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Sentinel Surveillance of Sexually Transmitted Infection Syndrome aetiologies and HPV genotypes among patients attending Primary Health Care Facilities in South Africa, April 2014 – September 2015

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The contents of this report are solely the responsibility of the authors

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Executive Summary

Background and Introduction

As part of the new National Aetiology Surveillance Programme of sexually transmitted infections (STIs), laboratory-based STI surveillance was undertaken in 36 Primary Health Clinics (PHCs) across nine South African provinces. The main objectives of the survey were to determine the microbial aetiologies of the three major STI syndromes, as well as the prevalence of HPV infection and individual genotypes among a sample of adolescent girls and young women accessing family planning services. Secondary objectives included determination of the prevalence of serologically-diagnosed co-infections: syphilis, infectious hepatitis B, and herpes simplex virus type 2 (HSV-2) among patients presenting with STI syndromes. Additionally, prevalence of human immunodeficiency virus (HIV) infection among STI patients and family planning clinic participants was ascertained.

Methods

The targeted sample size was 25 cases of each STI syndrome per PHC, which equated to 900 cases of each syndrome nationally. Surveillance was conducted for Male Urethritis Syndrome (MUS); Vaginal Discharge Syndrome (VDS) and Genital Ulcer Syndrome (GUS); and high-risk human papillomavirus (HPV) infection in adolescent girls and young women. The aetiological study commenced in April 2014 and ended in September 2015, which was the period of co-operative agreement between the NICD, NDoH and CDC. STI syndromes were diagnosed and classified in accordance with the newly revised 2014 national Standard Treatment Guidelines for STIs at Primary Health Care Centres. Eligible and consenting STI and family planning clinic attendees were enrolled anonymously into the survey, completed a healthcare worker administered questionnaire and provided relevant clinical specimens, which included genital swab specimens and blood samples. STI and family planning clinic attendees could have more than one syndrome diagnosed at enrolment. Laboratory testing was performed at the NICD's STI Reference Laboratory at the Centre for HIV & STIs, as well as at the Institute of Infectious Disease and Molecular Medicine, University of Cape Town.

Findings

Not all clinics had achieved the intended target for sampling by the end of the study period.

A total of 1,512 STI patients, accounting for 801 episodes of VDS (89% of target), 540 episodes of MUS (60% of target) and 171 episodes of GUS (19% of target) were recruited at four PHCs in each of the nine provinces. The prevalence of detectable aetiological pathogens was determined for each of the three STI syndromes. Among patients with genital discharge, *Neisseria gonorrhoeae* was detected in 72.6% MUS & 10.6% VDS cases; *Chlamydia trachomatis* in 20.2% MUS & 14.2% VDS cases; *Trichomonas vaginalis* in 4.6% MUS & 15.8% VDS cases; *Mycoplasma genitalium* in 6.1% MUS & 8.3% VDS cases. The commonest causes of VDS were bacterial vaginosis (BV) and candidiasis (CA), accounting for 405 (50.6%) and 140 (17.5%) cases, respectively. Overall, 179 VDS cases (22.3%) had BV-STI co-infections and 36 (4.5%) were co-infected with CA and STI pathogens. Hence, STI co-infections were prevalent in a significant proportion of those with BV (179/405; 44.2%) and CA (36/140; 25.7%); the STI pathogen most commonly implicated being *Trichomonas vaginalis*, in over 40% of co-infections. Among GUS cases, only 82 of 171 had detectable STI pathogens: HSV in (47.9%), *Treponema pallidum* in 12 (7.0%) and 1 case each (0.6%) of chancroid (*Haemophilus ducreyi*) and lymphogranuloma venereum (*Chlamydia trachomatis* L1-3). Donovanosis was not detected in any of the nine provinces.

A total of 240 endocervical swab samples (27% of target) from 18-20 year-old females accessing family planning services were tested for HPV infection. HPV infection was detected in 178 (73.9%) females, and 127 (51.2%) of these were infected with high-risk genotypes.

Seroprevalence of syphilis, defined by a positive rapid plasma reagin (RPR) test, was 11.1% among GUS, 4.7% among MUS, and 3.5% among VDS patients. Serological evidence of active syphilis, defined by proxy of RPR ≥ 1:4, was detected in 7.6% of GUS, 2.8% of MUS and 1.2% of VDS cases. Seroprevalence of HSV-2 ranged from 43.5% for MUS to 78.4% for GUS. Rates of HIV co-infection were as follows: 64.3% in GUS, 41.9% in VDS and 24.6% in MUS patients. There was a significant association between HPV and HIV co-infection: the HIV co-infection rate among females infected with high-risk HPV genotypes was 28.1%. There was a significant association between HIV seropositivity and all STI syndromes ($p<0.0001$). Additionally HIV seropositivity was significantly associated with HSV-2 seropositivity ($p<0.0001$).

Conclusions

Neisseria gonorrhoeae is the predominant cause of MUS; and syndromic management with dual antimicrobial therapy, which also covers *Chlamydia trachomatis*, the second most common pathogen, is rational. Surveillance for antimicrobial resistance in *Neisseria gonorrhoeae* is essential. Herpes simplex virus is the commonest detectable cause of genital ulceration, validating the continued use of acyclovir in syndromic management; however, the cause of ulceration in nearly 46% of patients without an STI diagnosis requires further research. The syndromic management of VDS remains complex: the commonest causes, bacterial vaginosis and candidiasis, are non-STI related; however, a significant proportion of patients with either condition were co-infected with STI pathogens particularly *Trichomonas vaginalis*. The cervical HPV prevalence in young women aged 18-20 years was high, as was the prevalence of HPV-16, which is associated with persistent infection and accounts for the majority of cervical cancer. The significant proportion of young women infected with HPV genotypes contained in the bivalent vaccine supports a large-scale roll-out of the vaccine. The HIV seroprevalence among STI patients is high, underlining the importance of linkage to universal HIV counselling and testing in primary healthcare settings.

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LIST OF ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
CT	*Chlamydia trachomatis*
CTL1-3	*Chlamydia trachomatis* serotypes L1-3
DHIS	District Health Information System
DISCA	District STI Quality of Care Assessment
GI	Granuloma inguinale
GUS	Genital Ulcer Syndrome
HAST	HIV/AIDS/STI/TB
HPV	Human papillomavirus
HIS	Health information system
HIV	Human immunodeficiency virus
MUS	Male Urethritis Syndrome
NDOH	National Department of Health
NG	*Neisseria gonorrhoeae*
NHLS	National Health Laboratory Service
NICD	National Institute for Communicable Diseases
NIDS	National Indicators Data Set
HBV	Hepatitis B virus
HD	*Haemophilus ducreyi*
HSV	Herpes simplex virus
MG	*Mycoplasma genitalium*
PHC	Primary healthcare
PID	Pelvic inflammatory disease
RPR	Rapid plasma reagin
SADHS	South African Demographic Health Survey
STIs	Sexually Transmitted Infections
TP	*Treponema pallidum*
TV	*Trichomonas vaginalis*
VDS	Vaginal Discharge Syndrome
WHO	World Health Organization

1. Background and Literature Review

Bacterial STIs cause significant morbidity in South Africa and may rarely cause death, for example from ruptured ectopic pregnancy secondary to tubal damage from *Neisseria gonorrhoeae* and *Chlamydia trachomatis* or foetal death from congenital syphilis.(1) Importantly, both ulcerative and genital discharge syndromes are key co-factors for augmenting human immunodeficiency (HIV) acquisition and have been shown to increase transmission risk by 2-fold to 5-fold in prospective studies.(2)

HPV is the most common STI and, importantly, specific types of "high-risk" HPV are the cause of cervical cancer.(3) Cervical cancer is the second most common cancer of South African women.(4) HPV is also causally associated with anogenital warts (acuminata condylomata).(5) The present vaccines, Cervarix and Gardasil, both vaccinate against the major two HPV types that cause cervical cancer, namely HPV-16 and HPV-18.(6, 7) There is also evidence of cross-protection against some of the other high-risk HPV types.(8) In addition to HPV-16 and 18 Gardasil also protects against HPV-6 and 11 which cause anogenital warts. HPV is also causally associated with approximately 60% orpharyngeal cancers.(9)

Sexually transmitted infections (STIs), including HPV, facilitate the transmission and acquisition of HIV, by increasing concentration of HIV target cells (CD4+ cells) for inflammatory STIs such as gonorrhoea and chlamydial infection.(2, 10) Genital ulcer disease, such as genital herpes and untreated early syphilis, may also act as a portal for human immunodeficiency virus (HIV) entry.(11) HIV-infected persons with newly diagnosed STIs are at greatly increased risk for transmitting HIV as HIV viral loads (VL) are increased in cervico-vaginal, seminal and ulcer-derived secretions in the presence of other STIs, such as gonorrhoea or herpes.(12-14) For a given plasma VL, HIV-infected individuals with STI are at greater risk of transmitting HIV than those individuals without STIs.(15)

Women co-infected with HIV are at substantially increased risk of HPV infection and HPV associated cancers.(15) In HIV positive women there is an increased prevalence of anogenital warts.(16) HIV positive women are more likely to have recurrence of both anogenital warts and high-grade cervical lesions after treatment compared to HIV negative women.(17) Anti-retroviral treatment does not impact on HPV associated disease.(4) Therefore as HIV positive women live longer they are at higher risk of HPV associated disease.

Within South Africa, STIs have been treated using the syndromic management approach in primary healthcare centres (PHCs) since the late 1990s. This approach is a tool to manage symptomatic STIs and has the advantage of providing same-day treatment according to treatment flow charts, which can easily be adhered to by nursing staff at every PHC entry point across the country.(18) Although laboratory testing of STI patients is not required for case management, the WHO recommends that periodic assessment of aetiologies of STI syndromes (e.g., MUS, VDS and GUS) should be considered a core STI surveillance activity, especially in countries where STI syndromic management and case reporting are routinely undertaken.(19) Lack of clinical samples has deskilled laboratory staff in terms of ability to diagnose STIs on an aetiological basis and to determine antimicrobial susceptibility profiles for bacteria such as *Neisseria gonorrhoeae*.(20) The syndromic approach generally works better for male-associated as compared to female-associated STI syndromes. The poor specificity of syndromes like VDS and lower abdominal pain syndrome (LAP) to predict the presence of STIs leads to over-diagnosis of STIs, unnecessary stigmatisation and potentially relationship difficulties. Importantly, the syndromic approach results in substantial over-prescribing of antimicrobial agents that may influence the development of antimicrobial resistance among both sexually transmitted and non-sexually transmitted bacteria.(21) Mathematical modelling has shown that syndromic management is the cheapest programmatic approach to the management of STIs, although there remains debate as to whether it is the most cost-effective.(22, 23)

The NDoH is responsible for producing STI guidelines in South Africa, using antimicrobials on the Essential Drugs List, which ideally should be used in both public and private facilities. The NDoH issued revised evidence-based STI treatment guidelines in mid-2008.(18) Key changes in the 2008 revision were the inclusion of acyclovir in the GUS treatment algorithm, the replacement of ciprofloxacin with either cefixime for the treatment of uncomplicated gonorrhoea or ceftriaxone for the treatment of complicated gonorrhoea, and finally the replacement of erythromycin with amoxicillin for the treatment of presumptive chlamydial infection in pregnant women with VDS.(24) It is estimated that at least half of STI care episodes are managed by the private sector, where the NDoH has less influence on prescribing practice.(25) An interview-based study conducted among general practitioners (GPs) in Gauteng over a decade ago highlighted poor knowledge of STI syndromic management. and less than half of prescriptions overall were judged to be effective.(26) In addition, for most STI syndromes, uninsured patients were offered significantly cheaper and less convenient antibiotic regimens. A more recent self-administered survey for private-sector nurses and doctors, revealed a significant lack of knowledge of common STI aetiologies and recommended treatment, especially among those who had not received formal STI training.(27) In 2015, the STI management guidelines were formally revised in response to the increase in *Neisseria gonorrhoeae* antimicrobial resistance observed worldwide.(28) A pre-emptive strategy of dual antimicrobial therapy was incorporated to curb the emergence of resistance in *Neisseria gonorrhoeae* to extended-spectrum cephalosporins. Specifically, oral cefixime was replaced with single doses of injectable ceftriaxone and oral azithromycin.

Due to the syndromic management principle of managing patients on the first day of presentation without the use of laboratory tests, it is not possible to determine the burden of individual STI pathogens in countries using the syndromic management approach; all one can measure is the number of STI syndromes diagnosed over time. While no STI surveillance systems exist within South Africa's private sector; at public sector PHCs, the numbers of total STI syndrome episodes and new episodes of MUS are recorded. However, no data are routinely recorded for other STI syndromes, including VDS and GUS. For this reason, a national sentinel clinical STI syndrome surveillance system was launched in November 2003. This surveillance system was designed by the former STI Reference Centre, now part of the newly created Centre for HIV and STIs at the National Institute for Communicable Diseases (a Division of the National Health Laboratory Service, NHLS), and funded through a co-operative agreement between the NICD and the U.S. Centers for Disease Control and Prevention (CDC). The surveillance system was implemented by the National Department of Health (NDoH) with technical assistance from NICD staff and operates at 270 clinical sites across South Africa.(1) The STI Reference Centre analysed and reported the data for the first year of the sentinel survey (April 2004 to March 2005); subsequent to this, the clinically-based sentinel surveillance system has been managed in entirety by the NDoH.

Between April 2004 and March 2005, 1,654,776 new STI episodes were treated in PHCs clinics throughout South Africa (Centre for HIV and STI, unpublished data). Incidence rates of new STI syndrome episodes, calculated per 1,000 population aged 15-49 years of age, demonstrated a national incidence rate of 63 per 1,000 population, with the highest incidence rates recorded in Limpopo (90 per 1,000 population), Kwa-Zulu Natal (87 per 1,000 population) and the Eastern Cape (73 per 1,000 population); the lowest incidence rate was recorded in the Western Cape (38 per 1,000). During the same time period, a total of 145,818 new STI syndrome episodes (46,222 in males, 99,596 in females, 8.8% of the national

total) were reported among 126,656 patients in the sentinel survey, with a peak in the 20-24 year old age group. In men with STIs, the most frequent syndromes were MUS and GUS, whereas for women they were VDS and LAP. The relative prevalence and incidence of MUS, the most reliable indicator syndrome for 'true' STIs, seen at the sentinel sites during 2004-05 is shown by province in **Table 1**. Clinical surveillance data on the distribution of STI syndromes (2000 – 2007) in Gauteng Province PHCs have revealed that MUS, VDS and GUS together comprise nearly 80% of all syndromes seen.(29)

Table 1. Primary health care MUS indicators by province in 2004/05

Province	**New episodes (n)**	**Relative prevalence of MUS among STI patients (%)**	**Incidence rate per 1,000 men aged 15 - 49 (95% CI)**
Eastern Cape	60,147	25.6	40.8 (39.8 - 41.8)
Free State	20,533	25.1	28.6 (28.2 - 29.0)
Gauteng	61,139	23.7	19.4 (18.9 - 19.9)
KwaZulu-Natal	121,972	26.7	50.2 (49.3 - 51.0)
Limpopo	59,409	24.6	50.1 (48.9 - 51.4)
Mpumalanga	40,227	39.5	47.9 (47.4 - 48.3)
North West	36,394	24.1	33.5 (32.4 - 34.5)
Northern Cape	7,364	32.7	33.7 (33.0 - 34.5)
Western Cape	32,062	30.1	23.5 (22.7 - 24.3)
National	**439,247**	**26.5**	**35.2 (34.2 - 36.3)**

Table 1. Primary health care male urethritis syndrome (MUS) indicators by province in 2004-05.

Note: the denominator for the relative prevalence of MUS includes males and females

An important component of the STI syndromic management approach is the requirement for regular STI aetiological and antimicrobial resistance surveys to ensure that the flow charts and treatment algorithms are still able to treat the majority of the pathogens causing the STI syndromes. Since 2007, the NICD has conducted aetiological STI surveys among STI patients attending PHCs in several major cities, including Cape Town, Bloemfontein, East London, Johannesburg, Kimberley, Nelspruit, Polokwane and Rustenburg. With the exception of Johannesburg (Gauteng), where annual STI aetiological surveys have been conducted by NICD/NHLS since 2007, surveys have been conducted by NICD/NHLS only once in each of the other cities over the past 5 years. These surveys have provided useful information, confirming the importance of *Neisseria gonorrhoeae* as the major cause of MUS, the fact that less than 50% of VDS cases are attributed to sexually transmitted pathogens, and that the vast majority of genital ulcers are herpetic in origin, i.e. due to herpes simplex virus type 2 (HSV-2).(30, 31)

Although *Treponema pallidum* is endemic in South Africa, it is now a relatively uncommon cause of GUS. Macrolide antimicrobials are not first-line therapy for the treatment of primary syphilis; but azithromycin has demonstrated clinical efficacy for early syphilis in nonrandomized studies.(32) Macrolide resistance has been ascribed to specific 23S ribosomal RNA mutations.(33, 34) In South Africa, analysis of 63 specimens collected between 2005 and 2010 from various cities in Gauteng, Free State and Northern Cape provinces as part of national microbiological surveillance did not reveal any macrolide resistance determinants.(35) Molecular sub-typing of *Treponema pallidum* has been used to identify clusters of infection, and better understand strain distribution and transmission dynamics of syphilis.(36) Molecular typing of 161 *Treponema pallidum* specimens obtained from cross-sectional surveys of GUS (1996-1998) showed significant strain diversity (35 subtypes) in Johannesburg, Durban and Cape Town.(37) This was ascribed to the long endemicity of syphilis in the country, the ongoing transmission of infection from large numbers of untreated individuals as well as the introduction of new strains from infected migrants.

Human papillomavirus (HPV) infection is the most common sexual transmitted infection, and in women its prevalence peaks during adolescence soon after sexual debut; and decreases with age. There are currently three vaccines registered to prevent HPV infection, namely Cervarix targeting HPV-16 and -18; Gardasil targeting HPV-6, -11, -16 and -18; and Gardasil-9 targeting HPV-6, -11, -16, -18, -31, -33, -52, -56 and -58.(38, 39) HPV-6 and 11 are associated with genital warts, while HPV-16, -18, -31, -33, -52, -56 and -58 are associated with cervical cancer. High-risk genotypes HPV-16 and 18 are associated with approximately 70% cervical cancer cases. The National Department of Health introduced national school based HPV vaccination programme in 2014 using a two-dose Cervarix schedule in public schools for girls of 9-10 years of age, and coverage of more than 90% was achieved.(40) As part of the HPV vaccination strategy in South Africa it is important to have baseline data on HPV in teenagers and young women so that the impact of vaccination on circulating HPV genotypes in the long term can be assessed. It will likely be several years before the anticipated public health outcome of decreasing prevalence of infection and disease caused by HPV types targeted by Cervarix is observed.

NICD aetiological surveys have not been linked to sites that form part of the national sentinel clinical STI syndrome surveillance system. For a number of reasons, it has also proven operationally difficult for NICD staff to conduct these surveys on a regular basis at sites outside Johannesburg. There would be tremendous benefit to linking a national STI aetiological surveillance system to the national sentinel clinical STI syndrome surveillance system as one could then determine more reliable estimates of the national burden of individual STI pathogens. This would be an important indicator tool to measure trends in sexual behaviour over time and would support on-going efforts to reduce the incidence of both HIV and STIs within South Africa as part of the National Strategic Plan on HIV, STIs and TB.(41)

2. STI MICROBIOLOGICAL SURVEILLANCE IN SOUTH AFRICA

In developing countries, STIs and their complications rank in the top five disease categories for which adults seek health care. Surveillance of the prevalence of STIs is as a key priority in public health. The national comprehensive surveillance system for STIs in South Africa is made up of three components namely:

- The National Indicators Data Set (NIDS) which contain 5 data elements on STIs and are collected from all primary health care (PHC) facilities and level-one hospitals in the country.

- The National Clinical Sentinel Surveillance (NCSS) programme under which detailed data are collected from a selected number of PHCs in the country.

- The National Microbiological Surveillance (NMS) programme, which is composed of periodic surveys of syndrome aetiology and drug resistance monitoring.

Accurately monitoring the incidence and prevalence of STIs among the general population and particularly STI patients is important in measuring the effects of disease control and prevention efforts. The syndromic approach to treatment of STIs has been vital in rationalizing and improving the management of STIs. STI control in low- income countries is shaped by case management guidelines promoting syndromic management. Periodic aetiological surveillance of STI syndromes is a critical component of the syndromic management approach of STIs as it validates existing algorithms and ensures that all major pathogens are covered.

In 2003-2004, Centre for HIV and STIs at NICD/NHLS assisted the South African National Department of Health (NDoH) establish a national sentinel surveillance programme for measuring STI syndromic patient presentations to 30 sentinel clinics per province (270 sites in total). The NDoH now runs this clinical syndrome-based programme and it would be significantly strengthened if a matching sentinel aetiological STI surveillance programme could be carried out at a proportion of these 270 sentinel sites (4 sites/province, 36 sites nationally). This would allow an estimation of the burden of individual STIs, rather than clinical syndromes, within patients attending the public sector services across the country and by province.

As part of the HPV vaccination strategy in South Africa it is important to have baseline data on HPV in key populations, such as young women attending public sector family planning clinics (FPCs) and women attending colposcopy clinics with high-grade cervical cytology results, so that the impact of vaccination can be assessed. There is very little data on the prevalence of HPV and HPV types in women in South Africa.

As part of the National Aetiology Surveillance Programme of sexually transmitted infections (STI), laboratory based STI surveillance was undertaken in 36 PHCs across nine South African provinces during April 2014 to September 2015. The main objectives of this survey were to determine the aetiology of three major STI syndromes, and the prevalence of HPV infection and individual genotypes among a convenience sample of young women accessing public sector family planning services at the same 36 sentinel sites used for STI aetiological surveillance. Secondary objectives were to determine the seroprevalence of sexually transmitted co-infections: active syphilis, infectious hepatitis B and herpes simplex virus type 2 (HSV-2) infection in STI patients, as well as the HIV co-infection rate among STI patients and family planning participants.

STI surveillance is one of the components of second generation HIV surveillance. As previously mentioned, the ability to develop national estimates of would be an important indicator tool to measure trends in sexual behaviour over time and would support on-going efforts to reduce the incidence of both HIV and STIs within South Africa as part of the National Strategic Plan on HIV, STIs and TB 2012-2016.(41) STI surveillance system must generate data linked in a meaningful way with STI programme implementation, and data generated must be used to revise national STI treatment guidelines as appropriate. HPV prevalence and genotype data will provide important vaccination data and enable future monitoring of trends in both the prevalence of HPV detection and the relative prevalence of vaccine-related HPV genotypes.

3. Project Goals and Objectives:

The objectives of the laboratory based STI surveillance were:

To provide supporting aetiological STI data to assist with the interpretation of the existing STI syndrome clinical data for three major STI syndromes (MUS, VDS and GUS) at the designated 36 sentinel public sector healthcare facility sites (4 sites/province) and analyse data by province and HIV status.

To determine co-infections with HIV, HSV-2, Hepatitis B and syphilis among patients with MUS, VDS and GUS and analyse data by province

Using *T. pallidum* DNA, to type the bacterium and determine the prevalence of key ribosomal RNA gene mutations associated with macrolide resistance.

To undertake detection and typing of HPV in endocervical swabs of women attending FPCs in order to determine the baseline prevalence of HPV infection and proportion of high-risk HPV genotypes

To determine co-infection with HIV among FPC attendees infected with HPV, and analyse data by province

In summary, the surveillance was undertaken to answer questions regarding the prevalence of various aetiologically-linked STI pathogens among three key STI syndromes (MUS, VDS, GUS) and the prevalence of co-infection with HIV, HSV-2, syphilis and hepatitis B in STI patients. Additionally, the findings of the survey will inform health policy makers about the prevalence of HPV among FPC attendees and the relative prevalence of various HPV genotypes among those with HPV infections. The project will also report on any associations that exist between HIV co-infection and, firstly, HPV detection and, secondly, the presence or absence of specific HPV genotypes.

The STI syndrome-related data may be used in conjunction with existing data from the national sentinel clinical STI syndrome surveillance system to estimate total numbers of individual STI infections presenting to primary healthcare clinics. For STIs, the estimates may also be used to predict the asymptomatic STI burden in the population based upon known data relating to the relative proportion of asymptomatic disease for each STI pathogen. These national estimates may assist the NDoH with monitoring the impact of HIV/STI interventions within South Africa. The HPV prevalence and genotyping data will provide important baseline data for young FPC attendees following the public sector introduction of an HPV vaccine.

4. Methods

4.1 Design

Cross sectional study at 36 sentinel sites in all nine provinces in South Africa

4.2 Eligibility criteria

Patients who met the following inclusion and exclusion criteria were eligible for enrolment in the survey

Inclusion criteria:

- patient presenting to the PHC with a new STI episode of MUS, VDS or GUS (STI aetiological surveillance) or female to the FPC for family planning services (HPV surveillance)
- aged 18 years and above (STI patients) or aged 18-20 (FPC attendees)
- able to read the patient information sheet/consent form
- not previously recruited in the same year's STI aetiological survey (STI patients only)
- not previously recruited in the same year's HPV survey (FPC attendees only)
- clinical evidence of MUS or VDS and/or GUS on examination (STI aetiological surveillance only)

Exclusion criteria:

- under 18 years of age (STI/FPC patients) or 21 years and over (FPC attendees)
- patient has previously attended the PHC for the same complaint in the past 2 weeks (STI aetiological surveillance)
- No clinical evidence of STI on examination (STI aetiological surveillance only)
- unable to read the patient information sheet/consent form
- STI patients previously recruited in the same year's STI aetiological survey
- FPC attendee previously recruited in the same year's HPV survey
- STI syndrome other than MUS, VDS, GUS (STI aetiological surveillance only)

*Pregnancy was not an exclusion criterion for participation in either the STI aetiological and FPC-based components of the survey

4.3 Site Selection

The total number of new STI episodes recorded in the 270 national sentinel sites between 2011 and May 2012 were obtained, and the highest performing facilities selected per province. The study was conducted at 36 of the 270 sentinel sites. For each province, 4 sites were selected (**Table 2**) by convenience sampling from the 30 existing provincial sentinel sites on the basis of pre-determined criteria:

(i) evidence of continued reporting of STI syndromes to the sentinel surveillance programme,
(ii) geographical representation within the province
(iii) rural versus urban facility.
(iv) the willingness of the staff at the sites to participate

4.4 Recruitment of participants and data collection procedures

Consecutive patients presenting with MUS, VDS or GUS at the 36 PHCs during the period April-2014 and September 2015 were invited to participate in the survey by trained PHC nurses. Each patient was given a patient information sheet to read (Appendix 1), and an opportunity to ask the nurse questions, following which they could consent in written format to participation in the survey and the collection of biological specimens (Appendices 2 & 3). Following eligibility assessment and informed consent procedures, eligible and consenting patients had a short clinical questionnaire (Appendix 4) administered by the attending nurse.

The questionnaire was limited to one page as it was important that enrolment of patients in the survey did not unnecessarily slow down clinical operations and increase patient waiting times during the period of the survey. The questionnaire recorded key demographic, STI clinical and sexual behavior variables (Table 3) based on the following information: the name of the sentinel site, the date of enrolment, the unique STI syndrome surveillance number for the patient (sticker), the patients' age, the patients' gender, the patients' ethnic group (defined as African, Coloured, Indian, White or Other), the patients' sexual orientation (defined as heterosexual, homosexual or bisexual), the STI syndrome(s) diagnosed in the patient (specifically, MUS, VDS and/or GUS), history of treatment of VDS/MUS/GUS without success in the past 3 months, referral from another clinic for persistent MUS/VDS/GUS, history of STI syndromes in the past one year, condom use at the last sexual intercourse, age of first sex (coitarche), sex with someone living in another South African province in the last 3 months, sex with someone living outside South Africa in the last 3 months and, finally, a checklist of the specimens collected from each patient.

All patients were managed according to routine standard of care practices in the clinic. This included antimicrobial treatment according to the national syndromic management flow charts from the nurse managing their condition, HIV/STI health education, HIV testing and counselling and condom provision.(18) In addition, the STI patients received partner notification slips to enable partner referral. The FPC attendees received the same family planning advice and assistance that they would have received from the nurse had not participated in the surveillance project.

Table 2. Selected sentinel sites: highest performing facilities based on total number of new STI episodes between 2011 and May 2012

Province	Facility	DataElementName	Number of new STI episodes in 18 month period	Urban/Rural
Eastern Cape	Nozuko Clinic	STI treated - new episode	1 301	Urban
	Algoa Park Clinic	STI treated - new episode	922	Urban
	Tombo CHC	STI treated - new episode	1 119	Rural
	Nkanya Clinic	STI treated - new episode	411	Rural
Free State	Khotalong Clinic	STI treated - new episode	438	Rural
	Kopanong Clinic	STI treated - new episode	447	Rural
	Qholaqhwe Clinic	STI treated - new episode	486	Rural
	Rearabetswe Clinic	STI treated - new episode	874	Rural
Gauteng	Daveyton Main CDC	STI treated - new episode	3 696	Urban
	Phillip Moyo CHC	STI treated - new episode	2 023	Urban
	Discoverers CHC	STI treated - new episode	3 192	Urban
	Bekkersdal West CHC	STI treated - new episode	2 393	Urban
KwaZulu-Natal	Osizweni 2 Clinic	STI treated - new episode	2 194	Urban
	Mpumalanga Clinic	STI treated - new episode	3 552	Urban
	Ntumeni Clinic	STI treated - new episode	899	Rural
	Port Edward Clinic	STI treated - new episode	936	Rural
Limpopo	Lephepane Clinic	STI treated - new episode	551	Rural
	Louis Trichardt Clinic	STI treated - new episode	1 932	Rural
	Tshisaulu Clinic	STI treated - new episode	1 046	Rural
	Shongoane Clinic	STI treated - new episode	739	rural
Mpumalanga	Shabalala Clinic	STI treated - new episode	1 757	Rural
	Tonga Block B Clinic	STI treated - new episode	1 092	Rural
	White River Clinic	STI treated - new episode	701	Rural
	Belfast Gateway Clinic	STI treated - new episode	924	Urban
North West	Hoekfontein (Mmakau) Clinic	STI treated - new episode	684	Rural
	Kunana Clinic	STI treated - new episode	352	Urban
	Promosa CHC	STI treated - new episode	846	Urban
	Ipelegeng Clinic	STI treated - new episode	486	Rural
Northern Cape	Beaconsfield Clinic	STI treated - new episode	2 037	Urban
	Galeshewe Day Hospital	STI treated - new episode	430	Urban
	Kagiso CHC	STI treated - new episode	936	Rural
	TshwaraganoGateway Clinic	STI treated - new episode	926	Rural
Western Cape	Citrusdal Clinic	STI treated - new episode	793	Rural
	Clanwilliam Clinic	STI treated - new episode	301	Rural
	Klawer Clinic	STI treated - new episode	344	Rural
	Moorreesburg Clinic	STI treated - new episode	317	Rural

Table 3. Information collected in the clinical questionnaire.

Unique Identifiers	Patient Information	Clinical Information	Behavioral Information
Name of sentinel site	Age	STI syndrome	Condom use at the last sex
Date of enrolment	Gender	History of unsuccessful treatment in the past 3 months	Age of first sex
Surveillance number	Ethnic group	Referral for persistent MUS/VDS/GUS	Sex with someone living in another South African province in the last 3 months
	Sexual orientation	History of STI syndrome in the last one year	Sex with someone living outside South Africa in the last 3 months

4.5 Specimen collection

As part of the survey, genital specimens and sera were collected from patients with MUS, VDS, GUS and from FPC attendees. All these specimens, as well as the clinical questionnaire, were linked to each other by a survey number which was delinked from any participant identifiers. The Centre for HIV and STIs undertook all laboratory-based testing of specimens.

For men with MUS, an endourethral swab was taken from each patient, applied to a glass slide, and then placed in a sterile plastic tube for multiplex polymerase chain reaction (M-PCR) testing at the Centre for HIV & STIs (NICD/NHLS). For women with VDS, a swab was collected from the lateral vaginal wall and- posterior fornix and a smear made on a glass microscope slide. An endocervical swab was subsequently taken for M-PCR testing and handled the same way as the M-PCR swab from MUS patients. Men and women with genital ulceration had two swabs taken; the first ulcer swab was smeared on a microscope slide for Giemsa staining for donovanosis and the second ulcer swab was placed in a sterile plastic tube for M-PCR testing. Serological testing required the collection of a 10 ml venous blood sample in a clotted blood tube from each patient.

For FPC attendees, an endocervical swab was collected from each and placed in a sterile plastic tube. Additionally, a 10ml venous blood sample was taken in a clotted blood tube.

All glass microscope slide specimens were placed unfixed in slide boxes. All swab specimens and clotted blood tubes were stored in cooler boxes with ice packs until collection by the NHLS courier.

4.6 Laboratory procedures

All specimens, with the exception of endocervical swabs from FPC participants, were couriered to the STI Section of the Centre for HIV and STIs (CHIVSTI), NICD/ NHLS, in Johannesburg. Swab specimens from FPC participants were couriered to the CHIVSTI Cape Town laboratory. Microscopy, STI multiplex polymerase chain reaction (M-PCR) assays and serological assays were performed at the Centre's Johannesburg laboratory and HPV detection and genotyping were undertaken at the Centre's Cape Town laboratory.

Molecular testing for STI pathogens:

DNA was extracted from swabs using an automated DNA extractor (X-tractor Gene, Qiagen, Hilden, Germany). A validated real-time in-house M-PCR assay for *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT), *Mycoplasma genitalium* (MG) and *Trichomonas vaginalis* (TV) on a RotorGene platform (Corbett Research, Sydney, Australia) was used to determine the aetiology of MUS (Male urethritis syndrome) and VDS (Vaginal discharge syndrome) cases. DNA extracted from ulcer swabs of GUS patients was similarly tested by M-PCR for herpes simplex virus (HSV), *Haemophilus ducreyi* (HD) and *Treponema pallidum* (TP). Any DNA extracts found to be positive for HSV were sub-typed into HSV-1 and HSV-2 using a commercial PCR

assay (Sacace Biotechnologies, Como, Italy). For those DNA extracts that were positive for *C. trachomatis*, a second in-house PCR was performed that is specific for *C. trachomatis* serovars L1-L3, the causative agent of lymphogranuloma venereum (LGV).

Microscopy: Slides were fixed by heat fixing (MUS/VDS slides) or by application of methanol (GUS slides) and stained with either Gram stain (MUS/VDS slides) or Giemsa stain (GUS slides). The MUS slides were examined for the presence of pus cells with (gonorrhoea) or without (non-gonococcal urethritis) evidence of intracellular or extracellular Gram negative diplococci. The VDS slide were examined for the content of vagina flora, using Nugent's scoring, in order to diagnose bacterial vaginosis and also for the presence of either spores of pseudohyphae consistent with the presence of *Candida* species. The GUS slides were examined for the presence of intracellular Donovan bodies which are pathognomic for donovanosis (granuloma inguinale).

Serology:

Sera from patients recruited with MUS, VDS or GUS were tested for the presence of syphilis using a rapid plasmin reagin (RPR) assay (Immutrep, Omega Diagnostics, Alva, Scotland), for the presence of anti-HSV-2 antibodies (HerpeSelect HSV-2 enzyme-linked immunosorbent assay, Focus Diagnostics, Cypress, CA, USA) and for the presence of Hepatitis B surface antigen (Determine Hepatitis B Surface Antigen assay, Alere Medical, Chiba, Japan). The sera of all patients, both STI clinic attendees and FPC attendees, were tested for the presence of anti-HIV antibodies using two rapid tests, specifically the Determine™ HIV-1/2 antibody test (Alere Medical, Chiba, Japan) and the Uni-gold™ HIV test (Trinity Biotech Plc, Bray, Ireland).

Treponema pallidum azithromycin resistance testing and sub-typing:

Samples that tested positive for *Treponema pallidum* (TP) on M-PCR testing were further analysed for the 23srRNA A2058G and A2059G mutations that result in azithromycin resistance.

Nucleic acid was extracted from the samples using the XtractorGene (Corbett) platform. The extracted DNA was amplified using the methodology as described by Lukehart et al. (33)with a few modifications (33). Briefly, a 50 µl reaction contained 5 µl PCR reaction buffer with $MgCl_2$ (Roche Diagnostics, Mannheim, Germany), 5 µl of the deoxynucleoside triphosphate (dNTP) mix (2 mM each of dGTP, dCTP, dATP and dTTP) (Roche Diagnostics), 0.2 µM each of the 23SS (5' – GTA CCG CAA ACC GAC ACA G – 3') and 23SF (5' – AGT CAA ACC GCC CAC CTA C – 3') primers, 2.5 U Taq polymerase (Roche Diagnostics) and 10 µl extracted DNA. The PCR was performed on the GeneAmp 9700 (Applied Biosystems, Foster City, CA, USA) or G-Storm (Vacutec) platforms. Denaturation was performed at 93ºC for 3 min followed by 45 amplification cycles (93ºC for 1 min, 63ºC for 2 min and 72ºC for 1 min) followed by an elongation step of 10 min at 72ºC. This resulted in a 628 bp fragment of the 23S rRNA gene. Amplicons were analysed on the Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany). The amplicons were then digested with *BsaI*- and *MboII*-restriction enzymes (New England Biolabs, Beverley, MA) to screen for the A2059G and A2058G mutations respectively (33, 34). Samples were sized on the Agilent 2100 Bioanalyzer and samples with no mutations resulted in a single 628 bp band, while the A2058G and A2059G mutations resulted in 2 band sizes. Positive controls that have a resistant phenotype included the Street 14 strain (A2058G mutation) and the A2059G-mutant strain. The Nichols wild-type strain which exhibits no azithromycin resistance mutations was used as the negative control.

Treponema pallidum sub-typing is based on 3 target genes: the acidic repeat protein (*arp*) gene, the *Treponema pallidum* repeat gene and more recently, the tp0548 gene (36, 42, 43).

Arp-gene analysis:

For *arp*-gene analysis, a previously published touchdown 2-stage PCR methodology was used with a few modifications (35, 44, 45). The arp N1 (5' – ATC TTT GCC GTC CCG TGT GC – 3') and arp N2 (5' – CCG AGT GGG ATG GCT GCT TC – 3') primers were used during amplification on the GeneAmp 9700 (Applied Biosystems) or G-Storm (Vacutec) platforms. The amplicons were analysed on the Agilent 2100 Bioanalyzer (Agilent Technologies). The number of *arp* repeats was estimated by comparing it to the Nichols control strain which has 14 short tandem repeats and an amplicon size of approximately 1155 bp.

Treponema pallidum repeat (*tpr*) gene analysis:

A previously described nested PCR followed by restriction enzyme digestion with *MseI*, resulted in a RFLP banding pattern when analysed on the Agilent 2100 Bioanalyzer that could be associated with different *tpr* types described previously (44).

tp0548 gene analysis:

tp0548 typing relies on sequencing to detect variability of the gene 131 bp downstream from the start codon. Originally only 8 subtypes were identified by Marra *et al*. (2010), but recently 4 more have been identified (Tian *et al*., 2014; Grillova *et al*., 2015) and the types now ranged from subtype a-l (36, 42, 43).

HPV testing and genotyping:

For HPV testing, DNA was extracted from the endocervical swabs using the MagNA Pure Compact nucleic acid extractor (Roche Diagnostics, Mannheim, Germany). Following DNA extraction, the Linear Array (LA) HPV Genotyping Test (Roche Molecular Systems, Inc., Branchburg, NJ, USA) was used to determine the HPV genotype distribution among all patients. The LA test amplifies the target HPV DNA of 37 different HPV genotypes. High-risk (HR) HPV types include HPV−16, −18, −31, −33, −35, −39, −45, −51, −52, −56, −58 and −59; probable or possible HRHPV types included HPV−26, −53, −66, −67, −68, −70, −73 and −82; and low-risk (LR) HPV types HPV-6, −11, −40, 42, −54, −55, −61, −62, −64, −69, −71, −72, −81, −83, −84, −89 (HPV-CP6108) and –IS39.(46) HPV-52 was recorded positive only in the absence of HPV types 33, 35 and 58 due to the combined probe used for these 4 types in the LA assay. The β-globin gene was amplified as a control for cell adequacy, extraction and amplification. Samples with a negative β-globin result and a positive HPV DNA result were considered valid and adequate for analyses.

4.7 Training and Quality assurance

The recruiting nurses from the facilities were trained at provincial meetings held across the country, as well as at follow-up visits to the respective PHCs by the Centre for HIV & STIs (NICD) staff during the recruitment period. The training incorporated the accurate completion of clinic working cards and laboratory tracking forms; revision of STI syndromic management guidelines; and the collection and transport of samples for STI testing.

All laboratory staff working on the project received training on the objectives of the surveillance project, the nature of the tests involved and the importance of maintaining strict confidentiality of all information.

4.8 Sample size calculation

For sample size calculation, the prevalence for each pathogen was calculated in the three distinct patient populations (MUS, VDS, GUS), based on specific prevalence as shown from **Table 4** below as well as general assumed prevalence without regard to a specific pathogen (**Table 5**). This was necessary to get a required maximum sample size which could then be applied for all pathogens.

Table 4. Sample size calculation based on specific pathogen prevalence.

Syndrome	**Prevalence (%)**	**Sample size (n) using 95% confidence level**		
		5% precision	8% precision	10% precision
1. MUS				
N. gonorrhoeae	75	288	133	72
C. trachomatis	15	196	77	49
T. vaginalis	5	73	29	18
M. genitalium	7	100	39	25
2. VDS				
N. gonorrhoeae	10	138	54	35
C. trachomatis	13	174	68	43
T. vaginalis	20	246	96	61
M. genitalium	7	100	39	25
Bacterial vaginosis	40	369	144	92
Candidiasis	17	217	85	54
3. GUS				
Herpes simplex	65	349	137	87
T. pallidum	5	73	29	18
H. ducreyi	1	15	6	4
C. trachomatis L1-L3	1	15	6	4

Table 5 shows sample sizes calculated using EPI INFO. This calculation used specific prevalences of 1%, 5%, 7%, 10%, 15% and 20% with varying precision levels of 5%, 8% and 10%. These figures gave minimum sample sizes assuming 95% confidence level. For this study, based on logistical and financial challenges, we settled for 20% prevalence and 8% precision – leading to a minimum sample size of 96.

Table 5: Sample size calculations based on general assumed prevalence without regard to a specific pathogen

Prevalence (%)	**Sample size (n) using 95% confidence level**		
	5% precision	8% precision	10% precision
1	15	6	4
5	73	29	18
7	100	39	25
10	138	54	35
15	196	77	49
20	246	96	61

If some sites were not able to collect the required numbers of specimens for each STI syndrome, other sites within the same province were asked to enrol more STI patients so that the provincial total would be 100 specimens per STI syndrome per survey (**Table 6**).

Table 6. Anticipated sample size for MUS, VDS and GUS cases, and FPC participants per province.

Province	No. of sentinel sites	MUS	VDS	GUS	Total syndromes	FPC participants
Eastern Cape	4	100	100	100	300	100
Free State	4	100	100	100	300	100
Gauteng	4	100	100	100	300	100
Kwa-Zulu Natal	4	100	100	100	300	100
Limpopo	4	100	100	100	300	100
Mpumalanga	4	100	100	100	300	100
North-west	4	100	100	100	300	100
Northern Cape	4	100	100	100	300	100
Western Cape	4	100	100	100	300	100
All provinces	36	900	900	900	2700	900

The aim was to recruit approximately 100 patients at each site, consisting of (i) 25 consecutive consenting FPC participants and (ii) up to 75 consecutive consenting STI patients presenting with the following syndromes: MUS, VDS and GUS (25 sets of specimens for each of the three STI syndromes per survey site) (**Table 5**). For GUS patients, 15 consecutive consenting males and 10 consecutive consenting females were to be selected at each recruiting site - more males than females as GUS is more frequently reported by male STI patients. It was anticipated that some MUS and VDS patients may also be enrolled as GUS cases if both STI syndromes were present. Accordingly, the number of STI patients enrolled would then be slightly less than the number of STI syndromes for which specimens were available for testing. The maximum number of patients to be enrolled per site would be 100 patients, i.e. 25 FPC participants and 75 STI patients. Enrolment of eligible participants was consecutive, and numbers of non-participants, required to assess representativeness of the annual sample, were kept in a clinic-based log.

4.9 Data Management and Statistical Methods

Data management was undertaken by staff at the Centre for HIV and STIs (NICD/NHLS). Completed clinical questionnaires and laboratory results were entered into a survey specific Access© [Microsoft Access 2010, Microsoft, Seattle Washington] database. Double data entry was conducted in order to minimize data entry errors. Data were exported into STATA 14® [Stata Corporation, College Texas] for analysis.

The enrolled participants were described using frequencies and proportions for categorical data, and medians and interquartile ranges (IQRs) for continuous variables. The relative prevalences of the different pathogens were determined as proportions. Fishers' exact and Pearsons' chi-square tests were used to test for associations. Although data were collected at site level within a province, the analysis was conducted at provincial level based on the assumption that the prevalence distribution in each site is the same and therefore representative of province level estimate. These statistics were presented at provincial level and, if no statistical significant differences were found, pooled to present an aggregated national level prevalence. The prevalence of co-infections by STI syndrome, HPV infection and HPV genotype status, as well as any associations between laboratory diagnosis and data from the clinical questionnaire, were determined using the Chi Square test with the level of significance defined as $p < 0.05$.

4.10 Ethical Considerations

Ethical approval for the study was granted by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (certificate number M120365). Following a discussion of study procedures with potential participants, clinic nurses obtained written informed consent to participate in the survey, answer questions in the clinical questionnaire and provide relevant specimens. All forms were written in English and the surveillance nurse gave necessary explanation in the vernacular language, where required.

5. Results

5.1 Recruitment rates

Between 06 March 2014 and 31 August 2015, 1668 participants were consented and enrolled at the 36 sentinel sites. The total number enrolled represented 46.3% of the overall target. The number of participants enrolled per province ranged from 74 in the EC to 290 in KZN, while the number enrolled per facility ranged from 5 at a facility in EC to 95 at a facility in KZN. The number of facilities and median number of participants per facility by province are presented in **Table 7** below. The median number recruited per facility is presented (instead of the mean), in order to demonstrate the variability in the number of participants enrolled by the four facilities in each province.

Recruitment rates were suboptimal and did not reach a total target of 400 participants for all syndromes surveyed in any of the provinces. **Table 7** shows the total number of new STI cases and total number of MUS cases seen in the four sentinel facilities by province during the period of surveillance. When compared to the expected relative distribution of STI syndromes based on clinical surveillance data, the total number of study participants recruited (which included FPC participants) did not reach even 10% in any of the provinces; and ranged from 1.8% (Gauteng) to 8.9% (Limpopo). The MUS recruitment rate was less than 5% of all MUS cases seen at the four sites in each province during the surveillance period; and ranged from 0.6% (Gauteng) to 3.7% (Free State). Only Mpumalanga and Kwa-Zulu Natal recruited close to the target of 100 MUS cases. Denominator data were not available for the Eastern Cape.

5.2 Description of participants

Of the 1668 participants enrolled, 611 (36.6%) were male STI patients, 850 (51.0%) female STI patients and 207 (12.4%) family planning clinic attendees. Of the participants enrolled, 1518 (91.0%) were black Africans. **Table 8** describes the demographic and behavioural characteristics of male and female STI patients. The median age (and IQR) of male and female STI patients was 27 (23-32) and 29 years (23-37), respectively, and this was significantly different. With respect to high risk sexual behaviours, there were statistically significant differences in median age at sexual debut (younger by 1 year in male STI group); and reported condom use at last sexual encounter (lower for the male STI group). Males were also more likely than females to have sexual partner from another province or another country. Over 40% of patients in both groups reported having an STI syndrome in the previous 12-month period (there was no significant difference between the two groups).

Table 7: Number of participants enrolled by facility and province

Province	Number of facilities	Total number of participants recruited per province for study	Total number of MUS cases recruited per province for study	Number of participants recruited per facility for study Median (range)	Total number of new STI cases presenting to 4 sentinel facilities per province during surveillance period	Total number of MUS cases presenting to 4 sentinel facilities per province during surveillance period
Eastern Cape	4	74	14	19 (5 - 31)	No information available	No information available
Free State	4	107	16	33 (7 - 35)	1,322	438
Gauteng	4	187	65	50 (24 – 63)	10,605	2,307
KwaZulu Natal	4	290	95	71 (54 - 95)	*4,341	*761
Limpopo	4	225	72	60 (34 - 71)	2,518	867
Mpumalanga	4	249	93	59 (52 - 80)	3,079	900
Northern Cape	4	194	52	46 (8 - 54)	3,248	391
North West	4	154	51	46 (23 - 80)	2,368	487
Western Cape	4	188	82	55 (14 - 64)	3,026	770
Total	36	1668	**540**	51 (5 - 95)	30,507	6,921

**Information not available for Osizweni 2 Clinic in Kwa Zulu Natal*

Table 8: Demographic and behavioural characteristics of STI patients by group

Characteristic	Male STI (611)	Female STI (850)	p-value (Male STI vs Female STI)
Age in years, (median, IQR)	27 (23-32)	29 (23-37)	<0.001
African race, (n, %)	549 (89.9)	774 (91.1)	0.740
Age in years at sexual debut (median, IQR)	17 (16-19)	18 (16-19)	0.015
Condom use at last sexual encounter, (n, %)	149 (24.4)	311 (36.5)	<0.001
Sexual partner from another province in the last 3 months, (n, %)	93 (15.2)	86 (10.1)	0.006
Sexual partner from another country in the last 3 months, (n, %)	56 (9.2)	51 (6.0)	0.046
History of any STI syndrome in the past 12 months, (n, %)	267 (43.7)	377 (44.4)	0.817

Table 9 describes the demographic and behavioural characteristics of 207 FPC participants aged 18-20 years. The median age at sexual debut was 17 years (IQR 16-18 years); over 50% reported condom use at last sexual encounter and approximately 12% had a history of STI in the preceding 12-month period.

Table 9: Demographic and behavioural characteristics of FPC participants by group

Characteristic	Family planning Clinic participants (207)
Age in years, (median, IQR)	19 (18-20)
African race, (n, %)	195 (94.2)
Age in years at sexual debut (median, IQR)	17 (16-18)
Condom use at last sexual encounter, (n, %)	111 (53.6)
Sexual partner from another province in the last 3 months, (n,%)	15 (7.2)
Sexual partner from another country in the last 3 months, (n,%)	3 (1.4)
History of any STI in the past 12 months, (n,%)	25 (12.1)

5.3 National Aetiological Results

Overall samples were taken for aetiological determination from 1,512 cases of STI syndromes. These cases included secondary STI syndromes (from patients who presented with more than one STI syndrome), as well as family planning participants presenting with STI syndromes. Aetiological data were analysed by STI syndrome.

MUS

Among 540 patients presenting with Male Urethritis Syndrome (**Table 10; Figure 1**), *Neisseria gonorrhoeae* was the most commonly detected aetiological agent (392, 72.6%), followed by *Chlamydia trachomatis* (109, 20.2%). Less than 10% of MUS cases were caused by *Mycoplasma genitalium*, and less than 5% by *Trichomonas vaginalis*. The majority of patients (345, 63.9%) had infections caused by single agents. Multiple pathogens were detected in 101 (18.7%) MUS patients: the majority of these mixed infections (98, 97%) were caused by *Neisseria gonorrhoeae* together with one or more STI pathogens, mostly *Chlamydia trachomatis* (68, 67.3%). No identifiable STI aetiology was found in 94 MUS patients (17.4%).

VDS

Among 801 women with Vaginal Discharge Syndrome (**Table 11; Figure 1**), only 295 (36.8%) had a detectable STI pathogen on testing: most STI-pathogen related infections were caused by *Trichomonas vaginalis* (126, 15.7%); followed by Chlamydia trachomatis (114, 14.2%). Only 85 symptomatic VDS cases (10.6%) were caused by *Neisseria gonorrhoeae*. Overall, single STI pathogens were detected in 219 (27.3%) VDS cases; and mixed infections with multiple (two or more) STI pathogens in 76 (9.5%). Therefore in 295 patients infected with STI pathogens, the majority (219/ 295, 74.2%) of infections were attributable to a single STI aetiology. Mixed infections with one or more STI pathogens were detected in 25.8% (76/295): of these, 39.5% (30/76) were co-infected with both *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

Most VDS cases were attributed to conditions that are not traditionally considered to be STIs: bacterial vaginosis (BV) was identified in 405 (50.6%), and vaginal candidiasis (CA) accounted for 140 (17.5%). An identifiable pathogen or cause was not found for 27% (216/ 801) of VDS cases.

Table 12 shows co-infection rates with STI and non-STI aetiologies among VDS patients. Overall 179 VDS cases (22.3%) had BV-STI co-infections; of these 126 (70%) were infected by a single STI pathogen. Thirty-six VDS cases (4.5%) had CA-STI co-infections; of these, the majority (29, 80.6%) were infected by a single STI pathogen. Therefore 179/405 patients with BV (44.2%) and 36/ 140 patients with CA (25.7%) had STI co-infections. The relative prevalence of STI pathogens detected in co-infections is presented in **Table 13**.

Demographic and behavioural data were available for 87/96 (90.6%) VDS patients infected with only STI pathogens; and 277/290 (95.5%) patients infected with BV and/or Candida without an STI pathogen (**Table 14**). The median age (and IQR) of women with non-STI causes of VDS was 29 years (24-36), whereas that of women harbouring one or more STI pathogens was 26 years (22-34); the difference was not statistically significant. There were no statistically significant differences in sexual behaviour or practices between the two groups. Microbial aetiology of VDS and STI pathogen prevalence, stratified by age (**Table 15; Figure 2**), shows that age is not an accurate predictor of infection with STI pathogens, including *Neisseria gonorrhoeae*; nor with non-STI related conditions such as bacterial vaginosis or candidiasis. There was a significant sustained downward trend in the prevalence of *Chlamydia trachomatis* with increasing age; the prevalence declined significantly in those aged 35 years and older.

GUS

For 171 GUS cases (**Table 16; Figure 3**), the major cause was herpes simplex virus (HSV) in 48% (82/171); followed by *Treponema pallidum* (TP) in 7.0% (12/171). Type-specific PCR revealed that all HSV infections were caused by herpes simplex virus type 2 (HSV-2). There was only 1 case each (0.6%) of *Haemophilus ducreyi* (HD) causing chancroid (Eastern Cape) and *Chlamydia trachomatis* L1-L3 (CT L1-L3) causing LGV (Mpumalanga). No cases of donovanosis were detected in any of the provinces. Most pathogen-detectable cases had a single aetiology (90/171, 52.6%). Only 3 cases had mixed aetiology: all were co-infected with HSV and one other pathogen, namely TP, HD and CTL1-L3, respectively. An ulcer-derived pathogen was not identified in 45.6% GUS cases (78/171).

Table 10: Prevalence of STI pathogens in MUS patients

Syndrome	NG (%)	CT (%)	TV (%)	MG (%)	No STI pathogen (%)	Single infections (%)	Mixed infections: multiple pathogens (%)
MUS (n=540)	392 (72.6)	109 (20.2)	25 (4.6)	33 (6.1)	94 (17.4)	345 (63.7)	101 (18.9)

Key: Male Urethritis Syndrome (MUS); *Neisseria gonorrhoeae* (NG); *Chlamydia trachomatis* (CT); *Trichomonas vaginalis* (TV); *Mycoplasma genitalium* (MG)

Table 11: Prevalence of aetiological agents (STI and non-STI) in VDS patients

Syndrome	NG (%)	CT (%)	TV (%)	MG (%)	BV (%)	CA (%)	No STI pathogen (%)	STI pathogen Detected (%)	Single infections: one STI pathogen	Mixed infections: multiple STI pathogens (%)
VDS (n=801)	85 (10.6)	114 (14.2)	126 (15.8)	66 (8.2)	405 (50.6)	140 (17.5)	216 (27)	295 (36.8)	219 (27.3)	76 (9.5)

Key: Vaginal Discharge Syndrome (VDS); *Neisseria gonorrhoeae* (NG); *Chlamydia trachomatis* (CT); *Trichomonas vaginalis* (TV); *Mycoplasma genitalium* (MG); Bacterial vaginosis (BV); Candidiasis (CA)

Figure 1: Distribution of aetiological pathogens among participants with MUS and VDS (N=540)

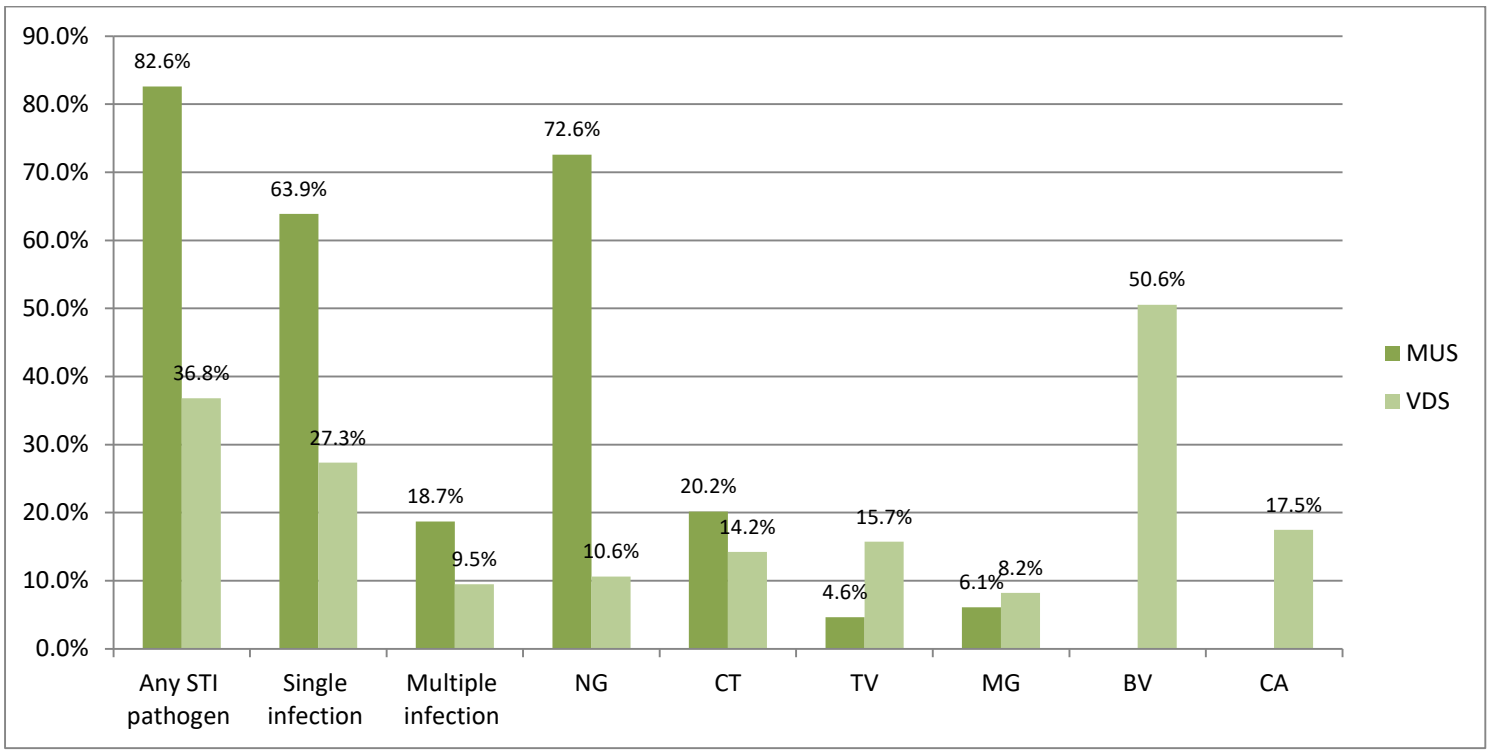


Table 12: Co-infections with STI and non-STI aetiologies among VDS patients

Syndrome	BV with STI* (n, %)	CA with STI* (n, %)	BV and/or CA with STI* (n, %)	BV and/or CA without STI* (n, %)	BV and CA ONLY (n, %)	STI* pathogen only (n, %)
VDS (n=801)	179 (22.3)	36 (4.5)	216 (26.8)	290 (36.2)	40 (5)	96 (12)

Key: Vaginal Discharge Syndrome (VDS

Bacterial vaginosis (BV); Candidiasis (CA)

*STI: refers to infection with one or more STI pathogens – *Neisseria gonorrhoeae* (NG); *Chlamydia trachomatis* (CT); *Trichomonas vaginalis* (TV); *Mycoplasma genitalium* (MG)

Table 13: Prevalence of STI pathogens among VDS patients with BV and CA

Infection	n	NG (%)	CT (%)	TV (%)	MG (%)
BV with STI	179	50 (28)	75 (42)	72 (40.2)	49 (27.4)
CA with STI	36	7 (19.4)	14 (39)	17 (47.2)	7 (19.4)

Neisseria gonorrhoeae (NG); *Chlamydia trachomatis* (CT); *Trichomonas vaginalis* (TV); *Mycoplasma genitalium* (MG); Bacterial vaginosis (BV)

Table 14: Demographic and behavioural characteristics of VDS patients stratified by cause

Variable	Women with one or more STI* pathogens only, and WITHOUT BV/CA (N=87)	Women with BV and/ or Candida WITHOUT STI* (N=277)	p-value
Age in years, (median, IQR)	26 (22- 34), n= 87	29 (24- 36), n=271	0.095
African race, (n, %)	77/87 (88.5)	249 (89.9)	0.796
Age in years at sexual debut (median, IQR)	18 (16- 18)	18 (16- 18	0.256
Condom use at last sexual encounter, (n, %)	36/85 (42.4)	97/264 (36.7)	0.248
Sexual partner from another province in the last 3 months, (n, %)	8/86 (9.3)	32/276 (11.6)	0.580
Sexual partner from another country in the last 3 months, (n, %)	6/86 (7.0)	18/273 (6.6)	0.681
History of any STI syndrome in the past 12 months, (n, %)	38/87 (43.7)	132/277 (47.7)	0.517

*STI: refers to infection with one or more STI pathogens – *Neisseria gonorrhoeae* (NG); *Chlamydia trachomatis* (CT); *Trichomonas vaginalis* (TV); *Mycoplasma genitalium* (MG)

Table 15: Microbial aetiology of VDS stratified by age

*Age categories	N	STI patho gen(s) only	%	NG	%	CT	%	TV	%	MG	%	BV only	%	CA only	%	BV and/ or CA without STI	%
18 - 20	63	11	17.5	13	20.6	18	28.6	10	15.9	7	11.1	11	17.5	3	4.8	16	25.4
20 - 21	62	10	16.1	11	17.7	15	24.2	16	25.8	4	6.5	9	14.5	6	9.7	20	32.3
22 - 23	67	6	9.0	9	13.4	15	22.4	4	6.0	13	19.4	18	26.9	3	4.5	26	38.8
24 - 25	77	12	15.6	11	14.3	16	20.8	13	16.9	8	10.4	14	18.2	9	11.7	29	37.7
26 - 28	87	8	9.2	9	10.3	11	12.6	15	17.2	5	5.7	29	33.3	8	9.2	43	49.4
29 - 31	78	12	15.4	6	7.7	11	14.1	9	11.5	8	10.3	15	19.2	8	10.3	30	38.5
32 - 34	67	6	9.0	5	7.5	9	13.4	8	11.9	5	7.5	16	23.9	7	10.4	26	38.8
35 - 37	55	2	3.6	7	12.7	3	5.5	10	18.2	2	3.6	15	27.3	4	7.3	21	38.2
38 - 40	35	2	5.7	2	5.7	2	5.7	7	20.0	3	8.6	13	37.1	3	8.6	16	45.7
41 - 43	31	4	12.9	3	9.7	1	3.2	5	16.1	1	3.2	9	29.0	3	9.7	13	41.9
44 - 46	29	3	10.3	1	3.4	0	0.0	7	24.1	1	3.4	7	24.1	1	3.4	8	27.6
47 - 49	28	3	10.7	1	3.6	0	0.0	6	21.4	3	10.7	7	25.0	0	0.0	7	25.0
50- 73**	55	6	10.3	1	3.8	3	6.4	8	14.1	0	0.0	10	16.7	5	9.0	16	28.2
Total	734	85	11.6	79	10.8	104	14.2	118	16.1	60	8.2	173	23.6	60	8.2	271	36.9

*23 participants for whom age was missing were excluded

** Age category included age 50 years & above and age missing

STI: refers to infection with one or more STI pathogens – *Neisseria gonorrhoeae* (NG); *Chlamydia trachomatis* (CT); *Trichomonas vaginalis* (TV); *Mycoplasma genitalium* (MG); Bacterial vaginosis (BV); Candidiasis (CA)

- p-value for the association between age and having STI pathogens only (i.e. comparing those with STI pathogens only and those with BV and/ or Candidiasis without STI) = 0.263
- p-value for the association between age category and NG infection (comparing those with NG infection and those with BV and/ or Candidiasis without STI)= 0.062
- p-value for the association between age category and CT infection (comparing those with CT infection and those with BV and/ or Candidiasis without STI) = < 0.001

Figure 2: The distribution of VDS aetiologies by age

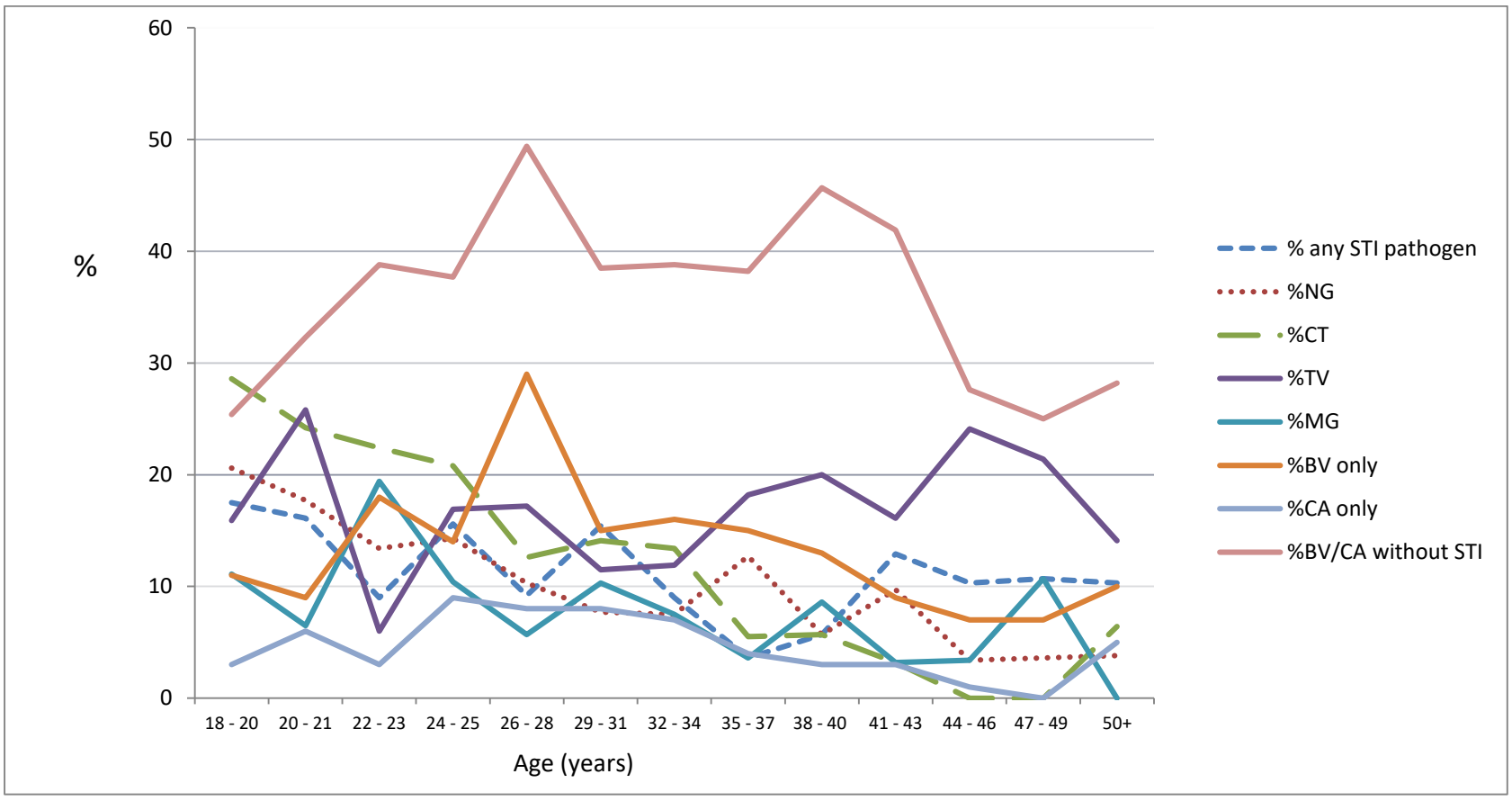
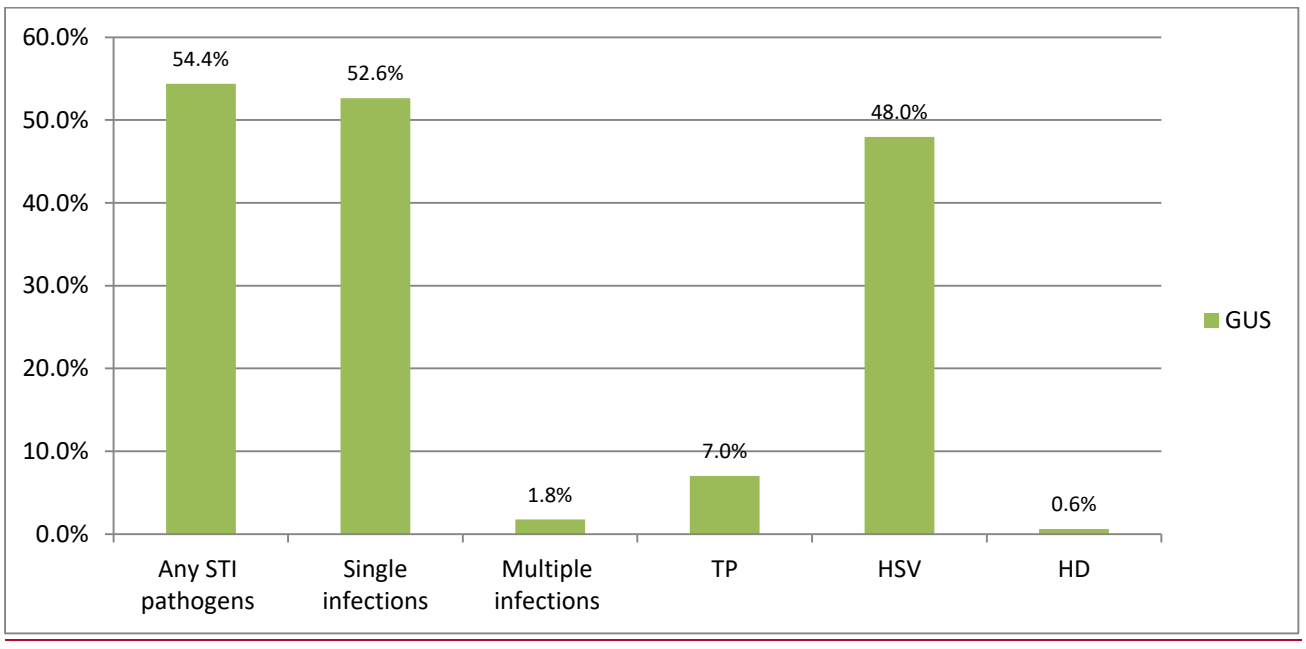


Table 16: Prevalence of STI pathogens in GUS

Syndrome	TP (%)	HSV (%)	HD (%)	LGV (%)	GI (%)	No STI pathogen (%)	Single infections (%)	Mixed infections: multiple pathogens (%)
GUS (n=171)	12 (7.0)	82 (47.9)	1 (0.6)	1 (0.6)	0 (0.0)	78 (45.6)	90 (52.6)	3 (1.8)

Key: Genital Ulcer Syndrome (GUS); Herpes simplex virus (HSV); *Treponema pallidum* (TP); *Haemophilus ducreyi* (HD); *Chlamydia trachomatis* L1-3 (CTL1-3); Granuloma inguinale (GI).

Figure 3: Distribution of STI pathogens among participants with GUS



5.4 National Serological Results

Syphilis (RPR) seroprevalence was highest at 11.1% (19/ 171) among GUS patients, followed by 4.7% (25/ 540) among MUS patients and 3.5% (28/ 801) among VDS patients. Active syphilis, defined by an RPR titre ≥ 4, was identified in 13 (7.6 %) of GUS, 15 (2.8 %) of MUS and 10 (1.2%) of VDS cases (**Table 17**). Among the 12 GUS patients whose ulcers were attributed to primary syphilis (i.e. TP PCR positive), 8 (66.7%) had positive RPR results, all with titres of ≥ 4. As reflected by our results, the sensitivity of RPR for primary syphilis is approximately 70%; it may be negative in the early stages of primary syphilis. The use of an RPR titre of 4 to define active syphilis has inherent limitations: some patients with sustained low fixed titres of 4 and 8 may have been "serofast" (and non-infectious) following successful therapy for syphilis.

The sero-prevalence of anti-HSV-2 antibodies was as follows: GUS patients 78.4 % (134/171), VDS 67.3 % (538/799) and among MUS cases 43.5 % (235/540). Of 82 GUS patients whose ulcers were caused by HSV-2, 66 (80.5%) had detectable HSV-2 antibodies; and 16 (19.5%) were seronegative for HSV-2.

Overall 53 of 1,399 patients (3.8%) presenting with STI syndromes had infectious hepatitis B, defined by a positive serological result for HBsAg. The sero-prevalence of HBsAg was 4.7% among MUS, 3.7% among GUS and 3.1% among VDS cases. The median age of these patients was 26 years (IQR 21-34). There was no significant difference in mean age when compared to the hepatitis B uninfected group (p = 0.5). Only four of 53 patients (7.5%) were ≤ 20 years of age at the time of enrolment, and would have been eligible for hepatitis B vaccination included in the expanded program on immunization schedule.

HIV co-infection rates were as follows: 64.3% (110/171) in GUS; 41.9 % (336/801) in VDS and 24.6% (133/540) in MUS (Table 14). There was a significant association between HIV seropositivity and all STI syndromes (p<0.001). Additionally HIV seropositivity was significantly associated with HSV-2 seropositivity (p<0.001).

Table 17: Serology: RPR, HSV-2, Hep BsAg, HIV co-infection rates by syndrome

Syndrome	RPR (%)	RPR≥1:4 (%)	HSV-2 (%)	HBs Ag (%)	HIV (%)
MUS=540	25 (4.7)	15 (2.8)	235 (43.5)	24 (4.7)	133 (24.6)
VDS=799	28 (3.5)	10 (1.2)	538 (67.3)	23 (3.1)	336 (41.9)
GUS=171	19 (11.1)	13 (7.6)	134 (78.4)	6 (3.7)	110 (64.3)

5.5 Distribution of STI aetiologies and seroprevalence of STI co-infections by province

The number of specimens tested for each syndrome varied widely between the provinces. The relatively small number of specimens from some provinces undermined the reliability of statistical methods to detect significant differences in the provincial prevalence of STI aetiologies.

Table A1 in Annex 1 shows the aetiology of MUS by province, as determined by M-PCR and seroprevalence of STI co-infections. The number of specimens tested ranged from 14 (Eastern Cape) to 95 (KwaZulu Natal). There was a significant difference in the prevalence of *Neisseria gonorrhoeae* between provinces: in Limpopo the pathogen accounted for 61.1% (44/72) MUS whereas in the Eastern Cape, it was present in 100% (14/14) of specimens (p = 0.004).

Table A2 in Annex 1 shows the aetiology of VDS by province, as determined by M-PCR and seroprevalence of STI co-infections. Among patients with VDS, there was a statistically significant difference in the provincial prevalence of bacterial vaginosis relative to STI pathogen prevalence: it was lowest in the Northern Cape (34.8%; 32/92); and highest in the Free State Province (62.1%; 36/58); there was a similar trend for the relative prevalence of STI pathogens. There was also a significant difference in HIV co-infection rates, which ranged from 23.2% for Gauteng to 57% for Mpumalanga (p < 0.001).

Table A3 in Annex 1 shows the aetiology of GUS by province, as determined by M-PCR and seroprevalence of STI co-infections. Statistical analyses were limited by small sample sizes for GUS from each province; and were therefore underpowered to detect significant differences. HSV-2 was the commonest cause per province, accounting for 41.2% (Northern Cape) to 66.7% (Free State). *Treponema pallidum* causing primary syphilis was a relatively uncommon cause. There was one case each of chancroid (Eastern Cape) and LGV (Mpumalanga). No cases of donovanosis were identified. There were no significant provincial differences in GUS aetiology or HIV co-infection rates.

5.6 Treponema pallidum macrolide resistance testing and molecular sub-typing

Table 18 depicts the results of *Treponema pallidum* (TP) macrolide resistance testing and sub-typing. Of the 13 samples that were TP-PCR positive the A2058G mutation, denoting azithromycin resistance, was identified in two (15.3%). Both samples were collected in Kwa-Zulu Natal from two different clinics. The A2059G mutation was not detected in any of the samples. One of the Eastern Cape samples proved to be non-typeable by all 3 sub-typing methods. With the exception of the samples collected from KZN, the subtypes found in the other provinces were all different from each other. In Gauteng and Northern Cape there were 2 samples that had unique tp0548-gene profiles (with associated mutations shown in Table 18) indicating that these could be novel sub-types that have not previously been described.

5.7 HPV testing and genotyping

A total of 241 endocervical samples from 18-20 year-old family planning clinic attendees were available for HPV testing and genotyping. HPV infection was detected in 73.9% (178/241). The prevalence of single genotype HPV infection was 19.9% (48/241); whereas multiple genotype (2-14) HPV infection was identified in 53.9% (130/241). HR-HPV infection was detected in 52.7% (127/241) women, probable HR-HPV infection in 27.0% (65/241) and LR-HPV infection in 53.5% (129/241). HPV-16 and HPV-66 were the two most frequently detected HPV types (10.8% and 10.4% respectively (**Figure 4**). The prevalence of infection with genotypes present in the quadrivalent HPV vaccine was as follows: 7.5% for HPV-6; 4.6% for HPV-11; 10.8% for HPV-16 and 7.9% for HPV-18 (**Table 19**). The proportion of females infected with one or more HPV types found in the bivalent vaccine (HPV-16/18) and quadrivalent vaccine (HPV-6/11/16/18) and nanovalent vaccine (HPV-6/11/16/18/31/33/52/56/58 was 18.3%; 25.7% and 43.6%, respectively (**Figure 5**).

Stratification and analysis of prevalence by province was limited by small sample sizes. The prevalence of HPV infection by province ranged between 55.6% (Western Cape) and 83.3% KwaZulu Natal (**Table A4 in Annex 2**).

HIV co-infection rates in HPV-infected females ranged from 27.7% among those infected with LR-HPV genotypes to 34.4% for those with probable HR-HPV (**Table 20**). HIV infection was significantly associated with all HPV infection.

Table 18: *Treponema pallidum* macrolide resistance testing and sub-typing

Specimen	TP Resistance testing		arp sub-type	tpr sub-type	TP0548 type	FINAL SUB-TYPE
	BsaI (A2059G)	MboII (A2058G)				
Street 14 (A2058G)	SENSITIVE	RESISTANT	ND	ND	ND	ND
A2059G mutant	RESISTANT	SENSITIVE	ND	ND	ND	ND
Nichols (wild-type)	SENSITIVE	SENSITIVE	14	a	a	**14a/a**
EC1-008	SENSITIVE	SENSITIVE	16	i	c	**16i/c**
EC2-012	SENSITIVE	SENSITIVE	X	X	X	**UNTYPABLE**
GP2-014	SENSITIVE	SENSITIVE	14	e	f	**14e/f**
GP4-006	SENSITIVE	SENSITIVE	6	m	f (T140C)	**6m/f (T140C)**
KZ1-028	SENSITIVE	**RESISTANT**	14	e	f	**14e/f**
KZ4-026	SENSITIVE	**RESISTANT**	14	e	f	**14e/f**
NW1-007	SENSITIVE	SENSITIVE	14	e	d	**14e/d**
NW4-007	SENSITIVE	SENSITIVE	13	e	f	**13e/f**
NC2-017	SENSITIVE	SENSITIVE	7	e	f (G158C & G164A)	**7e/f (G158C & G164A)**
NC2-018	SENSITIVE	SENSITIVE	14	e	f	**14e/f**
WC1-008	SENSITIVE	SENSITIVE	14	e	c	**14e/c**
WC1-011	SENSITIVE	SENSITIVE	15	i	c	**15i/c**
WC4-009	SENSITIVE	SENSITIVE	12	e	d	**12e/d**

Figure 4. The prevalence of individual human papillomavirus genotypes in young women attending family planning clinics in South Africa

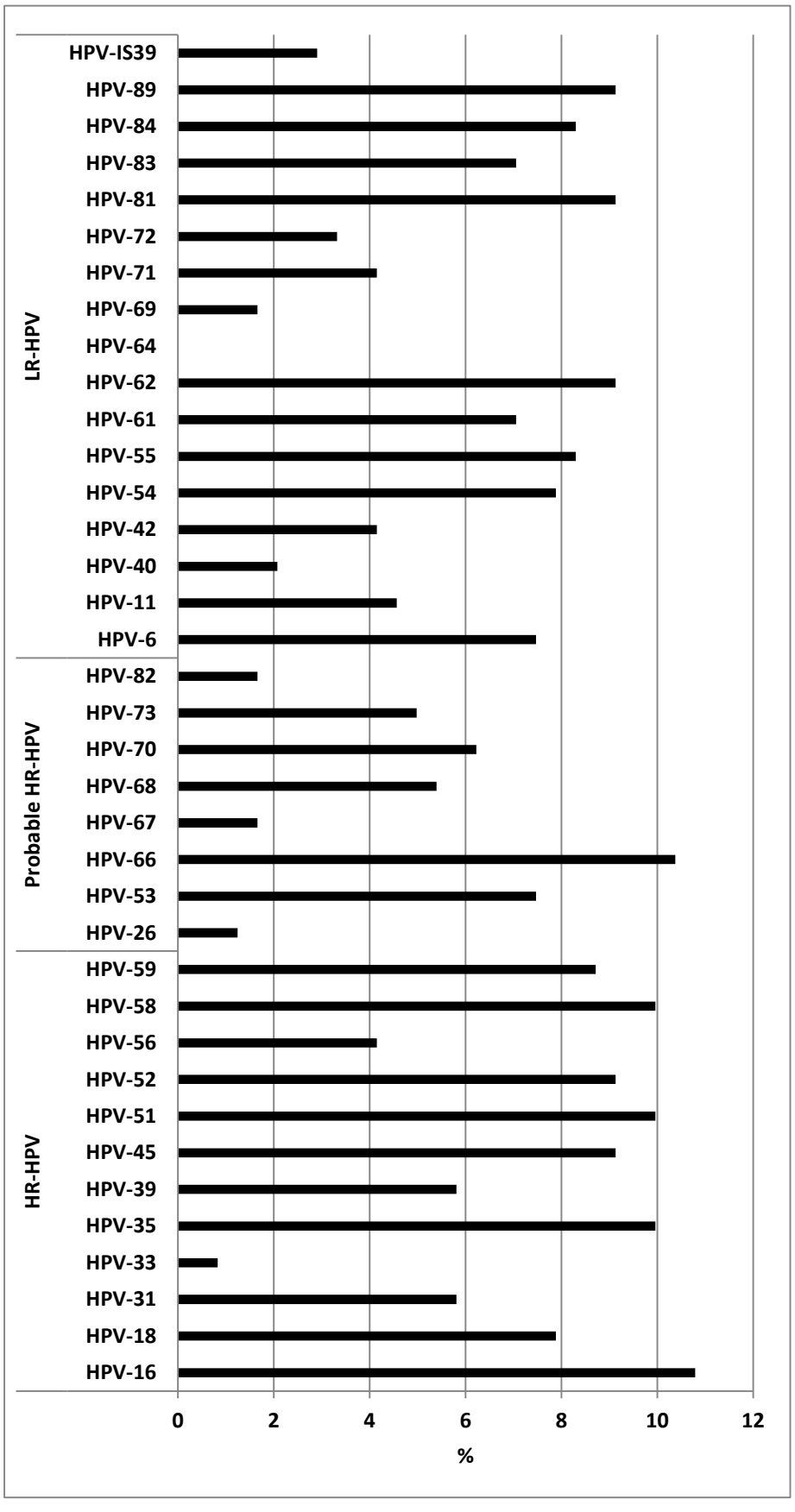


Figure 5. The Prevalence of HPV types targeted by Cervarix, Gardasil and Gardasil-9 human papillomavirus vaccines in South African women

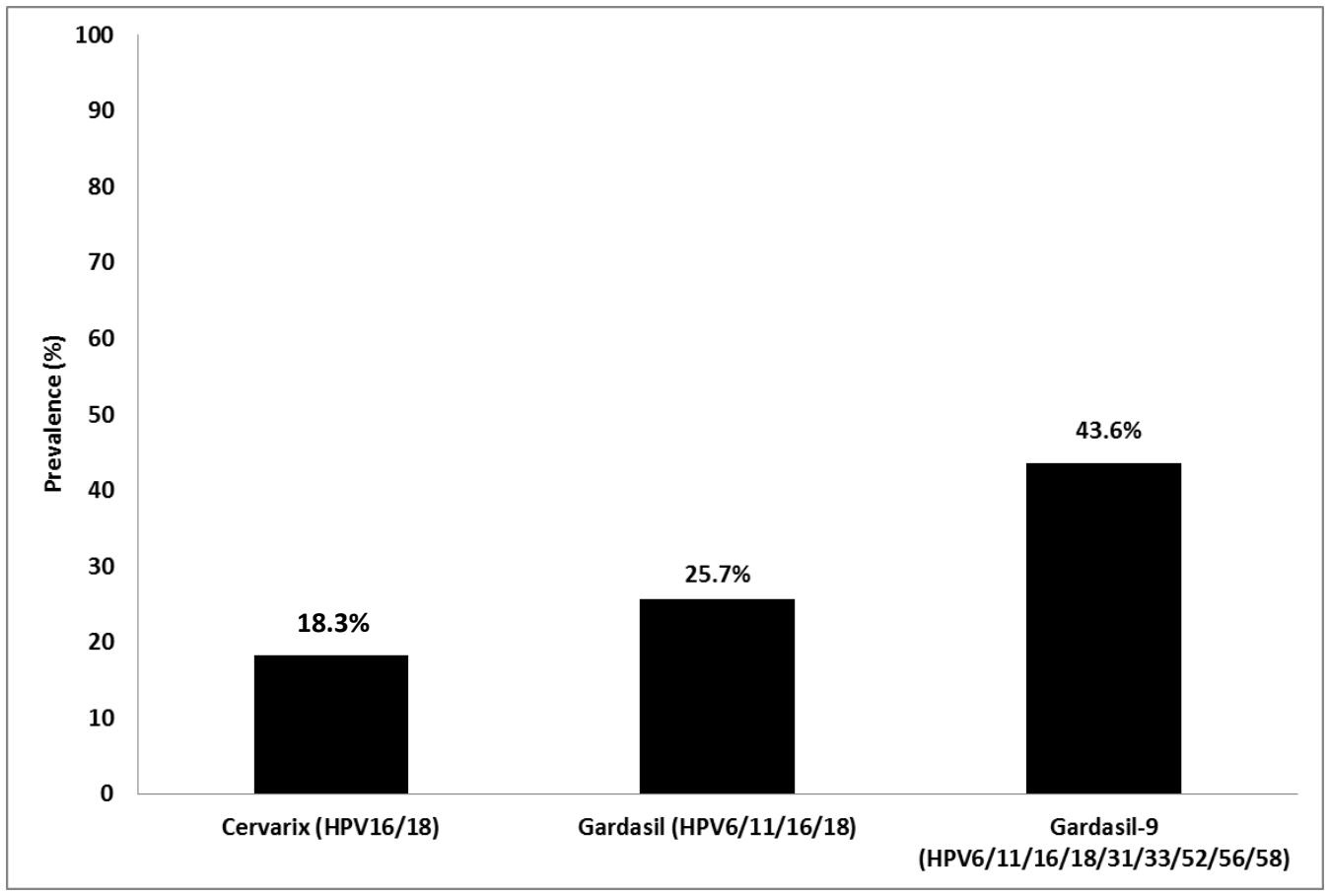


Table 20: Prevalence of HIV co-infection in HPV-infected females

	HR-HPV	**Probable HR-HPV**	**LR-HPV**	**HPV-16/18**
HIV co-infection rate (%)	28.1	34.4	27.6	32.5
X^2 p-value for association	< 0.001	< 0.001	< 0.001	0.01

6. DISCUSSION

6.1 General discussion

This surveillance study provides a snapshot of STI Syndrome aetiologies across South Africa in 2014-2015.

Overall the study found that majority of participants enrolled with STI syndromes were young and reported high risk sexual behaviour, with male STI service attendees being significantly younger and exhibiting more high risk sexual behaviour, such as younger age at sexual debut and unprotected sex at last sexual encounter. Males were also more likely to have partners from other regions of the country or continent. Mobile populations, such as long-distance truck drivers and migrant male workers in Africa are described as a priority or key populations with respect to spread of STIs and HIV in Africa, and intervention programs are needed to address risk behaviours in these population groups.(47, 48) Additionally, in a study of sexual relationship dynamics, male gender, younger age and lowest income bracket were associated with partner concurrency among black South Africans.(49)

Male Urethritis Syndrome

Aetiological testing of samples from MUS patients shows that *Neisseria gonorrhoeae* is the predominant cause of male urethritis syndrome in all provinces. Based on our data, syndromic management for MUS in the South African public health sector should include cover for the two leading causes, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Untreated infection with *Neisseria gonorrhoeae*, an obligate human pathogen, is associated with a fivefold increase in HIV transmission, as well as longterm complications such as infertility.(50) Furthermore, the gonococcus has displayed an alarming propensity to acquire resistance, through genetic mechanisms (both chromosomal and plasmid-mediated), to all sequential first-line antimicrobial agents used over the years.(51)

Azithromycin used in dual therapy for *Neisseria gonorrhoeae* provides empiric cover for *Chlamydia trachomatis* infection. *Chlamydia trachomatis* is a recognized cause of both symptomatic and asymptomatic urethritis, and reports from the USA have estimated a prevalence of 5-20% varying by region, age and symptom status. (52)

Trichomonas vaginalis was detected in less than 5% of men presenting with urethritis. A study conducted in a Johannesburg primary healthcare centre revealed a decreasing prevalence of *T. vaginalis* as a cause of MUS from 13.4% to 4.8% between 2007 and 2012.(53) These findings validate the current syndromic approach to reserve metronidazole treatment for those whose partners report vaginal discharge, or for those whose symptoms have persistent following first-line treatment for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.(28)

Mycoplasma genitalium is emerging as a significant cause of male urethritis, for which treatment should ideally be included in syndromic management algorithms.(54) There is increasing evidence that a 1g dose of azithromycin may be ineffective for eradication due to primary or induced resistance to the antimicrobial; and alternative therapeutic regimens such as prolonged higher-dose azithromycin or the newer fluoroquinolones have been suggested.(54, 55) Data from Australia reveal that the presence of 23srRNA gene mutations are correlated to clinical treatment failure with azithromycin, and azithromycin efficacy has declined significantly in Melbourne between 2005 and 2009.(56, 57) Future surveillance should include testing for the prevalence of azithromycin resistance mutations in *Mycoplasma genitalium* from urogenital samples.

Vaginal Discharge Syndrome

The surveillance shows that *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are not predominant causes of symptomatic vaginal discharge syndrome.

Trichomonas vaginalis was the predominant sexually-transmitted cause of VDS among our female STI patients. Although surveillance has revealed that the prevalence of *T. vaginalis* as a cause of symptomatic genital discharge in Johannesburg patients declined between 2007 and 2012, the highest prevalence was reported in women, particularly in those who were pregnant and older than 40 years.(53)

The prevalence of *Mycoplasma genitalium* among our symptomatic female patients is comparable to the relatively high prevalence seen in a previous VDS surveillance study at a Johannesburg primary healthcare centre.(30) The organism has been associated with long-term complications such as pelvic inflammatory disease and reproductive morbidity.(58) Additionally, a study of rural South African women demonstrated evidence of

asymptomatic infection at genital and rectal sites, as well as the presence of macrolide resistance mutations in a minority of *M genitalium* samples.(59) As azithromycin therapy is the cornerstone of syndromic management for this infection, ongoing surveillance is needed to detect the emergence and spread of macrolide resistance.

In our study, bacterial vaginosis (BV) was the leading cause of VDS, and prevalent in over 50% of females. Although the condition, which is linked to dysbiosis of normal vaginal flora, is not considered to be a traditional STI, a systematic review and meta-analysis of sexual risk factors revealed that BV was associated with behavioural risk factors such as new or multiple male partners or any female partner.(60) Additionally, BV is not seen in truly sexually naïve women without coital or non-coital sexual experience.(61, 62) Investigation of female same-sex partnerships highlighted the association between incident BV and sexual exchange of vaginal microflora between females.(63) Consistent condom use is strongly protective against incident and recurrent BV.(60) Furthermore, BV is associated with acquisition of other STI pathogens and HIV.(64-66) Our data reveal that there is no significant difference in median age of women infected with STI pathogens and those having BV with or without Candidiasis; and there were no differences in sexual risk behaviour, for the characteristics assessed, between the two groups. Further analyses of those infected only with *N. gonorrhoeae* or *C. trachomatis* revealed that there appears to be no appropriate age cut-off for therapy directed solely against these two infections in VDS management guidelines. A significant proportion of women with BV were co-infected with one or more STI pathogens – predominantly *Chlamydia trachomatis* and *Trichomonas vaginalis*. It has been postulated that *T. vaginalis* causes an alteration in the vaginal ecosystem that favours the development of BV.(67) These findings suggest that BV is associated with risk factors for traditional STI infections, and that the syndromic management algorithm for VDS should be reconfigured to remove non-specific variables such as age and include specific sexual risk characteristics that could be used to categorize patients into STI and non-STI groups for treatment.

Vulvovaginal candidiasis was present in 17.5% of VDS cases. Factors associated with symptomatic vulvovaginal candidiasis in women presenting to a rural primary healthcare facility in Kwa-Zulu Natal included pregnancy and advanced HIV infection.(68, 69) Only a minority of patients (5%) presented with co-existing BV and Candidiasis. This is in keeping with other South African studies of VDS.(67, 69) The negative association between BV and vulvovaginal candidiasis has been attributed to alteration of vaginal pH in BV, which creates an unfavourable environment for Candida colonization and infection.

Genital Ulcer Syndrome

The small sample size of GUS cases from all provinces was a limiting factor in data analysis and interpretation. Our data revealed that HSV-2 remains the leading cause of pathogen-detectable GUD in Gauteng (70), and this validates the use of anti-viral therapy in the syndromic management guidelines since 2008. The WHO recommends inclusion of anti-herpes therapy in GUD treatment algorithms if HSV is responsible for 30% or more of GUD.(71) A change in epidemiology to HSV-1 has not been observed, as is the case in high-income countries where genital HSV-1 has been identified as the leading cause of incident GUD, especially in young heterosexual women and MSM.(72) This change in Western Europe and USA has been attributed to a rise in socio-economic conditions with a corresponding reduction in childhood acquisition if HSV-1, as well as a transformation in sexual practices. The low prevalence of HSV-1 infection in our STI patients implies childhood acquisition of this virus, and that oro-genital sexual intercourse is not a popular practice among patients accessing STI services at PHCs. Approximately 80% of genital herpes cases were HSV-2 antibody positive, and represented clinically apparent reactivation disease. The prevalence of first-episode HSV-2 was 20%. There appears to be a decline in the relative prevalence of HSV-related GUD in South Africa. Since 2004, there has been increased access to anti-retroviral therapy (ART) for HIV-infected individuals and a scale-up of ART coverage in the South African public healthcare sector. Anti-retroviral therapy is not associated with reduced HSV shedding (73); however, it may lead to a decrease in clinically apparent HSV reactivation disease.(74) Additionally, male medical circumcision, which is a component of the HIV Comprehensive Care and Treatment package, is associated with a reduction in incident HSV ulceration.(75)

Primary syphilis and LGV are relatively uncommon causes of GUD in the largely heterosexual populations accessing STI services in South African primary healthcare centres. However, these pathogens are increasingly being recognised among MSM in Western Europe, where there has been a

resurgence of syphilis as the predominant cause of GUD, and a rising incidence of LGV proctitis. (76, 77) An important component of future STI surveillance strategies will be the incorporation of aetiological monitoring in key populations.

From 2000 onwards, following the successful implementation of syndromic management as recommended by the WHO, the proportion of GUD caused by *Haemophilus ducreyi* has declined substantially worldwide.(78) Accordingly, chancroid, a predominant cause of GUD in South Africa in the 1980s and early 1990s (responsible for up to 70% of genital ulceration), has been virtually eliminated.

Donovanosis has a limited geographical distribution and is rarely detected as a cause of GUD in South Africa. Previous cases have been mostly located in rural Kwa Zulu Natal, and may have facilitated the acquisition and spread of the HIV epidemic in these areas.(79) The condition may be missed as diagnosis is made using relatively insensitive phenotypic methods. Commercial PCR assays have not yet been developed for use.

Treponema pallidum macrolide resistance testing and sub-typing

The few samples containing *Treponema pallidum* DNA in this study revealed an A2058G mutation prevalence of 15%. The A2059G mutation was not identified in any of the samples. The WHO recommends azithromycin use for syphilis only in special circumstances and when resistance is unlikely.(80) Azithromycin was incorporated into the South African STI syndromic management guidelines in 2014, specifically for treatment of genital discharge syndromes and associated longterm complications. Antimicrobial selection pressure among at-risk patients may lead to increased macrolide resistance in *Treponema pallidum*, and this should be monitored in future surveillance studies. Single dose azithromycin therapy has been used for syphilis treatment in Europe, North America, China and Uganda owing to ease and convenience of administration and cost-effectiveness.(81, 82). Resistance to the drug due to the A2058G 23SrRNA was first reported among MSM in San Francisco, where patients had presented clinical treatment failure for primary syphilis following receipt of single high-dose azithromycin.(33, 83). It was subsequently described in other US cities, Ireland and in China, where resistance was associated with prior macrolide therapy prescribed for a non-syphilitic indication.(33, 84) Ribosomal mutation A2059G, also linked to clinical treatment failure with macrolide therapy, was first described in the Czech Republic.(34)

The *Treponema pallidum* strains from our study were diverse and not clustered. The two macrolide-resistant *Treponema pallidum* samples belonged to the 14e/f sub-type; however this sub-type was not uniformly associated with the A2058G mutation. The small sample size and the lack of detailed demographic data did not allow us to determine epidemiological links among cases. In Canada and China, where recent resurgences in syphilis have been described, a single subtype was identified in the majority of samples tested.(85, 86) In the USA, during 2007 to 2009, samples collected from across the country revealed a high prevalence (62%) of 14d subtype and its association with infection in MSM.(87) Muller et al subtyped 60 *Treponema pallidum* specimens collected in the surveillance period 2005-2010 from patients in Gauteng, Northern Cape and Free State Provinces of South Africa.(35) Subtype 14d was the predominated in 43% of specimens; however, similar to our findings, there was a high genetic diversity among samples.

HPV genotyping

Data on the prevalence of human papillomavirus (HPV) infections and associated-HPV types in adolescents and young women in South Africa is limited. According to the National Cancer Registry, cervical cancer is the second most common cancer among all South African women, and the commonest among black South African women, with age standardized incidence of 21.67 per 100 000 (95% CI: 21.06-22.27) in 2011.(88) Cervical cancer incidence is likely to be under-reported in most South African Provinces. The high HPV prevalence (73.9%) among young women attending family planning clinics and, in particular, an HPV-16 prevalence of >10%, is worrying. Even though the majority of HPV infections are cleared spontaneously, HR-HPV types are reported to be more likely to persist, especially HPV-16. The median age of onset of sexual activity among FPC participants in our study was 17 years, and over 10% gave a history of STIs within the preceding year. Both HPV prevalence and acquisition are associated with early sexual debut (89), highlighting the importance of vaccinating young girls prior to onset of sexual risk behaviour. Approximately 18% of females were infected with one or more HPV genotypes found in the bivalent vaccine that is being administered to public health school-going girls aged 9-12 years. These findings encourage the continuation of large scale roll-out of HPV vaccination to South African girls and a catch-up vaccination program for adolescents and young women in order to prevent HPV-associated disease. The high prevalence of high risk HPV types targeted by the nanovalent HPV vaccine suggests that young South African women would benefit from this vaccine. The currently available HPV vaccines are prophylactic and are not effective in already infected women. Therefore, continuation of cervical cancer screening programs is essential

in order to reduce cervical cancer through early detection of precancerous lesions among HPV-infected women, women who have not been vaccinated or only partially vaccinated, and those infected with HR-HPV types not targeted by HPV vaccines in use.

There was a significant association between all HPV infection and HIV co-infection among FPC participants: almost one-third of young females infected with HR-HPV -16/-18 were HIV-infected. It has been established that concurrent HIV infection predisposes to co-infection with multiple HPV genotypes.(90) Additionally, HIV-associated immune suppression may favour persistence of HPV infection and the development of cervical precancerous lesions.(91)

Continuous monitoring of prevalent HPV types in South African women is important to obtain baseline data and evaluate the impact of HPV vaccination. Rural and urban health settings across provinces could be targeted in order to get a representative sample.

Recruitment for surveillance was limited by age restriction (18-22 years), and by the fact that women were not comfortable with specimens collected under speculum examination. Studies have shown that self-sampled vaginal specimens transported dry have good agreement with clinician-collected specimens, and similar sensitivity for the detection of cervical intraepithelial lesions (92, 93). In general, self-sampling in women was found to be highly acceptable and safe (94, 95); and this strategy could be employed to improve recruitment and participation in future HPV surveillance studies.

HIV Co-infection

The HIV prevalence among patients presenting with STI syndromes is significantly higher than the UNAIDS 2013 estimated prevalence of 19.1% for adults aged 15-49 years in the general South African population.(96) HIV co-infection rates in STI patients reflect the epidemiological synergy between STIs and HIV: there is common sexual behaviour and transmissibility with facilitation of both HIV transmission and acquisition in patients with STIs. Factors that contribute to this synergy include disruption of genital mucosal epithelium, recruitment of inflammatory HIV target cells, an altered vaginal ecosystem (bacterial vaginosis), and increased HIV viral load in genital secretions.(15) It has been demonstrated that genital ulcer disease and HSV-2 seropositivity are associated with HIV acquisition and seroconversion, and an increased HIV viral load.(97) The risk of HIV transmission is estimated to be highest during the stage of early HIV infection (recent seroconversion).(82) Probabilistic modelling of male-to-female HIV transmission per coital act has shown that determinants of HIV transmission include acute HIV infection and STI episodes that increase the seminal HIV viral load by 3-5 $\log_{10}$ copies/ml; and that interventions designed to reduce viral excretion below the threshold for HIV transmission could potentially decrease transmission probability.(98) A recent meta-analysis has revealed that effect of STI co-infections on HIV viral load is largely mitigated by anti-retroviral therapy; and thus STIs are unlikely to decrease the effectiveness of HIV treatment as prevention.(99) All these factors underscore the importance of linkage to universal HIV testing and treatment for STI patients; and support the recently adopted national policy of early ARV initiation for those who are HIV-infected, regardless of CD4+ cell count.

Syndromic management: pros & cons and future strategy

In 2003, the World Health Organization advocated the adoption of syndromic management for STIs, particularly in resource-constrained regions with limited access to laboratory diagnostic facilities, in an effort to establish standardized national treatment protocols.(71) In South Africa, strengths of syndromic management have been most apparent in the treatment of MUS and GUS. Male Urethritis Syndrome is predominantly associated with *Neisseria gonorrhoeae*, and therefore the inclusion of appropriate dual antimicrobial therapy for this rapidly evolving pathogen is justified. This dual therapy also includes cover for *Chlamydia trachomatis*, the second most prevalent pathogen, which is also implicated in the majority of co-infections with *N. gonorrhoeae*. The inclusion of anti-viral therapy is justified in Genital Ulcer Syndrome management algorithm, as the prevalence of HSV-2, the most predominant ulcer-associated pathogen, far exceeds 30%. Furthermore, the addition of macrolide therapy to the GUS algorithm in the early 2000s has led to the virtual elimination of chancroid in South Africa, the commonest cause of GUS in the latter part of the last century.(78) However, nearly 46% of GUS cases had no identifiable STI aetiology; which implies that a significant proportion of patients may be over-treated with anti-viral therapy. It is important, therefore, to investigate the causes of persistent genital ulcer disease unresponsive to syndromic management, and refer patients for further care.

Syndromic management is not perfect and the issues and imitations of syndromic management for VDS have been discussed earlier. In South Africa, non-STI pathogens such as BV and Candidiasis account for the majority of VDS cases. The symptom of abnormal vaginal discharge may not be predictive of cervical infection, and speculum examination (which may be inconsistently practised at primary healthcare level) becomes essential for the detection of cervicitis caused by STI pathogens such as *N. gonorrhoeae* and *C. trachomatis*. The prevalence of these STI pathogens is relatively low in females with symptomatic VDS, therefore over-treatment and the use of unnecessary antimicrobials is a concern. It is necessary to identify associated risk factors for STI pathogens; so that those women with a positive risk assessment for cervicitis are offered appropriate treatment. Our study did not identify specific risk factors among the demographic and behavioural characteristics assessed: age was poorly predictive of infection with specific STI pathogens. There remains a need to identify the main risk factors based on sexual behavioural patterns, and increase the sensitivity and specificity of the vaginal discharge algorithm for STI pathogens.

Although syndromic management is a preferred and more feasible public heath approach for STI management in resource-limited settings, a major limitation is that it does not treat asymptomatic infections, which comprise the bulk of STIs. A relatively high burden of asymptomatic infections with non-ulcerative urethritis pathogens has been demonstrated in men accessing mobile screening services in a South African informal settlement.(100) Over 90% of infections were asymptomatic, and almost one-third of males were HIV-infected. Additionally, STI pathogens implicated in VDS mostly cause asymptomatic infections. A cross-sectional study in rural South Africa found relatively high prevalence rates of genital chlamydia (16%) and gonorrhoea (10%) in women attending ante-natal or family planning clinics; and revealed that most women with urogenital infections were asymptomatic.(101) The prevalence of asymptomatic STI pathogens was reported to be higher in females than males in HIV-infected patients seeking HIV care at a South African treatment centre; and highlighted the need for screening STI diagnostics in this population.(102) Another study conducted in a Kwa-Zulu Natal ante-natal clinic reported high prevalence and incidence rates of STI infections in pregnancy and at 3 month post-partum, respectively.(103) More than 50% of these infections were asymptomatic and emphasized that this was an additional at-risk population for which STI screening strategies were required. The CDC recommends that all pregnant young (< 25 years) females, and older women with specific risk factors be routinely screened at the first ante-natal visit for urogenital gonorrhoea and chlamydia infections.(104)

Screening for asymptomatic STI infections may need to be extended to include extra-genital sites, particularly among key populations e.g. MSM and young women, in whom gonococcal pharyngeal infections are commonly detected.(105) Pharyngeal gonorrhoea may be especially difficult to eradicate due to differential concentrations of antimicrobials at that anatomic site, and also the potential for horizontal genetic transfer of resistant determinants from commensal *Neisseria* species residing in the pharynx, leading to the emergence of antimicrobial resistance.(51) A study among men-who-have-sex-with-men (MSM) in Cape Town revealed that asymptomatic infection with *N. gonorrhoeae* and *C. trachomatis* was more common than symptomatic infection irrespective of anatomical site; furthermore, extra-genital (rectal) infection was more common than urethral infection.(106) The researchers were also able to identify specific behavioural risk patterns associated with asymptomatic STI infection.

Evidence from these studies supports the case for a selective screening approach for asymptomatic STIs in high-risk/ key populations. These populations need to be clearly defined and may include MSM, sex workers, mobile populations, adolescent girls and young women. Sexual risk screening tools could be developed and validated for each key population group. There is also a need for validation of suitable rapid/ point-of-care STI diagnostic assays having acceptable performance characteristics, for use with relatively non-invasive specimens such as self-collected genital swabs or urine specimens, and also extra-genital specimens. These would be used in conjunction with a behavioural risk-assessment tool, to identify those who should be prioritised for STI screening.

An additional significant limitation of syndromic management is that it does not enable the study of local STI epidemiology, and the monitoring of antimicrobial susceptibility trends for rapidly evolving pathogens such as *Neisseria gonorrhoeae*. Antimicrobial resistance monitoring is essential to validate current syndromic management guidelines which recommend dual therapy for gonorrhoea: ceftriaxone (an injectable extended-spectrum cephalosporin) and azithromycin (a long-acting macrolide).(28) Analysis of *Neisseria gonorrhoeae* antimicrobial resistance trends in Gauteng 2008 – 2015, has revealed that penicillin, tetracycline and ciprofloxacin are unlikely to be included in any future genital discharge treatment algorithms.(107) Periodic STI aetiological surveys conducted by NICD/ NHLS have not confirmed the emergence and spread of extended-spectrum cephalosporin

resistance in *Neisseria gonorrheae* isolates from patients with genital discharge syndrome presenting to sentinel surveillance sites. However, case reports of cefixime resistance and subsequent treatment failure have been identified among MSM in South Africa.(108) It is essential that antimicrobial resistance surveillance is extended to include key populations such as MSM and sex workers.

6.2 Study Limitations

The survey had a number of important limitations.

A key limitation of the study was the suboptimal recruitment of participants which did not allow for robust statistical analyses. The target sample sizes were not met for syndromic STI or HPV surveillance in any of the provinces. Recruitment rates were lowest for the Eastern Cape, Free State and North-West provinces. It is important that the reasons attributed to surveillance gaps be addressed, so that they are not encountered in future surveillance studies conducted within a limited time-period. Prior to commencement of surveillance, centralised training in each of the provinces was organised by NDoH, and conducted by NICD and iTech. There are several possible reasons for low recruitment rates in provinces; one of these may have been the failure of cascaded training and the scaled-down supervision received by healthcare workers at facility level. Some management and senior administrative personnel who attended centralised training sessions may not have provided the necessary feedback or training to clinical staff who were ultimately responsible for recruitment and sample collection. Clinic staff at some facilities were entirely unaware of the study or specimen-taking protocol well into the period of surveillance. Some provincial STI co-ordinators were not involved in the various aspects of implementation, and did not accompany the surveillance team on clinic visits. Some facilities were under-resourced and lacked suitably qualified nursing staff; others experienced high staff turnover and new staff members were not adequately trained for study participation. The NICD, as one of the implementing partners, monitored recruitment rates by facility and province, and these data were communicated on a weekly basis to provincial HAST managers.

Another reason for low participant numbers was non-adherence to correct completion of clinical questionnaires, and missing questionnaires. A significant number (n=97) of clinical questionnaires were missing (and did not accompany clinical specimens), or were rejected for various reasons: participants were not within the right age category; participant consent form for laboratory testing was not received. This may be attributed to a lack of consistent, ongoing training of surveillance officers with appropriate feedback.

The small numbers of STI syndrome cases recruited in all clinics over a 15-month period highlights a major concern that the data presented may not be representative of patients with each syndrome attending each facility.

Another important limitation was the sampling methods used. Convenience sampling, of what were meant to be four high burden clinics per province, was done based on the availability of resources. In sampling this way, an assumption is made that distribution of STI pathogens in high burden clinical sentinel sites and low burden ones is the same. Random sampling of the 270 sentinel sites would have been a better approach for nationally representative data.

The lack of questions or data elements on coverage of HIV prevention, care and treatment in the survey questionnaire was another limitation. The survey questionnaire did not include questions about male medical circumcision (MMC) coverage, HIV testing, entry into care, antiretroviral therapy use and viral suppression. This information would have informed the STI programme on progress and gaps in the area of HIV/STI integration which is essential, given high prevalence of HIV co-infection among STI patients and FPC attendees.

7. Conclusions

Syndromic management of STIs at primary healthcare level remains a pragmatic and cost-effective public health intervention in South Africa, particularly in areas having limited access to laboratory facilities and low patient return rates. *Neisseria gonorrhoeae* is the predominant cause of MUS; and syndromic management with dual antimicrobial therapy, which also covers *Chlamydia trachomatis*, the second most common pathogen, is rational. Ongoing surveillance for resistant gonorrhoea is essential, particularly in key populations. Herpes simplex virus is the commonest detectable cause of genital ulceration, validating the continued use of acyclovir in syndromic management; however, the cause of ulceration in nearly 46% of patients without an STI diagnosis requires further research. The syndromic management of VDS remains complex: the commonest causes, bacterial vaginosis and candidiasis, are non-STI related; however, a significant proportion of patients with either condition were co-infected with STI pathogens particularly *Trichomonas vaginalis*. Of the demographic and behavioural characteristics assessed for VDS; none, including patient age-category, had a significant association with specific STI pathogens. Future surveillance studies need to analyse additional sexual behavioural patterns, in order to further refine the VDS algorithm and increase its predictive value for STIs. The cervical HPV prevalence in young women aged 18-20 years was high, as was the prevalence of HPV-16, which is associated with persistent infection and accounts for the majority of cervical cancer. The significant proportion of young women infected with HPV genotypes contained in the bivalent vaccine supports a large-scale roll-out of the vaccine. The HIV seroprevalence among STI patients is high, underlining the importance of linkage to universal HIV counselling and testing in primary healthcare settings. A major limitation of STI syndromic management is that it does not treat asymptomatic STIs, which comprise a large proportion of infections, particularly in key population groups. Additional interventions, such as a validated sexual risk screening tool and targeted rapid point-of-care diagnostic testing for those deemed to be at highest risk for STI, are needed. A limitation of this study was the under-recruitment of participants and failure to meet target sample sizes for all STI syndromes in all provinces. Thus it was under-powered to detect significant differences in STI aetiologies among provinces.

8. Recommendations

Based on findings from this survey the following is recommended in order to improve the prevention, care and treatment of STIs in South Africa.

- Preventive interventions are needed to address sexual risk behaviours, particularly in young sexually active males who have identifiable risk factors that make them vulnerable to STIs.

- *Neisseria gonorrhoeae* is the predominant cause of MUS. It is essential to monitor antimicrobial susceptibility trends to currently used antibiotics for this rapidly evolving pathogen that has the capacity to readily acquire antimicrobial resistance. Antimicrobial resistance surveillance should be extended to key populations such as men-who-have-sex-with-men (MSM), among whom cephalosporin-resistant gonococcal infections in South Africa have been identified. Patients with persistent urethral discharge unresponsive to syndromic management should be referred for further investigation.

- The syndromic management algorithm for VDS should be reconfigured to remove non-specific variables such as age, for categorizing patients into STI and non-STI groups. Additional sexual risk behaviours that increase the predictive value of the algorithm for STI pathogens need to be identified and included. Speculum examination for the detection of cervicitis (and treatment of associated STI pathogens) is an important component of syndromic management.

- Although inclusion of anti-viral therapy for Herpes simplex virus in the GUS syndromic management algorithm is justified, a significant proportion of ulcers have no identifiable STI aetiology. This requires further research and it is advisable to refer persistent genital ulcer disease unresponsive to syndromic management for further investigation.

- A key limitation of syndromic management is that it does not identify and treat asymptomatic STIs that comprise the bulk of infections. Strategies are needed to define key population groups who could be targeted for screening using a validated sexual behaviour risk-assessment tool. The use of rapid diagnostic tests with acceptable performance characteristics should be evaluated for this purpose.

- The high HPV prevalence, particularly of high-risk (HR) HPV types, in young females supports the large-scale roll-out of HPV vaccine. The high prevalence of HR-HPV types targeted by the nanovalent HPV vaccine suggests that use of this vaccine would be beneficial in the prevention of cervical cancer, which is the commonest cancer among black South African women.

- The HIV prevalence among patients presenting with STI syndromes is 2-3 times the estimated prevalence among adults aged 15-49 in the general population. The linkage to universal HIV counselling and testing services is essential for these patients at primary healthcare level. Future surveys will need to collect data on coverage of these services among STI clinic attendees.

- Future aetiological surveillance studies should ensure that participation at facility level is maximised by decentralising training and engaging directly with managers and healthcare workers at facility level. A suitable train-the-trainer (TTT) approach needs to be adopted to cater for high staff turnover. Provincial managers, who play an important role in surveillance co-ordination, should be engaged and involved from inception to completion. Ongoing training and feedback to participating facilities is essential for optimising patient recruitment and ensuring adequate specimen collection. Regular scheduled visits to participating facilities by the surveillance team and consistent monitoring of performance indicators are important.

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ANNEX 1: PROVINCIAL AETIOLOGICAL RESULTS BY SYNDROME

Table A1: MUS Aetiologies and co-infections stratified by province 2014-2015 (n=540)

Province	No. enrolled	Any STI pathogen	NG n (%)	CT n (%)	TV n (%)	Mg n (%)	STI pathogens not detected	HIV + n (%)	HBV sAg+ n (%)	HSV2 + n (%)	RPR + n (%)	Titre≥1:4 n (%)
Eastern Cape	14	14 (100)	14 (100)	2 (14.3)	0 (0.0)	0 (0.0)	0 (0.0)	5 (37.5)	1 (7.1)	8 (57.1)	1 (7.1)	1 (7.1)
Free State	16	15 (93.8)	14 (87.5)	1 (6.3)	1 (6.3)	0 (0.0)	1 (6.3)	0 (0.0)	1 (6.3)	4 (25.0)	1 (6.3)	0 (0.0)
Gauteng	65	46 (70.8)	41 (63.1)	10 (15.4)	3 (4.6)	6 (9.2)	18 (27.7)	14 (21.5)	2 (3.1)	27 (41.5)	3 (4.6)	3 (4.6)
KwaZulu Natal	95	70 (73.7)	64 (67.4)	16 (16.8)	6 (6.3)	4 (4.2)	25 (26.3)	27 (28.4)	4 (4.2)	41 (43.2)	4 (4.2)	2 (2.0)
Limpopo	72	59 (81.9)	44 (61.1)	15 (20.8)	1 (1.4)	7 (9.7)	11 (15.3)	19 (26.4)	7 (9.7)	30 (41.7)	0 (0.0)	0 (0.0)
Mpumalanga	93	85 (91.4)	75 (80.7)	26 (28.0)	2 (2.2)	5 (5.4)	7 (7.5)	29 (31.2)	7 (7.5)	48 (51.6)	3 (3.2)	1 (1.1)
North-West	52	44 (84.6)	35 (67.3)	15 (28.9)	5 (9.6)	3 (5.8)	8 (15.7)	13 (25.0)	2 (3.9)	26 (50.0)	2 (3.9)	2 (3.9)
Northern Cape	51	43 (84.3)	41 (80.4)	6 (11.8)	4 (7.8)	1 (2.0)	8 (15.7)	16 (31.4)	0 (0.0)	19 (37.3)	4 (7.8)	3 (5.9)
Western Cape	82	71 (86.6)	64 (78.1)	18 (22.0)	3 (3.7)	7 (8.5)	11 (13.4)	10 (12.2)	3 (3.7)	32 (39.0)	7 (8.5)	3 (3.7)
All	**540**	**447 (82.8)**	**392 (72.6)**	**109 (20.2)**	**25 (4.6)**	**33 (6.1)**	**89 (16.5)**	**133 (24.6)**	**27 (5.0)**	**235 (43.5)**	**25 (4.6)**	**15 (2.8)**
X^2 p-value for association by province		0.004	0.004	0.145*	0.336*	0.577*	0.004*	0.025	0.300*	0.400*	0.212*	0.297*

Key: Male Urethritis syndrome (MUS); Demonimators for percentages are those enrolled. *Neisseria gonorrhoeae* (NG)- 4 not tested; *Chlamydia trachomatis* (CT)- 5 not tested; *Trichomonas vaginalis* (TV)- 4 not tested; *Mycoplasma genitalium* (MG)- 4 not tested; HIV- 9 not tested, HBV – 9 not tested, HSV2- 11 not tested, RPR – 13 not tested, *Fisher's exact p-value

Table A2: VDS Aetiologies and co-infections stratified by province 2014-2015 (N=801)

Province	No. enrolled	Any STI pathogen	NG n (%)	CT n (%)	TV n (%)	Mg n (%)	BV n (%)	CA n (%)	STI pathogens not detected	HIV+ n (%)	HBV sAg+ n (%)	HSV2+ n (%)	RPR+ n (%)	Titre>= 1:4
Eastern Cape	39	12 (30.8)	3 (7.7)	7 (18)	6 (15.4)	1 (2.6)	19 (48.7)	5 (12.8)	45 (41.3)	16 (41.0)	1 (2.6)	20(51.3)	3 (7.7)	1(2.6)
Free State	58	32 (55.2)	9 (15.5)	12 (20.7)	12 (20.7)	10 (17.2)	36 (62.1)	11 (19.0)	14 (35.9)	31 (53.5)	1 (1.7)	43 (74.1)	1 (1.7)	0 (0.0)
Gauteng	82	28 (34.2)	11 (13.4)	7 (8.5)	11 (13.4)	4 (4.9)	33 (40.2)	16 (19.5)	19 (32.8)	19 (23.2)	0 (0.0)	51(62.2)	0 (0.0)	0 (0.0)
KwaZulu Natal	109	43 (39.5)	10 (9.2)	19 (17.4)	17 (15.6)	9 (8.3)	64 (58.7)	17 (15.6)	25 (30.8)	49 (45.0)	4 (3.7)	75(68.8)	3 (2.8)	1(0.9)
Limpopo	127	37 (29.1)	13 (10.2)	16 (12.6)	19 (15)	8 (6.3)	58 (45.7)	32 (25.2)	57 (44.9)	53 (41.7)	8 (6.3)	95(74.8)	5 (3.9)	2 (1.6)
Mpumalanga	112	54 (48.2)	16 (14.3)	16 (14.3)	24(21.4)	16 (14.3)	68 (60.7)	19 (17.0)	35 (31.3)	64 (57.1)	3 (2.7)	86(76.8)	5 (4.5)	2 (1.8)
North-West	103	32 (31.1)	6 (5.8)	10 (9.7)	14 (13.6)	7 (6.8)	47 (45.5)	12 (11.7)	36 (35)	46 (44.7)	3 (2.9)	64 (62.1)	1 (1.9)	0 (0.0)
Northern Cape	92	25 (27.2)	4 (4.4)	12 (13.0)	8 (8.7)	4 (4.4)	32 (34.8)	18 (19.6)	29 (31.5)	33 (35.9)	0 (0.0)	59(64.1)	0 (0.0)	0 (0.0)
Western Cape	79	32 (40.5)	13 (16.5)	15 (19.0)	15 (19.0)	7 (8.9)	48 (60.8)	10 (12.7)	30 (38)	25 (31.7)	4 (5.1)	45(57.0)	9(11.4)	3 (3.8)
All	**801**	**295 (36.8)**	**85 (10.6)**	**114 (14.2)**	**126 (15.7)**	**66 (8.2)**	**405 (50.5)**	**140 (17.5)**	**290 (36.2)**	**336 (42)**	**24 (3.0)**	**538 (67.2)**	**28 (3.5)**	**9 (1.1)**
X^2 p-value for association by province		0.002	0.096	0.335	0.374	0.027	<0.001	0.237	0.337	<0.001	0.157	0.010	0.002*	0.222*

Vaginal discharge syndrome (VDS); Demonimators for percentages are those enrolled. *Neisseria gonorrhoea* (NG)- 11 not tested; *Chlamydia trachoma* (CT)- 11 not tested; *Trichomonas vaginalis* (TV)- 11 not tested; *Mycoplasma genitalium* (MG)- 11 not tested , mycoplasma genitalium (MG)- 11 not tested); Bacterial vaginosis (BV)= 53 (19 from NW, 23 from NW) not tested, Candida (CA)= 53 (19 from NW, 23 from NW) not tested, HIV- 17 not tested, HBV – 17 not tested, HSV2- 20 not tested, RPR – 17 not tested, *Fisher's exact p-value

Table A3: GUS aetiologies and co-infections stratified by province 2014-2015 (N=171)

Province	Number Enrolled	TP n (%)	HSV n (%)	HD n (%)	CTL1-3 n n (%)	STI pathogen not detected	HIV+ n (%)	HBV sAg+ n (%)	HSV2 + n (%)	RPR+ n (%)	RPR titre >1:4
Eastern Cape	5	1 (20.0)	3 (60.0)	1 (20.0)	0 (0.0)	1 (20)	4 (80.0)	0 (0.0)	5 (100)	1 (20.0)	1 (20)
Free State	6	0 (0.0)	4 (66.7)	0 (0.0)	0 (0.0)	2 (33.3)	5 (83.3)	0 (0.0)	5 (83.3)	1 (16.7)	1 (16.7)
Gauteng	22	2 (9.1)	11 (50.0)	0 (0.0)	0 (0.0)	10 (45.5)	11 (50.0)	0 (0.0)	18 (81.2)	1 (4.5)	1 (4.5)
KwaZulu Natal	38	2 (5.3)	19 (50.0)	0 (0.0)	0 (0.0)	17 (44.7)	28 (73.6)	2 (5.3)	31 (81.6)	7 (18.4)	4 (10.5)
Limpopo	23	0 (0.0)	11 (47.8)	0 (0.0)	0 (0.0)	12 (52.2)	14 (60.9)	1 (4.4)	17 (73.9)	2 (8.7)	2 (8.7)
Mpumalanga	22	0 (0.0)	12 (54.6)	0 (0.0)	1 (4.5)	10 (45.5)	11 (50.0)	0 (0.0)	17 (77.3)	0 (0.0)	0 (0)
North-West	18	2 (11.1)	6 (33.3)	0 (0.0)	0 (0.00	10 (55.6)	12 (66.7)	2(11.1)	11 (61.1)	1 (5.6)	1(5.6)
Northern Cape	17	2 (11.8)	7 (41.2)	0 (0.0)	0 (0.0)	8 (47.1)	14 (82.4)	1 (5.9)	15 (88.2)	1 (5.9)	0 (0)
Western Cape	20	3 (15.0)	9 (45.0)	0 (0.0)	0 (0.0)	8 (40.0)	11 (55.0)	1 (5.0)	15 (75.0)	5 (20.0)	5 (20)
All	**171**	**14 (7.0)**	**82 (48)**	**1 (0.6)**	**1 (0.6)**	**78 (45.6)**	**110 (64.3)**	**7 (4.1)**	**134 (78.4)**	**19 (11.1)**	**15 (8.8)**
Fisher's exact X^2 p-value for association by province		0.241	0.905	.	.	0.946	0.267	0.783	0.689	0.127	0.099

Genital ulcer syndrome (GUS); *treponema pallidum* (TP)- 2 not tested, ulcer-derived *herpes simplex virus* (HSV)-2 not tested, *Haemophilus ducreyi* (HD)- 2 not tested ; *chlamydia trachomatis* L1-3 (CTL1-3) – 2 not tested ; *granuloma inguinale* (GI). HIV- 2 not tested, HBV –2 not tested, HSV2- 3 not tested, RPR – 22 (11 from Limpopo, 5 from Gauteng and 4 from NW) not tested.

ANNEX 2: PROVINCIAL HPV PREVALENCE BY GENOTYPE

Table A4: Prevalence of HPV infection and genotypes contained in quadrivalent HPV vaccine in FPC participants, stratified by province

Provinces	N	HPV prevalence		HPV-6		HPV-11		HPV-16		HPV-18	
		n	%	n	%	n	%	n	%	n	%
Eastern Cape	28	19	67.9	3	10.7	0	0.0	2	7.1	2	7.1
Free State	30	21	70.0	4	13.3	1	3.3	3	10.0	3	10.0
Gauteng	26	21	80.8	3	11.5	1	3.8	2	7.7	2	7.7
KwaZulu-Natal	60	50	83.3	4	6.7	4	6.7	10	16.7	7	11.7
Limpopo	8	8	100.0	1	12.5	0	0.0	1	12.5	1	12.5
Mpumalanga	39	28	71.8	3	7.7	1	2.6	3	7.7	3	7.7
North West	35	20	57.1	0	0.0	3	8.6	1	2.9	1	2.9
Northern Cape	6	6	100.0	0	0.0	0	0.0	4	66.7	0	0.0
Western Cape	9	5	55.6	0	0.0	1	11.1	0	0.0	0	0.0
All Provinces	**241**	**178**	**73.9**	**18**	**7.5**	**11**	**4.6**	**26**	**10.8**	**19**	**7.9**

Appendix 1 – STI Aetiological Surveillance Protocol NMS-002

Version 4 – 2nd October 2013

Flesch-Kincaid Grade Level 6.8

PATIENT INFORMATION SHEET

General information about the survey

Good day. We are asking you to take part a national survey for sexually transmitted infections (STIs). The survey will inform the National Department of Health (NDoH) about the types and burden of STIs in South Africa. The survey is run by the National Institute of Communicable Diseases (NICD) in collaboration with the NDoH and the US Centers for Disease Control and Prevention. The survey has been approved by a South African Ethics Committee. This survey will be conducted among 3,600 patients attending 36 clinics across South Africa.

Do I have to take part in this survey?

No, taking part is voluntary. If you choose not to take part, your care at the clinic today will not be affected in any way. You may also ask the nurse if you wish to stop being part of the survey at any time today without this affecting your care. You will be given a copy of this form with your survey number on it. Please keep this in case you wish to withdraw from the survey. Withdrawal will only be possible up until 3 months from today.

What do I have to do?

We would like to take some genital and blood specimens from you to test for STIs, including HIV. You will also be asked some questions by a nurse. The survey will take about 20-30 minutes to complete. You will only be asked to take part for today. We will not need to see you again.

What specimens will I need to provide?

- From **MEN** with genital discharges ('drop'), we require a swab from the tube where you pass urine.
- From **WOMEN** with genital discharges, we require swabs from inside the vagina.
- From **MEN/WOMEN** with genital ulcers, we require a swab from the ulcer.
- We require a 10 ml (2 teaspoons) blood specimen from **EVERYONE**

Normally, these swabs and blood would not be taken and so they are experimental. The specimens will be tested at the NICD in Johannesburg.

Will taking the specimens be harmful?

Taking these specimens will not harm you in any way. You may experience minor discomfort and possibly mild pain which will last only a few seconds. You are free to stop at any stage during the sampling procedure.

What are the benefits of taking part in the survey?

You will not benefit yourself from taking part. However, the results of the survey will be used to ensure that the treatment for STIs in South Africa is correct. Other patients, and possibly yourself, will benefit from the survey results in the future as some STI treatments may be improved.

Is taking part confidential and what will happen to the results?

Taking part is completely confidential. None of the samples we take from you will be labeled with your name or any other information to identify you. Therefore, you cannot receive any of the results. The results will be analyzed at the NICD and a report written for the NDoH.

If I take part, will my treatment be affected in any way?

You will receive treatment for your medical condition in the same way as normal at the clinic.

If you have any questions about the survey survey-related injuries or withdrawing the survey at a later date, you may contact Prof David Lewis (Principal Investigator), Tel: (011) 555-0468

If you have any questions about your rights as a participant, you may contact the Ethics Committee Chairperson, Professor Peter Cleaton-Jones, Tel: (011) 717 2301

Appendix 2 - STI Aetiological Surveillance Protocol NMS-002

Version 2 – 11th December 2012

Flesch-Kincaid Grade Level 7.2

PATIENT CONSENT FORM

I have read the patient information sheet.

I understand the contents of patient information sheet.

I agree to participate in the STI survey

I agree to provide specimens as explained in the patient information sheet.

I understand that I will receive the usual standard of care offered by this clinic.

I understand that I will be asked to have a rapid HIV test in the clinic today.

Patient name: _____

Signature: _____

Date: _____

Name of the person obtaining consent: _____

Job: _____

Signature: _____

Date: _____

Principal Investigator: Prof David Lewis

Tel: (011) 555-0468

Appendix 3 - PERMISSION TO STORE SAMPLES

Version 3 – 2nd October 2013

Flesch-Kincaid Grade Level 7.2

STI Aetiological Surveillance Protocol NMS-002

We would like to store "left-over" samples such as swabs, blood and organisms from the survey. These samples will be stored in freezers belonging to the NICD. The samples will be stored by means of a code, without any personal information, in an access controlled area.
Samples will only be used for research related to sexually transmitted infections (STIs). Your personal genes will not be tested in any way.
If someone wants to use these samples in a future study, they would need to first get permission from an Ethics Committee in South Africa.
You can choose not to have your samples stored, and still be part of the current STI survey.
Professor David Lewis (Principal Investigator), Tel: (011) 555 0468

- ❑ I agree to have my samples saved for future studies.
- ❑ I do not want my samples saved for future studies.

If you give permission to store you samples today and later wish to have them destroyed, please contact Prof David Lewis (Principal Investigator), Tel: (011) 555-0468

_____ _____
Subject's Signature Date

Name of the person obtaining consent: _____

Job: _____

Signature: _____

Date: _____

Appendix 4 – CLINICAL QUESTIONNAIRE

Version 3 – 11th December 2012

Name of facility: ……

Date: ……

Survey No: *Place sticker*

PATIENT INFORMATION

1. STI clinic male ☐ STI clinic female ☐

FPC female under 21 years ☐

2. Age: ……. (years)

3. Ethnic group:

African ☐ Coloured ☐ Indian☐

White ☐ Other ☐

4. Sexual orientation:

Heterosexual☐ Homosexual☐ Bisexual☐

CLINICAL INFORMATION

5. STI syndrome(s) diagnosed today:
(tick all applicable)

VDS ☐ MUS ☐ GUS ☐ None ☐

LAP ☐ SSW ☐ GW ☐

VDS, vaginal discharge; MUS, male urethritis; GUS, genital ulcer; LAP, lower abdominal pain; SSW, scrotal swelling; GW, genital warts

6. Treated <u>without</u> success for the same STI syndrome in the past 3 months?

Yes ☐ No ☐ N/A ☐

7. Referral from another clinic for persistent MUS, VDS or GUS?

Yes ☐ No ☐ N/A ☐

8. History of STI syndrome in last one year?

None ☐ MUS ☐ SSW ☐

VDS ☐ LAP ☐ GUS ☐ GW ☐

9. Condom use at last sex:

Yes ☐ No ☐ Can't remember ☐

10. Age of first sex? ……(years)

11. Sex with someone living in <u>another</u> province (last 3 months):

Yes ☐ No ☐

If yes, which province(s)?

EC ☐ FS ☐ GP ☐ KZN ☐ LP ☐ MP ☐ NC ☐ NWP☐ WC ☐

12. Sex with someone living <u>outside of South Africa</u> (last 3 months):

Yes ☐ No ☐

If yes, which country(s)? ……

CHECKLIST FOR SPECIMENS

For MUS patients:

Endourethral smear on slide	□
Endourethral swab	□
10ml venous blood	□

For VDS patients:

Vaginal smear on slide	□
Endocervical swab	□
10ml venous blood	□

For GUS patients

Ulcer smear on slide	□
Ulcer swab	□
10ml venous blood	□

For FPC patients:

Endocervical swab	□
10ml venous blood	