AETIOLOGICAL SURVEILLANCE OF SEXUALLY TRANSMITTED INFECTION SYNDROMES AT SENTINEL SITES: GERMS-SA 2014-2016

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Executive Summary

Sentinel aetiological surveillance of sexually transmitted infection (STI) syndromes was conducted at primary healthcare facilities in four South African provinces during the period 2014-2016. *Neisseria gonorrhoeae* was the predominant cause of male urethritis syndrome (MUS), and syndromic management with dual antimicrobial therapy that also covers *Chlamydia trachomatis*, the second most common pathogen, is necessary. Continued surveillance for antimicrobial resistance in *N. gonorrhoeae* is essential. Herpes simplex virus was the commonest detectable cause of genital ulceration, supporting the continued use of acyclovir in syndromic management. The syndromic management of vaginal discharge syndrome (VDS) remains complex because the commonest causes - bacterial vaginosis and candidiasis - are not considered to be STIs. However, a significant proportion of patients with either condition were co-infected with STI pathogens. The HIV seroprevalence among STI patients was high, underlining the importance of linkage to universal HIV counselling and testing in primary healthcare settings.

Background

In South Africa, sexually transmitted infections (STIs) are managed principally at primary healthcare facilities (PHCs) using standard syndromic management guidelines.[1] National clinical STI syndrome surveillance is conducted by the National Department of Health (NDoH) at 270 surveillance sites across the country. Clinical surveillance data on the distribution of STI syndromes in Gauteng Province public health clinics (PHCs) during the period 2000 to 2007 revealed that male urethritis syndrome (MUS), vaginal discharge syndrome (VDS) and genital ulcer syndrome (GUS) combined constitute nearly 80% of all syndromes seen.[2]

Periodic aetiological surveillance of the three main STI syndromes is critical in terms of validating the existing treatment algorithms. The STI Section of the National Institute for Communicable Diseases (NICD) has conducted regular aetiological and antimicrobial resistance surveillance at sentinel PHCs since 2007. During 2014 to 2016, STI aetiological surveillance was conducted in the following provinces: Gauteng (Alexandra Health Centre), Mpumalanga (Kabokweni and Hluvukani Clinics), KwaZulu-Natal (Eastboom Community Health Centre in Pietermaritzburg and Phoenix Clinic in Durban) and Eastern Cape (Gqebera Clinic).

The primary objectives of this surveillance were to determine the aetiologies of the three major STI syndromes (MUS, GUS, VDS) and the antimicrobial susceptibility profiles of *Neisseria gonorrhoeae* isolates. Secondary objectives were to determine syphilis, herpes simplex type 2 (HSV-2) and HIV co-infections among patients presenting with STI syndromes.

Methods

Consecutive consenting patients presenting with MUS, VDS or GUS at the selected PHCs between January 2014 and December 2016 were included in the surveillance. Inclusion criteria were STI patients aged 18 years and above with a new episode of clinically confirmed MUS, VDS and/or GUS. The target sample size per site was as follows: 100 cases each of MUS and GUS and approximately 150-200 cases of MUS (in order to obtain at least 100 viable gonococcal isolates from each site). Following eligibility and informed consent procedures, a nurse-administered questionnaire was used to document demographic and clinical information. Swabs were used for the sampling of genital discharge (vaginal, endocervical, urethral) and genital ulcers. Additionally, a 10 ml specimen of venous blood was collected from each participant. Laboratory testing was performed using the diagnostic assays shown in Table 1. Data from a survey-specific database were imported into STATA 14® [Stata Corporation, College Texas] for analysis.

Table 1. Specimen types and laboratory testing by sexually transmitted infection (STI) syndrome, South Africa, 2014 – 2016.

Syndrome	Specimen	Test
Male urethritis syndrome (MUS)	Endourethral smear	Gram stain for Gram-negative diplococci
	Endourethral swab (Dacron)	In-house multiplex real-time (RT) PCR for discharge pathogens
	Endourethral swab in Amies transport medium	*Neisseria gonorrhoeae* culture and antimicrobial susceptibility. E-test MIC (bioMerieux): cefixime, ceftriaxone, ciprofloxacin Agar dilution MIC: penicillin, tetracycline, azithromycin
Vaginal discharge syndrome (VDS)	Vaginal smear	Gram stain: Nugent score (bacterial vaginosis); yeast (candidiasis)
	Endocervical swab	In-house multiplex RT PCR for discharge pathogens
Genital ulcer syndrome (GUS)	Ulcer smear	Giemsa stain for *Klebsiella granulomatis* (granuloma inguinale)
	Ulcer swab	In-house multiplex RT PCR for ulcer pathogens Commercial PCR (Sacace Biotechnologies) for HSV-1 & 2 subtyping LGV-specific in-house RT PCR for *Chlamydia trachomatis* L1-3 serovars
All participants	10 ml venous blood for serology	HSV-2 (Focus HerpeSelect 2 IgG) HIV (Trinity Biotech Unigold; Determine Alere diagnostics) RPR (Immutrep Omega diagnostics)

Results

Patient demographic and clinical characteristics

Of 1824 participants, 962 (52.6%) were male (Table 2). Median age of participants was 27 years (IQR 23-32 years) and the majority were of black African ethnicity (99.4%) and of heterosexual orientation (98.9%). With respect to high risk sexual behaviours, median age at sexual debut was 17 years (IQR 16-19 years)

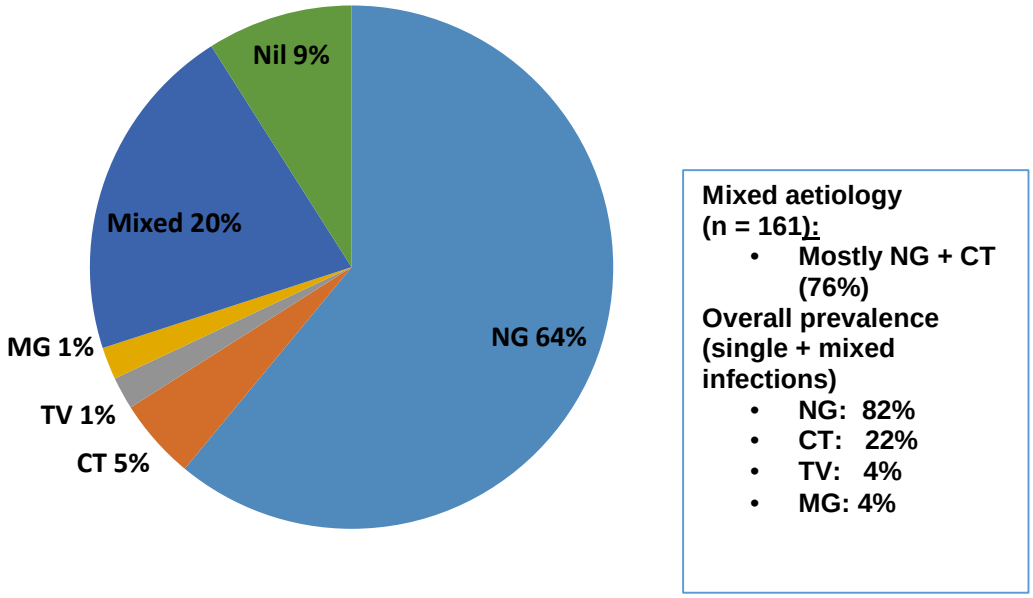
and self-reported condom use at last sexual encounter was low (17.6%). Almost one third of participants (28.7%) had been diagnosed with an STI syndrome within the preceding 12-month period.

Table 2. Demographic and clinical characteristics of participants enrolled into national sexually transmitted infection (STI) syndrome surveillance, South Africa, 2014 – 2016.

Variable	All (N = 1824)
N	962 (52.7)
Current age median (IQR)	27 (23- 32)
Black Africans	1813 (99.4)
(n, %)	
Age at sexual debut	17 (16- 19)
Median (IQR)	
Condom use	322 (17.6)
(n, %)	
Sex with someone outside province	292 (16.0)
(n, %)	
Sex with someone outside country	214 (11.7)
(n, %)	
STI syndrome diagnosed in the past 12 months	523 (28.7)
(n, %)	
Heterosexual orientation	1803 (98.9)
(n, %)	
Main syndrome diagnosed:	
Male urethritis syndrome	808 (44.3)
Vaginal discharge syndrome	757 (41.5)
Genital ulcer syndrome	366 (20.1)
>=2 syndromes	107 (5.9)

STI Syndrome aetiologies

Male urethritis syndrome: Among 808 patients presenting with MUS, *N. gonorrhoeae* was the commonest cause (666, 82.4%; 95% CI 79.6 – 84.9) followed by *Chlamydia trachomatis* (178, 22.0%; 95% CI 19.3 - 25) (Figure 1). The majority of patients (578, 71.5%; 95% CI 68.3 – 74.5) had infections caused by single agents. *Trichomonas vaginalis* and *Mycoplasma genitalium* accounted for less than 5% of MUS. Multiple pathogens were detected in approximately 20% (161; 95% CI 17.3 – 22.8). The majority of these mixed infections (150, 93.2%) were caused by *N. gonorrhoeae* together with one or more STI pathogens, mostly *C. trachomatis* (123,76.4%). An STI pathogen was detected in approximately 91% of specimens (739; 95%CI 89.3-93.2) and less than 10% (69; 95% CI 6.8 – 10.7) had no identifiable STI aetiology.



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

Figure 1. Relative prevalence of sexually transmitted infection (STI) pathogens in patients presenting with male urethritis syndrome (MUS), South Africa, 2014 – 2016, (N = 808).

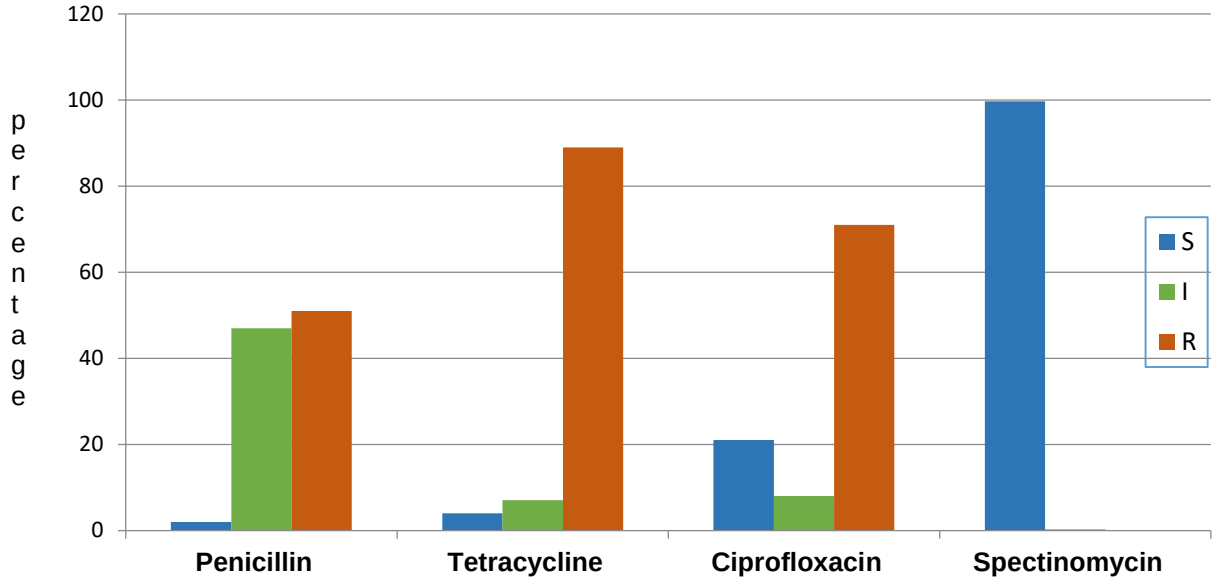
Neisseria gonorrhoeae antimicrobial susceptibility profiles: *Neisseria gonorrhoeae* minimum inhibitory concentrations (MICs) to extended-spectrum cephalosporins and azithromycin were available for 339 viable culture isolates from male urethral discharge specimens collected in 2016 (Table 3). All isolates demonstrated low ES-cephalosporin MICs that were within the susceptible range. MICs to azithromycin showed that 99.4% isolates were susceptible to azithromycin with only two (0.6%) isolates from Mpumalanga and KwaZulu-Natal provinces, respectively, exhibiting intermediate resistance (MIC = 0.5 µg/ml). The susceptibility profiles to the other antimicrobials tested are shown in Figure 2. High-level resistance rates were as follows: 51% for penicillin; 88% for tetracycline and 70% for ciprofloxacin. All isolates, except for one that displayed an intermediately-resistant MIC of 64 µg/ml, were susceptible to spectinomycin.

Table 3. *Neisseria gonorrhoeae* minimum inhibitory concentrations (MICs) to extended-spectrum cephalosporins and azithromycin. National sexually transmitted infection (STI) syndrome surveillance, South Africa, 2014 – 2016, (n = 339).

Antimicrobial	MIC	MIC	Maximum MIC	% with MIC = 0.125	% with MIC = 0.25	% WITH MIC ≥ 0.5
Cefixime	< 0.016	< 0.016	0.016	0	0	0
Ceftriaxone	0.003	0.006	0.032	0	0	0

Antimicrobial	MIC	MIC	Maximum MIC	% with MIC ≤ 0.25	% with MIC = 0.5	% with MIC ≥ 1
Azithromycin	0.128	0.25	0.5	99.4	0.6	0

Interpretive criteria used: CLSI for extended-spectrum cephalosporins; EUCAST for azithromycin; MICs in µg/ml



Interpretive criteria used: CLSI. S = sensitive; IR = intermediately-resistant; R = resistant

Figure 2. Neisseria gonorrhoeae antimicrobial susceptibility profiles, national sexually transmitted infection (STI) syndrome surveillance, South Africa, 2014 – 2016, (n = 330).

Vaginal discharge syndrome: Among 756 women with VDS (Figure 3), less than 50% had a detectable STI pathogen in single or mixed infections (330; 95% CI 40.1 – 47.1). The commonest STI aetiology was *N. gonorrhoeae* (140, 18.5%; 95%CI 15.9 – 21.4), followed by *C. trachomatis* (134, 17.7%; 95% CI 15.2 – 20.6). *T. vaginalis* accounted for less than 15% of infections, and *M. genitalium* less than 10%. Overall, single STI pathogens were detected in 234 VDS cases (31%; 95% CI 27.7 – 34.3); and mixed infections with multiple (two or more) STI pathogens in 96 (13%; 95% CI 10.5 – 15.3).

Most VDS cases were attributed to conditions that are not traditionally considered to be STIs. Bacterial vaginosis (BV) was identified in 427/752 (56%; 95% CI 52.8 – 59.9) cases, and vulvovaginal candidiasis (CA) accounted for 167 (22%; 95% CI 19.2 – 25.1) cases. An identifiable pathogen or cause was not found for 144 (19%; 95% CI = 16.4 - 22) of VDS cases.

A significant proportion of VDS patients had co-infection with STI and non-STI aetiologies. Only 98/752 (13%) of VDS cases tested for all causes had a sole STI aetiology. The rest (232/752, 31%) had an STI plus BV and/or CA.

Overall, 205 VDS cases (27%) had BV-STI co-infections, and sixty-five VDS cases (8.5%) had CA-STI co-infections. Therefore, 205/427 patients with BV (48%; 95% CI 43.3 – 52.8) and 65/167 patients with CA (39%; 95% CI 31.8 – 46.6) had STI co-infections.

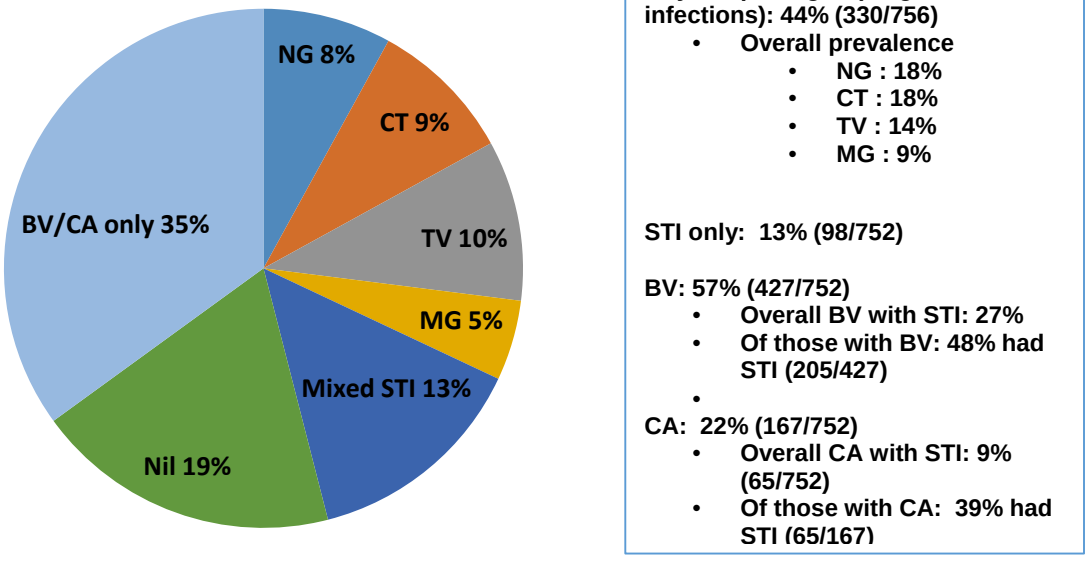
The median age (and IQR) of women with non-STI causes of VDS was 27 years (22-33), and that of women harbouring one or more STI pathogens was also 27 years (23-31).

The relative prevalence of STI pathogens detected in co-infections is presented in Table 4. The commonest STI pathogen in BV co-infected patients was *N. gonorrhoeae* (93/205, 45%). The commonest STI pathogen in CA-STI co-infected patients was *C. trachomatis* (29/65, 45%).

Table 4. Prevalence of sexually transmitted infection (STI) pathogens among vaginal discharge syndrome (VDS) patients with bacterial vaginosis (BV) and vulvovaginal candidiasis (CA), national STI syndrome surveillance, South Africa, 2014 – 2016.

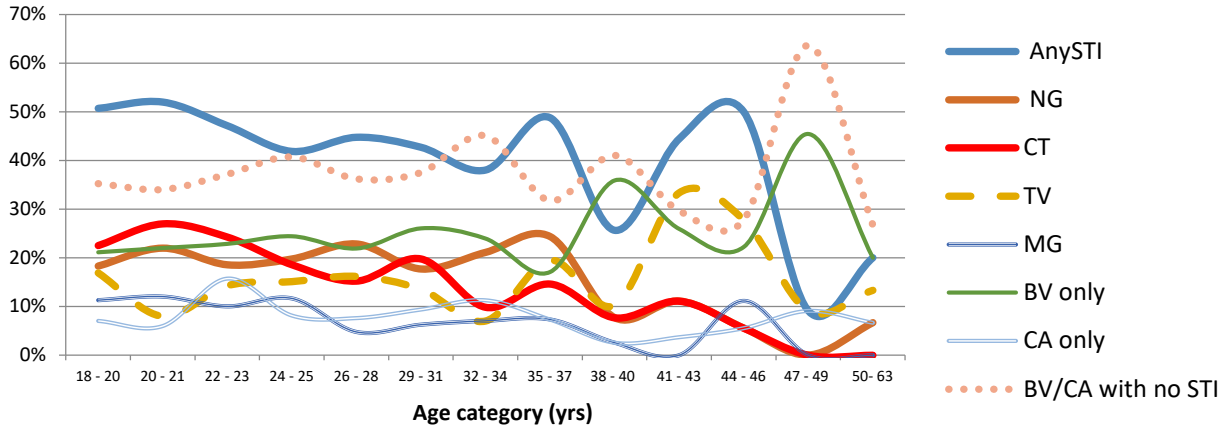
Infection	n	*Neisseria gonorrhoeae* (%)	*Chlamydia trachomatis* (%)	*Trichomonas vaginalis* (%)	*Mycoplasma genitalium* (%)
BV with STI	205	93 (45)	88 (43)	55 (27)	44 (21)
CA with STI	65	23 (35)	29 (45)	19 (29)	15 (23)

Microbial aetiology of VDS and STI pathogen prevalence, stratified by age, shows that age is not an accurate predictor of infection with STI pathogens, including *N. gonorrhoeae*, or with non-STI related conditions such as bacterial vaginosis or candidiasis (Figure 4). There was a significant sustained downward trend in the prevalence of *C. trachomatis* with increasing age in which the prevalence declined significantly in those aged 35 years and older.



Key: *Neisseria gonorrhoeae* (NG); *Chlamydia trachomatis* (CT); *Trichomonas vaginalis* (TV); *Mycoplasma genitalium* (MG); bacterial vaginosis (BV); vulvovaginal candidiasis (CA)

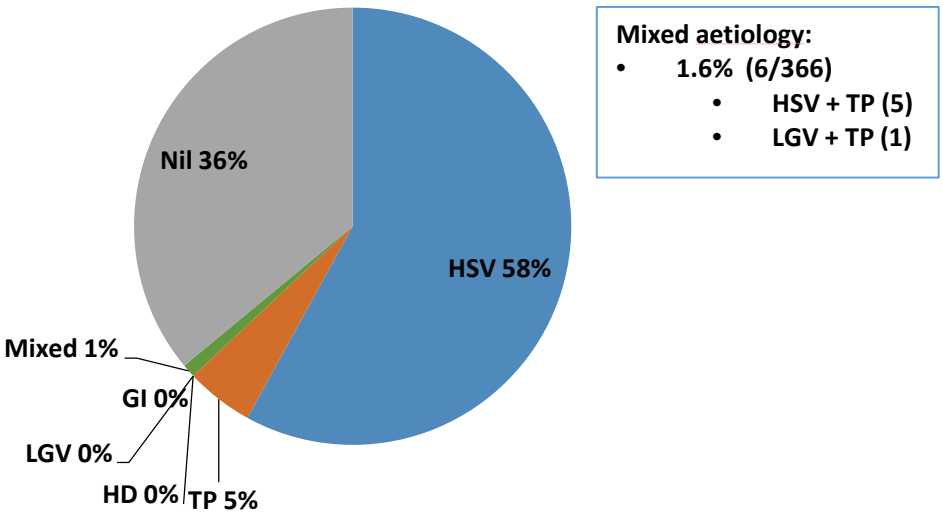
Figure 3. Relative prevalence of vaginal discharge syndrome (VDS) aetiologies, national sexually transmitted infection (STI) syndrome surveillance, South Africa, 2014 – 2016, (N = 752).



Neisseria gonorrhoeae (NG); *Chlamydia trachomatis* (CT); *Trichomonas vaginalis* (TV); *Mycoplasma genitalium* (MG); bacterial vaginosis (BV); vulvovaginal candidiasis (CA)

Figure 4. Distribution of vaginal discharge syndrome (VDS) aetiologies by age. National sexually transmitted infection (STI) syndrome surveillance, South Africa, 2014 – 2016.

Genital ulcer syndrome: Among 366 GUS cases (Figure 5), the major cause was herpes simplex virus (HSV) in 59.3% (217/366; 95% CI 54 - 64), followed by *Treponema pallidum* (TP) in 6% (22/366; 95% CI 4 - 9) of cases. Type-specific PCR revealed that 99.0% (215/217) HSV infections were caused by HSV-2. Of two HSV-1 infected ulcers from Gauteng and Mpumalanga provinces, one was co-infected with HSV-2. There was only 1 case each (0.6%) of *Haemophilus ducreyi* (HD) causing chancroid (Eastern Cape) and lymphogranuloma venereum (LGV) caused by *C. trachomatis* L1-L3 (Mpumalanga). No cases of granuloma inguinale were detected in any of the provinces. Most pathogen-detectable cases had a single aetiology (228/366, 62.3%). Only 6 cases had mixed aetiology. They were all were co-infected with HSV and one other pathogen, namely TP (5), HD and LGV.[1] An ulcer-derived pathogen was not identified in 36.1% of GUS cases (132; 95% CI 31.3 – 41.1).



Herpes simplex virus (HSV); *Treponema pallidum* (TP); lymphogranuloma venereum (LGV); granuloma inguinale (GI)

Figure 5. Relative prevalence of sexually transmitted infection (STI) pathogens in patients presenting with genital ulcer syndrome (GUS), South Africa, 2014 – 2016.

Serological results

Syphilis (RPR) seroprevalence was highest at 10.2% among GUS patients (37/364; 95% CI 7.4 – 13.7), followed by 2.9% in MUS (23/791; 95% CI 1.9 – 4.3) and 3% (22/742; 95% CI 2.0 – 4.5) in VDS patients. Active syphilis, defined by an RPR titre ≥ 4, was identified in 7.4% of GUS (95% CI 5.1 – 10.6), 2.4 % of MUS (95% CI 1.5 – 2.7) and 2.2% (95% CI 1.3 – 3.4) of VDS cases. Among the 22 GUS patients whose ulcers were attributed to primary syphilis, 19 (86%; 95% CI 62.8 – 96.0) had positive RPR results and 15 (68%; 95% CI 44.7 – 85.0) had RPR titres of $\underline{\geq}$ 4. The sero-prevalence of anti-HSV-2 antibodies among the GUS patients whose ulcers were caused by HSV-2 was 82% (178/217; 95% CI 76.3 – 86.6). HIV co-infection rates were as follows: 57.3% (208/363; 95% CI 52.1 – 62.3) in GUS; 47.2% (350/742; 95% CI 43.6 – 50.8) in VDS and 26.6% (211/794; 95% CI 23.6 – 29.8) in MUS. There was a significant association between HIV seropositivity and all STI syndromes ($p<0.001$).

Discussion and Conclusions

This surveillance study provides a snapshot of STI syndrome aetiologies across several South African provinces during the period 2014 to 2016. Overall, the study found that the majority of participants enrolled with STI syndromes were young and reported high-risk sexual behaviour, such as young age at sexual debut and unprotected sex at last sexual encounter.

Neisseria gonorrhoeae was the predominant cause of male urethritis syndrome. Based on these data, syndromic management for MUS in the South African public health sector should include cover for the two leading causes, *N. gonorrhoeae* and *C. trachomatis*. In 2015, the national STI syndromic management guidelines were formally revised in response to the increase in *N. gonorrhoeae* antimicrobial resistance observed worldwide, as well as reports of cefixime resistance in South Africa.[3] A pre-emptive strategy of dual antimicrobial therapy was incorporated to curb the emergence of resistance in *N. gonorrhoeae* to extended-spectrum cephalosporins. Specifically, oral cefixime was replaced with single doses of injectable ceftriaxone and oral azithromycin. Azithromycin used in dual therapy for *N. gonorrhoeae* also provides empiric cover for *C. trachomatis* infection. *Trichomonas vaginalis* was detected in less than 5% of men presenting with urethritis. These findings support the current syndromic approach to reserve metronidazole treatment for those whose partners report vaginal discharge, or for those whose symptoms persist following first-line treatment for *N. gonorrhoeae* and *C. trachomatis*.

Bacterial vaginosis was the leading cause of VDS and was prevalent in over 50% of females. Although the condition, which is associated with dysbiosis of the vaginal microbiome, is not considered to be a traditional STI, systematic review and meta-analysis of sexual risk factors have revealed that the epidemiological profile of BV is similar to that of established STIs.[4] The current VDS management algorithm has an age cut-off of 35 years and older for treatment of BV and *Candida* only, with exclusion of antimicrobial therapy for *N. gonorrhoeae* and *C. trachomatis* in older patients. These data reveal that there is no significant difference in median age of women infected with STI pathogens and those having BV or candidiasis. 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 management guidelines. A significant proportion of women with BV were co-infected with one or more STI pathogens. These findings suggest that BV is associated with risk factors for traditional STI infections, and that the management algorithm for VDS should be reconfigured to remove non-specific variables such as age and include specific sexual risk characteristics that increase the predictive value of the algorithm for STI pathogens.

Herpes simplex virus-2 remains the leading cause of pathogen-detectable GUD in Gauteng, and this supports the use of anti-viral therapy in the syndromic management guidelines. A change in epidemiology to HSV-1 has not been observed. Approximately 80% of genital herpes cases were HSV-2 antibody positive and represented clinically apparent reactivation disease. Primary syphilis and LGV are relatively uncommon causes of GUD in the predominantly heterosexual populations accessing STI services in South African primary healthcare centres. In keeping with epidemiological trends worldwide, chancroid, a predominant cause of GUD in South Africa in the late 20th century (responsible for up to 70% of genital ulceration), is now only detected sporadically.[5] The significant proportion of cases without an identifiable ulcer-derived aetiology requires further research.

The HIV prevalence among patients presenting with STI syndromes is significantly higher than the UNAIDS 2015 estimated prevalence of 19.1% for adults aged 15-49 years in the general South African population. This underscores the importance of linkage to universal HIV testing and treatment for STI patients and supports the recently adopted national policy of early ARV initiation for those who are HIV-infected.

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References

1. National Department of Health. Sexually Transmitted Infections Management Guidelines 2015. Adapted from: Standard Treatment Guidelines and Essential Drugs List PHC. National Department of Health, Republic of South Africa.
2. National Department of Health, Epidemiological Comments. 2008; 3(3)
3. Lewis D, Sriruttan C, Muller E et al. Phenotypic and genotypic characterization of the first two cases of extended-spectrum cephalosporin resistant *Neisseria gonorrhoeae* infection in South Africa and association with cefixime treatment failure. *J Antimicrob Chemother* 2013; 68(6) 1267-70.
4. Fethers K, Fairley K, Hocking J et al. Sexual risk factors and bacterial vaginosis: a systematic review and meta-analysis. *Clin Infect Dis* 2008; 47: 1426-35.
5. Gonzalez-Beiras C, Marks M, Chen CY, Roberts S, Mitja O. Epidemiology of *Haemophilus ducreyi* Infections. *Emerg Infect Dis*. 2016; 22(1): 1-8.