The Zimbabwe STI Etiology Study: Design, Methods, Study Population

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Words in summary: 54

Words in abstract: 301

Words in manuscript: 3,436

Tables: 1

Keywords: STI, Etiology, Syndromic Management, Methods

Acknowledgements:

We thank Prof. Ahmed Latif (Public Health Consultant, Brisbane, Australia) and Dr. Danuta Kaspzryk (University of Washington, School of Nursing) for their careful review of the manuscript.

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This study could not have been conducted without the gracious support and collaboration of the staff and patients of the following clinics:

Harare: Mbare and Budiriro clinics

Bulawayo: Nkulumane and Khami Road Clinics

Beitbridge: Dulibadzimu Clinic

Gutu: Gutu Rural Hospital

The Zimbabwe STI Etiology Study was supported by funds from the President's Emergency Plan for AIDS Relief (PEPFAR) through a cooperative agreement between the U.S. Centers for Disease Control and Prevention and the University of Zimbabwe Department of Community Medicine SEAM Project under the terms of Cooperative Agreement Number IU2GGH000315-01.

Summary: A study of the etiology of STI syndromes in Zimbabwe using a traveling team of trained study nurses resulted in timely patient enrollment, high quality and completeness of data and laboratory samples, and positive rapport between study nurses and clinic staff, suggesting a model for conducting future STI etiology studies at sentinel sites.

Abstract

Background: In most developing countries, sexually transmitted infections (STI) are managed syndromically with combinations of antibiotics to which the most prevalent etiologic agents are sensitive. Periodic surveys are necessary to establish the actual prevalence of etiologic agents to validate the appropriateness of syndromic treatment guidelines.

Methods: We conducted a study among patients served by a regionally and ethnically diverse sample of clinics in Zimbabwe to assess the etiology of urethral discharge in men, vaginal discharge in women, and genital ulcer disease in women and men. Patients were enrolled sequentially by a traveling team of designated study nurses. This manuscript provides details of the study methodology and presents demographic and sexual history data that were collected using a standardized questionnaire. Etiology outcome data will be presented in subsequent articles.

Results: Over a period of 10 months, 600 patients were enrolled in 6 clinics, 200 in each of the syndrome categories. Among the clinics there were significant differences in gender, ethnicity, and sexual history. Overall, 35.9% of participants reported a history of STI and 29.5% were HIV positive by self-report. Observations during the study period confirmed that the study team was well integrated in regular clinic operations and performed both research and clinical duties consistent with the study protocol. Record review indicated over 90% data completeness and

high concordance between paper and electronic data files. Specimen collection was complete for all but 2 patients.

Conclusions: Enrollment at multiple clinics increases study subject diversity with potentially important consequences for study outcomes. A large proportion of patients presenting with STIs in Zimbabwe reported to be HIV infected, indicating a high risk for ongoing HIV transmission. Finally, process outcomes of the study suggest that a traveling team approach as used in this study may serve as a model for conducting STI studies in sentinel sites.

Introduction

According to the 2012 estimates from the World Health Organization (WHO), the global burden of sexually transmitted infections (STI) comprises 357 million incident bacterial/protozoan infections among adults 15-49 years, including 131 million *Chlamydia trachomatis* infections, 78 million *Neisseria gonorrhoeae* infections, 143 million *Trichomonas vaginalis* infections, and 5.6 million *Treponema pallidum* infections, respectively.[1] Sub-Saharan Africa represents 13.4% of the world's population[2], but has historically accounted for a disproportionate number of STIs, including 20% of global gonococcal infections, and 32% of syphilis and trichomoniasis cases.[3]

In many countries, including most developing nations, the treatment of STI-associated syndromes, including urethritis in men, vaginal discharge in women, and genital ulcer disease in both men and women has been the mainstay of STI control and prevention. Syndromic management involves the administration of a combination of antimicrobials to patients presenting with STI syndromes when diagnostic tests to differentiate between the major etiologic agents associated with these syndromes are not available. WHO has published guidelines for STI syndromic management, the most recent in 2003[4], and most countries employing syndromic management base their country guidelines on the WHO recommendations. Thus the Zimbabwe STI treatment guidelines as published in 2012[5], recommend ceftriaxone or kanamycin in combination with doxycycline or azithromycin for men presenting with urethral discharge and women with vaginal discharge to treat gonococcal and

chlamydial infections. In addition, women presenting with vaginal discharge are also given metronidazole to treat trichomoniasis and bacterial vaginosis. Men and women presenting with genital ulcer disease are given a combination of benzathine penicillin, erythromycin and acyclovir to treat syphilis, chancroid, and genital herpes, respectively.[5]

The underlying epidemiology of STIs is changing. For example, chancroid appears to be a decreasing cause and genital herpes an increasing cause of genital ulcer disease in sub-Saharan Africa.[6-8] Also, micro-organisms such as *Mycoplasma genitalium* are increasingly recognized as a cause of male urethritis[9] and may result in pelvic inflammatory disease among women.[10] Thus, periodic surveys are necessary to keep track of the changing STI epidemiological landscape and keep syndromic management guidelines up to date. In addition, given the challenges of antimicrobial susceptibility, in particular for gonococcal infections, surveillance of gonococcal resistance patterns is also indicated. Indeed, recent studies of gonococcal resistance have been published from southern Africa including one study from Zimbabwe.[11] By contrast, there has been a paucity of basic STI etiologic studies in Africa and no recent comprehensive studies have been conducted outside South Africa[12], Botswana.[6], and Zambia.[7] Existing studies often originate from large public health laboratories and specialty clinics associated with them, thus limiting regional representativeness. To the extent that studies involve regional and rural clinics, they often rely on specimen collection by inadequately trained and supervised staff with competing clinical duties. [ref]. To overcome these limitations, we developed a study model that relied on a traveling team of trained nurses to collect data and specimens

in a study assessing the etiology of the genital discharge syndromes and genital ulcer disease among 600 men and women presenting with these conditions in a regionally diverse selection of STI clinics in Zimbabwe.

In this first article, we describe the study design and methods and also present data describing the enrolled population and the quality and completeness of data collection. Etiologic testing data, will be presented elsewhere. [include refs here]

Methods

Ethics Statement

The study protocol, including consent forms and questionnaires was reviewed and approved by the institutional review boards of the University of Zimbabwe (Joint Research and Ethics Committee of Parirenyatwa Central Hospital), the Zimbabwe Medical Research Council and the U.S. Centers for Disease Control and Prevention.

Study Design, Patient Selection, and Study Procedures

Study Design

The overarching goal of the study was to determine the current etiology of STI syndromes in Zimbabwe. Specific aims were to determine the prevalence of *Neisseria gonorrhoeae* , *Chlamydia trachomatis*, *Trichomonas vaginalis*, and *Mycoplasma genitalium* among men and women with urethral and vaginal discharge respectively and the prevalence of bacterial vaginosis and yeast infections among women with vaginal discharge. For men and women presenting with genital ulcer disease we intended to determine the prevalence of *Treponema pallidum*,

Haemophilus ducreyi, lymphogranuloma venereum strains of *Chlamydia trachomatis*, and herpes simplex virus type 1 and 2 (HSV-1 and HSV-2) infections.

Secondary aims included the determination of the prevalence and awareness of infection status of human immunodeficiency virus (HIV) infection among men and women with STI syndromes, the association of HIV with STI syndromes and pathogens, and the prevalence of gonorrhea and chlamydia among patients with genital ulcer disease.

Two countervailing principles guided the development of the study. On the one hand we intended to study a population representative of Zimbabwean patients presenting with STIs at urban and regional clinics and to have a sample size that would allow a robust assessment of the relative proportion of pathogens associated with the respective STI syndromes. On the other hand, these goals were restricted by a limitation of resources and time to conduct the study. Thus, we followed a pragmatic approach to identify study clinics and determine appropriate sample sizes.

Clinic Selection

In Zimbabwe, STI syndromes are reported from polyclinics to district and provincial health offices and from there to the Zimbabwe Ministry of Health and Child Care Head Office. We examined 2012 surveillance statistics to identify a regionally diverse sample of clinics with high numbers of reported STI syndromes. After an initial round of site visits to determine the logistics and feasibility of conducting the study, we selected 6 geographically diverse clinics: Mbare and Budiriro clinics in

Harare, the country's capital city; Khami Road and Nkulumane clinics in Bulawayo the second largest city in the southwestern part of the country and ethnically distinct from Harare; Dulibadzimu clinic in Beitbridge on the border with South Africa, an important hub for truck drivers and sex workers, and finally Gutu Road Hospital in Gutu in Masvingo Provice, a predominantly rural clinic. These clinics were deemed to be sufficiently representative of risk populations both regionally and ethnically to yield meaningful and generalizable results. To enhance generalizability, we aimed to enroll 200 patients in each of the three regions. More details on clinic selection can be found in the study protocol, available online.[13]

Sample Size

Based on statistical considerations detailed in the study protocol[13], as well as the limited resources available to the study, we felt that sample sizes of 200 each for urethral discharge and vaginal discharge syndromes would strike an acceptable balance between available study resources and the need for precision. For genital ulcer disease with lower expected frequencies for chancroid, syphilis, and lymphogranuloma venereum, a larger sample size would have been desirable, but was not deemed feasible within the time and budgetary constraints of the project given the overall lower prevalence of genital ulcer disease in comparison to the discharge syndromes. We thus aimed to enroll 200 subjects in this category as well; 100 men and 100 women. Since the study population was in essence a convenience sample, we did not intend to apply sample weights. Importantly, we did not consider clustering of pathogens by study site in calculating sample sizes. In the presentation

of the study results, we will therefore be careful in presenting clinic comparisons as well as list caveats of combining the data.

Patient Selection

During the enrollment period at each clinic, sexually active men and women aged 18-55 years were eligible for the study if presenting with a chief compliant of urethral discharge (men), vaginal discharge (women) or a genital ulceration (men and women). Patients were ineligible if they did not speak English, Shona or Ndebele (the 3 major languages in Zimbabwe), if they were unable to provide consent, if they had received antibiotics for STI treatment or for other reasons in the previous 4 weeks, or if they had been previously enrolled.

Study Team

A team of 3 nurses from the Zimbabwe Community Health Intervention Research (ZiCHIRe) program in Harare was trained in study procedures and deployed sequentially for a period of 10-17 weeks to each of the 6 study sites, starting in the Harare clinics, then moving to Bulawayo and finally to Beitbridge and Gutu. The start of enrollment at each site was preceded by at least two site visits involving study leadership, including the team lead (VK), the study's lead consultant (CAR) and senior researchers representing the Ministry of Health and Child Care (AM and MM). The primary purpose of these visits was to properly inform clinic leadership and staff of the upcoming study, to obtain their support and to review study logistics, including the availability of a study room, basic materials, a refrigerator, etc. To enhance the usefulness of team presence at the clinic, team staff members

were tasked with regular clinic duties if enrollment was slow and were also encouraged to involve regular clinic staff with patient enrollment as a teaching opportunity. During the enrollment period, each clinic was visited at least once by the study leadership (CAR, MM, AM) to review clinic procedures, data management and specimen processing. A final visit to each of the clinics by the study leadership was conducted after completion of the study to provide study results and additional training.

Study Procedures

Upon establishing eligibility criteria, patients underwent a consenting process and were asked to sign the informed consent form[13] covering all study procedures, except HIV testing for which a separate consent line was included. The study nurse then completed a paper-copy questionnaire that included demographic information, a sexual history, description of symptoms, history of STI/HIV, and current use of medications. The study questionnaire is available online.[13] Next the patient underwent a genital examination and specimens were taken as follows: men with urethral discharge: smear for Gram stain and a first-voided urine sample; women with vaginal discharge: Ph testing by strip method, smear for Gram stain and 4 vaginal swabs; men and women with genital ulcer disease: 1 swab of ulcer base as well as 2 vaginal swabs for women and a first-voided urine sample for men. Blood specimens were collected from all patients with genital ulcer disease for syphilis testing and from consenting patients for HIV testing. Whole blood samples were refrigerated until shipment. Because of the expected time lag between sample

collection and laboratory testing (see below), test results were not communicated to the study participants, with the exception of HIV and syphilis results. In addition, some study participants were tested for HIV using rapid tests per clinic standard of care and followed up when found to be positive. However, these procedures fell outside the study protocol.

All patients were treated for their presenting STI syndrome according to the 2012 Zimbabwe STI treatment guidelines.[5] In case of medication stock-outs at the clinic, the team was provided with recommended medications for study patients.

All findings and actions were documented on the paper questionnaire.[13] After completion of the visit, paper data were reviewed by the lead study nurse and then transcribed into a computer-based data system on handheld devices. Data were uploaded at least daily from study sites to an online secure central database.

All patients received US $5 for their time and participation, which in most clinics was equal to what they paid for their clinic visit. In addition, clinics also received US $5 for each patient enrolled to help defray the costs of staff time and other operating expenses.

Laboratory Procedures

All patient specimens were kept refrigerated after collection and shipped in a cooler box with cooling packs by courier the same day or overnight to the receiving laboratory at Wilkins Hospital in Harare, where all samples were kept refrigerated until further processing.

A number of tests were conducted at the receiving laboratory, including Gram-staining and reading of the air-dried smears, HIV serologic testing by HIV rapid test using the standard testing algorithm[a] in Zimbabwe, treponemal testing by SD Bioline DUO rapid test (Standard Diagnostics Inc, Gyeonggi-do, Republic of Korea) and TPHA, and non-treponemal testing by RPR.

All discharge and ulcer samples were stored in a -70^{0}F freezer and batched for shipment to the STI reference laboratory at the National Institute for Communicable Diseases (NICD) in Johannesburg, South Africa. Using an in-house developed multiplex polymerase chain reaction (M-PCR) test procedure, discharge specimens were tested for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas* vaginalis, and *Mycoplasma genitalium* and ulcer specimens were tested for *Treponema pallidum*, *Haemophilus ducreyi*, LGV-associated strains of *Chlamydia trachomatis* and HSV-1 and HSV-2.[12]

Urine aliquots and vaginal samples of all patients (including patients with genital ulcer disease) were shipped by the Wilkins receiving laboratory to two local laboratories for testing for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* by nucleic acid amplification testing (NAAT) using Becton Dickenson ProbeTec (BD Molecular Diagnostics, Franklin Lakes, NJ, USA) at the University of Zimbabwe/University of California San Francisco laboratory in the OB/GYNE department of the University of Zimbabwe School of Medicine and using GeneXpert

[a] HIV testing in Zimbabwe follows a standard algorithm of the following HIV rapid tests: 1) initial test by First Response HIV1-2-O; 2) confirmatory test by Alere Determine HIV1/2 if the initial test is positive; and 3) INSTI HIV1/HIV2 is used as a tie breaker if the initial and confirmatory tests are discrepant.

(Cepheid, Sunnyvale, CA, USA) at the Flowcytometry laboratory in Harare. All testing was done using standard operating procedures. The use of multiple platforms for gonorrhea and chlamydia testing served a dual purpose of quality control as well as support for expanding local STI testing capacity. More detail on laboratory procedures can be found in the online supplement.[13]

Quality-Control Measures

As mentioned above, regular clinic visits were conducted by the study leadership (CAR, MM, AM) to review clinic procedures, data management and specimen processing.

The receiving laboratory at Wilkins hospital kept running logs to document specimen shipment from the study sites and to the participating laboratories. All paper records were shipped to the receiving laboratory at Wilkins hospital and stored in locled file cabinets.The study consultant (CAR) performed a review of a random sample (10%) of paper records after the completion of the study. Electronic data were periodically analyzed to assess data completion and integrity and identified problems were communicated with the study team.

Statistical Methods

Data on participant demographics and sexual health and STI history were analyzed using SAS software (Cary, NC, USA). Tests for statistical significance included the Chi Square test for categorical variables and Student's T-test for continuous variables.

Funding

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Results

Study enrollment started on June 8, 2014 and was completed on April 25, 2015. Enrollment lasted 17 weeks in Harare (N=212), 10 weeks in Bulawayo (N=211) and 12 weeks in Beitbridge and Gutu (N=177). Recruitment in Beitbridge/Gutu was limited due to slow enrollment at the Gutu site and resource constraints limiting the time the study team could spend in the field. By recruiting slightly more patients at the Harare and Bulawayo sites, overall recruitment targets were met with a total of 600 patients enrolled, 200 in each of the syndrome categories. The main characteristics of the study population are summarized by enrollment site in Table 1. Syndromes were evenly distributed by city, however statistically significant differences by clinic occurred, mostly because more women with vaginal discharge were enrolled in one of the Bulawayo clinics and more men with urethral discharge were enrolled in the other (Table 1, $p<0.01$).

As expected, there were significant ethnic differences among enrolled participants between clinics in Harare (predominantly Shona) and Bulawayo (predominantly Ndebele) reflecting the general population characteristics in these regions (Table 1).

Also, there were more participants characterized as “other” ethnicity in one of the Bulawayo clinics and in the Beitbridge clinic.

The mean age of the study populations was 28.6 years (median 27 years). While there were no significant differences for age by study site, women were generally younger (27.7 years) than men (29.4 years, $p<0.01$).

In terms of sexual history, the mean number of sex partners for the past 3 months in the entire study population was 2.1 (median 1). Participants in the Khami Road (Bulawayo) and Dulibadzimu (Beitbridge) clinics were significantly more likely to report more than 1 partner in the past 3 months, i.e., respectively 39.6% and 36.1% compared to 24.5% for the entire study group ($p<0.001$). However, commercial sex was reported by a considerably lower proportion of study participants and did not differ among study sites.

A (lifetime) history of any STI other than HIV was reported by 35.9% of the study population, with higher proportions among participants recruited at the Khami Road (46.2%) clinic.

Condom use at last sex with a main partner was reported by 24.2% of participants with no differences among clinics. However, condom use at last sex with a non-main partner (reported by 40.9% overall) was reported at higher frequency by patients recruited at the Khami Road (54.3%) and Dulibadzimu (47.6%) clinics.

Finally, by self-report, 29.5% of study participants were HIV positive, 29.2% were HIV negative, and 41.3% reported unknown status, with a small, yet significant variation by study site ($p<0.05$). HIV testing data will be presented elsewhere.

Measures of Study Process

Qualitative and quantitative review of collected data indicated high levels of data completion and record review indicated high concordance between paper and electronic data files. As can be seen in Table 1, data were complete for all demographic and most risk factor categories. In addition, specimen collection was complete with only few specimens not analyzable. Specifically, testing for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* was completed for 598/600 patients, with insufficient samples for two men with genital ulcer disease. However, M-PCR testing for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalis*, and *Trichomonas vaginalis* was completed on all 400 men and women presenting with genital discharge, and M-PCR testing for *Treponema pallidum*, *Haemophilus ducreyi*, *Chlamydia trachomatis* LGV strains, HSV-1 and HSV-2 was completed on all 200 patients with genital ulcer disease.

Regular site visits by study investigators confirmed the adequacy of study set-up at each of the clinics and adherence to study procedures. As per study guidelines, study staff performed non-study clinic duties when enrollment was slow, contributing to good rapport between clinic and study staff and greatly enhancing study acceptance at the clinic level.

Discussion

This article reports on the methodology, population characteristics and process outcomes of the Zimbabwe STI Etiology Study. The data presented in this report allow for a number of general conclusions.

First, the difference in patient characteristics and STI syndromes by study site, including ethnic and gender differences as well as differences by risk factors and self-reported HIV status and STD history, confirm the rationale for selecting the study population from geographically diverse regions. At the same time, these differences also point to a potential weakness of this study in that we cannot assume that the 6 clinics were representative of the country and that sampling more clinics in other regions would not have enhanced the diversity of the study population. In the articles detailing the study outcomes, we will explore how the results vary by recruitment site and thus provide a measure of representativeness of the overall study population, or lack thereof. In this context, we point out that we were not successful in recruiting more patients at the rural Gutu site. Low recruitment was solely the result of the lack of patients presenting with STIs at this site during the recruitment time frame.

Second, the high level of self-reported HIV infection (29.5%), will allow us to explore the relationship between HIV and other sexually transmitted infections. This relationship may indicate a high level of co-occurrence as a result of the same antecedent high-risk behaviors, or a higher level of genital infections resulting from (advanced) HIV infection, including genital herpes recurrence, vaginal yeast

infections, and bacterial vaginosis. Regardless of the nature of this relationship, HIV/STI co-infections enhance the transmission of HIV and should be a focus of study.

Finally, process outcomes of the study, including timely patient enrollment, completeness and quality of data and laboratory samples, and observed rapport between study nurses and clinic staff, confirm the advantages of the study team approach. Alternative models that rely on existing clinic staff trained in study protocols with some form of central oversight have the advantage of being cheaper and may have less impact on overall clinic operations. However, relying on already overextended clinic staff and resources for study purposes has been shown to result in compromising efficiency and quality, compounded by lack of training and local study oversight.[11] Thus, the team approach as described in our study, while requiring a greater investment, appears to be superior both in terms of study efficiency and quality, and to ultimately have less negative and probably more positive impact on clinic operations. We suggest that this approach may serve as a model for conducting future STI etiology studies at sentinel sites.

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Table 1. Zimbabwe STI Etiology Study: Characteristics of Study Population by Enrollment Site

	Clinical Sites														
	Harare				Bulawayo				Beitbridge/Gutu						
	Mbare	%	Budiriro	%	Nkulumane	%	Khami Road	%	Dulibadzimu	%	Gutu Road	%	Total	%	P
Total	157	26.2	55	9.2	105	17.5	106	17.7	166	27.7	11	1.8	600		
Syndrome															<0.01
Discharge Women	53	33.8	16	29.1	50	47.6	19	17.9	59	35.5	3	27.3	200	33.3	
Discharge Men	51	32.5	20	36.4	24	22.9	44	41.5	58	34.9	3	27.3	200	33.3	
Ulcer Women	23	14.6	10	18.2	19	18.1	17	16.0	27	16.3	4	36.4	100	16.7	
Ulcer Men	30	19.1	9	16.4	12	11.4	26	24.5	22	13.3	1	9.1	100	16.7	
Gender															<0.001
Female	76	48.4	26	47.3	69	65.7	36	34.0	86	51.8	7	63.6	300	50.0	
Male	81	51.6	29	52.7	36	34.3	70	66.0	80	48.2	4	36.4	300	50.0	
Ethnicity															<0.0001
Shona	110	70.1	36	65.5	32	30.5	35	33.0	109	65.7	11	100.0	333	55.5	
Ndebele	29	18.5	13	23.6	61	58.1	51	48.1	25	15.1	0	0.0	179	29.8	
Other	18	11.5	6	10.9	12	11.4	20	18.9	32	19.3	0	0.0	88	14.7	
Age															
Mean	29.0		29.4		28.4		28		28.1		32.5		28.6		NS
Median	28		28		27		27		27		34		27		
Age Category															NS
15-19	9	5.7	3	5.5	9	8.6	6	5.7	8	4.8	1	9.1	36	6.0	
20-24	39	24.8	14	25.5	30	28.6	39	36.8	46	27.7	1	9.1	169	28.2	
25-29	46	29.3	15	27.3	22	21.0	22	20.8	50	30.1	3	27.3	158	26.3	
30-34	32	20.4	11	20.0	27	25.7	21	19.8	34	20.5	2	18.2	127	21.2	
35-39	15	9.6	8	14.5	7	6.7	10	9.4	19	11.4	1	9.1	60	10.0	
40-44	9	5.7	0	0.0	6	5.7	6	5.7	7	4.2	2	18.2	30	5.0	
>=45	7	4.5	4	7.3	4	3.8	2	1.9	2	1.2	1	9.1	20	3.3	
Number of Partners in Previous 3 Months															
Mean	1.1		2.2		1		2.9		3.2		1.2		2.1		<0.01
Median	1		1		1		1		1		1		1		
Range	0-3		0-35		0-2		0-70		0-50		0-3		0-70		
More than 1 Partner in Previous 3 Months															<0.0001
No	137	87.3	43	78.2	94	89.5	64	60.4	106	63.9	9	81.8	453	75.5	
Yes	20	12.7	12	21.8	11	10.5	42	39.6	60	36.1	2	18.2	147	24.5	
Commercial Sex in Previous 3 Months															
No	135	86.0	41	74.5	86	81.9	95	89.6	145	87.3	11	100.0	513	85.5	0.06
Yes	22	14.0	14	25.5	19	18.1	11	10.4	21	12.7	0	0.0	87	14.5	
Condom Use Last Sex Main Partner															NS*
No	118	76.1	44	81.5	70	73.7	80	75.5	123	74.1	10	90.9	445	75.8	
Yes	37	23.9	10	18.5	25	26.3	26	24.5	43	25.9	1	9.1	142	24.2	
Not Applicable	2		1		10		0		0		0		13		
Condom Use Last Sex Casual Partner															<0.0001*
No	63	55.8	40	88.9	68	71.6	48	45.7	87	52.4	10	90.9	316	59.1	
Yes	50	44.2	5	11.1	27	28.4	57	54.3	79	47.6	1	9.1	219	40.9	
Not Applicable	44		10		10		1		0		0		65		
Perceived HIV Status															<0.05
Negative	46	29.3	16	29.1	37	35.2	23	21.7	48	28.9	5	45.5	175	29.2	
Positive	41	26.1	10	18.2	38	36.2	30	28.3	56	33.7	2	18.2	177	29.5	
Unknown	70	44.6	29	52.7	30	28.6	53	50.0	62	37.3	4	36.4	248	41.3	
STD History															NS*
No	98	65.3	33	67.3	72	68.6	56	53.8	106	63.9	10	90.9	375	64.1	
Yes	52	34.7	16	32.7	33	31.4	48	46.2	60	36.1	1	9.1	210	35.9	
Missing	7		6		0		2	1.9	0		0		15		

*Percentages and Chi-square analysis limited to non-missing data

Due to rounding errors, not all percentages add to 100%