosexual, transgender women and bisexual). Further details are described in Table 2.

In the anorectum, the prevalence of CT infection was almost twice (19%) as common as for NG (9.6%); this was true for the overall study population and within each sexual identity group. In the oropharynx, the prevalence of NG was slightly higher than for CT in all cases except for the bisexual sub-group. Comparing across sexual identities, the prevalence of each single and composite outcome infection was more common in TW participants, followed by Gay/Homosexual and less common among bisexuals in most of the cases, though these differences were not statistically significant. The only statistically significant difference was for either pharyngeal infection, p-value <0.05, see Table 2.

Overall, the prevalence of each of the four outcomes of the study decreases with increasing age. The test for trends over age group was significant (p<0.05) only in the following cases: within gay/homosexual for anal chlamydiasis, within gay/homosexual and transgender women for anal gonorrhea, and within transgender women for pharyngeal gonorrhea. For pharyngeal chlamydiasis a borderline statistical significance for trend (p=0.08) was found within TW. The graphical description of trends is shown in Figure 1.

Statistical models for outcomes and laboratory quality assurance

The six final multivariate models are presented in table 3. Briefly, the higher age group consistently remained inversely associated to all single and composite outcomes. For the oral infection outcomes, both oral sex and compensated sex were associated with higher proportions of infection though they were not statistically significant. For the anal infection outcomes, the number of sexual partners showed an erratic pattern of association and not statistically significant in most of the cases while condomless receptive anal intercourse remained directly and significantly associated with the infections in the models where it was included. The results of the laboratory quality assurance performed on 10% of the samples showed 100% of correlation (Data not shown).

Discussion

Using a snowball-like peer-based referral approach, we were able to reach a group of MSM and TW at high-risk as evidenced by their high prevalence of CT and NG infections. Using both technician-assisted pharyngeal swabs and self-collected anal swabs we found that close to 25% of the study population had anal infection by either CT or NG, while close to 10% had oropharynx infection by the same bacteria. The prevalence of these infections tends to decrease with age. In these regards, these findings constitute a very important update in the Peruvian scenario and become one of the few available studies in Latin America.

Gonorrhea prevalence in anorectal samples was higher than previously reported in HIV and STIs sentinel surveillance studies conducted among MSM and TW by the Peruvian Minister of Health during 1996-2002, this report detailed a decrease in anal NG infection from 5.1% in 1996 to 0.2% in 2002,[29] However, their method for gonorrhea diagnostics were the regular culture of urethral secretion and it is very well known that culture only detects viable bacteria in people with active infection.[30] Moreover, culturing NG from highly-contaminated samples can be very difficult. NAAT and especially TMA are a more reasonable approach for the rapid and accurate diagnosis of anal and pharyngeal gonorrhea without the limitations of culture.[31] However, one limitation of the NAAT technology is that current non-active infections can result in a positive test since the method assesses the presence of RNA and or DNA instead of live bacteria.[32]

Chlamydial infection prevalence also was quite high in this group of MSM and TW, especially in anorectal samples, where we found the highest prevalence of infection. A previous report of Peruvian people living with HIV who were tested for pharyngeal chlamydia infection did not find any positive cases.[33] Other studies among MSM and young men in Peru only detected chlamydia in urine samples.[34] A previous country-level study conducted among young Peruvian adults from the general population found a prevalence of 4.2% for CT in urogenital samples. [35] In this regards, our study adds important data to better understand the epidemiology of CT infection in Peru.

Prevalence of chlamydia and gonorrhea decreases with age and this can be explained by an increase in the awareness of STI infections and decreases in the number of sex partners with the age.[4, 36] Even if not treated, chlamydia can be cleared in certain group of infected people.[37] This inverse association between age and chlamydial infection has been also reported in other studies.[38, 39]

This is the first study in Peru that uses TMA technology for testing CT and NG infection in extragenital sites and our study highlight the importance of extragenital screening of CT and NG. Currently, the Peruvian STI Management Guidelines[19] establish that all female and male sex workers are subject to Periodic Medical Assessment, this monthly strategy includes STI assessment based on a syndromic approach. If urethral discharge is found, gonorrhea culture must be performed and treatment for chlamydia and gonorrhea should be offered. There is no further testing for chlamydial infection; and asymptomatic cases for both CT and NG infections, especially in extra-genital sites, can easily be missed and neglected. According to the current CDC recommendations NAAT testing should be available for CT and NG diagnosis.[31]

There are some limitations in our analysis. The participants tested were not randomly selected and all of them belong to high-risk groups (since they originally were enrolled for a trial implementation) from low-income neighborhoods in the metropolitan area of Lima, thus our results can show higher prevalences than random MSM or TW could have, and are not necessarily generalizable to all MSM and TW in Lima or Peru. However, given that this is a hidden population, the ethnographic techniques initially used to identify all of the MSM and TW within a neighborhood represent a nearly saturated sample for each community included.

At the moment there are not FDA-cleared CT/NG tests available for non-urogenital sites but the CT/NG Aptima Combo-2 test has shown good performance when used for pharyngeal and anal samples. [15, 40, 41]

In conclusion, infection by *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in extragenital sites (anorectum and oropharynx) are very common among MSM and TW in Lima, Peru and both infections need to be incorporated into the regular STI testing programs in Peru. The overall prevalence of CT and NG tends to decrease over age although this needs confirmation with further longitudinal analysis. Screening and treatment for extragenital CT and NG at non-health care facilities located in the actual venues where this populations are by means of temporary mobile teams is feasible through a combination of coordinated work with the community, use of NAAT technology and previous ethnographic work. These approaches should be used by the Ministry of health in order to better control STIs in Lima and possibly in other cities of Peru.

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Disclaimer

All authors: no conflicts

Table 1: Overall study population characteristics and their bivariate associations with anal and pharyngeal infections caused by C trachomatis and N gonorrhoeae among 718 MSM/TW participants in Lima, Peru 2008-2009.

		Total enrolled (N=718)		Provided anal swabs (N=701)				Provided throat swabs (N=712)			
				(CT+ = 133)		(NG+ = 67)		(CT+ = 34)		(NG+ = 46)	
Characteristics		n	%	n	%	n	%	n	%	n	%
Demographic characteristics											
Age											
Median (IQR)		29	(23-35)	**26**	**(22-30)**	**24**	**(21-29)**	**25**	**(22-29)**	**25**	**(23-29)**
Tertile 1: 18-25		261	(36.4)	**61**	**(24.1)**	**37**	**(14.6)**	**18**	**(7.0)**	**24**	**(9.3)**
Tertile 2: 26-33		242	(33.7)	**50**	**(21.2)**	**25**	**(10.6)**	**12**	**(5.0)**	**16**	**(6.7)**
Tertile 3: 34-45		215	(29.9)	**22**	**(10.4)**	**5**	**(2.4)**	**4**	**(1.9)**	**6**	**(2.8)**
Educational attainment											
At least some beyond HS		204	(28.4)	42	(21.1)	19	(9.6)	9	(4.5)	15	(7.5)
Full/partially attended HS		448	(62.4)	83	(19.0)	38	(8.7)	22	(4.9)	24	(5.4)
Elementary or none		66	(9.2)	8	(12.1)	10	(15.2)	3	(4.6)	7	(10.6)
Self-reported sexual identity											
Gay/Homosexual		465	(64.8)	87	(19.2)	39	(8.6)	19	(4.1)	*25*	*(5.4)*
Transgender women		208	(29.0)	41	(20.1)	25	(12.3)	14	(6.7)	*20*	*(9.6)*
Bisexual/Heterosexual		45	(6.2)	5	(11.4)	3	(6.8)	1	(2.3)	*1*	*(2.3)*
Sexual Characteristics											
Sexual role during anal sex											
Pasivo		485	(67.5)	*89*	*(18.8)*	45	(9.5)	23	(4.8)	30	(6.2)
Moderno		188	(26.2)	*41*	*(22.2)*	20	(10.8)	9	(4.8)	13	(7.0)
Activo		45	(6.3)	*3*	*(7.0)*	2	(4.7)	2	(4.4)	3	(6.7)
Sexual debut											
Median (IQR)		14	(12-16)	14	(12-16)	14	(12-15)	13	(12-15)	14	(13-17)
Tertile 1: 13 or younger		294	(41.0)	61	(21.0)	*28*	*(9.7)*	18	(6.2)	15	(5.1)
Tertile 2: 14-15		188	(26.2)	38	(20.8)	*24*	*(13.1)*	9	(4.8)	14	(7.5)
Tertile 3: 16 or older		236	(32.8)	34	(14.9)	*15*	*(6.6)*	7	(3.0)	17	(7.3)
Sexual partners over the last 6 mo*											
Median (IQR)		5	(2-15)	5	(2-15)	**7**	**(4-30)**	**7**	**(3-30)**	6	(2-18)
1 partner		111	(16.1)	14	(12.8)	**5**	**(4.6)**	2	(1.9)	8	(7.4)
2 partners		117	(16.9)	29	(25.7)	**4**	**(3.5)**	5	(4.3)	5	(4.3)
3-10 partners		262	(37.9)	52	(20.4)	**28**	**(11.0)**	10	(3.8)	16	(6.1)
More than 10 partners		201	(29.1)	37	(18.7)	**26**	**(13.1)**	13	(6.5)	14	(7.0)
Sexual behaviors over the last 6 mo											
C-IAI with men	Yes	131	(18.3)	18	(14.2)	**5**	**(3.9)**	4	(3.1)	8	(6.2)
	No	587	(81.7)	115	(20.0)	**62**	**(10.8)**	30	(5.2)	38	(6.5)
C-RAI sex with men	Yes	469	(65.3)	**97**	**(21.2)**	49	(10.7)	23	(5.0)	30	(6.5)
	No	249	(34.7)	**36**	**(14.8)**	18	(7.4)	11	(4.4)	16	(6.5)
Oral sex with men	Yes	472	(65.7)	92	(19.7)	46	(9.9)	25	(5.3)	33	(7.0)
	No	246	(34.3)	41	(17.5)	21	(8.9)	9	(3.7)	13	(5.4)

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Compensated sex	Yes	394	(54.9)	73	(19.0)	**47**	**(12.2)**	23	(5.9)	26	(6.6)
	No	324	(45.1)	60	(19.0)	**20**	**(6.3)**	11	(3.4)	20	(6.3)

IQR: Interquartile range; p<0.05 for bolded values; p<0.10 for underlined values; *n=691 for this variable

C-RAI: Condomless receptive anal intercourse C-IAI: Condomless insertive anal intercourse

CT+: Positive result for Chlamydia trachomatis infection NG+: Positive result for Neisseria gonorrhoeae

Values between parentheses are percentages in row display except for the following variables: age, age at sexual debut and number of partners

Bivariate associations between categorical variables tested either using chi square or Fisher's exact test according to expected values

Differences in the distribution of age, age at sexual debut and number of partners between subjects with and without each of the infections tested using Wilcoxon's Rank Sum Test

This table 1 only includes data about positive cases for each infection under study. However, the statistical comparisons made reflect positive versus negative cases.

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Table 2: Prevalence of anal and pharyngeal infections caused by C trachomatis and/or N gonorrhoeae among 718 MSM/TW participants and sub-groups by self-reported sexual identity

Type of infection	Total enrolled (N=718)		Gay/Homosexual (N=465)		Transgender women (N=208)		Bisexual/Hetero (N=45)		p value
	n/N	%	n/N	%	n/N	%	n/N	%	
Anal									
C trachomatis	133/701	(19.0)	87/453	(19.2)	41/204	(20.1)	5/44	(11.4)	0.40
N gonorrhoeae	67/701	(9.6)	39/453	(8.6)	25/204	(12.3)	3/44	(6.8)	0.28
Either	169/701	(24.1)	105/453	(23.2)	57/204	(27.9)	7/44	(15.9)	0.18
Both concurrently	31/701	(4.4)	21/453	(4.6)	9/204	(4.4)	1/44	(2.3)	0.77
Pharingeal									
C trachomatis	34/712	(4.8)	19/460	(4.1)	14/208	(6.7)	1/44	(2.3)	0.25
N gonorrhoeae	46/712	(6.5)	25/460	(5.4)	20/208	(9.6)	1/44	(2.3)	0.06
Either	73/712	(10.2)	41/460	(8.9)	30/208	(14.4)	2/44	(4.6)	0.04
Both concurrently	7/712	(1.0)	3/460	(0.7)	4/208	(1.9)	0/44	(0.0)	0.24

p value from Chi square or Fisher's exact test to compare prevalence of infections between sex identity/gender groupsValues between parentheses are percentages in column display

Out 718 participants, 701 provided anal swabs and 712 pharyngeal swabs

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Table 3: Multivariable models for each specific single or composite either infection by anatomic site among 718 MSM/TW participants in Lima, Peru 2008-2009.

Anatomic site	Variables included and their categories		Infection					
			C Trachomatis		N gonorrhoeae		Either bacteria	
			PR (95%CI)	p	PR (95%CI)	p	PR (95%CI)	p
Anal	C-RAI	Yes	1.4 (1.0-2.0)	0.07	1.6 (0.9-2.7)	0.11	1.4 (1.0-1.8)	0.03
		No	Ref		Ref		Ref	
	Partners	>10	1.4 (0.8-2.5)	0.25	2.6 (1.0-6.5)	0.04	1.5 (1.0-2.5)	0.07
		3-10	1.6 (0.9-2.7)	0.11	2.3 (0.9-5.7)	0.08	1.5 (0.9-2.3)	0.12
		2	2.1 (1.2-3.7)	0.01	0.8 (0.2-2.2)	0.74	1.7 (1.0-2.8)	0.05
		1	Ref		Ref		Ref	
	Age (years)	34-45	0.5 (0.3-0.7)	<0.01	0.2 (0.1-0.5)	<0.01	0.4 (0.2-0.6)	<0.01
		26-33	0.9 (0.7-1.3)	0.52	0.8 (0.5-1.2)	0.28	0.8 (0.6-1.1)	0.18
		18-25	Ref		Ref		Ref	
	Sample size		675		675		675	
	Number of events		132		63		164	
	AIC		654.5		397.1		723.3	
	BIC		686.1		428.7		754.9	

Anatomic site	Variables included and their categories		Infection					
			C Trachomatis		N gonorrhoeae		Either bacteria	
			PR (95%CI)	p	PR (95%CI)	p	PR (95%CI)	p
Pharingeal	Oral sex	Yes	1.4 (0.7-2.9)	0.38	1.3 (0.7-2.4)	0.45	1.2 (0.8-2.0)	0.38
		No	Ref		Ref		Ref	
	Age (years)	34-45	0.3 (0.1-0.8)	0.02	0.3 (0.1-0.7)	<0.01	0.3 (0.2-0.6)	<0.01
		26-33	0.7 (0.3-1.4)	0.32	0.7 (0.4-1.3)	0.26	0.7 (0.5-1.2)	0.22
		18-25	Ref		Ref		Ref	
	Sample size		686		686		686	
	Number of events		30		43		67	
	AIC		272.9		339.4		464.9	
	BIC		291.1		357.7		483.1	

C-RAI: Condomless receptive anal intercourse

Partners: Number of sexual partners reported over the last 6 months

Number of events: Number of subjects with the specific outcome modeled

AIC: Akaike Information Criterion BIC: Bayesian Information Criterion

Oral sex: No distinction in the specific role during oral sex, just engagement over the last 6 months

PR: Adjusted Prevalence Ratios along wigh 95%-level confidence interval

Ref: Specific sub-category used as baseline reference for the analysis

NA: Variable not included in the model

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Competing Interests

No, there are no competing interests

Contributorship Statement

SRL monitored data collection for the whole study, drafted and revised the paper; ERS and KAK wrote the statistical analysis plan, cleaned and analyzed the data, drafted and revised the paper; JAF supervised the laboratory testing; ASS monitored the data collection and revised the paper; JTG monitored data collection and supervised ethical issues of the study; JDK assessed the biological and treatment component; TJC and CFC designed the study and revised the paper.

Data Sharing Statement

No additional data available.

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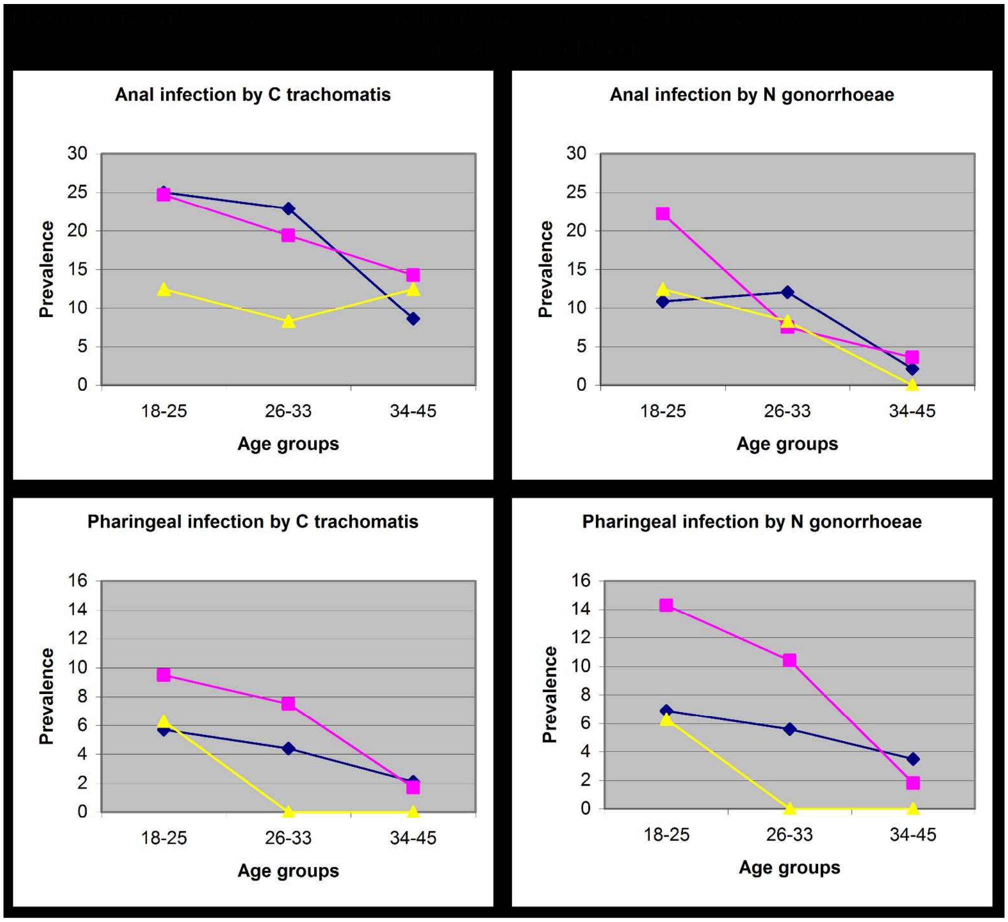

Legend: Pink = Transgender women; Blue = Gay/Homosexual; Yellow = Bisexual
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Prevalence of Chlamydia trachomatis and Neisseria gonorrhoeae Infections in Anal and Pharyngeal Sites among a community-based sample of Men who Have Sex with Men and Transgender Women in Lima, Peru

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High Prevalences of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections in Anal and Pharyngeal Sites among a community-based sample of Men who Have Sex with Men and Transgender Women in Lima, Peru

Short Title: Chlamydia and Gonorrhea in Anorectum and Oropharynx

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Abstract

Objectives: This study aimed to characterize the epidemiology of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae (NG)* infections among men who have sex with men (MSM) and transgender women (TW) in Lima, Peru.

Setting: Cross sectional study in Lima, Peru

Participants: We recruited a group of 510 MSM and 208 TW for a subsequent community-based randomized controlled trial. The presence of CT and NG were evaluated using Aptima Combo2 in pharyngeal and anal swabs. We also explored correlates of these infections.

Primary and secondary outcome measures: Study endpoints included overall prevalence of C. trachomatis and N. gonorrhoeae in anal and pharyngeal sites.

Results: Overall prevalence of *C. trachomatis* was 19.0% (95% CI: 16.1-22.1) and 4.8% (95% CI: 3.3-6.6) in anal and pharyngeal sites respectively, while prevalence of *N. gonorrhoeae* was 9.6% (95% CI: 7.5-12.0) and 6.5% (95% CI: 4.8-8.5) in anal and pharyngeal sites, respectively.

Conclusions: The prevalence of each infection declined significantly among participants older than 34 years ($p<0.05$). Efforts towards prevention and treatment of extra-urogenital chlamydial and gonococcal infections in high risk populations like MSM and TW in Lima, Peru are warranted.

Keywords: Chlamydia, Gonorrhea, anorectal, oropharynx, co-infection, MSM, Peru

Strengths and limitations of this study:

- First study in Latin America to describe the prevalence of non-genital infections in high-risk MSM and transgender populations
- Comprehensive assessment of anal and pharyngeal CT and NG infections.
- Ethnographic sampling might preclude generalizability of our findings to populations outside of the geographic areas under study.
- Oral sex was accessed in-depth; therefore it is not possible to link specific oral sex practices and pharyngeal infections

Key messages

- Currently, in Peru there is no guidelines-based testing for chlamydial or gonorrheal infections in extra-genital sites. Moreover, since these are mostly asymptomatic, then missed and neglected cases are highly likely to occur. Screening should be offered as part of the regular medical care to high-risk populations and enhanced by non-traditional approaches as described here.
- Rectal and pharyngeal infections by Chlamydia trachomatis and Neisseria gonorrhoeae are common among MSM and TW populations in Lima, with prevalences ranging roughly from 10% to 20% in high-risk subpopulations. Their overall prevalences tend to decrease over age although further confirmation with longitudinal studies is warranted.
- Detection of these infections among asymptomatic MSM and TW in Lima, Peru is feasible using a combination of community outreach by mobile units in non-health care settings and NAAT technology. This will lead to a better understanding of the epidemiology of this diseases, and therefore better disease control and prevention.

Introduction

The most common curable genital sexually transmitted infections (STI) worldwide are those caused by *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG).[1] They remain a major public health challenge because of their high prevalence and incidence, especially among high risk populations such as men who have sex with men (MSM) and transgender women (TW).[2, 3] Simultaneous testing for both bacteria is highly recommended and many current diagnostic approaches combine the detection of both agents since co-infection is common.[4, 5]

With regard to CT and NG infections in extragenital sites, their transmission from the urogenital tract of one individual to the oropharynx of another and vice versa has been previously reported.[6-8] Common sexual practices among MSM and TW such as fellatio, anilingus and both receptive and insertive condomless anal intercourse can facilitate CT and NG infections in exposed anatomical sites. These facts highlight the importance and complexities of the transmission of these bacteria given the potential of the anorectum and oropharynx to be infected or colonized and then to serve as silent reservoirs and new sources of infections. However, many public health programs in low and middle-income countries such as Peru still do not routinely screen for NG and CT in anorectal and pharyngeal sites. [5, 9, 10]

The importance and potential impact of screening for CT and NG in non-genital sites, for high-risk men and women, has been shown in several previous studies.[11, 12] CT and NG infections in the oropharynx and/or the anorectum are much more frequent among MSM and TW than in heterosexual men. [6, 13-17] The inclusion of CT and NG screening in some STI programs at public STI clinics worldwide has showed a significant increase in their detection especially when testing is targeted and based upon reported sexual practices and STI past history in these high-risk populations.[6, 10, 18]

Our study aimed to characterize the epidemiology of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae (NG)* infections among men who have sex with men (MSM) and transgender women (TW) in Lima, Peru.

Methods

Study Design, Study Population and Setting:

We analyzed the baseline data of the "Comunidades Positivas and Enhanced Partner Therapy Trial" (ClinicalTrials.gov identifier: NCT00670163) collected during 2008-2009 for a larger clinical trial which was implemented in Lima, Peru until 2012. This timeframe included planning, implementation and data analysis. We recruited 718 participants who were 18 to 45 years old, born biologically male, who lived and/or socialized in the study neighborhoods, had anal or oral sex with other men in the previous 12 months and expressed sexual preference for men. HIV status was not considered for enrollment purposes. Recruitment used a snowball-like, peer-based referral approach in sites previously identified by ethnographic methods. This sample size was defined *a priori* to meet the aims of the original trial. Among the trial participants, 701 agreed to provide self-collected anal swabs and 712 agreed to provide technician-assisted pharyngeal swabs for screening. We offered free treatment to all participants resulting with a positive test for CT and/or NG according to the Peruvian STI Management Guidelines (Azithromycin, 1.0 g single dose orally for CT and Cefixime, 400 mg single dose orally for NG).[19]

Data collection:

Mobile study teams went to each study neighborhood for 1 to 2 weeks to implement all study procedures using temporary offices. Once the participant volunteered and consented in written to participate in the study, they were asked to respond to a questionnaire using a Computer-Assisted Personal Interview (CAPI) system.[20] The questionnaire collected information on demographic characteristics, general health and health care seeking behavior, exposure to HIV/STI prevention messages, HIV testing history, sexual risk behaviors, and substance use and took approximately 60 minutes to complete. After completing the questionnaire, all participants went through pre-test counseling for STIs, including HIV infection, with a trained counselor according to the Peruvian STI Management guidelines.[19]

Laboratory Procedures:

We trained all participants to self-collect anal swabs, using pictures and mirrors for guidance. Trained technicians collected pharyngeal swabs by swabbing the tonsils. We initially stored all collected samples in Aptima collection tubes and then transported them to our laboratory facilities in Lima where nucleic acid amplification testing (NAAT) was performed. Specifically, the detection of *CT* and/or *NG* infections was performed by means of a Transcription Mediated Amplification (TMA) with the Aptima Combo2 CT/NG test (Gen Probe Incorporated, San Diego, CA) following the manufacturer's directions. NAAT technology is well known for being highly sensitive and specific.[15, 18, 21, 22] The results were given to the participants within two weeks of sample collection, along with post-test counseling and treatment as necessary according to Peruvian STI Management Guidelines.[19] Laboratory quality assurance was performed on a randomly selected 10% of the samples and conducted by the Laboratory of the San Francisco Department of Public Health in California.

Variables and Statistical Analysis:

Outcomes: The four outcome variables of interest for this analysis were anorectal chlamydiasis, anorectal gonorrhoea, oropharyngeal chlamydiasis and oropharyngeal gonorrhoea. We calculated their prevalences and 95% confidence intervals overall and for each of the three self-reported sexual and gender identity sub-groups (Gay/Homosexual, Male-to-Female transgender women, and Bisexual/Heterosexual). Composite outcomes for any infection and concomitant co-infection at each anatomical site were also calculated. The concomitant co-infection outcome was generated in order to gain statistical power for later modeling analysis, especially for the oral infection outcomes given their very low absolute numbers.

Correlates: The following variables were used to describe the study population and analyzed to explore their associations with the outcome variables: age, age at sexual debut, and number of sexual partners over the last 6 months (given their distribution departure from normality, these were re-categorized in tertiles), educational attainment, self-reported sexual/gender identity, preferred role during anal sex, engagement in condomless anal intercourse, engagement in oral sex (without differentiation if giving or receiving) , and ever engaging in compensated sex. Oral sex and condomless anal intercourse were summary binary variables constructed from responses provided by participants when asked for specific sexual behaviors with up to 3 most recent sexual partners in the past 6 months.

Statistical analysis: We tested associations between categorized variables and each of the four outcomes by using either Chi square's test or Fisher's exact test when needed. Differences in the distribution of numeric variables between subjects with and without infection were assessed using the Wilcoxon's Rank Sum test. Within each sexual/gender identity group, we also assessed the linear trend of the prevalence of each infection across age groups using the Chi square test for linear trends. To model the outcomes as a function of the exposure (correlate) variables of a priori interest we fit multivariable Log-Binomial Models under the

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Generalized Linear Models framework using a logarithmic link and binomial family settings.[23] This approach estimates Prevalence Ratios (PR)which are more appropriate for a cross-sectional study like the present.[24] Moreover, in comparison to other alternatives, such as the Cox or Poisson regression models, the PR obtained are less prone to errors in interval estimation as well as less biased.[25, 26]

The selection and inclusion of variables into the final multivariate models relied on a conceptual framework developed by the authors following guidelines previously described for epidemiological applications.[27] Conceptually, we considered three hierarchical levels of variables related to the outcome: a distal level (age and education), an intermediate level (sexual identity, sexual role, number of partners and age at sexual debut) and a proximal level (compensated sex and either condomless receptive anal intercourse or oral sex-the latter according to the outcome under analysis). Each anal infection outcome was regressed on each variable at a time and only one variable from each level was selected according to its best fit measured by the lowest reported value of Akaike Information Criterion (AIC) for the distal and intermediate levels, or the Bayesian Information Criterion (BIC) for the proximal level. We adopted this approach given the specific properties of each information criterion: AIC tends to favor the best model among a set of candidate models while BIC tends to favor the "one true model" among a set of candidate models.[28, 29] For the oral infections outcomes we followed the same approach but later further limited the number of correlates in the final model to avoid overfitting and stick to the proximal and distal levels only. In general, in order to avoid overfitting and instability in the final models we limited the number of variables to include in the models in such a way that there is about between 10-16 events in the outcome per each dichotomous or dummy.[30] We assessed potential collinearity among predictors by measuring uncentered variance inflation factors.

Ethical approval:

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The study was approved by the IRB of the University of California at Los Angeles and for the Institutional Ethics Committee of the Universidad Peruana Cayetano Heredia.

Results

Participants Characteristics:

During 2008 and 2009, we enrolled 718 participants in Lima and surrounding areas. Participant age range was 18 to 45 years old. Most of them were born in the coastal region of Peru: 53.6% (385/718) in Lima and 32.2% (232/718) in other coastal cities. The remaining 14.1% (101/718) were born in non-coastal. Almost 80% of participants were concurrently working and/or studying (568/718). The mean age of sexual debut was 14 years. Most participants self-reported as gay or homosexual (64.8%), and having preference for a pasivo sexual role (67.5%). The median number of sexual partners in the last six months was 5.. Further details are described in Table 1.

Infections by anatomical site, infectious agent and sexual identity:

Anal swabs were provided by 97.6% of participants (701/718) and 99.2% (712/718) provided pharyngeal swabs for CT and NG testing. Overall, anal infection by *Chlamydia trachomatis* was 19.0% (95% CI: 16.1-22.1) while pharyngeal infection was 4.8% (95% CI: 3.3-6.6). The prevalence of *Neisseria gonorrhoeae* was 9.6% (95% CI: 7.5-12.0) for anal infection and 6.5% (95% CI: 4.8-8.5) for pharyngeal infection. This pattern of higher CT infection in anorectum and higher NG infection in pharinx was evident within each sexual identity subgroup (Gay/homosexual, transgender women and bisexual). Further details are described in Table 2.

In the anorectum, the prevalence of CT infection was almost twice (19%) as common as for NG (9.6%); this was true for the overall study population and within each sexual identity group. In the oropharynx, the prevalence of NG was slightly higher than for CT in all cases except for the bisexual sub-group. Comparing across sexual identities, the prevalence of each single and composite outcome infection was more common in TW participants, followed by Gay/Homosexual and less common among bisexuals in most of the cases, though these differences were not statistically significant. The only statistically significant difference was for either pharyngeal infection, p-value <0.05, see Table 2.

Overall, the prevalence of each of the four outcomes of the study decreases with increasing age. The test for trends over age group was significant (p<0.05) only in the following cases: within gay/homosexual for anal chlamydiasis, within gay/homosexual and transgender women for anal gonorrhea, and within transgender women for pharyngeal gonorrhea. For pharyngeal chlamydiasis a borderline statistical significance for trend (p=0.08) was found within TW. The graphical description of trends is shown in Figure 1.

Statistical models for outcomes and laboratory quality assurance

The six final multivariate models are presented in table 3. Briefly, the higher age group consistently remained inversely associated to all single and composite outcomes. For the oral infection outcomes, both oral sex and compensated sex were associated with higher proportions of infection though they were not statistically significant. For the anal infection outcomes, the number of sexual partners showed an erratic pattern of association and not statistically significant in most of the cases while condomless receptive anal intercourse remained directly and significantly associated with the infections in the models where it was included. The results of the laboratory quality assurance performed on 10% of the samples showed 100% of correlation (Data not shown).

Discussion

Using a snowball-like peer-based referral approach, we were able to reach a group of MSM and TW at high-risk as evidenced by their high prevalence of CT and NG infections. Using both technician-assisted pharyngeal swabs and self-collected anal swabs we found that close to 25% of the study population had anal infection by either CT or NG, while close to 10% had oropharynx infection by the same bacteria. The prevalence of these infections tends to decrease with age. In these regards, these findings constitute a very important update in the Peruvian scenario and become one of the few available studies in Latin America.

Gonorrhea prevalence in anorectal samples was higher than previously reported in HIV and STIs sentinel surveillance studies conducted among MSM and TW by the Peruvian Minister of Health during 1996-2002, this report detailed a decrease in anal NG infection from 5.1% in 1996 to 0.2% in 2002,[31] However, their method for gonorrhea diagnostics were the regular culture of urethral secretion and it is very well known that culture only detects viable bacteria in people with active infection.[32] Moreover, culturing NG from highly-contaminated samples can be very difficult. NAAT and especially TMA are a more reasonable approach for the rapid and accurate diagnosis of anal and pharyngeal gonorrhea without the limitations of culture.[33] However, one limitation of the NAAT technology is that current non-active infections can result in a positive test since the method assesses the presence of RNA and or DNA instead of live bacteria.[34]

Chlamydial infection prevalence also was quite high in this group of MSM and TW, especially in anorectal samples, where we found the highest prevalence of infection. A previous report of Peruvian people living with HIV who were tested for pharyngeal chlamydia infection did not find any positive cases.[35] Other studies among MSM and young men in Peru only detected chlamydia in urine samples.[36] A previous country-level study conducted among young Peruvian adults from the general population found a prevalence of 4.2% for CT in urogenital samples. [37] In this regards, our study adds important data to better understand the epidemiology of CT infection in Peru.

Prevalence of chlamydia and gonorrhea decreases with age and this can be explained by an increase in the awareness of STI infections and decreases in the number of sex partners with the age.[4, 38] Even if not treated, chlamydia can be cleared in certain group of infected people.[39] This inverse association between age and chlamydial infection has been also reported in other studies.[40, 41]

This is the first study in Peru that uses TMA technology for testing CT and NG infection in extragenital sites and our study highlight the importance of extragenital screening of CT and NG. Currently, the Peruvian STI Management Guidelines[19] establish that all female and male sex workers are subject to Periodic Medical Assessment, this monthly strategy includes STI assessment based on a syndromic approach. If urethral discharge is found, gonorrhea culture must be performed and treatment for chlamydia and gonorrhea should be offered. There is no further testing for chlamydial infection; and asymptomatic cases for both CT and NG infections, especially in extra-genital sites, can easily be missed and neglected. According to the current CDC recommendations NAAT testing should be available for CT and NG diagnosis.[33]

There are some limitations in our analysis. The participants tested were not randomly selected and all of them belong to high-risk groups (since they originally were enrolled for a trial implementation) from low-income neighborhoods in the metropolitan area of Lima, thus our results can show higher prevalences than random MSM or TW could have, and are not necessarily generalizable to all MSM and TW in Lima or Peru. However, given that this is a hidden population, the ethnographic techniques initially used to identify all of the MSM and TW within a neighborhood represent a nearly saturated sample for each community included.

At the moment there are not FDA-cleared CT/NG tests available for non-urogenital sites but the CT/NG Aptima Combo-2 test has shown good performance when used for pharyngeal and anal samples. [15, 42, 43]

In conclusion, infection by *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in extragenital sites (anorectum and oropharynx) are very common among MSM and TW in Lima, Peru and both infections need to be incorporated into the regular STI testing programs in Peru. The overall prevalence of CT and NG tends to decrease over age although this needs confirmation with further longitudinal analysis. Screening and treatment for extragenital CT and NG at non-health care facilities located in the actual venues where this populations are by means of temporary mobile teams is feasible through a combination of coordinated work with the community, use of NAAT technology and previous ethnographic work. These approaches should be used by the Ministry of health in order to better control STIs in Lima and possibly in other cities of Peru.

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Disclaimer

All authors: no conflicts

Table 1: Overall study population characteristics and their bivariate associations with anal and pharyngeal infections caused by C trachomatis and N gonorrhoeae among 718 MSM/TW participants in Lima, Peru 2008-2009.

	Total enrolled (N=718)		Provided anal swabs (N=701)										Provided throat swabs (N=712)										
			C trachomatis					N gonorrhoeae					C trachomatis					N gonorrhoeae					
			Positive = 133		Negative = 568			Positive = 67		Negative = 634			Positive = 34		Negative = 678			Positive = 46		Negative = 666			
Characteristics	n (%)		n (%)		n (%)			n (%)		n (%)			n (%)		n (%)			n (%)		n (%)			
Demographic characteristics																							
Age																							
Median (IQR)	29	(23-35)	26	(22-30)	29	(24-36)	<0.05	24	(21-29)	29	(23-36)	<0.05	25	(22-29)	29	(23-36)	<0.05	25	(23-29)	29	(23-36)	<0.05	
Tertile 1: 18-25	261	(36.4)	61	(24.1)	192	(75.9)		37	(14.6)	216	(85.4)		18	(7.0)	241	(93.0)		24	(9.3)	235	(90.7)		
Tertile 2: 26-33	242	(33.7)	50	(21.2)	186	(78.8)	<0.05	25	(10.6)	211	(89.4)	<0.05	12	(5.0)	227	(95.0)	0.05	16	(6.7)	223	(93.3)	<0.05	
Tertile 3: 34-45	215	(29.9)	22	(10.4)	190	(89.6)		5	(2.4)	207	(97.6)		4	(1.9)	210	(98.1)		6	(2.8)	208	(97.2)		
Educational attainment																							
At least some beyond HS	204	(28.4)	42	(21.1)	157	(78.9)		19	(9.6)	180	(90.4)		9	(4.5)	192	(95.5)		15	(7.5)	186	(92.5)		
Full/partially attended HS	448	(62.4)	83	(19.0)	353	(81.0)	0.27	38	(8.7)	398	(91.3)	0.25	22	(4.9)	423	(95.1)	0.96	24	(5.4)	421	(94.6)	0.22	
Elementary or none	66	(9.2)	8	(12.1)	58	(87.9)		10	(15.2)	56	(84.8)		3	(4.6)	63	(95.4)		7	(10.6)	59	(89.4)		
Self-reported sexual identity																							
Gay/Homosexual	465	(64.8)	87	(19.2)	366	(80.8)		39	(8.6)	414	(91.4)		19	(4.1)	441	(95.9)		25	(5.4)	435	(94.6)		
Transgender women	208	(29.0)	41	(20.1)	163	(79.9)	0.40	25	(12.3)	179	(87.7)	0.28	14	(6.7)	194	(93.3)	0.25	20	(9.6)	188	(90.4)	0.06	
Bisexual/Heterosexual	45	(6.2)	5	(11.4)	39	(88.6)		3	(6.8)	41	(93.2)		1	(2.3)	43	(97.7)		1	(2.3)	43	(97.7)		
Sexual Characteristics																							
Sexual role during anal sex																							
Pasivo	485	(67.5)	89	(18.8)	384	(81.2)		45	(9.5)	428	(90.5)		23	(4.8)	458	(95.2)		30	(6.2)	451	(93.8)		
Moderno	188	(26.2)	41	(22.2)	144	(77.8)	0.07	20	(10.8)	165	(89.2)	0.46	9	(4.8)	177	(95.2)	0.99	13	(7.0)	173	(93.0)	0.94	
Activo	45	(6.3)	3	(7.0)	40	(93.0)		2	(4.7)	41	(95.3)		2	(4.4)	43	(95.6)		3	(6.7)	42	(93.3)		
Sexual debut																							
Median (IQR)	14	(12-16)	14	(12-16)	14	(12-16)	0.12	14	(12-15)	14	(12-16)	0.24	13	(12-15)	14	(12-16)	0.15	14	(13-17)	14	(12-16)	0.50	
Tertile 1: 13 or younger	294	(41.0)	61	(21.0)	229	(79.0)		28	(9.7)	262	(90.3)		18	(6.2)	274	(93.8)		15	(5.1)	277	(94.9)		
Tertile 2: 14-15	188	(26.2)	38	(20.8)	145	(79.2)	0.16	24	(13.1)	159	(86.9)	0.08	9	(4.8)	179	(95.2)	0.25	14	(7.5)	174	(92.5)	0.49	
Tertile 3: 16 or older	236	(32.8)	34	(14.9)	194	(85.1)		15	(6.6)	213	(93.4)		7	(3.0)	225	(97.0)		17	(7.3)	215	(92.7)		
Sexual partners over the last 6 mo*																							
Median (IQR)	5	(2-15)	5	(2-15)	4	(2-15)	0.67	7	(4-30)	4	(2-15)	<0.05	7	(3-30)	4	(2-15)	<0.05	6	(2-18)	4	(2-15)	0.56	
1 partner	111	(16.1)	14	(12.8)	95	(87.2)	0.11	5	(4.6)	104	(95.4)	<0.05	2	(1.9)	106	(98.1)	0.26	8	(7.4)	100	(92.6)	0.75	

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2 partners		117	(16.9)	29	(25.7)	84	(74.3)		4	(3.5)	109	(96.5)		5	(4.3)	112	(95.7)		5	(4.3)	112	(95.7)	
3-10 partners		262	(37.9)	52	(20.4)	203	(79.6)		28	(11.0)	227	(89.0)		10	(3.8)	251	(96.2)		16	(6.1)	245	(93.9)	
More than 10 partners		201	(29.1)	37	(18.7)	161	(81.3)		26	(13.1)	172	(86.9)		13	(6.5)	187	(93.5)		14	(7.0)	186	(93.0)	
Sexual behaviors over the last 6 mo																							
C-IAI with men	Yes	131	(18.3)	18	(14.2)	109	(85.8)	0.13	5	(3.9)	122	(96.1)	<0.05	4	(3.1)	126	(96.9)	0.32	8	(6.2)	122	(93.8)	0.88
	No	587	(81.7)	115	(20.0)	459	(80.0)		62	(10.8)	512	(89.2)		30	(5.2)	552	(94.8)		38	(6.5)	544	(93.5)	
C-RAI sex with men	Yes	469	(65.3)	97	(21.2)	360	(78.8)	<0.05	49	(10.7)	408	(89.3)	0.15	23	(5.0)	441	(95.0)	0.76	30	(6.5)	434	(93.5)	0.99
	No	249	(34.7)	36	(14.8)	208	(85.2)		18	(7.4)	226	(92.6)		11	(4.4)	237	(95.6)		16	(6.5)	232	(93.5)	
Oral sex with men	Yes	472	(65.7)	92	(19.7)	374	(80.3)	0.46	46	(9.9)	420	(90.1)	0.69	25	(5.3)	446	(94.7)	0.35	33	(7.0)	438	(93.0)	0.41
	No	246	(34.3)	41	(17.5)	194	(82.5)		21	(8.9)	214	(91.1)		9	(3.7)	232	(96.3)		13	(5.4)	228	(94.6)	
Compensated sex	Yes	394	(54.9)	73	(19.0)	312	(81.0)	0.99	47	(12.2)	338	(87.8)	<0.05	23	(5.9)	369	(94.1)	0.13	26	(6.6)	366	(93.4)	0.84
	No	324	(45.1)	60	(19.0)	256	(81.0)		20	(6.3)	296	(93.7)		11	(3.4)	309	(96.6)		20	(6.3)	300	(93.7)	

IQR: Interquartile range; p<0.05 for bolded values; p<0.10 for underlined values; *n=691 for this variable

C-RAI: Condomless receptive anal intercourse   C-IAI: Condomless insertive anal intercourse

CT+: Positive result for Chlamydia trachomatis infection   NG+: Positive result for Neisseria gonorrhoeae

Values between parentheses are percentages in row display except for the following variables: age, age at sexual debut and number of partners

Bivariate associations between categorical variables tested either using chi square or Fisher's exact test according to expected values

Differences in the distribution of age, age at sexual debut and number of partners between subjects with and without each of the infections tested using Wilcoxon's Rank Sum Test

This table 1 only includes data about positive cases for each infection under study. However, the statistical comparisons made reflect positive versus negative cases.

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Table 2: Prevalence of anal and pharyngeal infections caused by C trachomatis and/or N gonorrhoeae among 718 MSM/TW participants and sub-groups by self-reported sexual identity

Type of infection	Total enrolled (N=718)		Gay/Homosexual (N=465)		Transgender women (N=208)		Bisexual/Hetero (N=45)		p value
	n/N	%	n/N	%	n/N	%	n/N	%	
Anal									
C trachomatis	133/701	(19.0)	87/453	(19.2)	41/204	(20.1)	5/44	(11.4)	0.40
N gonorrhoeae	67/701	(9.6)	39/453	(8.6)	25/204	(12.3)	3/44	(6.8)	0.28
Either	169/701	(24.1)	105/453	(23.2)	57/204	(27.9)	7/44	(15.9)	0.18
Both concurrently	31/701	(4.4)	21/453	(4.6)	9/204	(4.4)	1/44	(2.3)	0.77
Pharingeal									
C trachomatis	34/712	(4.8)	19/460	(4.1)	14/208	(6.7)	1/44	(2.3)	0.25
N gonorrhoeae	46/712	(6.5)	25/460	(5.4)	20/208	(9.6)	1/44	(2.3)	0.06
Either	73/712	(10.2)	41/460	(8.9)	30/208	(14.4)	2/44	(4.6)	0.04
Both concurrently	7/712	(1.0)	3/460	(0.7)	4/208	(1.9)	0/44	(0.0)	0.24

p value from Chi square or Fisher's exact test to compare prevalence of infections between sex identity/gender groups

Values between parentheses are percentages in column display

Out 718 participants, 701 provided anal swabs and 712 pharyngeal swabs

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Table 3: Multivariable models for each specific single or composite either infection by anatomic site among 718 MSM/TW participants in Lima, Peru 2008-2009.

Anatomic site	Variables included and their categories		Infection: C Trachomatis PR (95%CI)	N gonorrhoeae PR (95%CI)	Either bacteria PR (95%CI)
Anal	C-RAI	Yes	1.4 (1.0-2.0)	NA	1.4 (1.0-1.9)
		No	Ref	NA	Ref
	Compensated sex	Yes	NA	1.4 (0.8-2.5)	NA
		No	NA	Ref	NA
	Partners	>10	1.4 (0.8-2.5)	2.0 (0.7-5.3)	1.5 (1.0-2.5)
		3-10	1.5 (0.9-2.7)	2.0 (0.8-5.1)	1.5 (0.9-2.3)
		2	2.1 (1.2-3.7)	0.7 (0.2-2.6)	1.7 (1.0-2.8)
		1	Ref	Ref	Ref
	Age (years)	34-45	0.4 (0.3-0.7)	0.2 (0.1-0.5)	0.4 (0.2-0.6)
		26-33	0.9 (0.6-1.2)	0.8 (0.5-1.2)	0.8 (0.6-1.1)
		18-25	Ref	Ref	Ref

Anatomic site	Variables included and their categories		Infection: C Trachomatis PR (95%CI)	N gonorrhoeae PR (95%CI)	Either bacteria PR (95%CI)
Pharingeal	Oral sex	Yes	NA	1.3 (0.7-2.4)	1.3 (0.8-2.0)
		No	NA	Ref	Ref
	Compensated sex	Yes	1.7 (0.8-3.4)	NA	NA
		No	Ref	NA	NA
	Age (years)	34-45	0.3 (0.1-0.8)	0.3 (0.1-0.7)	0.3 (0.2-0.6)
		26-33	0.7 (0.4-1.5)	0.7 (0.4-1.3)	0.7 (0.5-1.2)
		18-25	Ref	Ref	Ref

C-RAI: Condomless receptive anal intercourse

Partners: Number of sexual partners reported over the last 6 months

Oral sex: No distinction in the specific role during oral sex, just engagement over the last 6 months

PR: Adjusted Prevalence Ratios along with 95%-level confidence interval

Ref: Specific sub-category used as baseline reference for the analysis

NA: Variable not included in the model

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Contributorship statement

SRL monitored data collection for the whole study, drafted and revised the paper; ERS and KAK wrote the statistical analysis plan, cleaned and analyzed the data, drafted and revised the paper; JAF supervised the laboratory testing; ASS monitored the data collection and revised the paper; JTG monitored data collection and supervised ethical issues of the study; JDK assessed the biological and treatment component; TJC and CFC designed the study and revised the paper.

Competing interests

No, there are no competing interests.

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Data sharing statement

No additional data available

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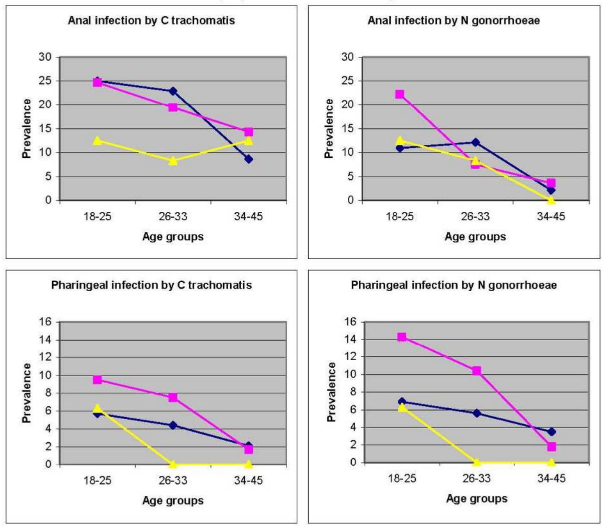
Figure 1: Trends of prevalence of anal and pharingeal infections over age group, stratified by agent and sexual identity



210x297mm (200 x 200 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract Yes, page 1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Yes, page 2
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Yes, page 5
Objectives	3	State specific objectives, including any prespecified hypotheses Yes, page 6
Methods		
Study design	4	Present key elements of study design early in the paper Yes, page 6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Yes, page 6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants Yes, page 6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Yes, pages 7/8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Yes page 8
Bias	9	Describe any efforts to address potential sources of bias Yes, page 9
Study size	10	Explain how the study size was arrived at Yes page 6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Yes pages 8/9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding Yes pages 8/9
		(*b*) Describe any methods used to examine subgroups and interactions Yes pages 8/9
		(*c*) Explain how missing data were addressed Yes pages 8/9
		(*d*) If applicable, describe analytical methods taking account of sampling strategy Yes pages 8/9
		(*e*) Describe any sensitivity analyses Yes pages 8/9
Results		

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Yes, page 10
		(b) Give reasons for non-participation at each stage Yes, page 10
		(c) Consider use of a flow diagram Yes, page 10
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Yes, page 10
		(b) Indicate number of participants with missing data for each variable of interest Yes, page 10
Outcome data	15*	Report numbers of outcome events or summary measures Yes, pages 10/15
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Yes, pages 10/15
		(*b*) Report category boundaries when continuous variables were categorized Yes, pages 10/15
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period Yes, pages 10/15
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Yes, Pages 10/11
Discussion		
Key results	18	Summarise key results with reference to study objectives Yes, pags 12/13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Yes page 13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Yes pages 13
Generalisability	21	Discuss the generalisability (external validity) of the study results Yes, page 13
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based Yes, page 14

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.