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INYUVESI YAKWAZULU-NATALI

Sexually Transmitted Infections among Men who have Sex with Men in the Durban Area, South Africa

Presented by

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Supervised by

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PLAGIARISM DECLARATION

I, **Kehinde Charles Mofolorunsho** hereby declare that

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As the candidate's supervisor, I Prof. Nathlee Abbai agree to the submission of this thesis.

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RESEARCH OUTPUTS

Published Manuscripts

1. **Mofolorunsho, Kehinde Charles**, Dorsamy, Vinogrin, Bagwandeen, Chauntelle and Abbai Nathlee Samantha. Prevalence of gonococcal and chlamydial infections among men who have sex with men in sub-Saharan Africa: protocol for a systematic review and meta-analysis. *Systematic Reviews* **12**, 141 (2023). https://doi.org/10.1186/s13643-023-02305-2.

Submitted Manuscripts

1. **Kehinde Charles Mofolorunsho**, Caitlin Ramnarain, Nonkululeko Mabaso, Nikita Nundlall, and Nathlee Abbai. Genotypes of *Chlamydia trachomatis* among men who have sex with men in Durban, South Africa. Under going revision for the Journal of Medical Laboratory Science & Technology of South Africa (JMLST#170).

2. **Kehinde Charles Mofolorunsho**, Nonkululeko Mabaso, Nikita Nundlall, Apiwe Nightingale, Makandwe Nyirenda and Nathlee Abbai. Prevalence and associated risk factors of chlamydia and gonorrhoea infections among Men who have Sex with Men in Durban, South Africa. Submitted to the African Journal of Reproductive Health.

3. **Kehinde Charles Mofolorunsho**, Nonkululeko Mabaso, Nikita Nundlall, Abidemi Oloranti Ojo, Errol Duncan Cason, and Nathlee Samantha Abbai. Comparison of the urinary microbiome in men who have sex with men with and without *Chlamydia trachomatis* infection. Submitted to the International Journal of Microbiology (ID Number: 6977803).

4. **Kehinde Charles Mofolorunsho**, Vinogrin Dorsamy, Chauntelle Bagwandeen, and Nathlee Samantha Abbai. Prevalence of gonococcal and chlamydial infections among men who have sex with men in sub-Saharan Africa: a systematic review and meta-analysis. Submitted to the Journal of Sexual Medicine.

Conference Presentations

1. **Mofolorunsho Kehinde**, Mabaso Nonkululeko, Nundlall Nikita, Nightingale Apiwe, Nyirenda Makandwe and Nathlee Abbai. *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections among Men who have Sex with Men Living in South Africa. Conference presentation at the 45th Global Congress on Infectious Diseases: Research on Diagnosis and Therapeutics, SciTech Central Conferences (28 November 2023).

DEDICATION

This PhD thesis is dedicated to my parents, Sir. Sam-Jones Jaiyeola Mofolorunsho (Late) and Philomena Bakuwa Mofolorunsho. Dad, you truly were an inspiration to me. You inspired me to achieve success. I wish you were here to celebrate this success with me. Mum, you have been a pillar of strength and a great teacher. I will forever be grateful to you two for the love and support you gave to me.

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Above all, I give glory to God Almighty for without Him I would never have come this far. I am forever grateful to God for preserving me, and giving me the strength and perseverance to accomplish this academic feat.

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ACRONYMS

ASVs	Amplicon Sequence Variants
BREC	Biomedical Research Ethics Committee
BV	Bacterial vaginosis
DNA	Deoxyribonucleic acid
DOAJ	Directory of Open Access Journals
gDNA	Genomic deoxyribonucleic acid
GRADE	Grading of Recommendations, Assessment, Development and Evaluation
HIV	Human Immunodeficiency Virus
IFN-γ	Interferon gamma
MeSH	Medical Subject Headings
MLST	Multi Locus Sequence Typing
MOMP	Major Outer Membrane Protein
MSM	Men who have sex with men
NCBI	National Centre for Biotechnology Information
NG	*Neisseria gonorrhoeae*
NGS	Next Generation Sequencing
NGU	Non-gonococcal urethritis
OTU	Operational Taxonomic Unit
PCoA	Principal Coordinates Analysis
PCR	Polymerase Chain Reaction

PEO	Population Exposure Outcomes
PID	Pelvic Inflammatory Disease
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analyses
PRISMA-P	Preferred Reporting Items for Systematic Reviews and Meta-analyses Protocols
PROSPERO	International Prospective Register of Systematic Reviews
qPCR	Quantitative Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphisms
rRNA	Ribosomal ribonucleic acid
STIs	Sexually Transmitted Infections
UTIs	Urinary Tract Infections
WHO	World Health Organization

ABSTRACT

Introduction: Gonorrhoea and chlamydia are amongst the most common sexually transmitted infections (STIs) worldwide, constituting a major public health problem. The incidence and prevalence of these infections are widespread, particularly in resource-poor countries due to the lack of access to health care facilities and ineffective diagnostic methods. In the African region, gonorrhoea and chlamydia have been reported to account for 11.4 and 12 million new cases per year, respectively. Gonorrhoea which is caused by the bacterium, *Neisseria gonorrhoeae*, and chlamydia, caused by the bacterium *Chlamydia trachomatis* have been found to be associated with serious health complications including infertility, pelvic inflammatory disease (PID), and epididymitis. Infections caused by these pathogens significantly increases the risk of Human Immunodeficiency Virus (HIV) transmission, particularly in men who have sex with men (MSM). Most MSM are asymptomatic, engaging in high-risk sexual behaviours, and have become an understudied group at greater risk for STI infections globally. *C. trachomatis* serovars have been linked to specific diseases, with serovars D-K detected in urogenital infections. In MSM, serovars reported were predominantly G, D and J. Microbiome variation has been found to be substantially affected by sexual practice. The bacterial composition of the male urinary microbiome is reported to influence the risk for the development of sexually transmitted infections. However, little is known about the urinary microbiome of MSM. The aim of this study was to determine the prevalence and risk factors associated with *N. gonorrhoeae* and *C. trachomatis* infections; characterize the distribution of *C. trachomatis* genotypes, and assess the bacterial composition of the urinary microbiome in a population of South African MSM.

Methods: This study which was in two parts, was conducted over a 3-year period. The first part involved a systematic review and meta-analysis on the prevalence of *N. gonorrhoeae* and *C. trachomatis* among MSM in sub-Saharan Africa while the second part involved a cross-sectional laboratory based study. This cross-sectional laboratory based study included sexually active MSM, 18 years and older, willing to provide written informed consent and a urine sample to test for *N. gonorrhoeae* and *C. trachomatis* infections. A total of 200 MSM from the King Edward VIII hospital and the Aurum Institute both in Durban, South Africa were enrolled into the study. Urine samples were provided by each participant, and 10ml of each urine sample was centrifuged and the recovered pellets subjected to further molecular analyses. With a commercially available kit, deoxyribonucleic acid (DNA) was extracted from the sample pellets following the manufacturer's protocol for the isolation of genomic DNA. *N. gonorrhoeae* and *C. trachomatis* were detected using the Applied Biosystems™ TaqMan® Assays. Amplification was performed on the QuantStudio 5 Real-time polymerase chain reaction (PCR) detection system. In addition, molecular genotyping of *C. trachomatis* positive samples was performed by an *omp1* gene semi-nested PCR followed by restriction fragment length polymorphism (RFLP) analysis. The amplified product was digested with *AluI*, *DdeI* and *HinfI*

restriction enzymes. Banding patterns obtained after digestion were used to determine the genotypes. The urinary microbiomes of study participants were characterized using *16S rRNA* (V3 and V4) gene sequencing on the Illumina MiSeq platform. Participants' demographics, sexual history, associated risk factors for each of the STIs as well as the barriers and facilitators to care were documented. All analyses were conducted using STATA 17.1 as well as the RStudio version 3.6.3 software.

Results: The overall result of the meta-analysis gave a pooled prevalence of 27% (95% CI, 19–39%), with an I^2 of 98% which indicates high heterogeneity amongst the studies. Subgroup analysis by country indicated that South Africa (n = 6) has a prevalence of 38%. Findings from our study showed that the prevalence of *N. gonorrhoeae* and *C. trachomatis* were 3.0% and 6.0%, respectively. Younger age was significantly associated with testing positive for *C. trachomatis* (p=0.037). Other factors significantly associated with testing positive for *C. trachomatis* included, education level, partner having other partners, sex practices, condom use and circumcision status (p<0.05). Being between the ages of 30-39 years old reduced the risk of acquiring *C. trachomatis* infection (OR: 0.10, 95% CI: 0.0120-0.7564, p=0.026). In addition, being circumcised reduced the risk of contracting *C. trachomatis* (adjusted OR: 0.01, 95% CI: 0.0005-0.3516, p=0.01). However, having between 2-4 sex partners increased the risk of testing positive for *C. trachomatis* (adjusted OR: 107.45, 95% CI: 1.3467-8573.3130, p=0.036). Cohabiting with one's sex partner, engaging in group sex, and drug use were significantly associated (p<0.05) with testing positive for *N. gonorrhoeae* infection. Fear and stigma were the main barriers to accessing health care in the studied population. The genotyping assays showed that genotype E was the most prevalent genotype present in 60% of the men infected with *C. trachomatis*. Other genotypes detected were Genotype I (30%) and Genotype J (10%). According to the bacterial taxonomic analysis, *Prevotella* and *Lactobacillus* were detected in urine samples of MSM. Alpha diversity metrics showed a slight increase in microbial diversity in positive samples. This was however not significant (ANOVA, P > 0.05). Principal coordinates analysis (PCoA) showed that the microbiome of *C. trachomatis* infected MSM was not clearly separated from those uninfected. Using normalized unweighted UniFrac dissimilarities, distinct bacterial communities were not detected between positive and negative samples (PERMANOVA $F_{1,22}$= 1.0284, R^2=0.047%, P=0.385).

Conclusion: This study showed that *C. trachomatis* was prevalent in the study population and this finding was confirmed by previous studies conducted among MSM in South Africa. A proportion of the population who tested positive for both STIs were asymptomatic. In addition, finding from our review suggests that the burden of *N. gonorrhoeae* and *C. trachomatis* among MSM in South Africa and the rest of sub-Saharan Africa is higher when compared with other regions. These highlights the need for routine screening of these infections among MSM in order to facilitate proper management since some MSM engage in risky sexual behaviour. Genotyping, using the PCR-RFLP technique

revealed the presence of three genotypes circulating in the study population. An important finding was the detection of genotype I. Since this genotype is common among women and heterosexual men, further research is needed to provide clarity as to why this genotype was present among MSM. The microbial composition of urine samples from our study participants showed diverse bacterial communities including *Gardnerella* and *Sneathia* which are associated with bacterial vaginosis in women. Therefore, more studies on the role of the female urinary microbiota in relation to MSM urinary health, needs to be conducted. This study adds to the growing body of literature on genitourinary infections in South African MSM and provides data that serves as a baseline for future research.

IQOQA

I-Gonorhoea ne-chlamydia ziphakathi kwezinye izifo ezivamile ezithathelwanayo emhlabeni wonke. Esifundeni i-Afrika, igonorrhoea nechlamydia kubikwe ukuthi zibhekene nezigameko ezintsha zezigidi eziyizi-11.4 neziyi-12 ngonyaka, ngokulandelana. Amadoda alala namanye amadoda (MSM) awanazimpawu zokugula, ukuzibandakanya nokuziphatha ngokocansi okuyingozi enkulu, nokuthi sekuphenduke kwaba yiqembu elingafundile elisengozini enkulu yezifo ezithathelwana ngokocansi emhlabeni. Lolu cwaningo beluhlose ukuhlonza ukwanda nezinto eziyingozi esihlobene neNisseria gonorroede (Ng) neChlamydia trachomatis (Ct) ukuthathelwana, ukubalula ukusatshalaliswa kweCt genotypes nebhakhtheriya ye-urinary microbiome kubantu baseNingizimu Afrika MSM. Lolu cwaningo obelugxile elabhorethi yezingxenye ezahlukahlukene lwenziwe esikhathini esiyiminyaka emi-3. I-MSM ethanda ucansi, eneminyaka eyi-18 nendala yabhaliswa, namasampula omchamo kwalethwa ukuba kuhlolwe Ng/Ct. Ukuhlola kwakubandakanya izindlela okungezona ezijwayelekile njengokuthatha iDNA, Qpcr, isemi-nested PCR, RFLP ukuhlaziya kanjalo futhi nokulandelana kofuzo lwe16S Rrna (V3 neV4) isakhifuzo sebhakhtheriya. Okutholakele ocwaningweni lwethu kukhombise ukuthi ukwanda kweNg neCt bekungu-3.0% no-6.0%, ngokulandelana. Abasebancane bebehlanganiswa kakhulu nokuhlolwa ukuthi banayo iCt ($p=0.037$). Ezinye izinto ezihlanganiswa kakhulu nokuhlolwa ukuba nayo iCt kubandakanyiwe, ukwenza ucansi, nesimo sokusoka ($p<0.05$). Ukusokwa kunciphisa ingozi yokuthola iCt ($p=0.01$). Kodwa-ke, ukuba nothandana nabo abaphakathi kwaba-2-4 kwandisa ingozi yokuthi uhlolwe kutholakale unayo iCt ($p=0.036$). Ukuzibandakanya ocansini nabaningi nokudla izidakamizwa kuhlanganiswe kakhulu ($p<0.05$) nokuhlolelwa ukuthi ukutheleleka ngeNg. Ukuhlolelwa i-genotype kwakhombisa ukuthi i-genotype E iyona kakhulu ephambili ekhona ngama-60% wamadoda atheleleke ngeCt. Ngokusho kokuhlaziya kwebhakhtheriya e-taxonomic, iPrevotell neLactobacillus kwatholakala kumasapula omchamo weMSM. Ukwehluka kwe-Alpha metrics kukhombise ukwenyuka kancane kokwehlukahlukana kwamagciwane kumasampula eCt. Lolu cwaningo lukhombise ukuthi iCt yayidlangile kubantu abacwaningiwe, eqhakambisa isidingo sokuhlolwa okujwayelekile kokutheleleka futhi nokwengeza ekwandeni kwengqikithi yezincwadi ngezifo ze-henitourinary eNingizimu Afrika ngeMSM nokuhlinzeka ngemininingo esebenza njengesisekelo socwaningo lwangomuso.

CHAPTER ONE

1.1 Thesis Overview

1.1.1 Thesis structure

This thesis is structured according to the guidelines stipulated by the College of Health Sciences of the University of KwaZulu-Natal for the thesis by manuscript format. Each manuscript is formatted according to the journal guidelines to which they were submitted. The thesis is guided by the following chapters:

Chapter 1: This chapter is a protocol for the systematic review and meta-analysis of literature on *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections in men who have sex with men from sub-Saharan Africa. It also describes the problem statement and rationale of the study, defining the aim and objectives. This manuscript titled "**Prevalence of gonococcal and chlamydial infections among men who have sex with men in sub-Saharan Africa: protocol for a systematic review and meta-analysis**" was submitted to Systematic Reviews and was accepted for publication on the 04th of August 2023.

Chapter 2: This chapter is entitled: "**Prevalence of gonococcal and chlamydial infections among men who have sex with men in sub-Saharan Africa: a systematic review and meta-analysis**". This article is a systematic review aimed at documenting the pooled prevalence of gonorrhoea and chlamydia in men who have sex with men in sub-Saharan Africa. This manuscript was submitted to the Journal of Sexual Medicine.

Chapter 3: This chapter is entitled: **"Prevalence and associated risk factors of chlamydia and gonorrhoea infections among Men who have Sex with Men in Durban, South Africa**". This chapter contains the manuscript detailing the prevalence of and risk factors associated with *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections in our study setting. This manuscript was submitted to the African Journal of Reproductive Health.

Chapter 4: This chapter described the genotypic diversity of *C. trachomatis* in the study cohort. This article titled "**Genotypes of *Chlamydia trachomatis* among men who have sex with men in Durban, South Africa**" was submitted to the Journal of Medical Laboratory Science & Technology of South Africa. A revision has been submitted back to the journal.

Chapter 5: This chapter is entitled: "**Comparison of the urinary microbiome in men who have sex with men with and without *Chlamydia trachomatis* infection**". This manuscript was submitted to the International Journal of Microbiology. This article assessed the bacterial composition of the urinary microbiome associated with *Chlamydia trachomatis* infection.

Chapter 6: This is the conclusion chapter which discussed relevant findings from the study in the broader context. The limitations of the study were highlighted as well.

1.1.2 Study design and Methodology

Ethical clearance for this study was obtained from the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (BREC/00002798/2021). This was a cross-sectional laboratory based study. This study which was conducted over a 3-year period was in two parts. The first part involved a systematic review and meta-analysis while the second part involved the cross-sectional laboratory based study. The target population for this study were men who have sex with men (MSM), 18 years and older residing in Durban. A total of 200 MSM were enrolled for this study. This was determined based on sample size calculations. The calculation used a 12.7% prevalence of STIs from a previous study and included a 15% non-refusal rate. The standard normal deviate was set at 1.96 (95% CI) and the degree of precision set at 0.05. All laboratory assays were conducted at the School of Clinical Medicine Research Laboratory of the Nelson R. Mandela School of Medicine, University of KwaZulu-Natal.

1.2 Introduction

Epidemiologic data on sexually transmitted infections (STIs) suggest a high burden of disease worldwide (Newman et al. 2015) contributing significantly to the global burden in public health and medical care (Unemo et al. 2017). Published reports have shown that more than 1 million STIs are acquired every day, and an estimated 376 million new infections with 1 of the 4 main curable STIs (chlamydia, gonorrhoea, syphilis, and trichomoniasis) occur every year in the world (WHO, 2018; Rowley et al. 2019). Evidence has shown that men who have sex with men (MSM) are at high risk of acquiring Human Immunodeficiency Virus (HIV) and other STIs, and have been identified as a population with reported increase in the incidence of chlamydia, gonorrhoea and syphilis infections (Rebe et al. 2013; Jansen et al. 2015; CDC, 2017).

Sexually transmitted infections (STIs) are a major public health problem worldwide and are caused by different pathogens including bacteria, viruses and protozoa and are particularly important as they can facilitate the spread of HIV and are associated with severe disease. These infections can cause severe

fetal and neonatal death, ectopic pregnancy, infertility, and genital neoplasia (Mohammed et al. 2016; Unemo et al. 2017).

The increased incidence of STIs has been attributed to various factors, including cultural, social, behavioural, economic and microbiological components. Data on the epidemiology of STIs suggest a high burden of disease worldwide with a global incidence estimated at over 350 million cases yearly; most of which occur in low income countries (De Schryver, 1990; Piot and Tezzo, 1990; Newman et al. 2015). Published estimates have shown that Africa remains the continent worst affected with STIs. In South Africa, modelled STI prevalence estimates are among the highest globally (Frank et al., 2023). Also, when compared to other African countries, the prevalence of STIs in South Africa is higher, accounting for 11 million cases being treated annually (McCormack et al. 2010).

Men who have sex with men (MSM) who engage in unprotected penile-anal intercourse with casual partners are at high risk of acquiring STIs (Werner et al. 2018) with observed increase in the incidence of chlamydia, gonorrhoea and syphilis infections having been reported in numerous countries (Jansen et al. 2015; Bremer et al. 2017; CDC, 2017; Public Health England, 2018). Factors associated with this increase include the loss of fear regarding HIV transmission because of the increased manageability of the infection, Internet use as an efficient way to find sex partners, increase in use of erectile dysfunction agents, and possibly the expanding role of oral sex in STI transmission (Illa et al. 2008; Werner et al. 2018).

Epidemiology

The World Health Organization (WHO) estimates that the total global number of new cases of the four main curable STIs, was nearly 500 million cases in adults aged 15 to 49 years old (WHO, 2012). In sub-Saharan Africa alone, 69 million cases of these STIs are reported annually making it a significant public health problem (Rours et al. 2006; White, 2009; Rours et al. 2010). They have been associated with a wide spectrum of complications such as urethritis and epididymitis in men and cervicitis in women (Walker et al. 2012). Population characteristics, including population size, age structure, ethnic makeup and net migration, affect the epidemiology of STIs (Hughes and Field, 2015).

Sexually transmitted infection rates among MSM have continued to increase across the United States and abroad (Fenton and Imrie, 2005; van der Bij et al. 2005). In Europe, MSM are disproportionately affected by HIV and other STIs (Beyrer et al. 2011; ECDC, 2014). In 2013, 29 157 people were newly diagnosed with HIV in the European Union/European Economic Area (EU/EEA) (ECDC/WHO, 2014). Sex between men was the most common mode of HIV transmission, representing 42% of newly diagnosed HIV cases. In 2012, HIV prevalence among MSM was at or above 5% in 15 EU/EEA countries (ECDC, 2013). Gonorrhoea and chlamydia incidence among MSM are also increasing

significantly in numerous countries although global data is lacking (Martí-Pastor et al. 2015; Stenger et al. 2015).

Men who have sex with men (MSM) in sub-Saharan Africa are a largely hidden population with disproportionate risk for HIV infection (King et al. 2013; Daniels et al. 2017; Pilgrim et al. 2019). Homosexual behaviour in relation to HIV and other STIs are not well-studied in these settings largely due to repressive government policies, criminalization, stigma and lack of basic epidemiological data describing these epidemics (Senior, 2010; King et al. 2020). However, as more studies measure HIV infection in sub-Saharan Africa, it is becoming clear that MSM have a considerably greater disease burden than men in the general population (Smith et al. 2009; Beyrer et al. 2010; Muraguri et al. 2012; King et al. 2013).

In South Africa and indeed most African countries, data on incidence of STIs among MSM are limited. However, few studies from the region show a high burden of STIs in this group of individuals. A study among MSM in Kenya reported a remarkably high burden of STI infection among HIV-1 negative MSM with 11(26%) of the 43 MSM screened testing positive for either *N. gonorrhoeae* or *C. trachomatis* infection (Sanders et al. 2010). Jones et al. in their study on STIs among South African MSM and transgender women (TGW) reported that STIs were highly prevalent among these sub-populations with the vast majority of STIs being asymptomatic (Jones et al. 2020). The current study was designed to provide data on the prevalence of *N. gonorrhoeae* and *C. trachomatis* infections in MSM.

Risk factors for STIs in MSM

The incidence of many STIs in gay, bisexual, and other MSM including primary and secondary syphilis and antimicrobial-resistant gonorrhoea is greater than reported cases in women and men who have sex with women only (Kirkcaldy et al. 2013; An et al. 2017; de Voux et al. 2017). The annual increases in reported cases of STIs could reflect increased frequency of behaviours that transmit both STIs and HIV (Solomon and Mayer, 2014; Solomon et al. 2014; Katz et al. 2016). Reasons for this relatively high incidence of STIs among MSM may be related to multiple factors, including behavioural and sexual network factors (Baggaley et al. 2010; Glick et al. 2012; Beyrer et al. 2013; Spicknall et al. 2017).

Risk of HIV and bacterial STIs transmission, is typically related to the high number of lifetime male partners, rate of partner exchange, unprotected anal intercourse (UAI) and the use of disinhibiting substances. Each of these risk taking behaviours influence an individual's probability of exposure to STIs (Mansergh et al. 2008; Beyrer et al. 2012a; Glick et al. 2012). In addition, MSM network characteristics such as high prevalence of STIs, interconnectedness and concurrency of sex partners, and possibly limited access to healthcare have been reported to affect the risk of acquiring a STI (Alvy

et al. 2011; Glick et al. 2012). Stigma, discrimination, and physical assault based on attraction to men are also associated with increased sexual risk behaviour among MSM (Balaji et al. 2017). Of these underlisted factors, UAI and the use of disinhibiting substances are two major factors that facilitate the spread of HIV and STIs among MSM (Mansergh et al. 2008; Beyrer et al. 2012a). Several studies have demonstrated high rates of HIV transmission through anal sex. A systematic review and meta-analysis on HIV transmission risks in anal sex, reported a 1.4% transmission probability per act of unprotective receptive anal intercourse (URAI) which was estimated to be roughly 18-times greater than that of vaginal intercourse (Grulich and Zablostka, 2010; Beyrer et al. 2012a; Baggaley et al. 2018). Another study reported a 20-fold increased risk of HIV seroconversion over 6 months among MSM who reported UAI when compared to those who did not (Baggaley et al. 2010; Morris and Little, 2011).

Acquisition of HIV and other STIs has been reported to be more directly related to the use of substances such as alcohol, crystal methamphetamine, and other recreational drugs in conjunction with un-protected sex. Among the drugs most highly associated with risk-taking behaviour is methamphetamine (Buchacz et al. 2005; CDC, 2006; Wilson et al. 2008). A study in the Netherlands identified partner age, HIV infection and sex following alcohol consumption as risk factors for incident STIs among MSM (Ramadhani et al. 2017). The study was designed to identify the associated risk factors for *N. gonorrhoeae* and *C. trachomatis* infections in MSM, now adding to the body of literature.

Factors affecting access to care for STIs in MSM

Despite available data indicating the relevance of prioritizing MSM in HIV related healthcare (Beyrer et al. 2012b; Ayala et al. 2013; Mayer et al. 2013; Wirtz et al. 2013), MSM are continuously being underserved in terms of appropriate healthcare due to non-existence of MSM friendly health services especially in sub Saharan Africa (Ntata et al. 2008; Beyrer et al. 2012b). Where such services are available, they are often suboptimal and may be exacerbated by sexual stigma and criminalization of homosexuality, which may ultimately contribute to the high prevalence and incidence of HIV and other STIs among MSM (Smith et al. 2009; Beyrer, 2010; Fay et al. 2011; Beyrer et al. 2012b; Beyrer et al. 2013).

Structural factors such as criminalization of same-sex, social marginalization, stigma and discrimination are key barriers to accessing social support, medical care, and HIV prevention services (Baral et al. 2009; Risher et al. 2013; Ballester-Arnal et al. 2014; Baral et al. 2014; Arreola et al. 2015). Stigma has played a role in health seeking behaviour where MSM with higher perceived stigma were less likely to pursue healthcare and have trouble disclosing their sexual orientation to healthcare providers (Fay et al. 2011). Perceived and experienced stigma and discrimination due to sexual preferences, and confidentiality concerns in healthcare settings are among structural barriers to accessing services among MSM especially in settings where male to male sex is illegal (Ntata et al. 2008; Wirtz et al. 2013). In

sub-Saharan Africa, MSM are a largely hidden population with disproportionate risk for HIV infection and are often highly stigmatized and in many countries, criminalized (Zulu et al. 2006; Baral et al. 2009; King et al. 2013; Okall et al. 2014). Studies from Malawi, Botswana and Namibia have demonstrated that MSM who had any interaction with healthcare had over two times greater odds of experiencing fear of seeking healthcare and over six times greater odds of having been denied health-care due to sexual orientation (Fay et al. 2011). This study now provides data that will be added to the body of literature regarding barriers as well as facilitators to accessing healthcare among MSM.

MSM-associated microbiomes

Human health has been described as the outcome of the complex interaction between the microbiome and its human host. Body sites such as the human skin, mouth, gastrointestinal tract and vagina have been reported to support diverse microbial communities including substantial numbers of uncharacterised and uncultivated species (Frank and Pace, 2001; Robinson et al. 2010; Vayssier-Taussat et al. 2014). These microbial communities are believed to play an important role in maintaining health. However, structural or metabolic perturbations of the microbiome may occur at these sites which could result in a variety of disease conditions including obesity, inflammatory bowel disease and Crohn's disease (Turnbaugh et al. 2006; Frank et al. 2007; Peterson et al. 2008; Turnbaugh et al. 2009). These alterations in the makeup of the human microbiota (dysbiosis) are often characterised by a loss of beneficial, commensal bacteria which protect against overgrowth of opportunistic pathogenic bacteria (Petersen and Round, 2014; Copeland et al. 2018; Hoggard et al. 2018).

Microbiome variation has also been found to be significantly influenced by sexual practice. Men who have sex with men (MSM) exhibit a distinct microbiome signature characterized by *Prevotella* enrichment and increased alpha diversity, which is linked with receptive anal intercourse in both males and females (Vujkovic-Cvijin et al. 2020). This vastly understudied population are at high risk for genitourinary diseases and other STIs. The urethra, rectum and pharynx are the common sites of STIs among this cohort due to unprotected oral/anal sexual behaviors (Workowski and Bolan, 2015; Jiang et al. 2022). Exposure to the gut and oral microbiome through these routes may lead to shifts in the urinary microbiota of MSM that increase predilection for genitourinary diseases (Sawhney et al. 2021). Majority of microbiome studies on MSM have focused on the gut microenvironment (Noguera-Julian et al. 2016; Kelley et al. 2017; Neff et al. 2018). Few studies, however, have provided data regarding the normal composition of the male urethral microbiomes or if these microbiomes are associated with male STIs (Sikalidis and Maykish 2020; Sawhney et al. 2021; Srinivasan et al. 2021). This study now provides data that will be added to the body of evidence regarding bacterial composition of the urethral microbiome in a cohort of MSM.

1.3 Systematic Review Protocol

Prevalence of gonococcal and chlamydial infections among men who have sex with men in sub-Saharan Africa: protocol for a systematic review and meta-analysis

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Systematic Reviews

PROTOCOL Open Access

Prevalence of gonococcal and chlamydial infections among men who have sex with men in sub-Saharan Africa: protocol for a systematic review and meta-analysis

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Abstract

Background Bacterial sexually transmitted infections (STIs) including *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are common in men who have sex with men (MSM). These infections increase the risk of acquiring and transmitting human immunodeficiency virus (HIV) in this key population. Access to MSM in many countries in sub-Saharan Africa remains generally difficult due to discrimination or criminalization of their sexual orientation which could lead to depression and risky sexual practices associated with prevalence. This protocol therefore proposes to undertake a systematic review and meta-analysis of literature on the prevalence of gonococcal and chlamydial infections among MSM in Sub-Saharan Africa.

Methods This review which aims to ascertain the pooled prevalence and risk factors of these infections in sub-Saharan Africa's MSM population will follow the Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA) guidelines. The search strategy will review relevant articles from the following databases: PubMed, Scopus, ISI Web of Science and the Directory of Open Access Journals (DOAJ). Articles screening for eligibility and data extraction will be conducted by two independent reviewers. All discrepancies will be resolved by the third and fourth reviewers. Heterogeneity in studies will be evaluated using the I^2 statistic and where heterogeneity is high and significant, a random effect model will be used to estimate the pooled prevalence. Publication bias will be assessed using the Doi plot. Extracted data will be analysed using MetaXL add-on for Microsoft excel. Data will be presented in tables and graphically presented in forest plots.

Discussion In this study, we anticipate being able to systematically determine the prevalence of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* among MSM as well as explore possible risk factors associated with prevalence. The outcomes of the systematic review and meta-analyses will serve to support researchers and public health stakeholders in identifying healthcare priorities and in addressing issues pertaining to the overall wellbeing of the MSM community.

Systematic review registration PROSPERO CRD42022327095

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Keywords Prevalence, Men who have sex with men, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, Sub-Saharan Africa

Background

Sexually transmitted infections (STIs), including gonorrhoea and chlamydia, cause significant morbidity and mortality worldwide [1]. These STIs are a variety of clinical syndromes caused by pathogens that can easily be transmitted through sexual contact [2] and can facilitate individual susceptibility to human immunodeficiency virus (HIV) acquisition and transmission [1, 3]. Globally, the rate of STIs is increasing despite biomedical advances and considerable public health efforts. In 2012, 498.9 million new cases of curable STIs were reported worldwide. Their burden is disproportionally higher in low- and middle-income countries, with sub-Saharan Africa contributing 93 million new cases [4, 5]. Vulnerable populations include men who have sex with men (MSM) [6].

Studies on MSM in sub-Saharan Africa have reported high prevalence of STIs including *Neisseria gonorrhoea* (Ng) and *Chlamydia trachomatis* (Ct) [7, 8]. These bacterial infections (Ng and Ct) among African MSM have prevalence reportedly ranging from 1% [9] to 23% [10]. Studies from Kenya have shown Ng prevalence among gay men and other MSM to be 9.3% [11]. In Senegal, Ng prevalence among MSM has been estimated at 5.5% [12, 13] while in Nigeria, a published study estimated a Ng prevalence of 23% and Ct prevalence of 16% [14].

Although much attention has been given to African MSM in recent times, access to MSM in many countries in sub-Saharan Africa remains generally difficult. This situation is largely due to stigma, discrimination and/or criminalization of their sexual orientation [15–17], leading to their reluctance in accessing healthcare services. Because MSM suffer significant stigma due to lack of social acceptance, they become depressed and may engage in risky sexual practices including alcohol and substance abuse which are associated with prevalence [18–20]. Almost two-third of African countries still criminalize same-sex sexual behaviour, with long prison sentences or even the death penalty [21]. These issues may explain why research on MSM in sub-Saharan Africa is lagging behind other parts of the world [22–24], contributing to the paucity of information on this key population at higher risk for STIs. Hence, a systematic review aimed at ascertaining the pooled prevalence of Ng and Ct, and their associated risk factors in MSM in sub-Saharan Africa is necessary. The result of which is likely to inform research and policies in identifying healthcare priorities for this population living in Africa.

Objectives

This protocol for a systematic review aims to document the pooled prevalence of infections by *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in MSM in sub-Saharan Africa. Other objectives are to:

- Describe chlamydial and gonococcal infections diagnosed by syndromic management or laboratory testing
- Identify the associated risk factors for the prevalent infections among MSM

Methods

This protocol is registered with the open access registry for systematic review protocols PROSPERO (CRD42022327095) and developed in accordance with the Preferred Reporting Items for Systematic Review and Meta-analyses for Protocols (PRISMA-P) 2015 checklist [25]. The planned systematic review will be reported according to the PRISMA 2020 statement [26].

Eligibility of research question

A Population Exposure Outcomes (PEO) framework (Table 1) will be used to evaluate the eligibility of the research question. Only studies reporting overall and/or anatomical site-specific prevalence of chlamydia and/or gonorrhoea in men who have sex with men will be included. No restriction will be applied for study design, diagnostic test type and year of publication.

Table 1 PEO (Population, Exposure, Outcomes) framework

Population	MSM in sub-Saharan Africa
Intervention/Exposure	Prevalence of chlamydia and gonorrhoea infections determined by syndromic management or laboratory testing for urethral/pharyngeal/rectal infection
Outcomes	Overall and anatomical site-specific prevalence rates, and risk factors for chlamydia and gonorrhoea

Identifying relevant studies

Electronic databases will be used to search articles in peer-reviewed journals from the following databases: PubMed, Scopus, ISI Web of Science, Directory of Open Access Journals (DOAJ). Search terms used and their synonyms were identified using the Medical Subject Headings (MeSH). The uniterms and Boolean operators in English to be used in the search strategies are (Men who have sex with men OR gay) AND ((*Neisseria gonorrhoeae* OR *N. gonorrhoeae* OR Gonorrhoeae infection OR Gonorrhoeae) AND (*Chlamydia trachomatis* OR *C. trachomatis* OR *Chlamydia infection* OR Chlamydia)) AND (Africa OR Sub-Saharan Africa OR Western Africa OR Eastern Africa OR Southern Africa OR Central Africa). Also, a combination of relevant key words with names of each of the countries in Sub-Saharan Africa will also be performed. The search strategy will be adapted for each of the databases to be searched. The search strategy was piloted in April 2022 to test the appropriateness of selected keywords and electronic databases as depicted in Table 2.

Study selection

The selection of eligible studies will be based on the following inclusion and exclusion criteria:

Inclusion criteria

1. Studies that describe data from MSM in sub-Saharan Africa
2. Studies that include men who have sex with men 15 years and older
3. Studies that quantified the prevalence of chlamydia and/or gonorrhoea
4. Original research written in English language
5. Studies that report prevalence based on an adequate numerator and denominator where actual diagnostic tests were employed

Exclusion criteria

1. Studies that assessed non-human subjects
2. Studies published in languages other than English
3. Studies without full text available
4. Studies that computed incident infections only
5. Studies conducted in other countries other than countries in Sub-Saharan Africa
6. Case reports, Short reports, Letters, Notes, Conference abstracts, Review articles

First, title and abstracts of retrieved articles will be screened. Titles and abstracts of all articles identified from the search will be independently screened by the primary author and a co-author, following removal of duplicates. Then, full article screening will be conducted for their eligibility. Eligible articles will be retrieved and exported to Endnote version 20 reference manager. A hand search of the reference list of all selected articles will also be performed in order to be more comprehensive in the search strategy. Disagreements between the two authors (if any) will be discussed and resolved with the third and fourth authors. Full article screening based on eligibility criteria will then be conducted independently by two authors and all discrepancies resolved by the third and fourth authors. The PRISMA flowchart will be used to report the screening results (Fig. 1).

Data extraction

A data extraction template will be designed using Microsoft Excel for collection of data from eligible studies. This data extraction template will be piloted and edited through an iterative process. Two authors will independently extract the data. The third and fourth author will independently verify all extracted data. The following data will be extracted: first author, year of publication, study period, country, geographic location, study design, setting, type of participants, sampling method, sample

Table 2 Potential search strategy on PubMed

Search	Query
#1	("sexual and gender minorities"[MeSH Terms] OR men who have sex with men[Text Word]) OR ("sexual and gender minorities"[MeSH Terms] OR "homosexuality"[MeSH Terms] OR gay[Text Word])
#2	((("neisseria gonorrhoeae"[MeSH Terms] OR Neisseria gonorrhoeae[Text Word]) OR ("neisseria gonorrhoeae"[MeSH Terms] OR N. gonorrhoeae[Text Word])) OR (gonorrhoeae[All Fields] AND ("infections"[MeSH Terms] OR infection[Text Word]))) OR gonorrhoeae[All Fields]
#3	((("chlamydia trachomatis"[MeSH Terms] OR Chlamydia trachomatis[Text Word]) OR ("chlamydia trachomatis"[MeSH Terms] OR C. trachomatis[Text Word])) OR ("chlamydia infections"[MeSH Terms] OR chlamydia infection[Text Word])) OR ("chlamydia"[MeSH Terms] OR chlamydia[Text Word])
#4	(((((africa"[MeSH Terms] OR Africa[Text Word]) OR ("africa south of the sahara"[MeSH Terms] OR Sub-Saharan Africa[Text Word])) OR ("africa, western"[MeSH Terms] OR Western Africa[Text Word])) OR ("africa, eastern"[MeSH Terms] OR Eastern Africa[Text Word])) OR ("africa, southern"[MeSH Terms] OR Southern Africa[Text Word])) OR ("africa, central"[MeSH Terms] OR Central Africa[Text Word])
#5	#1 AND #2 AND #3 AND #4

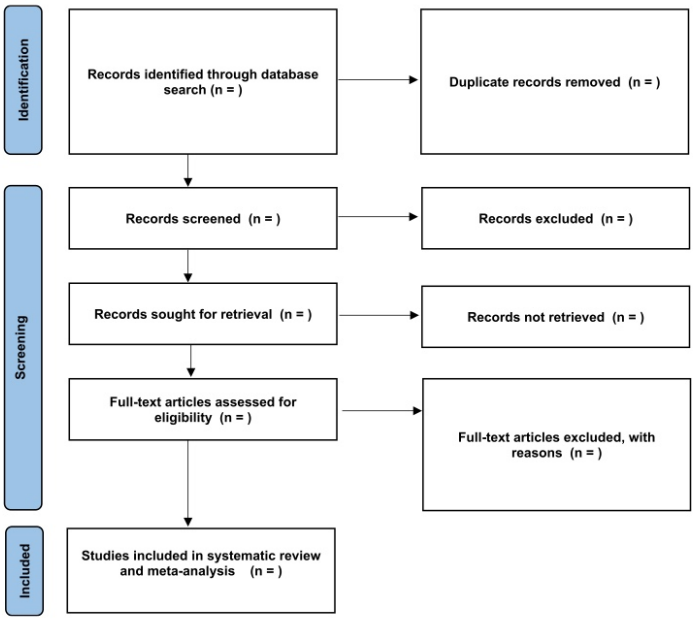

Fig. 1 PRISMA flow diagram describing selection of studies for systematic review of gonorrhoea and chlamydia prevalence among MSM in sub-Saharan Africa [26]

Table 3 Data extraction tool

1. First author
2. Year of publication
3. Study period
4. Country
5. Geographic location
6. Study design
7. Setting
8. Type of participants
9. Sampling method
10. Sample size
11. Age
12. Mean age
13. Specimen type
14. Anatomical site
15. Laboratory diagnosis method
16. Prevalence of chlamydia and/or gonorrhoeae

size, age, median age, specimen type, anatomical site, laboratory diagnosis method, prevalence of chlamydia and/or gonorrhoeae (Table 3). In case of missing or incomplete information, authors will contact the authors of the publications to request further particulars.

Risk of bias and quality assessment

The risk of bias tool for prevalence studies will be used to evaluate the quality and risk of bias of the included studies for the review and meta-analysis. This tool which was developed by Hoy and colleagues uses a 10-item rating scale to assess the internal and external validity of studies [27]. Each of the 10 items will be rated as either low or high risk of bias and the overall risk of bias will then be determined according to the number of high risk of bias per study (low: ≤2; moderate: 3–4; and high: ≥5) [28]. Insufficient information related to 10 items will be regarded as high risk of bias [29, 30].

The quality of evidence provided by the included studies will be established using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) tool taking into account the risk of bias, indirectness of evidence, inconsistencies, imprecision and publication bias. Following assessment, the overall certainty in evidence will be categorized into four: High (the true effect is similar to the estimated effect), Moderate (where the

true effect is probably close to the estimated effect), Low (where the true effect might be markedly different from the estimated effect) and Very low (where the true effect is probably markedly different from the estimated effect).

Data synthesis and analysis

The extracted data will be analysed using the MetaXL add-on for Microsoft excel [31, 32]. The data will be presented using tables and the results will be graphically presented in forest plots. The heterogeneity in the studies will be evaluated using the I^2 statistic. Where heterogeneity is high and significant across the included studies, a random effect model will be used to determine the pooled prevalence estimate. Subgroup analysis and meta-regression will be conducted to detect possible sources of heterogeneity according to study characteristics such as study design, sample size, country, diagnostic test and HIV status. Publication bias will be assessed using the Doi plot [33].

Discussion

In this study, we expect to be able to systematically determine the prevalence of Ng and Ct among MSM. Furthermore, we will explore possible risk factors associated with prevalence. The results section of the systematic review and meta-analysis will include a description of all studies, results of all analyses including planned subgroup analyses. We will in the discussion section summarize the main findings and their implications. We will also compare our findings with others and discuss limitations of the study.

This study is necessary and of importance considering that STIs remain a major public health problem in Africa, and the role of MSM in the transmission dynamics is increasingly being recognized [34]. Published estimates indicate that Africa remains the continent most affected by STI/HIV [35]. MSM, particularly those from Africa, are at increased risk for STIs such as HIV, Ng, Ct and syphilis [34, 36, 37] and may experience significant barriers to quality health care due to widespread sigma, criminalization [38] and ridicule by healthcare workers [22]. This situation is compounded by the general lack of data on MSM prevalence of STI/HIV and risk factors in the African setting [34, 35]. Therefore, in order to adequately address and contain the STI epidemic and the associated burden in this key population, more research is required.

The outcomes of the systematic review and meta-analyses will be disseminated through publication in peer-reviewed journals. The findings of this study will serve to support researchers and public health stakeholders in identifying healthcare priorities and in addressing issues pertaining to the overall wellbeing of the MSM population.

Abbreviations

STIs	Sexually transmitted infections
HIV	Human immunodeficiency virus
MSM	Men who have sex with men
Ng	*Neisseria gonorrhoeae*
Ct	*Chlamydia trachomatis*
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analyses
PRISMA-P	Preferred Reporting Items for Systematic Reviews and Meta-analyses Protocols
PEO	Population Exposure Outcomes
DOAJ	Directory of Open Access Journals
MeSH	Medical Subject Headings
GRADE	Grading of Recommendations, Assessment, Development and Evaluation

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Authors' contributions
This systematic review protocol was conceptualized by KCM and NSA. KCM, VD, CB and NSA participated in the design of the study. KCM prepared the original draft and VD, CB and NSA contributed to the review and editing of this protocol. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analysed during this investigation will be included in the published systematic review article and will be available upon request.

Declarations

Ethics approval and consent to participate
Not applicable. This systematic review will not consist of human participants.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Publisher's Note

1.4 Rationale

Sexually transmitted infections are commonly asymptomatic in this population and remain undiagnosed and where untreated, these infections could contribute to high HIV rates as prior exposure to STIs has been reported to be associated with increased HIV vulnerability among MSM. HIV among South African MSM is high with an estimated prevalence ranging between 10-43% (Rebe et al. 2015). This calls for interventions in this key population and one valid strategy for a reduced HIV transmission is the treatment of STIs. However, the burden of STIs in South African MSM remains unknown as no systematic surveillance is occurring due to the lack of clinical services as a result of high numbers of MSM attendees (WHO, 2011; Rebe et al. 2015). This study will therefore investigate the prevalence and risk factors for STIs in a population of MSM in the Durban area of South Africa. Study objectives will include the comparison of the urinary microbiome in the presence and absence of *C. trachomatis* infection.

1.5 Aims and Objectives

Aim

The aim of this study was to investigate the prevalence and risk factors for sexually transmitted infections in men who have sex with men in the Durban area of South Africa.

Objectives

- To determine the prevalence of *N. gonorrhoeae* and *C. trachomatis* infections in MSM
- To determine the risk factors associated with the prevalent infections in the studied population
- To identify barriers and facilitators with access to care for STIs
- To describe the genotypic diversity of *C. trachomatis* in the studied population
- To compare the urinary microbiome in the presence and absence of *C. trachomatis* infection

Mofolorunsho et al. (2023) showed that bacterial sexually transmitted infections (*N. gonorrhoeae* and *C. trachomatis*) are common among sub-Saharan African MSM. However, access to MSM in many countries in the region remains generally difficult due to discrimination which could lead to depression and risky sexual practices, contributing to the paucity of information regarding this key population including prevalence data on *N. gonorrhoeae* and *C. trachomatis* infections. Therefore, our aim was to undertake a systematic review and meta-analysis of literature on the prevalence of gonococcal and chlamydial infections among MSM in sub-Saharan Africa. Chapter two details the pooled prevalence of gonorrhoea and chlamydia in MSM in sub-Saharan Africa.

CHAPTER TWO

Prevalence of gonococcal and chlamydial infections among men who have sex with men in sub-Saharan Africa: a systematic review and meta-analysis

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Abstract

Background

Men who have sex with men (MSM) are disproportionately affected by sexually transmitted infections (STIs) including *Neisseria gonorrhoeae* (Ng) and *Chlamydial trachomatis* (Ct). The lack of robust data on STI among African MSM has limited the development of evidence-based screening strategies.

Aim

The objective of this study was to conduct a systematic review aimed at documenting the pooled prevalence of *N. gonorrhoeae*/*C. trachomatis* among MSM in sub-Saharan Africa.

Methods

This systematic review was performed according to the Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA) 2020 guidelines. Eligible studies reported on the prevalence of *N. gonorrhoeae* and or *C. trachomatis* among MSM population in sub-Saharan Africa and were available in full. Relevant articles from the following databases were searched: PubMed, Scopus, ISI Web of Science and the Directory of Open Access Journals (DOAJ). Publication bias was assessed using Hoy tool, the Doi plot and LFK ratio. Due to heterogeneity amongst studies subgroup analyses were performed using MetaXL add-on tool for Microsoft excel.

Outcome

Prevalence for *N. gonorrhoeae*/*C. trachomatis* infections may have clinical implications in the MSM population.

Results

The initial search yielded 525 articles and 20 were selected for inclusion. Of the 20 studies selected, 6 were cross-sectional, 4 had a prospective cohort study design and 1 was an epidemiological study. The pooled prevalence of *N. gonorrhoeae*/*C. trachomatis* in sub-Saharan African MSM was 27% (95% CI, 19-39%), with an I^2 of 98% which indicated high heterogeneity amongst the studies. Subgroup analysis by country indicated that South Africa (n = 6) has a prevalence of 38% (25-50%).

Clinical implications

Stakeholders in the public health sector should prioritize MSM healthcare needs and address issues pertaining to their overall wellbeing.

Limitations

Limited data reporting on prevalence of *N. gonorrhoeae*/*C. trachomatis* were available. Some of the studies included in this analysis used a cross-sectional design and as such, may not be representative of MSM.

Conclusion

This is the first study to systematically review the available literature on the prevalence of STIs in the MSM population from sub-Saharan Africa. This study showed that the burden of infection in this population is higher when compared with other regions.

Keywords

Men who have sex with men, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, Sub-Saharan Africa

Introduction

Men who have sex with men (MSM) are disproportionately affected by sexually transmitted infections (STIs) including *Neisseria gonorrhoeae* and *Chlamydia trachomatis*,[1-4] accounting for most patients diagnosed with rectal *N. gonorrhoeae* or *C. trachomatis*.[5] Since STIs are often asymptomatic and remain frequently undiagnosed and untreated,[6] they continue to pose a significant public health challenge globally. Sexually transmitted infections exert a substantial disease burden,[7] and are acquired by more than a million individuals daily, worldwide.[8] An estimated 374 million new infections with one of four curable STIs (gonorrhoea, chlamydia, syphilis and trichomoniasis) are acquired each year.[8]

N. gonorrhoeae and *C. trachomatis* infections are the two most prevalent curable STIs worldwide.[9-11] Approximately 87 million new cases of gonorrhoea were reported to have occurred amongst 15–49-year olds in 2016, with an incidence rate of 26 cases per 1000 men while an estimated 127.2 million new cases of chlamydia occurred globally every year.[12] In Africa, gonorrhoea and chlamydia accounted for 11.4 and 12 million new cases per year, respectively.[13,14]

Gonorrhoea, which is caused by *N. gonorrhoeae* is highly prevalent in less-developed countries and lower.[12,15] The prevalence of which varies by anatomic sites (whether urethral, rectal, or oropharyngeal)[16] and the methods of detection e.g. Gram's stain, standard culture, and molecular test.[17] Chlamydia, an equally concerning infection,[18] has been on the rise since 1995 and it is now the most pervasive STI.[10,19,20] This sexually transmitted infectious disease is caused by *C. trachomatis* with high rates among untreated asymptomatic patients.[21] *C. trachomatis* have been implicated in serious complications such as pelvic inflammatory disease (PID), ectopic pregnancy, tubal infertility, and chronic pelvic pain.[22,23] *C. trachomatis* infection has also been associated with non-gonococcal urethritis and epididymitis.[24]

MSM have in recent years become the group at greater risk of acquiring STIs worldwide: higher than female sex workers, the heterosexual populations, as well as the general population.[25-28] Extragenital STIs in MSM are frequent,[6,29.30] mostly asymptomatic,[16] and where undetected and untreated, can contribute substantially to the further spread.[31] Factors such as increased numbers of sexual partners, increased condom-less anal sex, and increased recreational drug use including chem-sex[32] facilitates the acquisition of STIs among this key population.

According to data from Europe, the United States and China, MSM have a high burden of HIV and other STIs.[33] In sub-Saharan Africa, data regarding bacterial STIs among MSM are sparse[34] since access to this sub-population in many countries of the region remains generally difficult, especially in light of their potential involvement in epidemiological research.[35] This observation is largely due to the

criminalization of their sexual orientation,[35,36] stigma and discrimination by healthcare workers.[36,37] Social and religious factors also play an important role in limiting research in this sub-population.[34]

The lack of robust data on STIs among African MSM[38] has limited the development of evidence-based screening strategies.[9] The objective of this study was to conduct a systematic review aimed at documenting the pooled prevalence of gonorrhoea and chlamydia in MSM in sub-Saharan Africa.

Methods

This systematic review was conducted according to the published protocol[39] registered (CRD42022327095) with the International Prospective Register of Systematic Reviews (PROSPERO). The present review was reported based on the Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA) 2020 guidelines.[40]

Search strategy

A systematic search of peer-reviewed articles was performed in all the electronic databases listed in the protocol. Search terms used and their synonyms were identified using the Medical Subject Headings (MeSH). The uniterms and Boolean operators in English used in the search strategies included (Men who have sex with men OR gay) AND ((*Neisseria gonorrhoeae* OR *N. gonorrhoeae* OR Gonorrhoeae infection OR Gonorrhoeae) AND (*Chlamydia trachomatis* OR *C. trachomatis* OR *Chlamydia infection* OR Chlamydia)) AND (Africa OR sub-Saharan Africa OR Western Africa OR Eastern Africa OR Southern Africa OR Central Africa). A combination of relevant key words with names of each of the countries in sub-Saharan Africa was also used in the search strategy.

Study selection

The selection of eligible studies was based on the criteria listed in the protocol.[39] Original research articles written in the English language that quantified the prevalence of gonorrhoea and/or chlamydia, and described data from sub-Saharan African MSM, 15 years and older were included. Studies were excluded if they assessed non-human subjects, computed incident infections only, were conducted in other countries other than countries in sub-Saharan Africa, were published in languages other than English, and were unavailable in full text. Case reports, short reports, letters, notes, conference abstracts, and review articles were also excluded.

Data extraction and quality assessment

Title and abstracts of retrieved articles were screened following removal of duplicates, and full article screening was conducted for their eligibility. Eligible articles were retrieved and exported to Endnote version 20 reference manager. A hand search of the reference list of all selected articles was also performed in order to be more comprehensive in the search strategy. Full article screening based on eligibility criteria was then conducted by two reviewers. For the collection of data from eligible studies, a data extraction sheet was designed using Microsoft Excel. This data extraction sheet was piloted, and edited through an iterative process.[39] Screening, data extraction and quality appraisal were independently performed by two reviewers (KCM and VD). Verification of all extracted data was conducted independently by the third and fourth reviewers (NA and CB). The study selection process was reported using a PRISMA flowchart.[40]

Risk of bias and quality assessment

The risk of bias tool for prevalence studies was used to evaluate the quality and risk of bias of the included studies for the review and meta-analysis. This tool uses a 10-item rating scale to assess the internal and external validity of studies.[41] (see Additional file 1: Table S1) Each of the 10 items was rated as either low or high risk of bias and the overall risk of bias was determined according to the number of high risk of bias per study (low: ≤2; moderate: 3–4; and high: ≥5).[42] Insufficient information related to 10 items were regarded as high risk of bias.[43,44] The quality of evidence provided by the included studies was established using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) [45] tool taking into account the risk of bias, indirectness of evidence, inconsistencies, imprecision and publication bias.

Data synthesis and analysis

All extracted data were analysed using the MetaXL add-on for Microsoft excel.[46,47] Data were presented using tables and the results were graphically presented in forest plots. The heterogeneity in the studies was determined using the *I2* statistic. Where heterogeneity is high and significant across the included studies, a random effect model was used to determine the pooled prevalence estimate. Subgroup analysis and meta-regression was conducted to detect possible sources of heterogeneity according to study characteristics such as study design, sample size, country, diagnostic test and HIV status. The Doi plot was used to assess Publication bias.[48]

Results

Characteristics of studies

Initial search retrieved 525 potential articles. Of the 525 articles, 28 were duplicates. A total of 38 articles were found eligible for full-text screening, of which 18 articles were excluded (Figure 1). Twenty articles that reported on the prevalence of gonorrhoea and chlamydia infections in MSM in sub-Saharan Africa were identified as fulfilling the criteria for inclusion in the analysis (Table 1).[34,38,49-66] All the studies were conducted in the major cities of the countries included; Togo,[59] Kenya,[50,53,58,62,64,66] South Africa,[34,38,54,61,65] Nigeria,[56,57] Tanzania,[52] Uganda,[55] Côte d'Ivoire,[51] and Senegal.[49] Two studies were conducted across 4 countries (Burkina Faso, Mali, Togo and Côte d'Ivoire).[60,63] The lowest prevalence rates for *N. gonorrhoeae* and *C. trachomatis* were reported from Uganda.[55] The highest prevalence rates for *N. gonorrhoeae* and *C. trachomatis* were reported from Kenya.[58,66] Included studies ranged in publication year from 2005 to 2023, and in sample size from 43 to 698 (Table 1).

Of the studies selected, 6 were cross-sectional, 4 studies had a prospective cohort study design and 1 was an epidemiological study (Table 1). The Respondent-Driven Sampling (RDS) technique was used by 8 studies to reach the MSM populations.[52,54-57,59,66] Majority of the studies (16) employed molecular tests for the diagnosis of *N. gonorrhoeae* and *C. trachomatis* infections[34,50-57,59-61,63-66] while one study used serology (Rapid Plasma Reagent Test) to test for infections.[38] The remaining studies combined molecular tests with either serology[49] or microscopy/culture tests.[58,62] Over half the studies (11/20; 55.0%) reported using both urine and rectal swabs specimens in testing for *N. gonorrhoeae* and *N. gonorrhoeae* (Table 1).

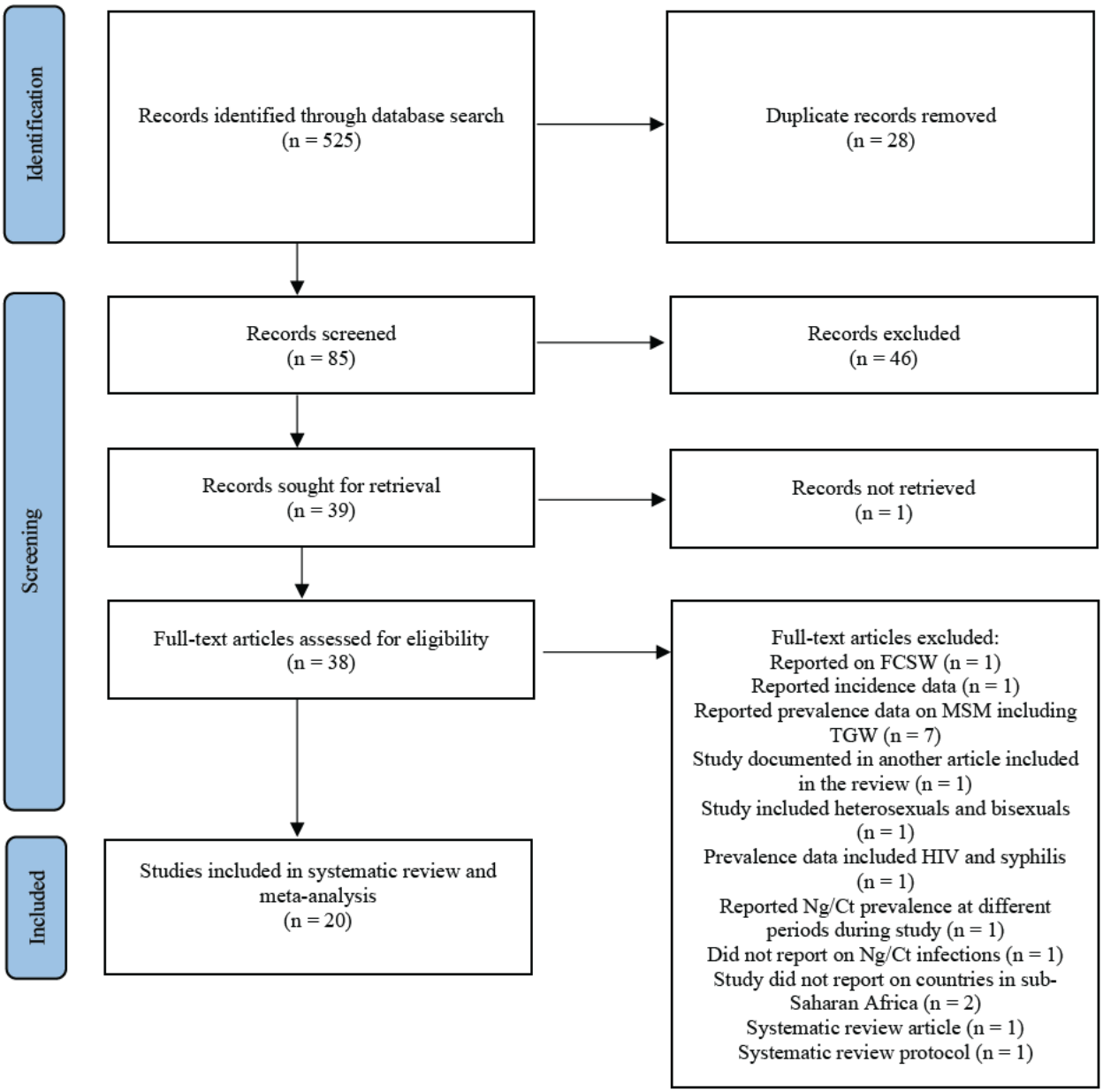

Figure 1. PRISMA flow diagram describing selection of studies for systematic review of gonorrhoea and chlamydia prevalence among MSM in Sub-Saharan Africa.[40]

Table 1 Characteristic of studies included in meta-analysis

Author	Country	Region	Study design	Sampling	Number of MSM tested	Diagnostic method used	Specimen	Infection	Prevalence(%)
Quilter (2018)[58]	Kenya	Kisumu	Not reported	Snowball	698	NAAT	Urine	Ng	47.2
						Microscopy	Rectal swab	Ct	25.0
Rebe (2015)[54]	South Africa	Cape Town	Cross-sectional	RDS	200	NAAT	Urine	Ng	16.0
							Rectal swab	Ct	12.0
							Pharyngeal swab		
Ramadhani (2016)[56]	Nigeria	Lagos	Prospective cohort	RDS	492	NAAT	Urine	Ng/Ct	11.4
		Abuja					Rectal swab		
Crowell (2018)[57]	Nigeria	Lagos	Cohort	RDS	420	NAAT	Urine	Ct	15.7
							Rectal swab		
Ross (2014)[52]	Tanzania	Dar es Salaam	Cross-sectional	RDS	220	NAAT	Urine	Ng/Ct	27.3
		Tanga					Rectal swab		
Ferré (2019)[59]	Togo	Lomé	Cross-sectional	RDS	207	NAAT	Rectal swab	Ng	11.6
		Kpalimé							
		Atakpamé						Ct	9.7
		Tsévié							
Kim (2016)[55]	Uganda	Kampala	Biobehavioural	RDS	295	NAAT	Urine	Ng	1.4
							Rectal swab	Ct	1.0
Laurent (2021)[63]	Burkina Faso	Ouagadougou	Prospective cohort	Not reported	598	NAAT	Urine	Ng	12.6
	Côte d'Ivoire	Abidjan					Rectal swab		
	Mali	Bamako					Pharyngeal swab	Ct	19.3
	Togo	Lomé							
Ngetsa (2020)[62]	Kenya	Coastal Kenya	Not reported	Not reported	104	NAAT	Rectal swab	Ng	9.6
						Culture		Ct	13.5
Mehta (2021)[64]	Kenya	Kisumu	Prospective cohort	Not reported	158	NAAT	Urine	Ng	1.9
							Rectal swab	Ct	7.7
De Baetselier (2020)[60]	Burkina Faso	Ouagadougou	Not reported	Not reported	497	NAAT	NR	Ng	11.5
	Togo	Lomé							
	Mali	Bamako						Ct	14.5
	Côte d'Ivoire	Abidjan							
Vuylsteke (2012)[51]	Côte d'Ivoire	Abidjan	Cross-sectional	Not reported	94	NAAT	Urine	Ng	12.8
							Rectal swab	Ct	3.2

Wade (2005)[49]	Senegal	Dakar Thiés Mbour Kaolack Saint-Louis	Epidemiological	Snowball	442	NAAT Serology	Urine Blood	Ng Ct	5.4 4.1
Jones (2020)[61]	South Africa	Cape Town Port Elizabeth	Prospective cohort	Not reported	292	NAAT	Urine Rectal swab	Ng Ct	2.3 10.8
Sanders (2014)[53]	Kenya	Coastal Kenya	Cohort	Not reported	244	NAAT	Urine Rectal swab	Ng Ct	1.6 6.1
Sanders (2010)[50]	Kenya	Coastal Kenya	Cohort	Not reported	43	NAAT	Urine Rectal swab	Ng Ct	2.0 12.0
Mwaniki (2023)[66]	Kenya	Nairobi	Cross-sectional	RDS	242	NAAT	Urine Rectal swab Pharyngeal swab	Ng Ct	14.9 58.7
Le Roux (2023)[65]	South Africa	Tshwane	Not reported	Snowball	199	NAAT	Urethral swab Rectal swab Pharyngeal swab	Ng Ct	17.1 18.1
Mashingaidze (2023)[34]	South Africa	Gauteng Western Cape KwaZulu-Natal North West Eastern Cape	Clinical trial	Not reported	173	NAAT	Urine Rectal swab	Ng Ct	8.1 26.0
Malefo (2023)[38]	South Africa	Tshwane North	Cross-sectional	RDS	200	Serology	Urethral swab Rectal swab Pharyngeal swab	Ng Ct	9.0 20.0

Abbreviations: NAAT, nucleic acid amplification tests; RDS, respondent driven sampling; Ng, *Neisseria gonorrhoeae*; Ct, *Chlamydia trachomatis*

Heterogeneity and publication bias

The included studies (20) were assessed for heterogeneity and publication bias. There was high heterogeneity for this finding; $I^2 = 98\%$ (Q = 885.25; $p = 0.001$). The Doi plot for publication bias with an LFK ratio was generated to assess the risk of bias. There was evidence of minor asymmetry with an LFK index of ±1.20 (see Additional file 2: Figure S1).

Sensitivity analysis

A sensitivity analysis of the twenty studies was conducted to evaluate the effect each study had on the pooled prevalence data (Table 2) The results showed little variation apart from the removal of the study De Baetselier et al., 2020[60] which reduced the prevalence by ~3%.

Table 2 Sensitivity analysis

Excluded study	Pooled Prevalence	LCI 95%	HCI 95%	Cochrane Q	p	I^2	I^2 LCI 95%	I^2 HCI 95%
Quilter et al. 2018	0.279	0.186	0.384	877.3	0.0	97.9	97.5	98.3
Rebe et al. 2015	0.274	0.184	0.374	885.0	0.0	98.0	97.5	98.4
Ramadhani et al. 2016	0.282	0.192	0.382	816.8	0.0	97.8	97.3	98.2
Crowell et al. 2018	0.278	0.187	0.379	861.3	0.0	97.9	97.4	98.3
Ross et al. 2014	0.272	0.183	0.371	884.6	0.0	98.0	97.5	98.4
Ferré et al. 2019	0.273	0.185	0.371	883.7	0.0	98.0	97.5	98.4
Kim et al. 2016	0.283	0.197	0.378	778.4	0.0	97.7	97.1	98.1
Laurent et al. 2021	0.276	0.183	0.380	883.9	0.0	98.0	97.5	98.4
Ngetsa et al. 2020	0.275	0.187	0.374	884.5	0.0	98.0	97.5	98.4
Mehta et al. 2021	0.279	0.190	0.378	871.1	0.0	97.9	97.4	98.3
De Baetselier et al. 2020	0.245	0.167	0.334	757.3	0.0	97.6	97.0	98.1
Vuylsteke et al. 2012	0.270	0.182	0.368	883.5	0.0	98.0	97.5	98.4
Wade et al. 2005	0.277	0.190	0.374	801.7	0.0	97.8	97.2	98.2
Jones et al. 2020	0.264	0.176	0.363	862.0	0.0	97.9	97.4	98.3
Sanders et al. 2014	0.284	0.194	0.384	860.1	0.0	97.9	97.4	98.3
Sanders et al. 2010	0.273	0.185	0.371	885.2	0.0	98.0	97.5	98.4
Mwaniki et al. 2023	0.258	0.184	0.340	626.3	0.0	97.1	96.4	97.7
Le Roux et al. 2023	0.269	0.181	0.366	867.1	0.0	97.9	97.4	98.3
Mashingaidze et al. 2023	0.268	0.181	0.365	862.6	0.0	97.9	97.4	98.3
Malefo et al., 2023	0.261	0.181	0.350	736.2	0.0	97.6	96.9	98.0

Pooled prevalence of *N. gonorrhoeae*/*C. trachomatis* among MSM

The prevalence estimates of *N. gonorrhoeae* and *C. trachomatis* among MSM is presented in a forest plot (Figure 2). The overall prevalence of the meta-analysis of 20 studies from the random effects model,[46] revealed that the pooled prevalence of *N. gonorrhoeae* and *C. trachomatis* among MSM in sub-Saharan Africa was 27% (95% CI, 19–39%). The I^2 value (98%) which suggest significant heterogeneity, is reflective of differences in the sampled populations in sub-Saharan Africa. Subgroup analysis by country (Figure 3) indicated that South Africa (n = 6) has a prevalence of 38% (95% CI; 25–50%). Kenya (n = 6) was 23% (95% CI; 5–45%), Nigeria (n = 2) was 13% (95% CI; 9-18%). Other countries (n = 6) were grouped together due to fewer studies conducted in these countries (Burkina Faso, Côte d'Ivoire, Mali, Senegal, Tanzania, Togo and Uganda), and prevalence was estimated at 41% (95% CI; 24–58%).

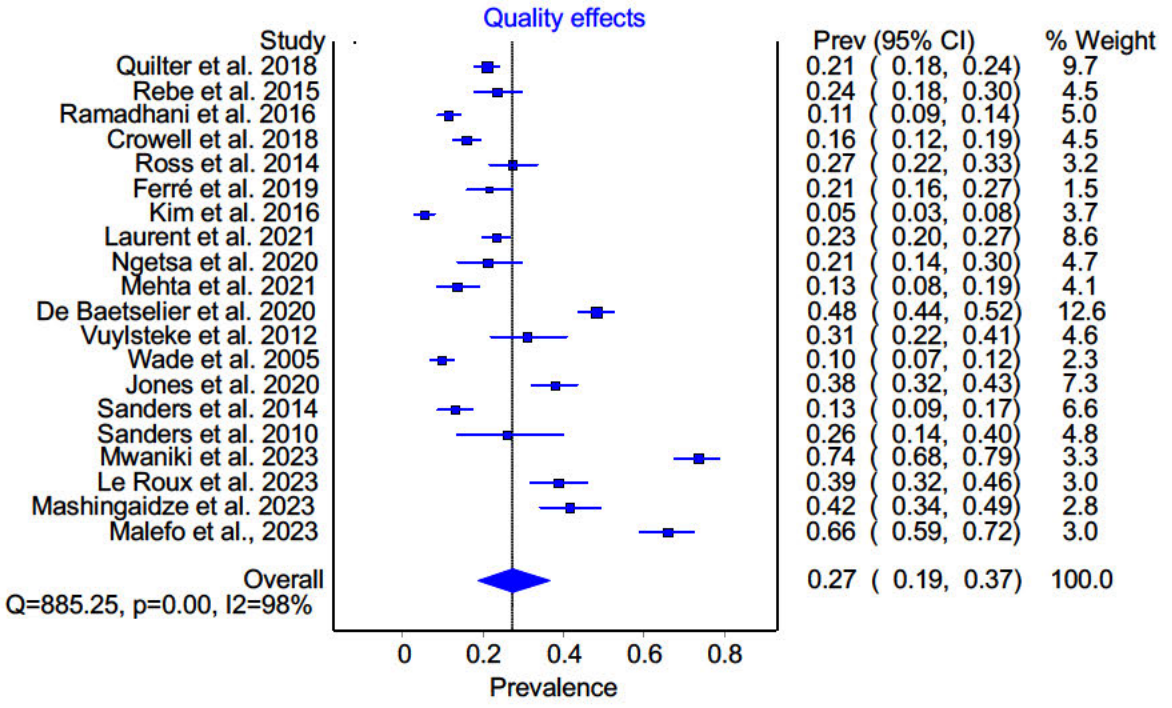

Figure 2. Forest plot of the pooled prevalence of *N. gonorrhoeae* and *C. trachomatis* among MSM in sub-Saharan Africa.

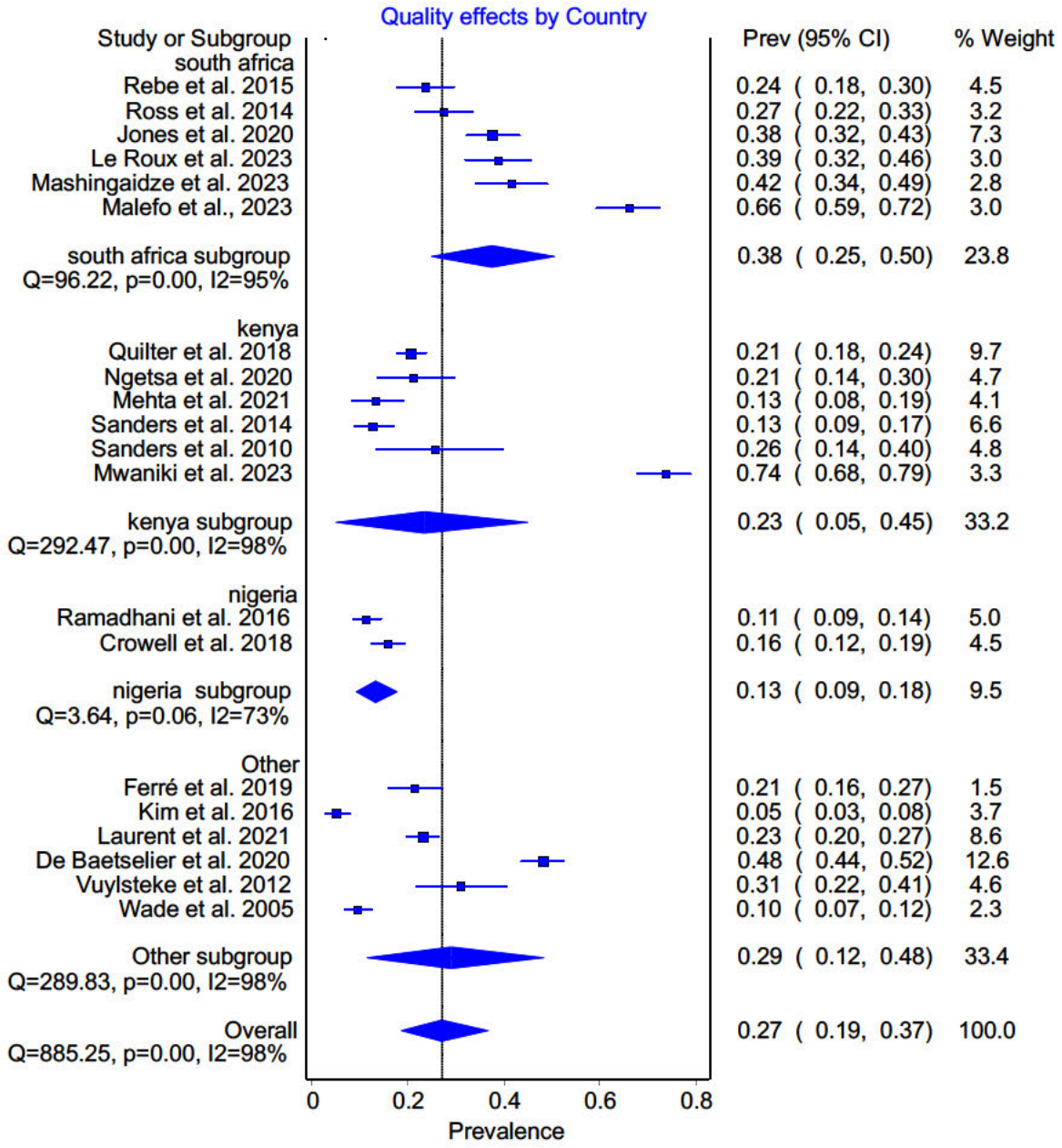

Figure 3. Subgroup analysis of *N. gonorrhoeae*/*C. trachomatis* prevalence by country of study.

Discussion

Men who have sex with men (MSM) infected with gonorrhoeae, and chlamydia are at greater risk of acquiring the human immunodeficiency virus (HIV).[67] To reduce the risk of transmission and acquisition of this infection, and to interrupt the spread of infection, screening of the MSM population and treatment for asymptomatic chlamydia and gonorrhoea must be prioritized.[68] To the best of our knowledge, this is the first attempt to estimate the pooled prevalence of *N. gonorrhoeae*/*C. trachomatis* among MSM in sub-Saharan Africa.

We conducted a systematic review and meta-analysis of studies on gonorrhoeae and chlamydia among MSM in sub-Saharan African countries. Overall, most of the studies employed molecular tests for the diagnosis of *N. gonorrhoeae* and *C. trachomatis* infections in MSM.[34,50-57,59-61,63-66] The nucleic acid amplification tests (NAATs) are the most sensitive techniques for the detection of *N. gonorrhoeae* [69] and *C. trachomatis*.[70] Most of these tests are based on polymerase chain reaction (PCR) and are of high specificity.[70] For *N. gonorrhoeae* NAATs, sensitivity and specificity is generally >95% and >99% in male first-catch urine.[71,72] Although NAATs remains the most reliable assays for the detection of pathogens, their use in low-income countries is greatly limited due to relatively high costs.[69] A number of studies have shown that, results of different NAATs were highly concordant.[73,74] Two fifths (8/20) of the studies used RDS as a sampling technique to reach MSM. The data from this review suggest that RDS is the most commonly used method for sampling MSM. This scientific method is used to recruit populations that are difficult to access.[35] It is characterized by the sample to be studied being created by the MSM themselves through chain referrals.[75-77] Although the RDS is a variant of "snowball sampling," it has been shown to produce unbiased estimates under certain conditions.[78-80]

The prevalence of *N. gonorrhoeae* varied from 1.4% to 47.2%.[55,58] The lowest prevalence rate of Ng was reported in Uganda in 2016,[55] while highest prevalence was reported in 2018 among MSM in Kenya.[58] For *C. trachomatis*, prevalence ranged from 1.0% in 2016 (Uganda)[55] to 58.7% in 2023(Kenya).[66] *Chlamydia trachomatis* is the most common bacterial STI with a global estimate of 105.7 million new infections occurring annually.[81] A systematic review by Dewart et al. on the prevalence of rectal *C. trachomatis* and *N. gonorrhoeae* in MSM and women revealed that although *C. trachomatis* and *N. gonorrhoeae* were the most prevalent STIs among MSM, chlamydia was more prevalent when compared with gonorrhoea.[9] Studies among community-recruited MSM have also shown that infection with gonorrhoea is far less common than chlamydia infection.[82,83] Contrasting trends, however, have been reported in other areas. A study conducted among MSM in the cities of Agadir and Fes in 2020 reported an overall prevalence of 11.3% and 13.3% for *C. trachomatis* and *N. gonorrhoeae* respectively.[84] The predominant asymptomatic presentation of *C. trachomatis*, which

allows for longer duration of infection and subsequent transmission, may be responsible for the high prevalence of the infection worldwide.[68,85]

Meta-analysis can obtain large sample size while providing a strong and reliable prevalence estimate[86] for *N. gonorrhoeae*/*C. trachomatis*. This study estimated the overall prevalence of *N. gonorrhoeae*/*C. trachomatis* among MSM in sub-Saharan Africa by reviewing the findings of 20 studies. The overall result of the meta-analysis gave a pooled prevalence of 27% (95% CI, 19–39%), with an I^2 of 98% which indicates high heterogeneity amongst the studies. This pooled prevalence is comparable with reports from Tanzania MSM with a prevalence rate of 27.3%.[52] A meta- analysis conducted by WHO reported that the global prevalence of *N. gonorrhoeae* and *C. trachomatis* among men was 0.6% and 2.7% respectively.[15] Also, in 2019, Rowley et al. performed a systematic review to estimate global prevalence of Ng/Ct in men. The prevalence estimates were; Ng 0.7% and Ct 2.7%.[12] Finding from our review suggests that the burden of *N. gonorrhoeae* and *C. trachomatis* among MSM in sub-Saharan Africa is higher when compared with other regions.

Regarding the analysis by country, South Africa had a high *N. gonorrhoeae*/*C. trachomatis* prevalence rate (38%), followed by Kenya (23%). Based on our analysis, *N. gonorrhoeae*/*C. trachomatis* prevalence was estimated to be the lowest in Nigeria (13%). Sexually transmitted infections have been reported to be highly prevalent among MSM in South Africa.[61,87] In South Africa, the prevalence of reported asymptomatic STIs varies wildly, ranging from 19% to 90%.[54,87,88] Among South African MSM, 91% of diagnosed rectal *N. gonorrhoeae* and/or *C. trachomatis* infections, were clinically asymptomatic.[61] A study conducted in Tshwane North, South Africa, among MSM reported 66% STI prevalence,[38] higher than in other studies conducted in Tanzania and Kenya. The results of the study conducted in Tanzania, among MSM, reported gonorrhoea, chlamydia and syphilis rates of 21%.[52] Similarly, a study on MSM from Kenya found that 26% tested positive for *C. trachomatis*, *N. gonorrhoeae* or both.[50] Also, few studies on MSM in South Africa have reported a prevalence of 10%–24% for Ct and 3%–55% for *N. gonorrhoeae* at any anatomic site regardless of presence of symptoms.[54,61,87] Although South Africa is the sole country in Africa where MSM rights are protected by the constitution,[89] making it much easier to access this key population, it still remains that *N. gonorrhoeae*/*C. trachomatis* is prevalent and the burden among South African MSM is high.

Kenya has been at the forefront in recognizing the vulnerabilities of MSM who feared legal authorities and had virtually no access to health services.[90] However, despite negative public debates and legal challenges,[91] the Kenyan ministry of health/national AIDS and STI control programme have recognized that MSM are a key population in need of urgent attention, and have demonstrated their willingness to work with them.[92] This is reflective in the number of research studies emanating from the country, some of which have been included in our study. In this study, the overall prevalence of *N. gonorrhoeae*/*C.*

trachomatis in Kenya was 23%. The observed prevalence is comparable to that observed among MSM in coastal Kenya (26%).[50] In contrast, the overall *N. gonorrhoeae*/*C. trachomatis* prevalence among MSM in our study was higher than that in the general population in Kenya, as reported in a study that found the prevalence of chlamydia and gonorrhoea were 16.8% and 7.1%, respectively.[93] This observation may be due to factors such as transactional sexual intercourse, unprotected anal intercourse, and being HIV positive, which have been found to be associated with *N. gonorrhoeae*/*C. trachomatis*.[58,94-96]

Our study showed that the overall prevalence of *N. gonorrhoeae*/*C. trachomatis* infections in Nigeria was 13%. The prevalence is higher in our study when compared with the 4.2% prevalence of both infections among MSM in Lagos.[97] Similarly, universal screening programmes deployed in Tanzania, Botswana and Kenya have reported high STI prevalence rates of between 12 and 20% in a relatively young MSM population.[52,98,99] This finding indicates that these infections are prevalent in the country. However, the observed prevalence should be treated with caution as it may not reflect the true prevalence of the infections due to the criminalization of homosexuality.[36] In many of the countries across sub-Saharan Africa, sexual intercourse between people of the same sex is criminalized, including in Nigeria.[100] In addition, MSM face significant social stigma and internalized homophobia that may poses as barriers to seeking healthcare services including screening for HIV and other STIs.[36,101] Unfortunately, we do not have enough studies to ascertain the true prevalence and therefore recommend further studies in order to ascertain the true the burden of STIs in Nigerian MSM. To achieve the WHO's goal of ending STI epidemics as major public health concerns,[13] countries need to know their STI burden to understand where and among which population groups new infections are occurring.[102] Only then can deliberate actions be effectively taken.

This is the first study to systematically review the available literature on the prevalence of Ng/Ct among MSM in sub-Saharan Africa. However, our study had limitations. First, limited data reporting on prevalence of *N. gonorrhoeae*/*C. trachomatis* were available. Second, some of the studies included in this analysis used a cross-sectional design and as such, may not be representative of MSM.

Conclusion

This systematic review and meta-analysis revealed a pooled prevalence of *N. gonorrhoeae*/*C. trachomatis* t in the MSM population to be 27% (95% CI, 19–39%). The prevalence of *N. gonorrhoeae*/*C. trachomatis* was high for South Africa (38%) and Kenya (23%). A plausible explanation for this observation is the fact that South Africa and Kenya remains the countries in sub-Saharan Africa that recognises the vulnerabilities of MSM, have demonstrated their willingness to support this key population. This makes it easier for MSM to access healthcare services and get tested. All efforts were made to find and analyse the available data so as to provide a unique perspective on

this issue, with the aim of informing policymakers on the need to prioritize MSM healthcare needs. Future studies should be conducted to determine true burden of STIs among MSM in sub-Saharan Africa.

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Author contributions

KCM and NSA conceptualized the study. KCM, VD, CB and NSA undertook the review. All authors read and approved the final manuscript.

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Conflicts of interests

The authors declare that they have no competing interests.

Data availability statement

All data generated or analysed during this investigation is included in the published systematic review article and will be available upon request.

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Supplementary material

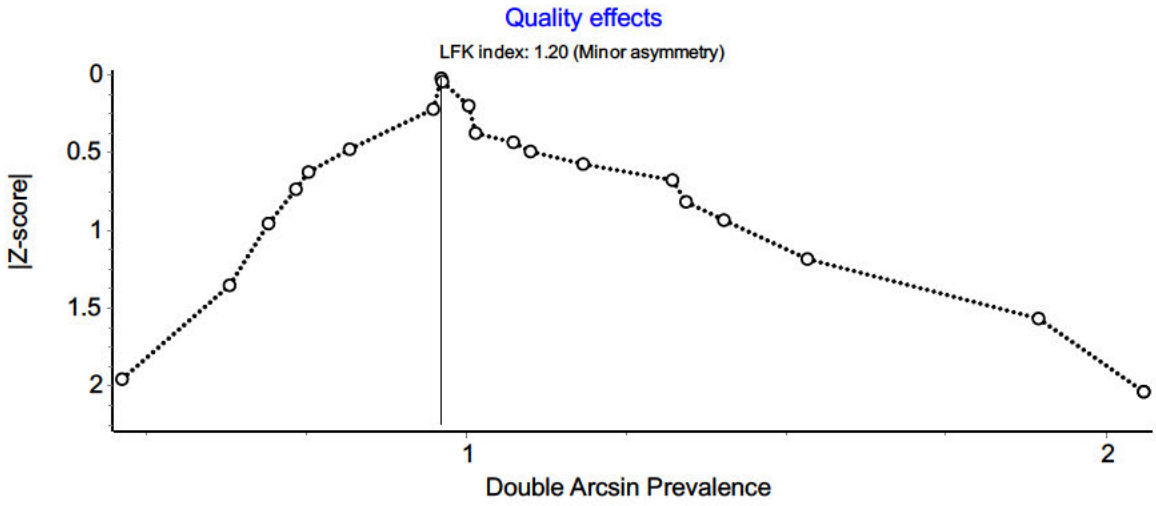

Figure S1. Doi plot analysis and LFK index of publication bias and asymmetry of overall pooled prevalence

Table S1 Risk of bias assessment of included studies using the Hoy tool[41]

Study ID	Representation	Sampling frame	Sample selection	Non-response bias	Data collection	Case Definition	Reliability and validity of study tool	Method of data collection	Numerator and denominator	Summary Assessment
Tafuma (2014)[54]	Not clear	Low risk	Low risk	Not clear	Low risk	Low risk	High risk	Low risk	Low risk	Medium risk
Quilter (2018)[59]	High risk	Not clear	Low risk	Not clear	Low risk	Low risk	Low risk	Low risk	Low risk	Medium risk
Rebe (2015)[55]	High risk	Low risk	Low risk	Not clear	Low risk	Low risk	High risk	Low risk	Low risk	Medium risk
Ramadhani (2016)[57]	High risk	Low risk	Low risk	Not clear	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Crowell (2018)[58]	High risk	Low risk	Low risk	Not clear	Low risk	Low risk	Not clear	Low risk	Low risk	Medium risk
Ross (2014)[52]	High risk	Not clear	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Ferré (2019)[60]	Low risk	Low risk	Low risk	Not clear	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Kim (2016)[56]	High risk	Low risk	Low risk	Not clear	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Laurent (2021)[65]	Not clear	Low risk	Not clear	Not clear	Low risk	Low risk	Low risk	Low risk	Low risk	Medium risk
Ngetsa (2020)[64]	Not clear	Low risk	Not clear	Not clear	Low risk	Low risk	High risk	Low risk	Low risk	Medium risk
Mehta (2021)[66]	High risk	Low risk	Not clear	Not clear	Low risk	Low risk	Low risk	Low risk	Low risk	Medium risk
De Baetselier (2020)[61]	Not clear	Not clear	Not clear	Not clear	Low risk	Low risk	Not clear	Low risk	Low risk	High risk
Vuylsteke (2012)[51]	High risk	Low risk	Not clear	Not clear	Low risk	High risk	Low risk	Low risk	Low risk	Medium risk
Wade (2005)[49]	Low risk	Low risk	Low risk	Not clear	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Maduna (2020)[63]	High risk	Low risk	Not clear	Not clear	Low risk	Low risk	Low risk	Low risk	Low risk	Medium risk
Jones (2020)[62]	High risk	High risk	Not clear	Not clear	Low risk	Low risk	Low risk	Low risk	Low risk	Medium risk
Diabaté (2023)[67]	Low risk	Low risk	High risk	High risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Sanders (2014)[53]	Not clear	Low risk	High risk	Not clear	Low risk	Low risk	Not clear	Low risk	Low risk	Medium risk
Sanders (2010)[50]	Not clear	Not clear	High risk	Not clear	Low risk	Low risk	Not clear	Low risk	Low risk	High risk
Mwaniki (2023)[69]	High risk	Low risk	Low risk	Not clear	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Le Roux (2023)[68]	Not clear	Low risk	Low risk	Not clear	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Mashingaidze (2023)[34]	Low risk	Low risk	Not clear	Not clear	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Malefo (2023)[38]	High risk	Low risk	Low risk	Not clear	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk

Risk of bias assessment tool: Yes (low risk); No (high risk)

1. Representation: Was the study population a close representation of the national population?
2. Sampling: Was the sampling frame a true or close representation of the target population?
3. Sample selection: was some form of sampling strategy used to select the sample?
4. Non-response bias: Was the likelihood of non-response bias minimal?
5. Data collection: Were data collected directly from the subjects?
6. Case definition: Was an acceptable case definition used in the study?
7. Reliability and validity of study tool: Was the reliability and validity of the study instrument that measured the parameter of interest determined?
8. Data collection: Was the same mode of data collection used for all subjects?
9. Numerators and denominators: Were the numerator(s) and denominator(s) for the parameter of interest appropriate?

The overall risk of bias was scored according to the number of high risk of bias per study: low (≤2), moderate (3–4), and high (≥5).

CHAPTER THREE

Prevalence and associated risk factors of chlamydia and *gonorrhoea* infections among Men who have Sex with Men in Durban, South Africa

Running title: Sexually transmitted infections among South African MSM

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Abstract

Despite significant research on the prevalence of STIs in South African men who have sex with men (MSM), recent data on the prevalence and risk factors for curable STI infections among this key populations are limited. This study determined the prevalence of and risk factors associated with *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections among MSM. The sample consisted of 200 MSM resident in Durban. Data were collected using a self-administered questionnaire, and urine samples were collected and tested for *N. gonorrhoeae* and *C. trachomatis*. The prevalence of *N. gonorrhoeae* and *C. trachomatis* were 3.0% and 6.0%, respectively. Younger age was significantly associated with testing positive for *C. trachomatis* (p=0.037). Being between the ages of 30-39 years old reduced the risk of acquiring *C. trachomatis* infection (OR: 0.10, 95% CI: 0.0120-0.7564, p=0.026). In addition, being circumcised reduced the risk of contracting *C. trachomatis* (adjusted OR: 0.01, 95% CI: 0.0005-0.3516, p=0.01). However, having between 2-4 sex partners increased the risk of testing positive for *C. trachomatis* (adjusted OR: 107.45, 95% CI: 1.3467-8573.3130, p=0.036). The following factors were significantly associated ($p<0.05$) with testing positive for *N. gonorrhoeae* infection: cohabiting with sex partner, engaging in group sex, and drug use. Fear and stigma were the main barriers to accessing health care in the studied population.

Keywords

MSM, Prevalence, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, South Africa

Introduction

Sexually transmitted infections (STIs) are a major public health problem affecting the health and lives of people globally[1,2]. Gonorrhoea and chlamydia are amongst the most common infectious diseases worldwide[2] and are acquired by more than one million individuals daily[3], constituting a major sexual and reproductive health challenge of tremendous magnitude[4]. They are capable of causing diseases like urethritis, vaginitis, cervicitis, and genital ulceration. Their etiological agents (*Neisseria gonorrhoeae* and *Chlamydia trachomatis*) can sometimes infect the rectum and pharynx causing serious complications, including pelvic inflammatory disease in women and orchitis in men[2]. The incidence and prevalence of these infections are widespread, particularly in resource-poor countries[5-7] where the rates of STIs remain high as the result of ineffective diagnostic methods and the lack of access to health care facilities[8].

The World Health Organization (WHO) in 2020, reported there were about 373.1 million infections from four of the most common curable STIs (128 million chlamydia cases, 82 million gonorrhoea cases, 156 million trichomoniasis cases, and 7.1 million syphilis cases)[9]. Other reports also indicated that 273 million prevalent cases of STIs occurred annually among adults between 15 and 49 years of age[5,6]. In the African region, gonorrhoea and chlamydia account for 11.4 and 12 million new cases per year, respectively[10,11]. These STIs are curable but can increase the risk of infection with the Human immunodeficiency virus (HIV), especially in men who have sex with men (MSM)[12-14].

Men who have sex with Men (MSM) are at greater risk of acquiring STIs compared to heterosexual populations[15-17]. This is usually due to their sexual network or behaviours (homosexual or bisexual behaviours)[18,19], resulting in rectal and urethral infections (mucosal inflammation and anogenital ulcers) mostly through insertive and receptive anal intercourses as well as possible transmission through oral-anal contact[20,21]. In recent years, MSM have become the group at highest risk for STI infections worldwide, higher than female sex workers and much higher than the general population[2]. Data from Europe, the United States of America and China have also shown that MSM are affected by a high STI burden[22]. In England, 77, 371 new STI cases were reported in MSM and the most common of the STIs were gonorrhoea (n = 33,853; 44%) and chlamydia (n = 23,187; 30%)[23].

Gonorrhoea is caused by the bacterium, *Neisseria gonorrhoeae* (*N. gonorrhoeae*) and transmission can occur by direct inoculation of infected mucosal secretions[24]. Chlamydia, a similarly behaving and equally concerning infection[25], is a sexually transmitted infectious disease caused by the bacterium *Chlamydia trachomatis* (*C. trachomatis*)[26]. Gonorrhoea and chlamydia are associated with an increased risk for HIV in men[27]. Although curable with antibiotics, these bacterial STIs are generally asymptomatic and as a result readily progress to symptoms affecting the urethra or spread within the body[28]. In addition to the associated symptoms, these STIs may promote reoccurrence of other STIs[29,30]

including HIV[31,32] through mucosal inflammation and ulceration of local tissues[27,33]. Studies have demonstrated that among MSM, the prevalence of rectal gonorrhoea and chlamydia ranges from 0.2% to 24% and 2.1 to 23%, respectively, while the prevalence of pharyngeal gonorrhoea and chlamydia ranges from 0.5% to 16.5% and 0% to 3.6%, respectively[34].

South Africa accounts for one of the highest HIV prevalence rates in the world; and other STIs continue to be endemic[35,36]. KwaZulu-Natal (KZN), one of the most densely populated provinces in South Africa, has been reported to be greatly affected by both the HIV and STI epidemics[37] particularly among MSM. In 2013, 42.7% of MSM in Durban were reported to have had symptoms of a STI in the last 12 months while an estimated 48.2% were living with HIV. This reported HIV prevalence is 2.58 times higher than that among non-MSM, aged 15 years and older in KZN[38-40].

Despite significant research on the prevalence of STIs in South African MSM[39,40-43], recent data on the prevalence and risk factors for curable STI infections among MSM populations in KZN are limited. This study investigated the prevalence of gonorrhoea and chlamydia among MSM in KZN. We also assessed risk factors associated with these STIs.

Methods

Ethics

Ethical clearance for this study was granted by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (BREC/00002798/2021).

Study design and population

This cross-sectional study including all laboratory assays were conducted at the School of Clinical Medicine Research Laboratory of the Nelson R. Mandela School of Medicine, University of KwaZulu-Natal. Participants were MSM recruited from the King Edward VIII hospital and the Aurum Institute both located in Durban, South Africa. The study participants were enrolled from October 2021 to July 2022 and included sexually active MSM, 18 years and older. Participants were requested to provide a urine sample.

Sample processing

The urine samples were processed within 24 hours of collection. Samples were refrigerated between 2-8°C until processed. A total of 10 ml of urine was centrifuged at 14 000x g for 45 minutes and the supernatant discarded. The recovered pellets were then subjected to further molecular analyses.

DNA extraction

Deoxyribonucliec acid (DNA) was extracted from the sample pellets using the commercially available, PureLink Microbiome Kit (ThermoFisher Scientific, United States), according to the manufacturers. Sample pellets were suspended in 800 µL of S1 lysis buffer by pipetting up and down. The sample was then transferred to the bead tube and 100 µL of S2 lysis enhancer was added to the bead tube, capped and homogenized with a vortex mixer. The tubes were incubated at 95°C for 10 minutes, followed by vortexing at a maximum speed for 7 minutes. The samples were then centrifuged at 14 000x g for 1 minute and 500 µL of the supernatant was transferred to a clean micro-centrifuge tube. To bind DNA to the column, 900 µL of binding buffer was added and vortexed briefly. Thereafter, 700 µL of the sample mixture was loaded onto a spin column-tube and centrifuged at 14 000x g for 1 minute. The flow through was discarded and the spin column was centrifuged at 14 000x g for 1 minute. Following this, the spin column was placed in a clean tube and 50 µL of S6 elution buffer was added. The tube was then incubated at room temperature for 1 minute. After incubation, the tube was centrifuged at 14 000x g for 1 minute, and the extracted DNA concentration was measured using a Nanodrop Spectrophotometer (ThermoFisher Scientific, United States). Extracted DNA from samples were stored at –20°C until subsequent analysis.

Detection of C. trachomatis* and *N. gonorrhoeae

PCR amplification was performed on the Quant Studio 5 real-time PCR detection system (ThermoFisher Scientific, Waltham, Massachusetts, United States of America), in a 96-well microtiter reaction plate. *C. trachomatis* and *N. gonorrhoeae* were detected using the Applied Biosystems™ TaqMan® Assays. The following commercial primers and probes (Ba04646249_S1) and (Ba04646252_S1) ThermoFisher Scientific, Waltham, Massachusetts, United States of America) were used for each organism respectively. The (Ba04646249_S1) targets the translocated actin-recruiting phosphoprotein gene of *C. trachomatis*, and (Ba04646252_S1) targets the hypothetical protein gene of *N. gonorrhoeae*. Each PCR reaction was performed in a final volume of 10 µL comprising: 1 µL FAM-labeled probe/primer mix, 5 µL Fast Start 4x probe master mix, (ThermoFisher, Part No. 4444434), 2 µL template DNA and 2 µL nuclease-free water. Non-template and positive controls (TaqMan™ Vaginal Microbiota Extraction Control; cat no. A32039) were also included. Amplification was performed at 95ºC for 30 seconds followed by 40 cycles comprising of denaturation at 95ºC for 30 seconds and annealing at 60ºC for 30 seconds. Detection of amplified fluorescent products was carried out at the end of the annealing phase. The raw fluorescent data that included the cycling threshold (CT) mean values were automatically generated by the Quant Studio 5 Real-time PCR system software.

Data analysis

The primary objective of this analysis was to determine the prevalence of *C. trachomatis* and *N. gonorrhoeae* in a sample of MSM individuals. The analysis further explored the associated risk factors

for each of the STIs as well as the barriers or facilitators to care. Sample size determination is explained in chapter four (methodology section).

Participant's age was measured in single ages at time of interview from last birthday. Categorisation of the data into broad age groups (18-29, 30-39, 40+) was also explored. Participant's education level completed was in the following categories: none, primary, high school, university or higher. The categories none and primary were later combined due to the very small numbers in those groups. Other explanatory variables included in the analysis were: employed (yes/no); having a regular partner (yes/no); cohabiting with partner (yes/no); number of life time partners (1, 2-4, >4); knowledge of partner having other partners (yes, no, don't know); condom use at last sex (always, frequent, sometimes, never); frequency of group sex (none, once, few times, weekly, monthly); receiving money for sex (no, once, few times, weekly, monthly); HIV status (negative, positive, don't know); substance use at last sex; knowledge and attitudes of access to sexual health clinics including preference to consult own doctor rather than use public health facilities for sexual health needs; experienced stigma due to sexual orientation (yes, no, no data); and being comfortable to disclose sexual orientation (yes, no , no data).

Descriptive characteristics of study participants were presented by *C. trachomatis*/*N. gonorrhoeae* status, as frequencies and percentages of the categorical variables. Comparisons in the descriptive characteristics were done using Chi squared tests. Univariable and adjusted multivariable logistic regression models were run for *C. trachomatis* and *N. gonorrhoeae* to test for associations with the explanatory variables. Model fit was checked using standard statistical tests for logistic regression models with detected influential variables dropped and models refitted. We used 5% and 10% significance levels with 95% confidence intervals to determine statistical significance. All analyses were conducted using STATA 17.1 software.

Results

Factors significantly associated with testing positive for C. trachomatis

Table 1 describes the characteristics of the study population according to *C. trachomatis* infection status. The prevalence of *C. trachomatis* in the study population was 6% (12/200). The majority of the men (67%) who tested positive for *C. trachomatis* were between the ages of 25-30 years old when compared to 25% who were between 18-24 years old and this was significant, p=0.037. A higher percentage (58.3%) of men who were positive for *C. trachomatis* had attended College/University when compared to 33.3% who had attended high school, p=0.006.

Three thirds (75.0%) of the men who had tested positive, reported that they did not know if their partners had other partners when compared to 25.0% who reported that their partners did not have other partners, p=0.032. A higher percentage (58.3%) of the men reported engaging in anal sex only in the past 30 days, 25.0% had engaged in anal and oral sex and this had a borderline significance, p=0.054. An estimated, 41.7% of the men reported that they had frequently used condoms when compared to 33.3% who reported sometimes using condoms and 25.0% who always used condoms, p=0.069. There was an equal proportion of men (50.0%) who were circumcised and uncircumcised in the *C. trachomatis* positive group, p=0.043 (Table 1).

Table 1: Characteristics of the study population according to *C. trachomatis* infection status

	C. trachomatis **Negative**	***C. trachomatis*** **Positive**	**Total**	**p-value**
	188 (94)	12 (6)	200	
Age group (years old)				0.037
18-24	21 (11.17)	3 (25)	24 (12)	
25-30	75 (39.89)	8 (66.67)	83 (41.5)	
31-44	78 (41.49)	1 (8.33)	79 (39.5)	
45+	14 (7.45)	0 (0)	14 (7)	
Level of education				0.006
Primary School	10 (5.32)	1 (8.33)	11 (5.5)	
High School	133 (70.74)	4 (33.33)	137 (68.5)	
College/University	31 (16.49)	7 (58.33)	38 (19)	
Refused to answer	14 (7.45)	0 (0)	14 (7)	
Employed				0.141
No	149 (79.26)	7 (58.33)	156 (78)	
Yes	39 (20.74)	5 (41.67)	44 (22)	
Has a regular sex partner				0.872
No	51 (27.13)	3 (25)	54 (27)	
Yes	137 (72.87)	9 (75)	146 (73)	
Cohabiting with partner				0.664
No	112 (59.57)	9 (75)	121 (60.5)	
Yes	71 (37.77)	3 (25)	74 (37)	
Refused to answer	5 (2.66)	0 (0)	5 (2.5)	
Number of sex partners in last 30 days				0.293
1	92 (48.94)	5 (41.67)	97 (48.5)	
2-4	66 (35.11)	3 (25)	69 (34.5)	
>4	7 (3.72)	1 (8.33)	8 (4)	
None	23 (12.23)	3 (25)	26 (13)	
Partner has other partners				0.032
No	53 (28.19)	3 (25)	56 (28)	
Yes	55 (29.26)	0 (0)	55 (27.5)	
Don’t know	80 (42.55)	9 (75)	89 (44.5)	
Sex practices in the past 30 days				0.054
Anal sex only	62 (32.98)	7 (58.33)	69 (34.5)	
Oral sex only	40 (21.28)	0 (0)	40 (20)	
Oral and anal sex	73 (38.83)	3 (25)	76 (38)	
None	13 (6.91)	2 (16.67)	15 (7.5)	

	C. trachomatis Negative	*C. trachomatis* Positive	Total	p-value
Condom use				0.069
Always	78 (41.49)	3 (25)	81 (40.5)	
Frequent	22 (11.7)	5 (41.67)	27 (13.5)	
Sometimes	76 (40.43)	4 (33.33)	80 (40)	
Never	12 (6.38)	0 (0)	12 (6)	
Frequency of group sex				0.573
Sometimes	39 (20.74)	1 (8.33)	40 (20)	
Frequently	10 (5.32)	1 (8.33)	11 (5.5)	
One time	55 (29.26)	3 (25)	58 (29)	
Never	84 (44.68)	7 (58.33)	91 (45.5)	
Trades sex for cash				0.896
Sometimes	49 (26.06)	3 (25)	52 (26)	
Frequently	19 (10.11)	0 (0)	19 (9.5)	
One time	53 (28.19)	4 (33.33)	57 (28.5)	
Never	67 (35.64)	5 (41.67)	72 (36)	
Has symptoms of STIs				0.738
No	92 (48.94)	5 (41.67)	97 (48.5)	
Yes	88 (46.81)	7 (58.33)	95 (47.5)	
Refused to answer	8 (4.26)	0 (0)	8 (4)	
Circumcised				0.043
No	34 (18.09)	6 (50)	40 (20)	
Yes	144 (76.6)	6 (50)	150 (75)	
Refused to answer	10 (5.32)	0 (0)	10 (5)	
HIV status				0.969
Negative	121 (64.36)	8 (66.67)	129 (64.5)	
Positive	53 (28.19)	3 (25)	56 (28)	
Did not know status	14 (7.45)	1 (8.33)	15 (7.5)	
Previously screened for STIs				0.622
Never	27 (14.36)	2 (16.67)	29 (14.5)	
Past 3 months	34 (18.09)	1 (8.33)	35 (17.5)	
More than 3 months ago	66 (35.11)	3 (25)	69 (34.5)	
Last month	61 (32.45)	6 (50)	67 (33.5)	
Previous STIs				0.681
None	11 (5.85)	1 (8.33)	12 (6)	
Chlamydia/Gonorrhoea	30 (15.96)	1 (8.33)	31 (15.5)	
Hepatitis B/C	13 (6.91)	0 (0)	13 (6.5)	
Herpes	7 (3.72)	1 (8.33)	8 (4)	
Syphilis	12 (6.38)	0 (0)	12 (6)	

	C. trachomatis Negative	*C. trachomatis* Positive	Total	p-value
Refused to answer	115 (61.17)	9 (75)	124 (62)	
Drug use				0.307
No	59 (31.55)	6 (50)	65 (32.66)	
Yes	126 (67.38)	6 (50)	132 (66.33)	
Refused to answer	2 (1.07)	0 (0)	2 (1.01)	

Significant risk factors for C. trachomatis infection

Table 2 describes the factors which are significantly associated with the risk for *C. trachomatis* infection. According to the univariate analysis, being between the ages of 30-39 years old reduced the risk of acquiring *C. trachomatis* infection (Odds Ratio [OR]: 0.10, 95% Confidence Interval [CI]: 0.0120-0.7564, p=0.026). With respect to condom use, men who frequently used condoms were at increased risk of acquiring *C. trachomatis* infection (OR: 6.67, 95% CI: 1.4791-30.0478, p=0.014). Being circumcised reduced the risk of being infected, (OR: 0.25, 95% CI: 0.0747-0.8033, p=0.02). After performing further adjustments, in the multivariable model, being between the ages of 30-39 years old still reduced the risk of acquiring *C. trachomatis* infection (OR: 0.62, 95% CI: 0.4224-0.8967, p=0.011). Similarly, being circumcised reduced the risk of infection (OR: 0.01, 95% CI: 0.0005-0.3516, p=0.01). Having between 2-4 sex partners in the past 30 days increased the risk of testing positive for infection (OR: 107.45, 95% CI: 1.3467-8573.3130, p=0.036) (Table 2).

Table 2: Risk factors for *C. trachomatis* infection

Univariable			
	Odds ratio	**95% Confidence Interval**	**P>z**
Age (years old)			
18-29	1.00		
30-39	0.10	0.0120-0.7564	0.026
40+	1.00	0.0000-0.0000	0
Condom use			
Always	1.00		
Frequently	6.67	1.4791-30.0478	0.014
Sometimes	1.54	0.3349-7.1162	0.578
Never	1.00	0.0000-0.0000	0
Is circumcised (yes)	0.25	0.0747-0.8033	0.02
Multivariable			
	Odds ratio	**95% Confidence Interval**	**P>z**
Age	0.62	0.4224-0.8967	0.011
Is circumcised (yes)	0.01	0.0005-0.3516	0.01
Number of sex partners in the past 30 days			
1			
2-4	107.45	1.3467-8573.3130	0.036
None	0.69	0.0191-24.9668	0.84

Factors significantly associated with testing N. gonorrhoeae positive

The prevalence of *N. gonorrhoeae* in the study population was 3% (6/200). According to the analysis, an equal proportion of men who tested positive for *N. gonorrhoeae* (33.3%) had attended High school and College/University and this was significant, p=0.047. A high proportion of men (83.3%) were cohabiting with their sex partners, cohabitation status was significantly associated with testing positive for infection, p=0.05. A high proportion of men (66.7%) had engaged in oral sex and 33.3% had engaged in anal sex and this was significant, p=0.034. In addition, a high proportion of men were engaging in group sex frequently, p=0.001. A high proportion of men (50%) were also engaging in sex for cash frequently, p=0.032. A high proportion of men (50%) who tested positive for infection were circumcised, p=0.033. A high proportion of men (50%) who tested positive for infection were also drug users, p=0.055 (Table 3).

Table 3: Characteristics of the study population according to *N. gonorrhoeae* infection status

	***N. gonorrhoeae* Negative**	***N. gonorrhoeae* Positive**	**Total**	**p-value**
	194 (97)	6 (3)	200	
Age group (years old)				0.829
18-24	23 (11.86)	1 (16.67)	24 (12)	
25-30	81 (41.75)	2 (33.33)	83 (41.5)	
31-44	76 (39.18)	3 (50)	79 (39.5)	
45+	14 (7.22)	0 (0)	14 (7)	
Level of education				0.047
Primary School	11 (5.67)	0 (0)	11 (5.5)	
High School	135 (69.59)	2 (33.33)	137 (68.5)	
College/University	36 (18.56)	2 (33.33)	38 (19)	
Refused to answer	12 (6.19)	2 (33.33)	14 (7)	
Employed				0.615
No	152 (78.35)	4 (66.67)	156 (78)	
Yes	42 (21.65)	2 (33.33)	44 (22)	
Has a regular sex partner				0.662
No	52 (26.8)	2 (33.33)		
Yes	142 (73.2)	4 (66.67)		
Cohabiting with partner				0.055
No	120 (61.86)	1 (16.67)	121 (60.5)	
Yes	69 (35.57)	5 (83.33)	74 (37)	
Refused to answer	5 (2.58)	0 (0)	5 (2.5)	
Number of sex partners in last 30 days				0.4
1	94 (48.45)	3 (50)	97 (48.5)	
2-4	68 (35.05)	1 (16.67)	69 (34.5)	
>4	8 (4.12)	0 (0)	8 (4)	
None	24 (12.37)	2 (33.33)	26 (13)	
Partner has other partners				0.496
No	55 (28.35)	1 (16.67)	56 (28)	
Yes	52 (26.8)	3 (50)	55 (27.5)	
Don’t know	87 (44.85)	2 (33.33)	89 (44.5)	
Sex practices in the past 30 days				0.034
Anal sex only	69 (35.57)	0 (0)	69 (34.5)	
Oral sex only	36 (18.56)	4 (66.67)	40 (20)	
Oral and anal sex	74 (38.14)	2 (33.33)	76 (38)	
None	15 (7.73)	0 (0)	15 (7.5)	

	N. gonorrhoeae Negative	*N. gonorrhoeae* Positive	Total	p-value
Condom use				0.133
Always	80 (41.24)	1 (16.67)	81 (40.5)	
Frequent	24 (12.37)	3 (50)	27 (13.5)	
Sometimes	78 (40.21)	2 (33.33)	80 (40)	
Never	12 (6.19)	0 (0)	12 (6)	
Frequency of group sex				0.001
Sometimes	39 (20.1)	1 (16.67)	40 (20)	
Frequently	8 (4.12)	3 (50)	11 (5.5)	
One time	56 (28.87)	2 (33.33)	58 (29)	
Never	91 (46.91)	0 (0)	91 (45.5)	
Trades sex for cash				0.032
Sometimes	51 (26.29)	1 (16.67)	52 (26)	
Frequently	17 (8.76)	2 (33.33)	19 (9.5)	
One time	54 (27.84)	3 (50)	57 (28.5)	
Never	72 (37.11)	0 (0)	72 (36)	
Has symptoms of STIs				0.178
No	95 (48.97)	2 (33.33)	97 (48.5)	
Yes	92 (47.42)	3 (50)	95 (47.5)	
Refused to answer	7 (3.61)	1 (16.67)	8 (4)	
Circumcised				0.033
No	39 (20.1)	1 (16.67)	40 (20)	
Yes	147 (75.77)	3 (50)	150 (75)	
Refused to answer	8 (4.12)	2 (33.33)	10 (5)	
HIV status				0.082
Negative	126 (64.95)	3 (50)	129 (64.5)	
Positive	55 (28.35)	1 (16.67)	56 (28)	
Did not know status	13 (6.7)	2 (33.33)	15 (7.5)	
Previously screened for STIs				0.141
Never	27 (13.92)	2 (33.33)	29 (14.5)	
Past 3 months	34 (17.53)	1 (16.67)	35 (17.5)	
More than 3 months ago	69 (35.57)	0 (0)	69 (34.5)	
Last month	64 (32.99)	3 (50)	67 (33.5)	
Previous STIs				0.182
None	12 (6.19)	0 (0)	12 (6)	
Chlamydia/Gonorrhoea	28 (14.43)	3 (50)	31 (15.5)	
Hepatitis B/C	12 (6.19)	1 (16.67)	13 (6.5)	
Herpes	8 (4.12)	0 (0)	8 (4)	
Syphilis	12 (6.19)	0 (0)	12 (6)	

	N. gonorrhoeae **Negative**	*N. gonorrhoeae* **Positive**	**Total**	**p-value**
Refused to answer	122 (62.89)	2 (33.33)	124 (62)	
Drug use				0.055
No	63 (32.64)	2 (33.33)	65 (32.66)	
Yes	129 (66.84)	3 (50)	132 (66.33)	
Refused to answer	1 (0.52)	1 (16.67)	2 (1.01)	

Barriers and facilitators to accessing care

The majority of the MSM were aware of sexual health clinics in the city (79%). In addition, the majority of the MSM (71.5%) were aware of the services that these clinics could provide to them. However, the majority of the MSM (62%) reported that they would prefer to seek care from a General Practitioner at a surgery. A high proportion, 76.5% reported that they would be comfortable disclosing their sexual behaviour to health care workers and 58.3% believed that their confidentiality would be protected. Almost half of the MSM, 49.5% had experienced stigma based on their sexual behaviour and 47.5% reported being afraid to go to health care centres due to stigma. The majority, 74% reported that having a trusted health care facility for MSM would encourage them to access care (Table 4).

Table 4: Factors related to accessing to health care services by the study population

Factors related to access to care	Overall (N=200)
Aware of sexual health clinics in the city	
No	37 (18.5%)
Yes	158 (79%)
Refused to answer	5 (2.5%)
Aware of sexual health services offered by clinics	
No	44 (22%)
Yes	143 (71.5%)
Refused to answer	13 (6.5%)
Comfortable disclosing sexual orientation to health care worker	
No	39 (19.5%)
Yes	153 (76.5%)
Refused to answer	8 (4%)
Prefer to seek care from General Practitioner at surgery	
No	50 (25%)
Yes	124 (62%)
Don't know	26 (13%)
Experienced stigma	
No	94 (47%)
Yes	99 (49.5%)
Refused to answer	7 (3.5%)
Fear stopped you from accessing health care	
No	97 (48.5%)
Yes	95 (47.5%)
Refused to answer	8 (4%)
Having a trusted and flexible sexual health clinic will encourage access to care	
Do not know	34 (17%)
No	18 (9%)
Yes	148 (74%)

Factors significantly associated with access to care according to C. trachomatis and N. gonorrhoeae status

According to the analysis, the majority of the men who tested positive for *C. trachomatis* (66.7%) were not aware of the sexual health services offered by the clinics in the city and this was significant, p=0.002. Of the men who tested positive for *N. gonorrhoeae*, 50% reported that they would prefer to access care from a General Practitioner based in a surgery, p=0.042. In addition, 33.3% of the men believed that having a trusted and flexible sexual health clinic will encourage access to care and this was significant, p=0.02 (Table 5).

Table 5: Factors associated with access to care according to *C. trachomatis* and *N. gonorrhoeae* status

	C. trachomatis **Negative**	***C. trachomatis*** **Positive**	**Total**	
	n (%)	**n (%)**	**n (%)**	**p-value**
Aware of sexual health clinics in the city				0.83
No	35 (18.62)	2 (16.67)	37 (18.5)	
Yes	148 (78.72)	10 (83.33)	158 (79)	
Refused to answer	5 (2.66)	0 (0)	5 (2.5)	
Aware of sexual health services offered by clinics				0.002
No	36 (19.15)	8 (66.67)	44 (22)	
Yes	139 (73.94)	4 (33.33)	143 (71.5)	
Refused to answer	13 (6.91)	0 (0)	13 (6.5)	
Prefer to seek care from General Practitioner at surgery				0.756
No	46 (24.47)	4 (33.33)	50 (25)	
Yes	117 (62.23)	7 (58.33)	124 (62)	
Don't know	25 (13.3)	1 (8.33)	26 (13)	
Comfortable disclosing sexual orientation to health care worker				0.158
No	34 (18.09)	5 (41.67)	39 (19.5)	
Yes	146 (77.66)	7 (58.33)	153 (76.5)	
Refused to answer	8 (4.26)	0 (0)	8 (4)	
Experienced stigma				0.171
No	85 (45.21)	9 (75)	94 (47)	
Yes	96 (51.06)	3 (25)	99 (49.5)	
Refused to answer	7 (3.72)	0 (0)	7 (3.5)	
Fear stopped you from accessing health care				0.546
No	91 (48.4)	6 (50)	97 (48.5)	
Yes	90 (47.87)	5 (41.67)	95 (47.5)	
Refused to answer	7 (3.72)	1 (8.33)	8 (4)	
Having a trusted and flexible sexual health clinic will encourage access to care				0.996
Do not know	17 (9.04)	1 (8.33)	18 (9)	
No	139 (73.94)	9 (75)	148 (74)	
Yes	32 (17.02)	2 (16.67)	34 (17)	
	N. gonorrhoeae **Negative**	***N. gonorrhoeae*** **Positive**	**Total**	**p-value**
	194 (97)	6 (3)	200	

Aware of sexual health clinics in the city				0.416
No	35 (18.04)	2 (33.33)	37 (18.5)	
Yes	154 (79.38)	4 (66.67)	158 (79)	
Refused to answer	5 (2.58)	0 (0)	5 (2.5)	
Aware of sexual health services offered by clinics				0.431
No	43 (22.16)	1 (16.67)	44 (22)	
Yes	139 (71.65)	4 (66.67)	143 (71.5)	
Refused to answer	12 (6.19)	1 (16.67)	13 (6.5)	
Prefer to seek care from General Practitioner at surgery				0.042
No	50 (25.77)	0 (0)	50 (25)	
Yes	121 (62.37)	3 (50)	124 (62)	
Don't know	23 (11.86)	3 (50)	26 (13)	
Comfortable disclosing sexual orientation to health care worker				0.493
No	39 (20.1)	0 (0)	39 (19.5)	
Yes	147 (75.77)	6 (100)	153 (76.5)	
Refused to answer	8 (4.12)	0 (0)	8 (4)	
Experienced stigma				0.075
No	90 (46.39)	4 (66.67)	94(47)	
Yes	98 (50.52)	1 (16.67)	99 (49.5)	
Refused to answer	6 (3.09)	1 (16.67)	7 (3.5)	
Fear stopped you from accessing health care				0.242
No	94 (48.45)	3 (50)	97 (48.5)	
Yes	93 (47.94)	2 (33.33)	95 (47.5)	
Refused to answer	7 (3.61)	1 (16.67)	8 (4)	
Having a trusted and flexible sexual health clinic will encourage access to care				0.02
No	18 (9.28)	0 (0)	18 (9)	
Yes	146 (75.26)	2 (33.33)	148 (74)	
Don't know	30 (15.46)	4 (66.67)	34 (17)	

Discussion

This study aimed to estimate the prevalence of *N. gonorrhoeae* and *C. trachomatis* among MSM living in Durban, South Africa. In this study, the prevalence of the individual STIs investigated among MSM were as follows: *N. gonorrhoeae*, 3.0% and *C. trachomatis*, 6.0%. The prevalence of *C. trachomatis* and *N. gonorrhoeae* observed in this study were consistent with those reported in a study among MSM in Marrakech, Morocco which reported a prevalence of 6.3% for *C. trachomatis* and 2.4% for *N. gonorrhoeae*[44]. Prevalence estimates from our study are also similar to those found in another study conducted among MSM in Tanga,Tanzania where prevalence rates of 7.5% and 2.5% were reported for chlamydia and gonorrhoea respectively[45]. Similarly, a study conducted among MSM in Hong Kong, China reported a prevalence of 4.7% for *C. trachomatis* and 0.2% for *N. gonorrhoeae*[46]. In other reported studies, *C. trachomatis* prevalence rates ranged from 2.2% to 26.0%[41,47-51] while prevalence estimates for *N. gonorrhoeae* ranged from 1.0% to 16.0%[25,47,52-54]. These reported studies are comparable with results from our systematic review and meta-analysis which showed a pooled prevalence of 27% for *N. gonorrhoeae*/*C. trachomatis* among MSM in sub-Saharan Africa.

Results from our study indicated that *C. trachomatis* is the most prevalent bacterial STI among MSM in South Africa. This is in line with previous studies conducted in South Africa[41,42]. Evidence from a systematic review by Dewart et al. had shown that although chlamydia and gonorrhoea are the most prevalent STIs among MSM, chlamydia is more prevalent when compared with gonorrhoea[55]. In South Africa, a study carried out in adult men and women found that amongst 15-49 year olds, men had more cases of *C. trachomatis* than women while the number of new cases of gonorrhoea was similar in both groups[56]. Another study conducted in Nairobi, Kenya among tertiary student MSM, reported a higher prevalence of chlamydia[54]. Studies among community-recruited MSM have also shown that infection with gonorrhoea are far less common than chlamydia infection[57,58]. Contrasting trends, however, have been reported in other areas. For instance, in England, MSM were most likely to contract *N. gonorrhoeae* than any other STI[23]. A study conducted by Ribeiro et al. in a population of MSM reported a prevalence rate of 10.75% for gonorrhoea and 7.59% for chlamydia[25]. Another study among MSM conducted in the city of Agadir in 2020 reported an overall prevalence of 11.3% and 13.3% for *C. trachomatis* and *N. gonorrhoeae*, respectively[59]. The predominant asymptomatic presentation of *C. trachomatis* which allows for longer duration of infection and subsequent transmission, may be responsible for the high prevalence of the infection worldwide[60,61].

The current standard for the management of STIs (chlamydia, gonorrhoea, syphilis and trichomoniasis) in South Africa and other developing countries is the syndromic approach, which depends on patients presenting with signs and symptoms for presumptive diagnosis and treatment of STIs without the use of laboratory tests[3]. This is especially concerning in low- and middle-income nations, which frequently have high-risk sexually active populations[62]. In this study, a considerable number of MSM who tested

positive for *N. gonorrhoeae* and *C. trachomatis* infections were asymptomatic. These findings suggest that syndrome-based STI management is an inadequate approach for reducing the burden of STIs in MSM, as this screening approach can create a false sense of security in asymptomatic patients, favouring the progression of silent STIs towards irreversible complications[11].

Of the MSM who reported previous history of STIs, 32% (64/200) reported that they had been diagnosed with at least one STI in the past. A study conducted by Budkaew et al. reported that a participant's previous history of diagnosed STIs was significantly associated with urethral gonorrhoeal infection[63]. Similarly, findings from other studies indicated that asymptomatic gonorrhoea or chlamydia infection was associated with a past exposure with at least one STI infection[47,49,64]. Bacterial STIs have been identified as a potential driver of HIV infection in MSM[65,66]. Several studies have established among MSM the association between STIs and increased risk of HIV infection[67-69]. A retrospective study conducted in San Francisco demonstrated that two or more prior rectal gonorrhoea or chlamydia infections among MSM were associated with 8 times increased risk of HIV seroconversion[27]. Furthermore, a study by Harney et al. found that a cumulated history of rectal gonorrhoea infection increased the risk of subsequent HIV infection among MSM[70].

Several risk factors such as unprotected sex[71], younger age[21,72,73], multiple sex partners[21,71,74,75], transactional sex and substance use[25,43,76] have been found to be associated with chlamydia and gonorrhoea infections. Knowledge of these associated risk factors are important as they play a crucial role in designing effective control measures[77]. In this present study, a range of factors were found to be associated with *C. trachomatis* and/or *N. gonorrhoeae* infections. We found that sex for cash and drug use were associated with testing positive for gonorrhoea. A similar study reported that inhaled drug use was significantly associated with higher rates of gonorrhoea and chlamydia infections[25]. Other studies have found that MSM having sexual contact with individuals who exchanged sex for money or drugs was significantly associated with high prevalence of gonorrhoea[43,76]. Persons who transact sex are not likely to be able to negotiate safe sex due to fear of losing out on transactional benefits[78]. This places them at risk for infection.

Previous studies have indicated that having a low level of education is associated with acquiring STIs[33,79,80]. This may be attributed to risk taking behaviour among the people with low education[81]. In our study however, 58.3% of MSM who tested positive for *C. trachomatis* had a university education (p=0.006), and of the men who tested positive for *N. gonorrhoeae*, an equal proportion (33.3%) attended high school and university (p=0.047). This finding, may be as a result of the unwillingness of MSM in seeking care due to stigma and discriminatory attitudes of healthcare workers which limits the uptake of health services[82] irrespective of educational status.

Engaging in anal/oral sex was also found to be significantly associated with testing positive for *N. gonorrhoeae* in this study. Engaging in anal/oral sex also showed a borderline significance with testing positive for *C. trachomatis* in our study. Previous studies in MSM have shown that anal sex is associated with anorectal gonorrhoea[83,84]. Other studies however have shown that anal sex is not associated with anorectal chlamydia in MSM[84,85]. In contrast, other STIs, including chlamydia, have been shown to be frequently transmitted through oral-genital or oral-rectal pathways[86].

Our study found that a high proportion of MSM engaged in group sex. Previous studies from Australia, the United Kingdom and the United States have documented that group sex was common among MSM[87-90]. Other studies have shown that between 25% and 55% of MSM who participate in group sex engaged in condomless anal sex[89,90,91-93], and were susceptible to contracting and transmitting STIs[94,95]. The relationship between group sex participation and STIs have also been discussed in previous studies[96-98]. In this study, group sex was significantly associated with testing positive for *N. gonorrhoeae.* A study by Rice et al. reported that participation in group sex was associated with more than twofold increased prevalence of *N. gonorrhoea* infection, but not with chlamydia[96]. According to van den Boom et al., group sex may be a higher-risk setting for the acquisition of STIs such as gonorrhoea[97].

A high proportion of MSM in our study were cohabiting with their sex partners. Furthermore, we found that cohabitation status was significantly associated with testing positive for *N. gonorrhoeae*. It has been reported that cohabiting and/or married MSM were less likely to use condoms than non-cohabiting/unmarried MSM[99]. More so, MSM who have sex in a married or cohabiting relationship are more likely to be monogamous or believe their partner is, which may cause them to believe they are less likely to contract HIV and STIs[100,101]. Previous research have indicated that MSM in committed relationships may see condoms as limiting sexual intimacy and indicating mistrust of a partner[100].

With respect to condom use, MSM who reported frequent use of condoms in this study were at increased risk of acquiring *C. trachomatis* infection (OR: 6.67, 95% CI: 1.4791-30.0478, p=0.014). A similar study among MSM in Lisbon demonstrated that despite the consistent use of condoms during anal sex, gonorrhoea and chlamydia transmission still occurred[25]. Our finding was however not consistent with reports from other studies where condom use significantly decreased the prevalence of chlamydia and gonorrhoea[63,102]. Although an invaluable tool in combating HIV, condom effectiveness in preventing other STIs has increasingly been questioned[25] since it does not adequately prevent infection caused by Hepatitis A virus[103].

With regards to circumcision, half of the MSM population who tested positive for *N. gonorrhoeae* infection were circumcised (p=0.033). For *C. trachomatis*, in the adjusted analysis, being circumcised

was shown to reduce the risk of infection (OR: 0.01, 95% CI: 0.0005-0.3516, p=0.01). There are inconsistent reports on the effect of circumcision on the incidence of STIs[104]. However, there is convincing evidence to show that male circumcision reduces the risk of HIV and STI infection[105-107]. A systematic review and meta-analysis conducted by Yuan et al., reported the association between male circumcision and HIV and other STIs among MSM in low- and middle-income countries. The authors found that circumcision was associated with 23% reduced odds of HIV infection[108] . Similarly, a meta-analyses conducted by Sharma and colleagues found that voluntary medical male circumcision (VMMC) may well reduce the risk of HIV infection by 20% among MSM[109]. Uncircumcised men can be at increased risk of STIs by reason of entry of pathogens through the inner surface of the foreskin[104]. A study conducted in Rustenburg, South Africa among circumcised and uncircumcised adult males reported that the prevalence of STIs was lower in the circumcised participants compared with those who were uncircumcised[110]. Furthermore, in a cohort study by Diseker et al., uncircumcised men were significantly more likely to have gonorrhoea than circumcised men[111]. As an approach to preventing HIV and other STIs, the WHO now recommends VMMC as one of the key prevention strategies[112].

In this study, our analysis found that younger age was significantly associated with testing positive for *C. trachomatis*. Most cases of chlamydia infection were found in MSM aged 25-30 years. This finding is in agreement with several previous studies which demonstrated that younger age was associated with having chlamydia and/or gonorrhoea infections[21,25,73,113,114]. This observed trend might be due to younger populations engaging in sexual practices with greater number of sexual contacts, as well as a higher probability of infection among those contacts[115]. Age was also associated with the risk of acquiring *C. trachomatis* in this study. In the univariate analysis conducted in our study, results showed that being between the ages of 30-39 years old reduced the risk of acquiring chlamydia infection (OR: 0.10, 95% CI: 0.0120-0.7564, p=0.026). After further adjustments, being within the age range 30 - 39 years old still reduced the risk for infection with *C. trachomatis* in the multivariate analysis (OR: 0.62, 95% CI: 0.4224-0.8967, p=0.011). A study conducted by Cunha et al. in a population of MSM in Brazil showed that for every additional 10 years of age, the prevalence of having at least one STI decreased by 22%[116]. On the other hand, several other studies have shown that younger age is associated with an increased risk for STI diagnosis[74,117].

Having multiple sex partners increases the likelihood of encountering an infected partner[118]. Having multiple sexual contacts has also been shown to predispose MSM to multiple concurrent STIs[75,119]. In the adjusted analysis conducted in our study, we found that MSM having between 2 to 4 sex partners in the past 30 days increased the risk of testing positive for *C. trachomatis* infection (OR: 107.45, 95% CI: 1.3467-8573.3130, p=0.036). Findings from a similar study by Ribeiro et al., reported a significant association between infection and having a higher number of concurrent sex partners[25]. Similarly, a study conducted in the United States found that MSM who reported multiple sexual partners had an

increased risk of incident bacterial STIs[120]. Other studies on MSM have indicated that having multiple sex partners is associated with having anorectal chlamydia and gonorrhoea infections[21,74,114]. This finding from our study underscores the need for tailored intervention focusing on the reduction of multiple sex partners among this key population. Intervention may employ sexual health approach aimed at reducing risk behaviours and promoting safer sex among MSM.

Majority of MSM in this study were generally well-informed about sexual health clinics in Durban and the services they provide. However, a high proportion (62%) of these participants would rather seek care from a general practitioner (GP) at a surgery. Half of the MSM who tested positive for *N. gonorrhoeae* preferred to access care from a GP based in a surgery (p=0.042). A study by Lea et al., reported that some gay men preferred accessing sexual health care via their GP due to ease of access and concerns about anonymity[121].

Research has indicated that MSM oftentimes experience discrimination from public sector healthcare workers[122,123], which negatively affects health-seeking behaviours[124]. In this study, almost half of the MSM reported being stigmatized based on their sexual behaviour while 47.5% reported being afraid to visit a health care clinic due to stigma. On the contrary, 58.3% believed that their confidentiality would be protected. A study in Vietnam reported that sexual stigma contributed to MSM's reluctance to actively access healthcare services[125]. Studies conducted in Africa have also highlighted on a number of challenges that MSM experience in accessing adequate medical care including negative patient-provider relationships[126-128]. A survey on stigma, health care access, and HIV knowledge among MSM conducted in Malawi, Namibia, and Botswana observed strong associations between MSM health-seeking behaviour and stigma/discrimination from healthcare workers[129]. Similarly, a study conducted in South Africa among MSM utilizing health services in South African cities found that stigma, discrimination, and negative health worker attitudes were major barriers to accessing health services[122]. Another study conducted in the South African cities of Bloemfontein and Mafikeng stated lack of confidentiality and concern around negative treatment from healthcare workers as reasons for MSM's reluctance to seek public sector healthcare and fear of disclosing their behaviours[82].

The strength of this study is that it fills a gap in the literature on the prevalence and risk factors associated with *C. trachomatis* and *N. gonorrhoeae* infection among MSM in South Africa as well as identified barriers to accessing healthcare services. A major limitation of this study is that it did not test for anorectal *C. trachomatis* and *N. gonorrhoeae*. Prevalence may have been substantially higher had the test been included. Data on demographic and behavioural characteristics as well as symptoms were self-reported, and as such might be subject to both recall and/or social desirability biases.

Conclusion

Our study provides evidence of STI prevalence rates, particularly the high rates of *C. trachomatis* infection among MSM resident in Durban. It also confirms previous research that *C. trachomatis* is the most common STI among MSM in South Africa. A percentage of MSM who tested positive for *N. gonorrhoeae* and *C. trachomatis* infections were asymptomatic. This poses a treatment challenge since South Africa employs the syndromic management approach. Syndromic management relies on patients presenting with signs and symptoms for presumptive diagnosis and treatment of STIs without the use of laboratory tests[3]. Given that *C. trachomatis* is the most prevalent STI amongst MSM in South Africa, point-of-care laboratory tests targeted at MSM, may be an alternative strategy. Also, considering the reluctance of MSM in seeking healthcare due to stigma and negative healthcare worker attitudes, we recommend the establishment of mobile STI screening services for the MSM population. The majority of MSM positive for *C. trachomatis* were between 25-30 years of age. A high proportion frequently engaged in group sex and tested positive for *N. gonorrhoeae*. A few factors were significantly associated with both *C. trachomatis* and *N. gonorrhoeae* infections. Fear and stigma were the main barriers to seeking medical care in this study. The results of this study not only contribute to the body of information showing the high burden of STIs in MSM populations worldwide[11], but also calls for more attention to be paid to younger MSM and healthcare workers' unfavourable attitudes towards this key population.

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Competing interest

The authors declare no conflict of interest.

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In chapter three, we observed that some of the participants who tested positive for *N. gonorrhoeae* and *C. trachomatis* infections were asymptomatic. We also observed a high prevalence (6.0%) of *C. trachomatis* infection among our study cohort. This finding supports previous research which reported *C. trachomatis* as the most common STI among South African MSM. Being young was significantly associated with testing positive for *C. trachomatis* and having between 2-4 sex partners increased the risk of infection with *C. trachomatis*. Factors such as engaging in group sex and drug use were significantly associated with testing positive for *N. gonorrhoeae* infection. Study findings indicates high disease burden in the study population. In South Africa, there are limited data on the genotypic diversity of *C. trachomatis*. In addition, very little is known about the characterization of *C. trachomatis* genotypes in South African MSM. The aim of the following chapter was to describe the distribution of *C. trachomatis* genotypes in a population of MSM from Durban, South Africa.

CHAPTER FOUR

Chlamydia trachomatis genotypes among men who have sex with men in Durban, South Africa

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Abstract

Introduction: Men who have sex with men (MSM) are at increased risk of acquiring sexually transmitted infections (STIs) including *Chlamydia trachomatis* (*C. trachomatis*). In South Africa, the genotypic diversity of *C. trachomatis* is understudied especially among this subpopulation. This study was undertaken to describe the genotypic diversity of *C. trachomatis* in a population of MSM from Durban, South Africa.

Methodology: In this study, a total of 200 urine samples collected from sexually active MSM in Durban were analysed for the presence of *C. trachomatis* using the Applied Biosystems™ TaqMan® Assay. The genotyping assay was performed on all samples testing positive for *C. trachomatis* by amplification and restriction digestion of the *omp*A gene with *AluI*, *DdeI*, and *HinfI.*

Results: The prevalence of *C. trachomatis* in the study population was 6% (12/200). The following factors were significantly associated with testing positive for *C. trachomatis*: age, education level, partner having other partners, sex practices, condom use and circumcision status ($p<0.05$). The *omp*A gene was amplified in 10 of the 12 samples. Genotype E was the most prevalent genotype present in 60% (6/10) of the men infected with *C. trachomatis*. The remaining genotypes detected were Genotype I (3/10; 30%) and Genotype J (1/10; 10%).

Conclusion: This study described the genotypic diversity of *C. trachomatis* associated with South African MSM. The PCR-RFLP technique revealed the presence of only three genotypes (E, I, and J) circulating in the MSM population of the study area.

Key words: *Chlamydia trachomatis*, Sexually transmitted infections, Genotypes, South Africa

Introduction

Chlamydia trachomatis (*C. trachomatis*), a Gram-negative, obligate intracellular bacterial pathogen, is the most prevalent bacterial sexually transmitted infection (STI) and the leading cause of STIs worldwide.[1-5] This human pathogen, which continues to cause substantial morbidity and economic loss worldwide,[6] infects 129 million persons per year and has shown a global prevalence of 1%–6%.[7-10] Despite efforts at reducing the spread of chlamydial infections, *C. trachomatis* remains the leading cause of preventable blindness worldwide[11] with an estimate of about 1.9 million cases of trachoma blindness and 125 million people at risk for the disease, according to the World Health Organization (WHO).[12]

Although infections caused by *C. trachomatis* are most often asymptomatic, they can lead to complications such as tubal infertility, pelvic inflammatory disease (PID) and ectopic pregnancy[13-19] in females, and non-gonococcal urethritis, proctitis and epididymitis in men[3,20-24] Infections can also result in trachoma[25] and lymphogranuloma venereum[17,19] including abortion, chorioamnionitis, premature rupture of the membrane, preterm labour, stillbirth, low birth weight and adverse neonatal sequelae.[18] In addition, infections caused by *C. trachomatis*, are associated with cervical cancer and significantly increases the risk of human immunodeficiency virus (HIV) transmission.[26-29]

The majority of chlamydial infection diagnoses are in young people between the ages of 15 and 24 years old[3] with adolescents younger than 20 years of age being at higher risk.[2,30] Reinfections are also common.[2] A study by Eng and Butler reported that 30–40% of sexually active teenagers were infected with *C. trachomatis*.[31] In women, *C. trachomatis* infection presents asymptomatically in 70–75% of cases while approximately 50% of infected men are asymptomatic[3,30,32,33] making diagnosis and treatment difficult.[34,35] In men aged 15-49 years, *Chlamydia* has been shown to represent a global prevalence of 4.2%.[17]

According to previous studies, most men who have sex with men (MSM) are asymptomatic.[36] These unrecognized, infected individuals serve as reservoirs and transmit infections to their sexual partners. In addition, chlamydial infections are associated with a 3–6-fold increase in the transmission and acquisition of HIV infection[37,38] Furthermore, the strains that infect this subpopulation are genetically distinct.[39]

Molecular epidemiological studies are essential to understand the genetic population structure and to gain insight into the transmission of *C. trachomatis*.[40-43] Furthermore, the temporal and geographical distribution of strains throughout the world has significant implications for vaccine development.[44] The established basis for defining *C. trachomatis* strains is variation in the *omp*A gene, which encodes the immunodominant major outer membrane protein.[45]

According to *omp*A gene molecular differences, *C. trachomatis* can be divided into 19 serovars based on antigenic properties of the major outer membrane protein (MOMP) with over 60 *omp*A genotypes.[46-

[51] The serovars are designated A through K, Ba, Da, Ia, Ja, and L1-3, and L2a while the *omp*A genotypes or strains are denoted by the same or by a number or letter after the conventional serovar name for new genotypes.[48] Among these, serovars A-C are associated with trachoma, serovars D-K are responsible for urogenital infections in adults and respiratory and chlamydial neonatal conjunctival infections worldwide. Serovars L1-L3 are associated with lymphogranuloma venereum[52-56] with the L2b–L2e genovariants associated with the development of proctitis in MSM.[57,58]

The distribution of the various genotypes of *C. trachomatis* have been identified in different regions and countries (Iran,[53] Argentina,[54] Cameroon,[59] Sweden,[60] United States of America,[61] and China[62]) of the world with the genotypes E (26–46%), D (9–20 %), F (14–24%),[62,63,64] G (10%) and K (5.8%)[65] being the most frequently detected genotypes among heterosexual populations. In MSM however, the most predominant *omp*A genovars reported are G, D and J.[65,66-69] Typing of *C. trachomatis* strains among high-risk groups, particularly MSM is important as it can be used to monitor the dynamical trends of sexual behaviours and reveal transmission pathways in the populations.[36,41]

Despite the high occurrence of *C. trachomatis* globally,[3,9,10] very little is known about the diversity of genotypes among MSM in South Africa. This study was therefore undertaken to describe the distribution of *C. trachomatis* genotypes in a population of MSM from Durban, South Africa.

Methodology

Study design and population

This cross-sectional study including all laboratory assays were conducted at the School of Clinical Medicine Research Laboratory of the Nelson R. Mandela School of Medicine, University of KwaZulu-Natal. Participants were MSM attending care at the King Edward VIII hospital and the Aurum Institute both located in Durban, South Africa. The study participants were enrolled from October 2021 to July 2022 and included sexually active MSM, 18 years and older. Participants were requested to provide urine samples.

Sample size calculation

The sample size (N) for this study was determined by calculation based on the formula

$N = Z_{1-\alpha/2}^2 p(1-p)/d^2$.[70]

Where $Z_{1-\alpha/2}$ is standard normal deviate set at 1.96 (95% CI), p is expected proportion in the target population based on previous studies (prevalence of STIs from previous study was 12.7%).[71] and d is the degree of precision set at 0.05; $N = (1.96)^2 \times (0.127 \times 0.873)/(0.05)^2 = 170$.

Adding 15% of the minimum sample size for the expected non-response rate, a sample size of 200 was estimated.

Sample processing

A total of 200 urine samples were collected. The samples were refrigerated between 2-8°C until processed. A total of 10 ml of urine was centrifuged at 14 000x g for 45 minutes and the supernatant discarded. The recovered pellets were then subjected to further molecular analyses. Samples were processed within 24 hours of collection.

DNA extraction

Deoxyribonucliec acid (DNA) was extracted from the sample pellets using the commercially available PureLink Microbiome Kit (ThermoFisher Scientific, United States), according to the manufacturer's protocol. Sample pellets were suspended in 800 µl of S1 lysis buffer by pipetting up and down. The sample was then transferred to the bead tube and 100 µl of S2 lysis enhancer was added to the bead tube, capped and homogenized with a vortex mixer. The tubes were incubated at 95°C for 10 minutes, followed by vortexing at a maximum speed for 7 minutes. The samples were then centrifuged (Eppendorf 5415C centrifuge) at 14 000x g for 1 minute and 500 µl of the supernatant was transferred to a clean micro-centrifuge tube. To bind DNA to the column, 900 µl of binding buffer was added and vortexed briefly. Thereafter, 700 µl of the sample mixture was loaded onto a spin column-tube and centrifuged at 14 000x g for 1 minute. The flow through was discarded and the spin column was centrifuged at 14 000x g for 1 minute. Following this, the spin column was placed in a clean tube and 50 µl of S6 elution buffer was added. The tube was then incubated at room temperature for 1 minute. After incubation, the tube was centrifuged at 14 000x g for 1 minute, and the extracted DNA concentration was measured using a Nanodrop Spectrophotometer (ThermoFisher Scientific, United States). Extracted DNA from samples were stored at –20°C for further testing. A flow diagram of the DNA extraction method used in this study is shown below (Figure 1).

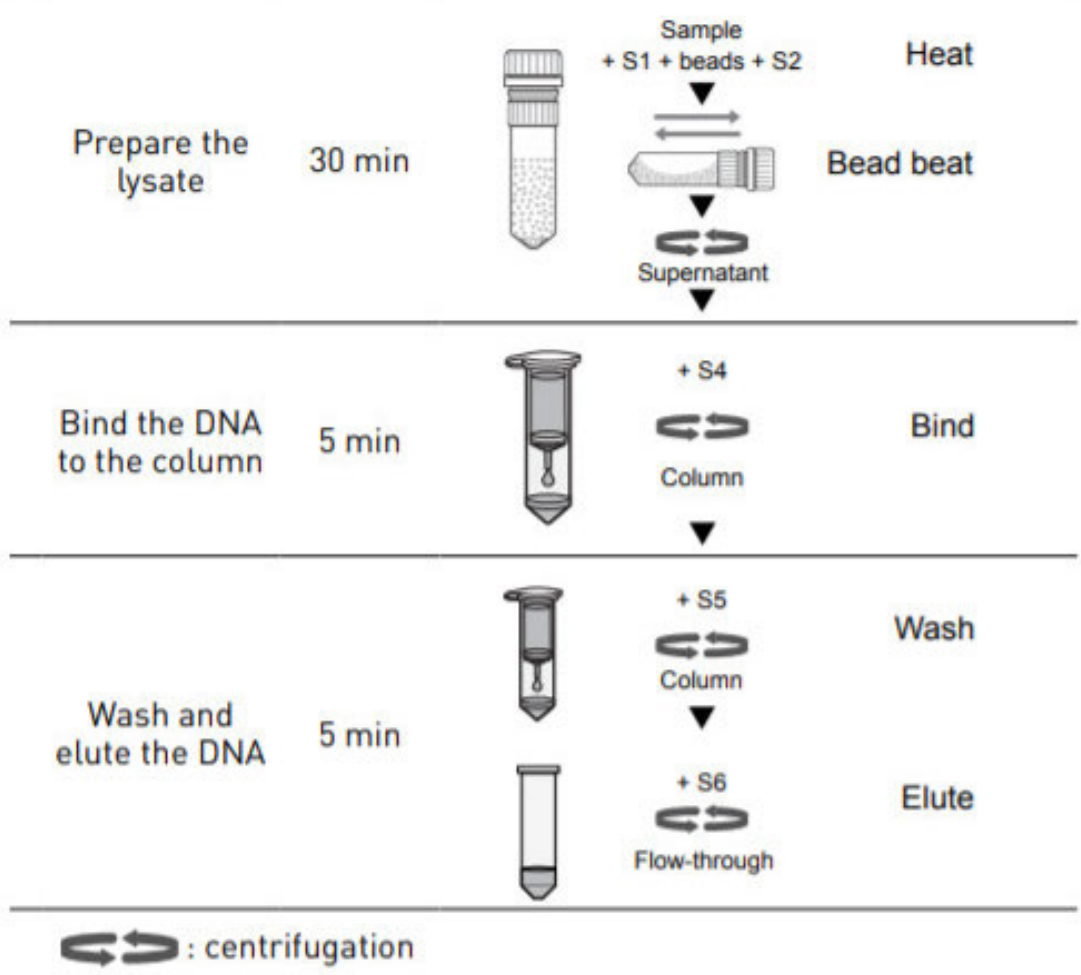

Figure 1: Overview of the DNA extraction process[72]

Detection of C. trachomatis

C. trachomatis was detected using the Applied Biosystems™ TaqMan® Assays. Commercial primers and probes (Ba04646249_S1) which targets the translocated actin-recruiting phosphoprotein of *C. trachomatis* were used. Amplification was performed on the Quant Studio 5 Real-time polymerase chain reaction (PCR) detection system (Thermo-Fisher Scientific, United States). Each reaction was performed in a final volume of 10 µl and included: 1 µl FAM-labelled probe/primer mix for individual targets, 5 µl Fast Start 4x probe master mix (Thermo-Fisher, Ba04646249_S1), 2 µl template DNA and 2 µl nuclease-free water. The runs included non-template control reactions. Amplification was performed under the following conditions: 1 cycle at 95°C for 30 seconds followed by 45 cycles of denaturation at 95°C for 30 seconds and annealing at 60°C for 30 seconds. Detection of fluorescent products were performed at the end of the annealing period. The raw fluorescent data was automatically generated by the Quant studio 5 Real-time PCR system software.

C. trachomatis genotyping

Molecular genotyping of *C. trachomatis* positive samples was performed by an *omp*A gene semi-nested PCR followed by restriction fragment length polymorphism (RFLP) analysis.[73] The first product of 1033 bp was amplified using the following paired primers at 200 nM concentrations: forward (SERO1A) (5'-ATGAAAAAACTCTGAAATCGG-3') and reverse (SERO2A) (5'-TTTCTAGATCTTCATTCTTGTT-3').[73] The reaction was performed in a final volume of 50 µl containing 16 µl of nuclease-free PCR water, 25 µl of the 2x DreamTaq PCR Master Mix (Thermo-Fisher Scientific, United States), 2 µl of each primer and 5 µl template DNA. Polymerase chain reaction (PCR) was performed with the following cycling conditions: initial denaturation at 94°C for 7 minutes, followed by 40 cycles of denaturation at 95°C for 1 minute, annealing at 45°C for 3 minutes and extension at 72°C for 3 minutes. An additional 7-minute extension at 72°C was performed at the end of the 40 cycles.[73] Following the first round PCR, 1 µl of the first-round PCR product was used for the semi-nested PCR, amplifying a 978 bp fragment. The second PCR round was performed with the same reagents and conditions as the first round with the following primers: reverse primer (SERO2A) from the first round and PCTM3 (5'-TCCTTGCAAGCTCTGCCTGTGGGGAATCCT-3').[73] After the PCR step, the amplified product was digested with *AluI*, *DdeI* and *HinfI* restriction enzymes and visualized after electrophoresis on a 2% agarose gel. *C. trachomatis* serovar identification was made by analysis of the specific restriction pattern.

Ethical approval

Ethical clearance for this study was granted by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (BREC/00002798/2021).

Data analysis

The statistical data analysis was conducted in a freely available Statistical Computing Environment, R software, version 3.6.3 using the RStudio platform. A descriptive analysis of the sociodemographic characteristics of the participants was carried out. Descriptive statistics of numerical measurements included mean and standard deviation. Categorical variables were described as counts and percentage frequencies. The Chi-square test was used to determine statistical significance, which was set at 0.05.

Results

Study population

The prevalence of *C. trachomatis* in the study population was 6% (12/200), Table 1. The majority of the men (67%) who tested positive for *C. trachomatis* were between the ages of 25-30 years old (Table 1). Among those aged 18-24 years old, 25.0% had chlamydial infection while 11.2% were negative for *C. trachomatis*. For those aged 31-44 years old, 8.3% were positive for *C. trachomatis* while 41.5% were without *C. trachomatis* infection; whereas no *C. trachomatis* infection was detected in MSM aged 45 years old and above. There was significant association between age and *C. trachomatis* status, p=0.037 (Table 1). A higher percentage (58.3%) of men who attended university were positive for *C. trachomatis* while 16.5% with same level of education were *C. trachomatis* negative. Of the participants who had high school education, 33.3% were positive for *C. trachomatis* infection while 70.7% were negative. For those who had primary school education, 5.3% were without *C. trachomatis* infection while 8.3% tested positive for *C. trachomatis*, p=0.006 (Table 1). For participants who do not know if their partner had other partners, *C. trachomatis* was detected in 75.0% while 42.6% were negative for the infection. For MSM who reported that their partner did not have other partners, 25.0% were positive for *C. trachomatis* while 28.2% were without chlamydial infection. No *C. trachomatis* was detected among participants who reported knowing that their partner has other partners, p=0.032. Among participants who engaged in anal sex only in the past 30 days, 58.3% had chlamydial infection while 33.0% were negative for *C. trachomatis*. For oral and anal sex in the past 30 days, 38.8% were without *C. trachomatis* infection while 25.0% were *C. trachomatis* positive, exhibited marginal significance, p=0.054 (Table 1). As presented in Table 1, 50.0% of MSM who were circumcised tested positive for *C. trachomatis* while 76.6% were negative for the infection. For those uncircumcised, 18.1% were *C. trachomatis* negative while 50.0% were infected with *C. trachomatis*. A significant association was observed between circumcision and *C. trachomatis* status (p=0.043). In the positive group, 25.0% (3/12) had more than one sex partner in the last 30 days. The same proportion of men (25.0%) engaged in group sex at least once within the same period. Of all the men who reported substance use, 66.3% (132/199) admitted to using illicit drugs during sex (Table 1).

Table 1. Characteristics of the study population according to *C. trachomatis* infection status

	C. trachomatis Negative (%)	*C. trachomatis* Positive (%)	Total	p-value
	188 (94)	12 (6)	200	
Age group (years old)				**0.037**
18-24	21 (11.17)	3 (25)	24 (12)	
25-30	75 (39.89)	8 (66.67)	83 (41.5)	
31-44	78 (41.49)	1 (8.33)	79 (39.5)	
45+	14 (7.45)	0 (0)	14 (7)	
Level of education				**0.006**
Primary School	10 (5.32)	1 (8.33)	11 (5.5)	
High School	133 (70.74)	4 (33.33)	137 (68.5)	
College/University	31 (16.49)	7 (58.33)	38 (19)	
Refused to answer	14 (7.45)	0 (0)	14 (7)	
Employed				0.141
No	149 (79.26)	7 (58.33)	156 (78)	
Yes	39 (20.74)	5 (41.67)	44 (22)	
Has a regular sex partner				0.872
No	51 (27.13)	3 (25)	54 (27)	
Yes	137 (72.87)	9 (75)	146 (73)	
Cohabiting with partner				0.664
No	112 (59.57)	9 (75)	121 (60.5)	
Yes	71 (37.77)	3 (25)	74 (37)	
Refused to answer	5 (2.66)	0 (0)	5 (2.5)	
Number of sex partners in last 30 days				0.293
1	92 (48.94)	5 (41.67)	97 (48.5)	
2-4	66 (35.11)	3 (25)	69 (34.5)	
>4	7 (3.72)	1 (8.33)	8 (4)	
None	23 (12.23)	3 (25)	26 (13)	
Partner has other partners				**0.032**
No	53 (28.19)	3 (25)	56 (28)	
Yes	55 (29.26)	0 (0)	55 (27.5)	
Don't know	80 (42.55)	9 (75)	89 (44.5)	
Sex practices in the past 30 days				**0.054**
Anal sex only	62 (32.98)	7 (58.33)	69 (34.5)	
Oral sex only	40 (21.28)	0 (0)	40 (20)	
Oral and anal sex	73 (38.83)	3 (25)	76 (38)	
None	13 (6.91)	2 (16.67)	15 (7.5)	
Condom use				0.069
Always	78 (41.49)	3 (25)	81 (40.5)	
Frequent	22 (11.7)	5 (41.67)	27 (13.5)	
Sometimes	76 (40.43)	4 (33.33)	80 (40)	
Never	12 (6.38)	0 (0)	12 (6)	

	C. trachomatis Negative (%)	*C. trachomatis* Positive (%)	Total	p-value
Frequency of group sex in the last 30 days				0.573
Sometimes	39 (20.74)	1 (8.33)	40 (20)	
Frequently	10 (5.32)	1 (8.33)	11 (5.5)	
One time	55 (29.26)	3 (25)	58 (29)	
Never	84 (44.68)	7 (58.33)	91 (45.5)	
Trades sex for cash				0.896
Sometimes	49 (26.06)	3 (25)	52 (26)	
Frequently	19 (10.11)	0 (0)	19 (9.5)	
One time	53 (28.19)	4 (33.33)	57 (28.5)	
Never	67 (35.64)	5 (41.67)	72 (36)	
Has symptoms of STIs				0.738
No	92 (48.94)	5 (41.67)	97 (48.5)	
Yes	88 (46.81)	7 (58.33)	95 (47.5)	
Refused to answer	8 (4.26)	0 (0)	8 (4)	
Circumcised				**0.043**
No	34 (18.09)	6 (50)	40 (20)	
Yes	144 (76.6)	6 (50)	150 (75)	
Refused to answer	10 (5.32)	0 (0)	10 (5)	
HIV status				0.969
Negative	121 (64.36)	8 (66.67)	129 (64.5)	
Positive	53 (28.19)	3 (25)	56 (28)	
Did not know status	14 (7.45)	1 (8.33)	15 (7.5)	
Previously screened for STIs				0.622
Never	27 (14.36)	2 (16.67)	29 (14.5)	
Past 3 months	34 (18.09)	1 (8.33)	35 (17.5)	
More than 3 months ago	66 (35.11)	3 (25)	69 (34.5)	
Last month	61 (32.45)	6 (50)	67 (33.5)	
Previous STIs				0.681
None	11 (5.85)	1 (8.33)	12 (6)	
Chlamydia/Gonorrhoea	30 (15.96)	1 (8.33)	31 (15.5)	
Hepatitis B/C	13 (6.91)	0 (0)	13 (6.5)	
Herpes	7 (3.72)	1 (8.33)	8 (4)	
Syphilis	12 (6.38)	0 (0)	12 (6)	
Refused to answer	115 (61.17)	9 (75)	124 (62)	
Drug use				0.307
No	59 (31.55)	6 (50)	65 (32.66)	
Yes	126 (67.38)	6 (50)	132 (66.33)	
Refused to answer	2 (1.07)	0 (0)	2 (1.01)	

Genotyping analysis

The DNA yields from this study were on average, 10 ng/µl and the A260/280 ratios fell between 1.8 and 2.0. Of the 12 *C. trachomatis* positive samples, only 10 samples produced positive amplification for the *omp*A gene as shown in Figure 2. Repeated attempts to amplify the 2 samples were unsuccessful. It is known that the samples were free from inhibitors since the samples were amplified using quantitative PCR.

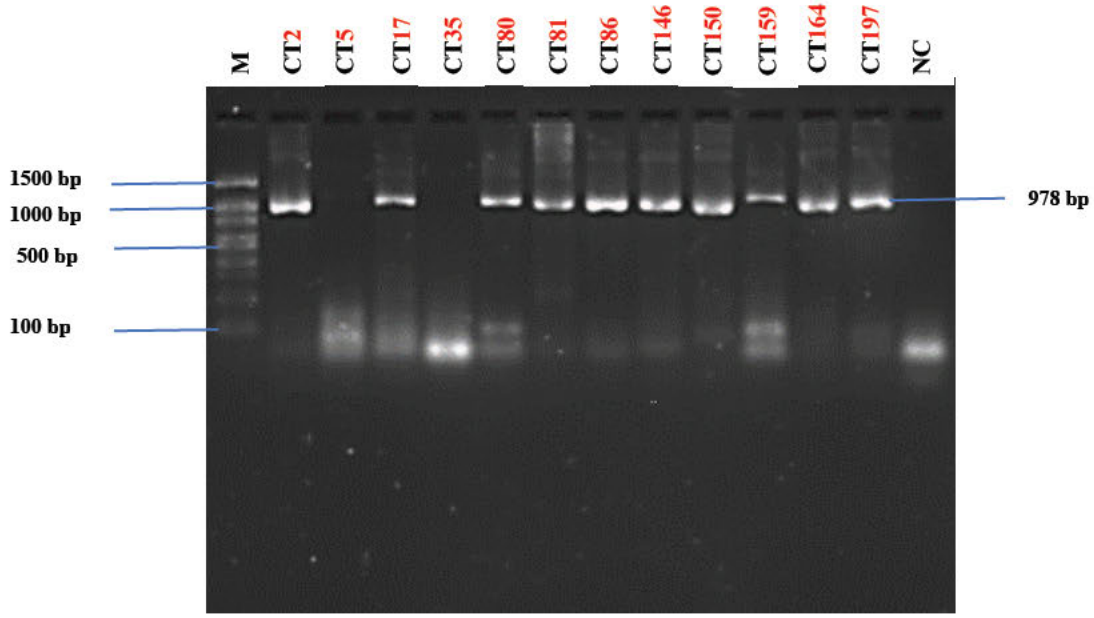

Figure 2: Agarose gel showing positive amplicons generated for the inner *Omp*A gene.

Lane M: Gene Ruler 100 bp DNA ladder (ThermoFisher Scientific, United States); lanes CT2 to CT197: amplified samples; and lane NC: Negative Control. A product size of 978 bp was observed in 10 of the 12 samples tested.

Of the 12 *C. trachomatis* positive samples, 10 (83.3%) were successfully typed and their distribution based on *AluI*, *DdeI* and *HinfI* digestions is presented in Table 2. *C. trachomatis* genotypes were determined by integrating the patterns from the three restriction enzyme profiles. The genotypes identified were as follows: E (n = 6/10; 60.0%), followed by I (n = 3/10; 30.0%) and J (n = 1/10; 10.0%), Table 2.

Table 2. Fragment sizes acquired after digestion of the *omp*A gene with *AluI*, *DdeI*, and *HinfI*. Genotypes identified after integrating the patterns from the three enzyme profiles.

Sample name	*AluI* fragment sizes (bp)	*DdeI* fragment sizes (bp)	*HinfI* fragment sizes (bp)	Genotype
CT 2	86, 302*, 458*, 500, 595, 1250	183*, 375, 450	495, 575	I
CT 17	138*, 225, 250, 495, 595, 1200	285*, 323*, 475	195, 225, 250, 425	I
CT 80	95*, 132*, 225*, 495, 595, 1200	350, 495	195, 225, 250, 430	E
CT 81	95*, 241*, 495, 595, 1225	275, 350, 450, 650	195, 225, 250, 425, 795	E
CT 86	95*, 225*, 495, 595, 850, 900, 1100	350, 460	195, 225, 260, 450	E
CT 146	95*, 225*, 495, 595, 850, 900, 1225	350, 460	195, 225, 250, 430	E
CT 150	138*, 302*, 495, 500, 600, 1225	183*, 375, 450	495, 575	I
CT 159	95*, 132*, 225*, 475, 500, 600, 1100	295, 425	470, 595	E
CT 164	138*, 305*, 495, 500, 600, 1250	195, 375, 450	480, 540*	J
CT 197	102*, 225*, 450, 495, 595, 1000, 1100	445, 650, 1000	195, 800	E

The band sizes of the digested fragments asterisked (*) indicate the published band sizes used to determine the specific genotypes for each sample with a positive amplicon. Band sizes in black indicate additional bands that were observed.

AluI Profile: All of the samples produced banding patterns for *AluI* (Figure 3). Bands at position 95 bp and 225 bp were observed in 4/10 (40.0%) of the samples. One sample (CT81) produced two fragment sizes at positions 95 bp and 241 bp, and another at positions 102 bp and 225 bp (CT197). For the remaining 4 samples, bands at positions 302 bp and 458 bp were observed in one sample (CT2) while the other three samples (CT17, CT150, and CT164) had at least one fragment size at position 138 bp (Table 2).

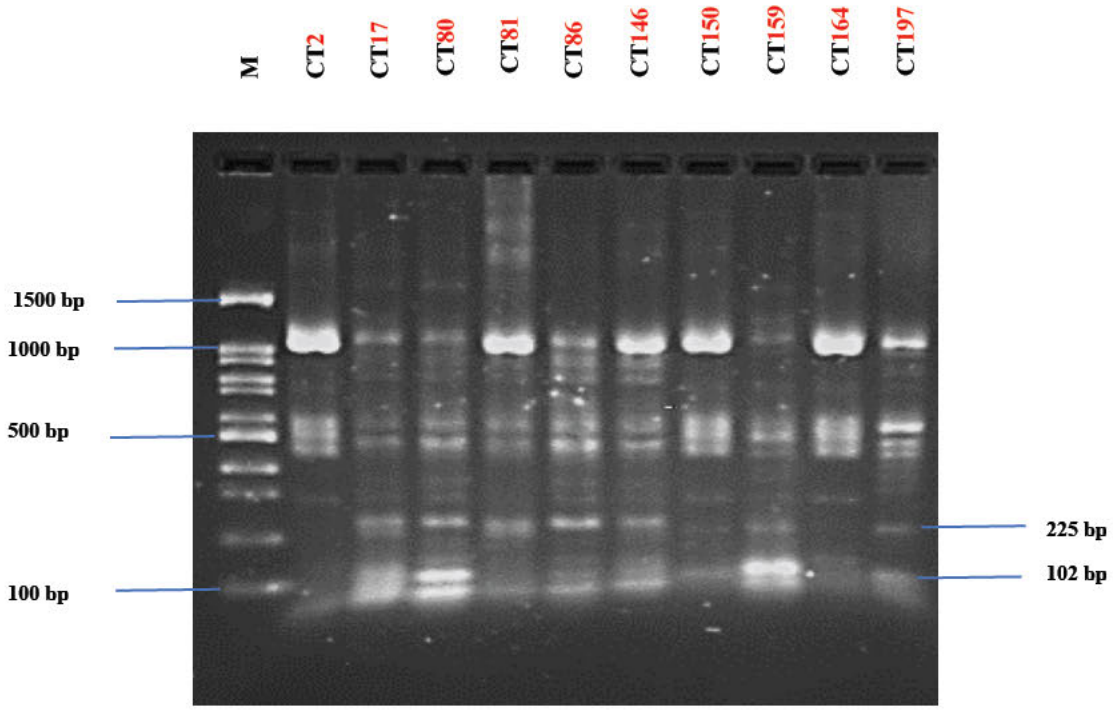

Figure 3: *AluI* RFLP pattern of the digested *Omp*A gene amplicon resolved on a 2% agarose gel. Lane M: Gene Ruler 100 bp DNA ladder (ThermoFisher Scientific, United States) and banding profiles of the samples.

DdeI Profile: For the *DdeI* reactions, one sample (CT17) produced two bands at positions 285 bp and 323 bp while two of the 10 samples (20.0%) produced an identical banding profile (i.e. band at 183 bp) (Table 2). Bands at positions 445 bp and 650 bp were also observed in sample CT197. However, two samples did not produce a banding pattern as gel lanes appeared blank (Figure 4).

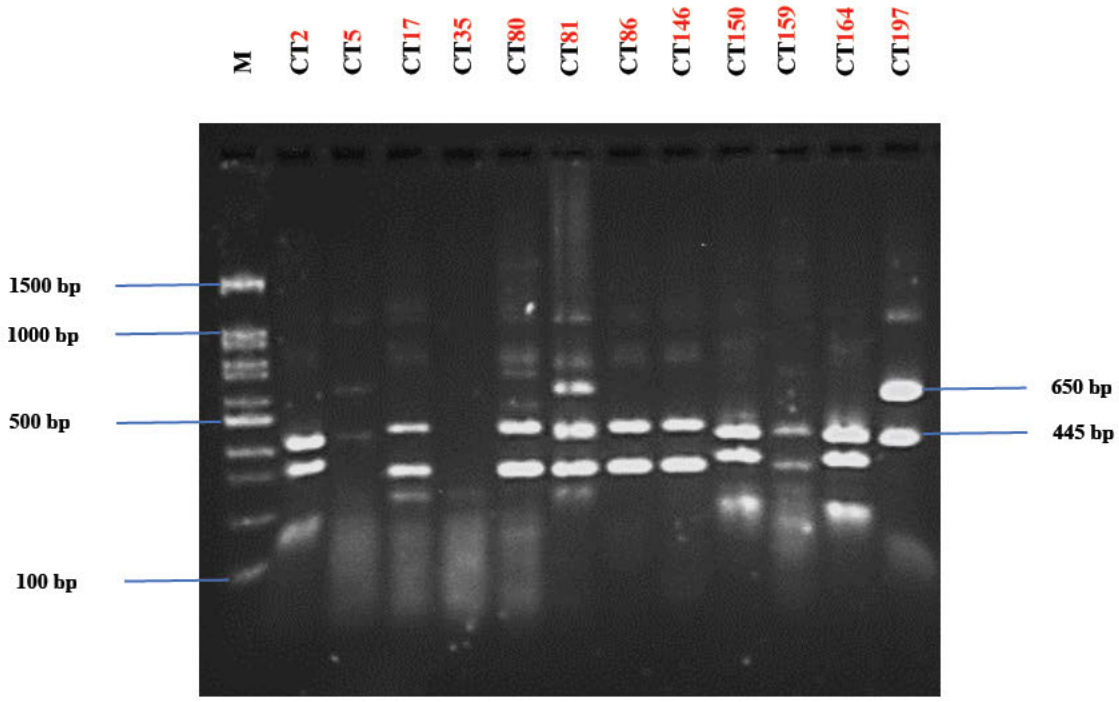

Figure 4: *DdeI* RFLP pattern of the digested *Omp1* gene amplicon resolved on a 2% agarose gel. Lane M: Gene Ruler 100 bp DNA ladder (ThermoFisher Scientific, United States) and banding profiles of the samples.

HinfI Profile: Based on the restriction digestion with *HinfI*, a similar binding profile was observed in some samples (Table 2). Two samples (CT197 and CT164), produced a different banding profile, one at positions 195 bp and 800 bp, and the other at position 540 bp. Two of the 10 samples (20.0%) did not produce any band (Figure 5).

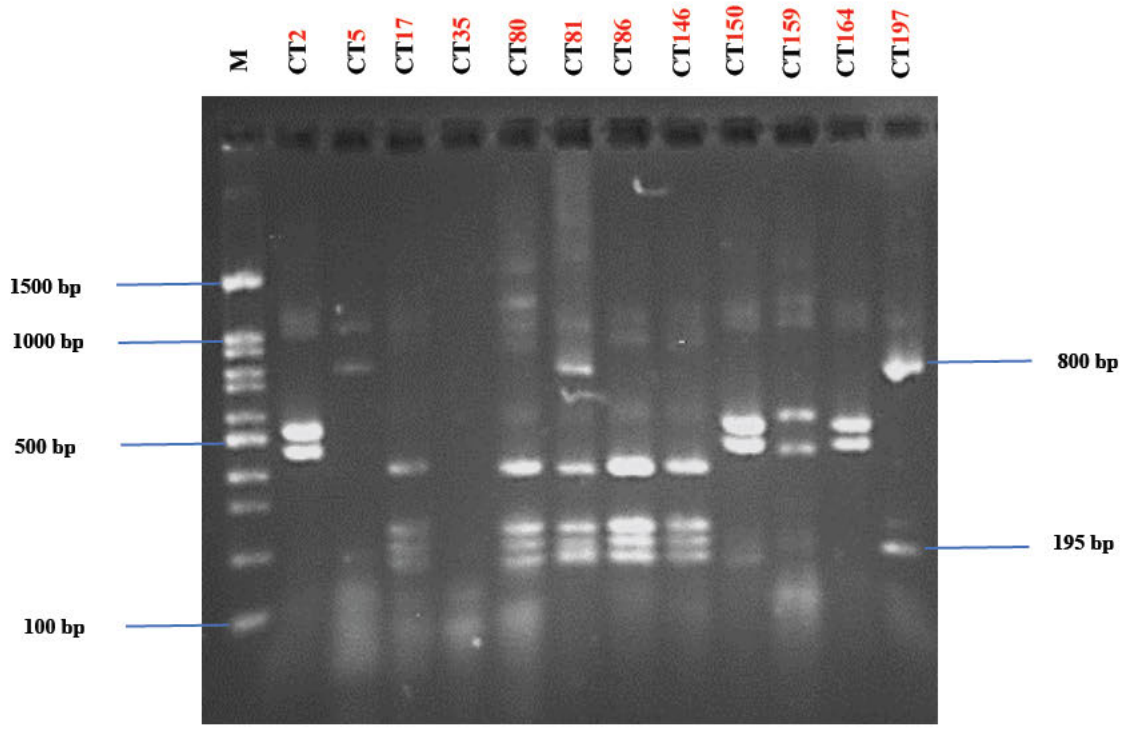

Figure 5: *HinfI* RFLP pattern of the digested *Omp1* gene amplicon resolved on a 2% agarose gel. Lane M: 100 bp DNA ladder (ThermoFisher Scientific, United States) and banding profiles of the samples.

Discussion

Sexually transmitted infections (STIs) including *C. trachomatis* are of major public health concern across the globe.[18,74] The associated epidemics of these infections among MSM are of global concern as well.[74] Men who have sex with men are at increased risk of acquiring STIs. This is due to their sexual network, behavioural or biologic factors.[75,76] In this study, the prevalence of *C. trachomatis* from the urethral site was 6% (12/200). This prevalence was higher than a recent study which reported a prevalence of 4.1% (61/1480) for urethral *C. trachomatis* in a population of MSM from Vietnam.[77] A previous study conducted in Brazil also reported a lower prevalence (2.2%; 6/273) for urethral *C. trachomatis* when compared to the current study.[78] The high prevalence observed in this study could be due to behavioural factors such as number of sexual partners, stimulant use before/during sex and unprotected anal intercourse (UAI). It is well known that UAI is a risk factor for *C. trachomatis* infection in MSM.[74] In our study we found that having multiple sex partners, and engaging in anal and group sex were associated with *C. trachomatis* infection. As a result, promoting condom use in this high-risk population will be crucial in preventing and controlling the spread of chlamydia and other STIs.[74] Additionally, our study found that younger age was significantly associated with testing positive for *C. trachomatis*. Most cases of chlamydia infection were found in MSM aged 25-30 years. This was followed by the 18-24 age group. This finding was in concordance with reports from other studies which demonstrated that younger age was associated with having chlamydia infection.[74,79,80,81] However, to the best of our knowledge, there are no available data from South Africa to compare our finding. This observation could be attributed to younger people participating in sexual practices that involve a greater number of sexual contacts.[80]

This present study investigated the distribution of *C. trachomatis* genotypes in asymptomatic MSM visiting clinics in Durban, South Africa. Typing of *C. trachomatis* by *ompA* genotyping is vital for molecular epidemiology and comparative studies of strains between STIs and trachoma populations, and remains the most widely used typing scheme among *Chlamydia* investigators.[82,83] This typing technique can be used in epidemiologic monitoring to evaluate changes in genotype distribution and reveal patterns of transmission in sexual networks.[84]

Based on the PCR-RFLP technique used in this study, three circulating genotypes of *C. trachomatis* were identified. In the population studied, genotypes E and I were the two most prevalent genotypes. Only one sample yielded the genotype, J. These results are similar to those reported in a previous study in which 8 *ompA* genotypes, including these three genotypes E, I, and J were identified from 203 MSM samples.[85] Another study conducted in Shenzhen, China also revealed the presence of these three genotypes among MSM.[86] On the basis of clinical syndromes, the genotypes (E, I, and J) detected from our study are responsible for respiratory and conjunctival infections,[52] as well as urogenital infections.[25,53] *C. trachomatis* has the ability to produce acute complications and long-term sequelae in

the upper genital tract.[30] In men, if untreated, *C. trachomatis* infections can lead to serious complications, including non-gonococcal urethritis, epididymitis and infertility.[23]

Published data from previous studies conducted in Europe and other countries of the world have shown that genotypes G and D are the most prevalent in MSM populations.[26,65,69,82,85,86,87-89] However, our study revealed genotype E as the most prevalent in MSM. The prevalence of this genotype is in accordance with the findings of earlier investigations on different patient groups[53, 54,59] and consistent with the findings from Spain where an infection with genotype E was associated with a large number of clinically asymptomatic MSM.[90] A plausible explanation for this observation is the lack of association between genotype and the presence of clinical symptoms.[69] A study by Klint et al found that certain genotypes not only appear to dominate among MSM population but also that some genetic variants are associated with infections in MSM. Moreover, strains can be shared with MSM populations around the globe through the internationalization of sexual contacts which implies that behaviour rather than cell tropism/other biological factors is the reason some strains are more common within an MSM community.[69]

An important finding from our study is the detection of genotype I in the MSM population. Although previous studies[60,61,65] have reported this genotype in heterosexual male patients as well as women,[30,43,84] only a few studies[85,86] have identified it among MSM. This finding may be an indication that some of these individuals are bisexual.

This study is not without its limitation. Firstly, the number of samples used for the genotyping analysis was low. Future studies recruiting a larger population of MSM are needed so that sound conclusions can be made regarding the distribution of genotypes amongst MSM in our local setting. In this study, the RFLP technique which is widely used in the epidemiological studies of *C. trachomatis* strains was employed. This typing method may not be the most appropriate method as the discriminatory power is known to be low and does not allow for identification of single nucleotide changes which are the most predominant variations in *ompA* gene.[91,92] A more effective and robust alternative for investigating the genetic diversity of *C. trachomatis*, would be the Multi Locus Sequence Typing (MLST) scheme as described by Smelov et al.,[83] which can be used for future studies by our research group.

Conclusion

The present study described the genotypic diversity of *C. trachomatis* associated with South African MSM. The PCR-RFLP technique revealed the presence of only three genotypes (E, I, and J) circulating in the MSM population of the study area. Findings from this study can serve as a baseline for further research aimed at distinguishing between new, recurrent, and persistent infections.

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Findings from chapter four, showed that our study cohort were at risk of acquiring sexually transmitted infections. Sex practices, condom use and circumcision status were among the factors significantly associated with testing positive for *C. trachomatis*. We identified three *C. trachomatis* genotypes (E, I, and J) circulating in our setting and study population. Of the three genotypes identified, E was the most prevalent. The highlight of this study was the detection of genotype I in the study population. Previous studies have found this genotype to be predominant in women and its presence in the studied population might be an indication that some of these men are bisexual. The urinary tract is colonized by microbial communities that impacts urinary health. In addition, variation in the microbiota has been reported to be significantly influenced by sexual practice. It has also been suggested that the bacterial composition of the male urinary microbiota is related to STIs. The following chapter assessed the bacterial composition of the urinary microbiome in South African MSM with and without *C. trachamatis* infection.

CHAPTER FIVE

Comparison of the Urinary Microbiome in Men who have Sex with Men with and without *Chlamydia trachomatis* Infection

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Abstract

The urinary tract is colonized by microbial communities that impacts urinary health. Previous studies have suggested that the bacterial composition of the male urinary microbiota is related to sexually transmitted infections (STIs). This study assessed the bacterial composition of the urinary microbiome in South African men who have sex with men (MSM) with and without *Chlamydia trachomatis* (*C. trachomatis*). This cross-sectional study used urine samples from MSM 18 years and older, attending care at the King Edward VIII hospital and the Aurum Institute in Durban, South Africa. A total of 200 samples were tested for *C. trachomatis* infection using the Applied Biosystems™ TaqMan® Assays. Urinary microbiomes were characterized using 16S rRNA (V3 and V4) gene sequencing on the Illumina MiSeq platform. The bacterial taxonomic analysis showed a higher abundance of *Streptococcus*, *Corynebacterium* and *Staphylococcus* in all the sequenced samples. Moreover, *Prevotella* and *Lactobacillus* were detected in the urine samples of the men. Alpha diversity metrics showed a slight increase in microbial diversity in positive samples; however, this was not significant (ANOVA, $P > 0.05$). Principal coordinates analysis (PCoA) showed that the microbiome of *C. trachomatis* infected MSM was not clearly separated from those uninfected. Distinct bacterial communities (Operational Taxonomic Unit level) were not detected between positive and negative samples (PERMANOVA $F_{1,22}= 1.0284$, $R^2=0.047\%$, $P=0.385$) using normalized unweighted UniFrac dissimilarities. The majority of microbiome studies on MSM to date have focused on the gut microenvironment. Few studies, however, have provided data regarding the normal composition of the male urethral microbiomes or if these microbiomes are associated with male STIs. This study adds to the growing body of knowledge highlighting the composition of the urinary microbiome in MSM with and without STIs.

Introduction

The human microbiome consists of approximately 38 trillion commensal and pathogenic microorganisms existing in the human body [1, 2]. The human body harbours complex microbial communities ranging from bacteria and eukaryotic viruses, to protozoa and fungi. These microorganisms are known to coexist with human organisms and are found in several body sites such as the skin, nasal tract, respiratory tract, gastrointestinal tract and urogenital tract [1, 3-6].

The human microbiome has been shown to contribute to the human condition [1], ensuring vital functions for the host and may affect health and disease susceptibility by regulating physiological homeostasis, energy metabolism, and immune-related bioprocesses [4, 6]. This community of microorganisms play an important role in maintaining health [7]. However, changes in the microbiome or dysbiosis can result in the development of several pathological conditions such as type II diabetes mellitus [8], Crohn's disease [9], non-alcoholic fatty liver disease [10], allergies [11], inflammatory bowel disease [12] and bacterial vaginosis [13].

Although comprehensive studies investigating the human microbiome have generated an unprecedented understanding of the microorganisms that colonize the human body [14], not all body sites were considered suitable for initial examination [15]. The urinary tract was initially thought to be sterile [16-19] and was not included in early microbiome research [17]. However, the existence of a urinary microbiome has been acknowledged over the last decade [19-21]. With the advent of evolving sensitive tools such as next-generation sequencing [16], the characterization of urine samples from human bladders discovered unique bacterial communities that make up the urinary microbiome [18, 19, 22].

Rich and complex microbial communities are present in the human urinary tract, and alterations in its composition (dysbiosis) are thought to be implicated in varying diverse urinary tract symptoms and disorders [23, 24]. Research has demonstrated that the urinary microbiome influences the risk for the development of urinary tract infections (UTIs), urinary incontinence, and persistent lower urinary tract symptoms in women [22, 25, 26]. Nevertheless, little is known about the urinary microbiome of males, especially those of men who have sex with men (MSM) [27]. The microbiome of the male urogenital tract is poorly described. On the other hand, it has been proposed that bacterial colonization of the male urethra may influence the risk of sexually transmitted infections (STIs) [7] including *Chlamydia trachomatis* (*C. trachomatis*).

C. trachomatis is an obligate intracellular bacterium that causes the disease chlamydia [28, 29]. Following infection with *C. trachomatis*, the pathogen migrates into the female upper genital tract [28-30], where it infects the cervix and urethral columnar epithelial cells, resulting in uncomplicated

cervicitis and urethritis [31]. Several studies have found that the genital microbiome plays a role in the host's susceptibility to chlamydial infection [7, 32-35].

Microbiome variation has been found to be significantly influenced by sexual practice. MSM exhibit a distinct microbiome signature characterized by *Prevotella* enrichment and increased alpha diversity, which is linked with receptive anal intercourse in both males and females [36]. It is assumed that certain genital microbiomes may cooperate with *C. trachomatis* to absorb indole from *Prevotella*, allowing the bacteria to evade the inhibition of IFN-γ [35, 37]. MSM are a vastly understudied population at high risk for genitourinary diseases such as chlamydia and other STIs [38, 39]. The urethra, rectum and pharynx are the common sites of STIs among this key population due to unprotected oral/anal sexual behaviors [38]. Exposure to the gut and oral microbiome through these routes may lead to shifts in the urinary microbiome of MSM that increase predilection for genitourinary diseases [27]. Majority of microbiome studies on MSM to date have focused on the gut microenvironment [40-42]. Few studies, however, have provided data regarding the normal composition of the male urethral microbiomes or if these microbiomes are associated with male STIs [7, 27, 43]. Hence, our study aimed to assess the bacterial composition of the urethral microbiome in a cohort of MSM with and without *C. trachomatis* infection.

Materials and Methods

Ethics statement

Ethical approval for this study was obtained from the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (BREC/00002798/2021).

Study population

This cross-sectional study used urine samples from sexually active MSM, 18 years and older, and attending care at the King Edward VIII Hospital and the Aurum Institute, both located in Durban, South Africa. All laboratory assays were conducted at the School of Clinical Medicine Research Laboratory of the Nelson R. Mandela School of Medicine, University of KwaZulu-Natal.

Sample processing and DNA Isolation

There were 200 available urine samples from study participants enrolled between October 2021 and July 2022. These samples were refrigerated between 2-8°C until processed. A total of 10 ml of each urine was centrifuged at 14 000x g for 45 minutes, and the supernatant discarded. The recovered pellets were then subjected to further molecular analyses. Deoxyribonucleic acid (DNA) was extracted from the sample pellets using the PureLink Microbiome Kit (ThermoFisher Scientific, United States), following the manufacturer's protocol, and quantified using the Nanodrop Spectrophotometer

(ThermoFisher Scientific, United States). The extracted DNA was stored at –20°C until subsequent analysis.

Detection of C. trachomatis

C. trachomatis was detected using the Applied Biosystems™ TaqMan® Assays. Commercial primers and probes (Ba04646249_S1) which targets the translocated actin-recruiting phosphoprotein of *C. trachomatis* were used. Amplification was performed on the Quant Studio 5 Real-time polymerase chain reaction (PCR) detection system (Thermo-Fisher Scientific, United States). Each reaction was performed in a final volume of 10 µl and included: 1 µl FAM-labelled probe/primer mix for individual targets, 5 µl Fast Start 4x probe master mix (Thermo-Fisher, Ba04646249_S1), 2 µl template DNA and 2 µl nuclease-free water. The runs included non-template control reactions. Amplification was performed under the following conditions: 1 cycle at 95°C for 30 seconds followed by 45 cycles of denaturation at 95°C for 30 seconds and annealing at 60°C for 30 seconds. At the end of the annealing period, samples with fluorescence were deemed positive for chlamydia.

Illumina 16S rRNA gene sequencing

The V3-V4 hypervariable regions of the 16S *ribosomal ribonucleic acid (rRNA)* gene was amplified using forward primer 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG- 3' and reverse primer 5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3' [44, 45]. Reactions were performed in a total volume of 25 µL, consisting of 25 ng gDNA template, 1.5 µL (0.3 µM) of each primer, 12.5 µL KAPA HiFi HotStart Ready-mix (1X) (ROCHE, Randburg, South Africa) and 7 µL nuclease-free water. The DNA library was prepared using the PCR products, following the Illumina TruSeq® Nano DNA Sample Preparation protocol. Sequencing was performed on an Illumina MiSeq, using a 2x 301 cycle paired-end approach.

Data processing and statistical analysis

The 16S rRNA gene sequences obtained from Illumina sequencing were analyzed using the QIIME 2 pipeline [46]. Sequencing remnants and adapter sequences were removed using Cutadapt [47]. The paired-end sequences were imported into QIIME 2, quality-controlled and combined using DADA2. DADA2 first truncated both the forward and reverse sequencing reads at positions in the reads supplied. These positions were determined based on a visual inspection of the quality distribution of the reads. Sequences were grouped into amplicon sequence variants (ASVs) based on 99% sequence similarity. The ASV table was built, chimeras were removed, and the taxonomy for the *16S rRNA* reads was assigned using the SILVA 132 [48] database. For taxonomic classification, the q2-feature-classifier

plugin was used to train naïve Bayes classifiers on reference data sets using Scikit-learn. Alpha rarefaction curve was plotted with 1000 sampling depths.

The processed gene sequence data was further analyzed using RStudio, an integrated development environment (IDE) for the programming language R [49]. The following R packages were used during the processing of the taxonomic data: Phyloseq [50], Tidyverse [51], Vegan [52], and Biomformat [53]. For beta diversity analysis, the taxonomic structures of the microbial communities were visualized using principal coordinate analysis (PCoA). A permutational analysis of variance (PERMANOVA) was used to assess beta diversity differences between different sampled groups. These analyses were performed with the "adonis" functions in vegan for R.

Results

In the current study, a total of 200 MSM were tested for *C. trachomatis* infection. Of the 200 men, 12 (6.0%) tested positive for *C. trachomatis*. An overview of study population demographics and clinical data has been published elsewhere [54]. Samples from 24 participants were subjected to sequencing. The selection was based on the fact that only 12 samples were positive for *C. trachomatis*. Therefore, to have a balanced number of samples, same amount of positive and negative samples were sequenced. Of these samples, 12 were from MSM positive for *C. trachomatis* infection, and 12 were from MSM who tested negative for *C. trachomatis* infection. However, one of the 12 positive samples was excluded from microbiome analysis, as the sample had insufficient amount of DNA. Therefore, a total of 23 samples were sequenced.

High-throughput sequencing yielded between 291 and 249 759 reads for the twenty-three samples. After quality filtering, denoising, merging and chimera filtering between 5 and 86 943 sequences remained (Supplementary Table 1). Rarefaction curves suggested that the sequencing depth was adequate to capture most of the prokaryotic diversity in most of the samples (Supplementary Figure 1).
Based on phylogenetic classification using the SILVA 16S rRNA gene database, sample diversity at the phylum level classified most reads as *Firmicutes*, *Actinobacteriota*, *Proteobacteria*, *Bacteroidota* and *Fusobacteriota* (Figure 1A, Supplementary Table 2). Differences in the distribution of these phyla among samples were also observed. *Firmicutes* were present in 22/23 of the urine samples, while *Actinobacteriota* and *Proteobacteria* were identified in 15/23 and 11/23 urine samples, respectively. *Bacteroidota* and *Fusobacteriota* were present in 7/23 and 6/23 urine samples. However, *Verrucomicrobiota* were detected in only 2/23 urine samples. Sample diversity at the genus level identified a diverse consortium in all samples (Figure 1D, Supplementary Table 3). Some of the more dominant genera in the samples included *Streptococcus, Corynebacterium, Staphylococcus*, *Prevotella*,

Gardnerella, and *Lactobacillus*. In contrast, sequences corresponding to *Chlamydia*, *Alloprevotella*, and *Parvimonas* were less abundant.

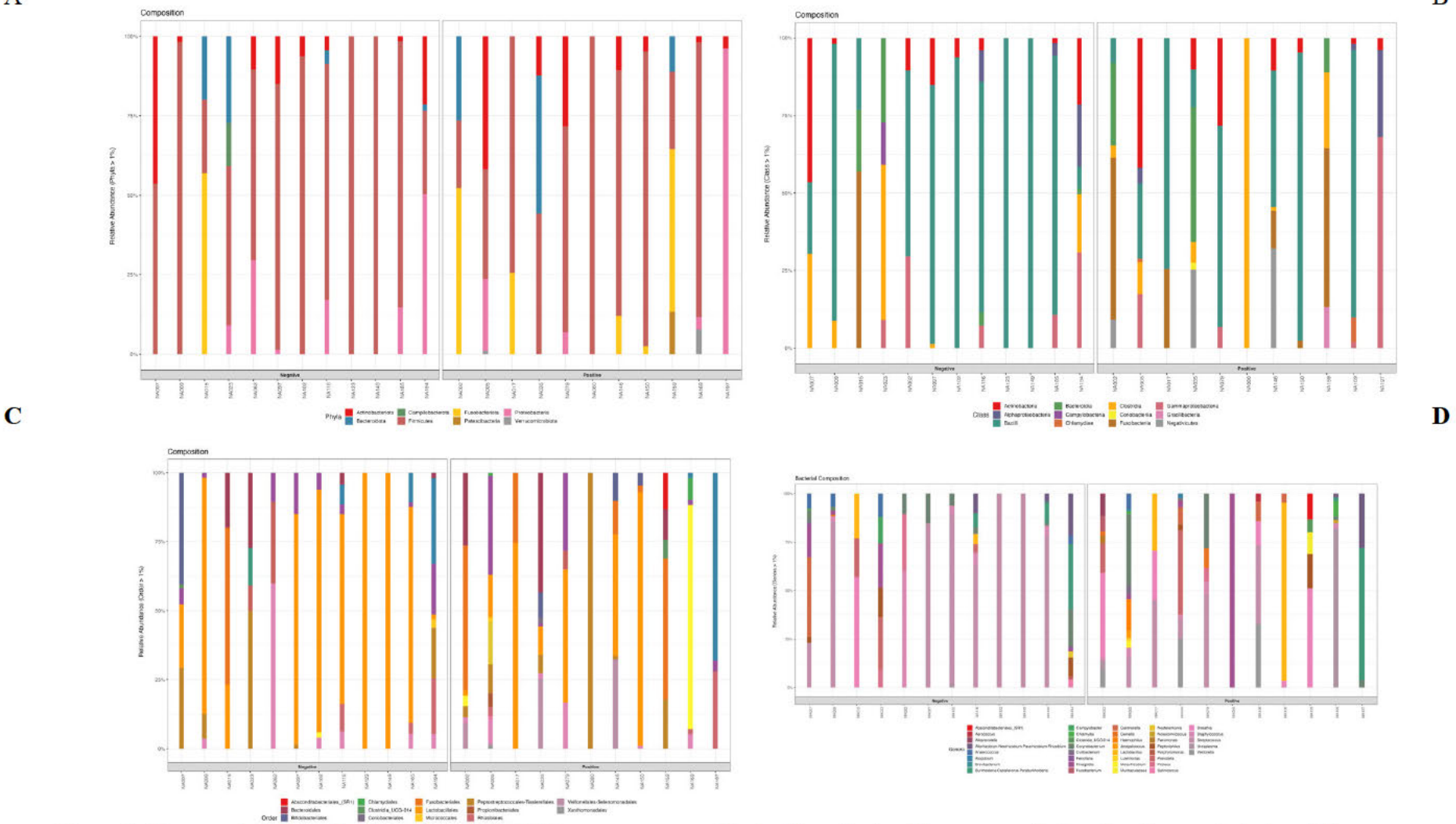

Figure 2: Taxonomic information based on *16S rRNA* gene sequences. Stacked bar charts of taxonomy relative abundances at phylum (A), class (B), order (C) and genus (D) level between *C. trachomatis* negative and *C. trachomatis* positive groups.

A total of 455 amplicon sequence variants (ASV) were identified across all samples. Of the total number of ASVs, 92 (representing 5.92% of the total number of sequences) were unique to the positive samples, 302 (16%) were unique to the negative samples and 61 (78.08%) were shared between both (Figure 2).

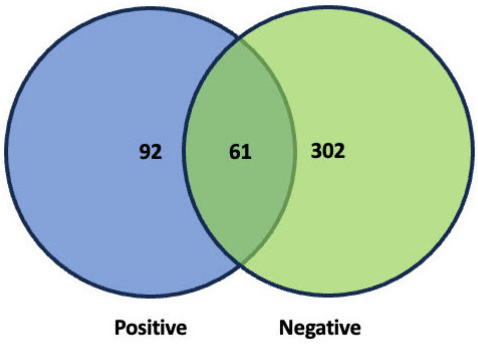

Figure 2: Venn diagram showing the number of unique and shared ASVs between samples from *C. trachomatis* infected (blue) and uninfected (green) groups.

Alpha diversity metrics (Richness, Shannon and Simpson) showed a slight increase in microbial diversity in positive samples; however, this was not significant (ANOVA, $P > 0.05$) (Figure 3).

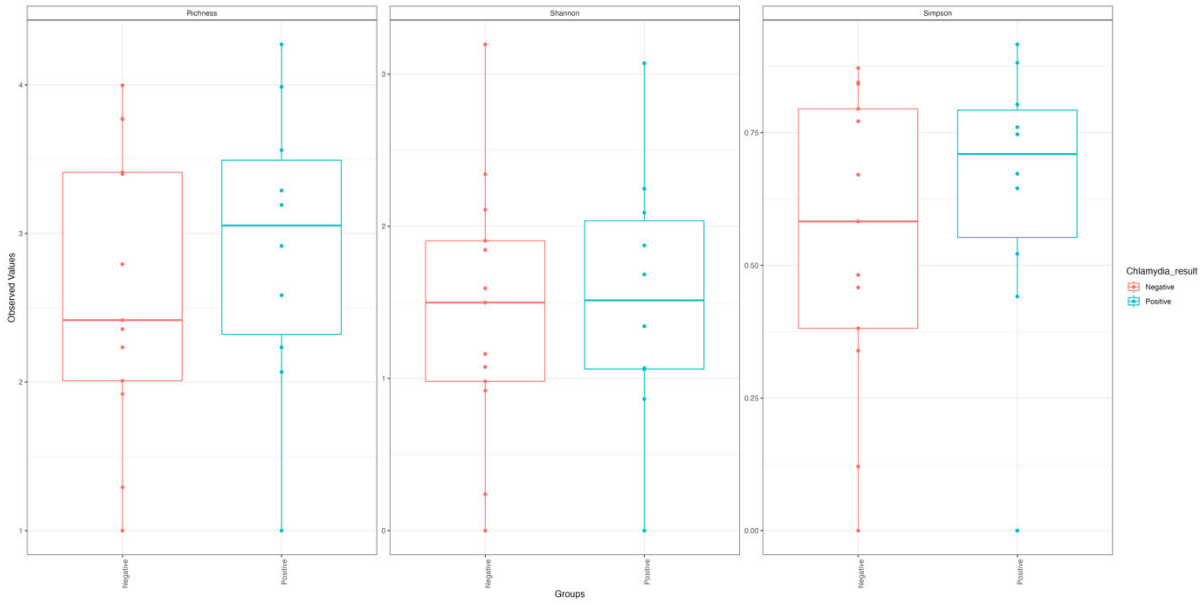

Figure 3: Alpha diversity boxplots of Richness, Shannon and Simpson indexes. Diversity measures of microbial species recovered from urine samples were not different between *C. trachomatis* positive and *C. trachomatis* negative groups.

Principal coordinates analysis (PCoA) showed that the microbiome of *C. trachomatis* infected MSM was not clearly separated from those uninfected. Overall, whether samples tested positive or negative was not important in explaining structural differences among bacterial communities (Figure 4). Distinct bacterial communities (Operational Taxonomic Unit level) were not detected between positive and negative samples (PERMANOVA $F_{1,22}$= 1.0284, R^2=0.047%, P=0.385) using normalized unweighted UniFrac dissimilarities.

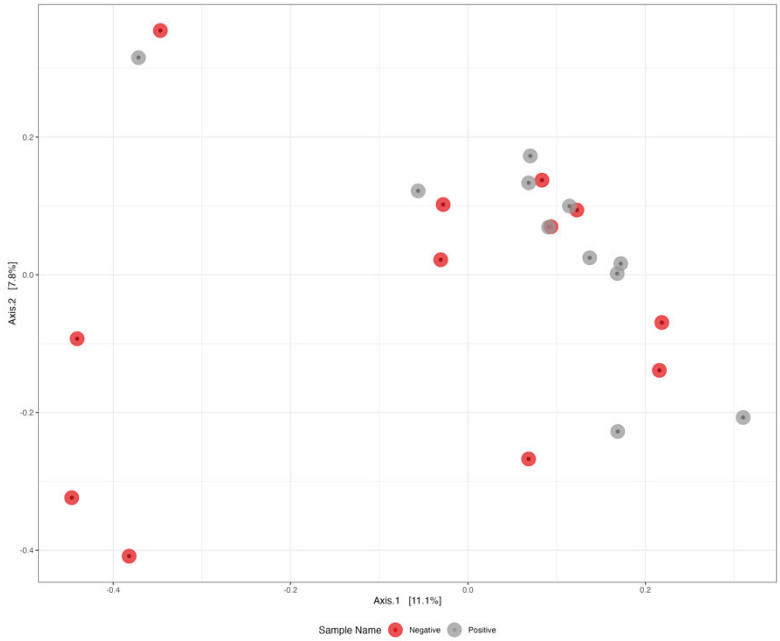

Figure 4: Principal Coordinates Analysis (PCoA) plot showing structural differences between bacterial communities based on unweighted Unifrac dissimilarity matrix. The grey dots indicate urine samples from MSM positive for *C. trachomatis* and the red dots correspond to urine samples from MSM negative for *C. trachomatis*. The percent variations explained by each principal coordinate are shown in parentheses.

Presence-absence results differed between MSM with and without *C. trachomatis* in 1 of the 5 groups analysed. The genera *Chlamydia* (p_{adj} = 0.001) had higher probabilities of presence in *C. trachomatis* positive MSM (Figure 5).

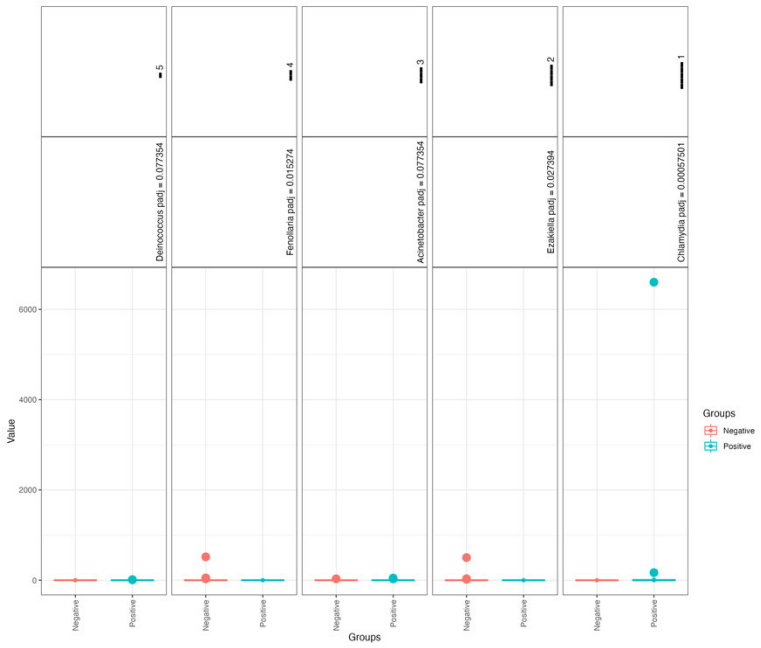

Figure 5: Presence-absence analyses identified only 1 genus with a significantly different probability of presence between MSM with and without *C. trachomatis*.

Figure 6 shows the log2 fold change (log2FC) of other identified bacterial taxa constituting the urine microbiome of MSM. A positive log2FC means a higher abundance for that taxa, while a negative log2FC means a lower abundance for that taxa. Of the 108 abundant taxa, 37 (red) were less abundant, and 71 (blue) were more abundant.

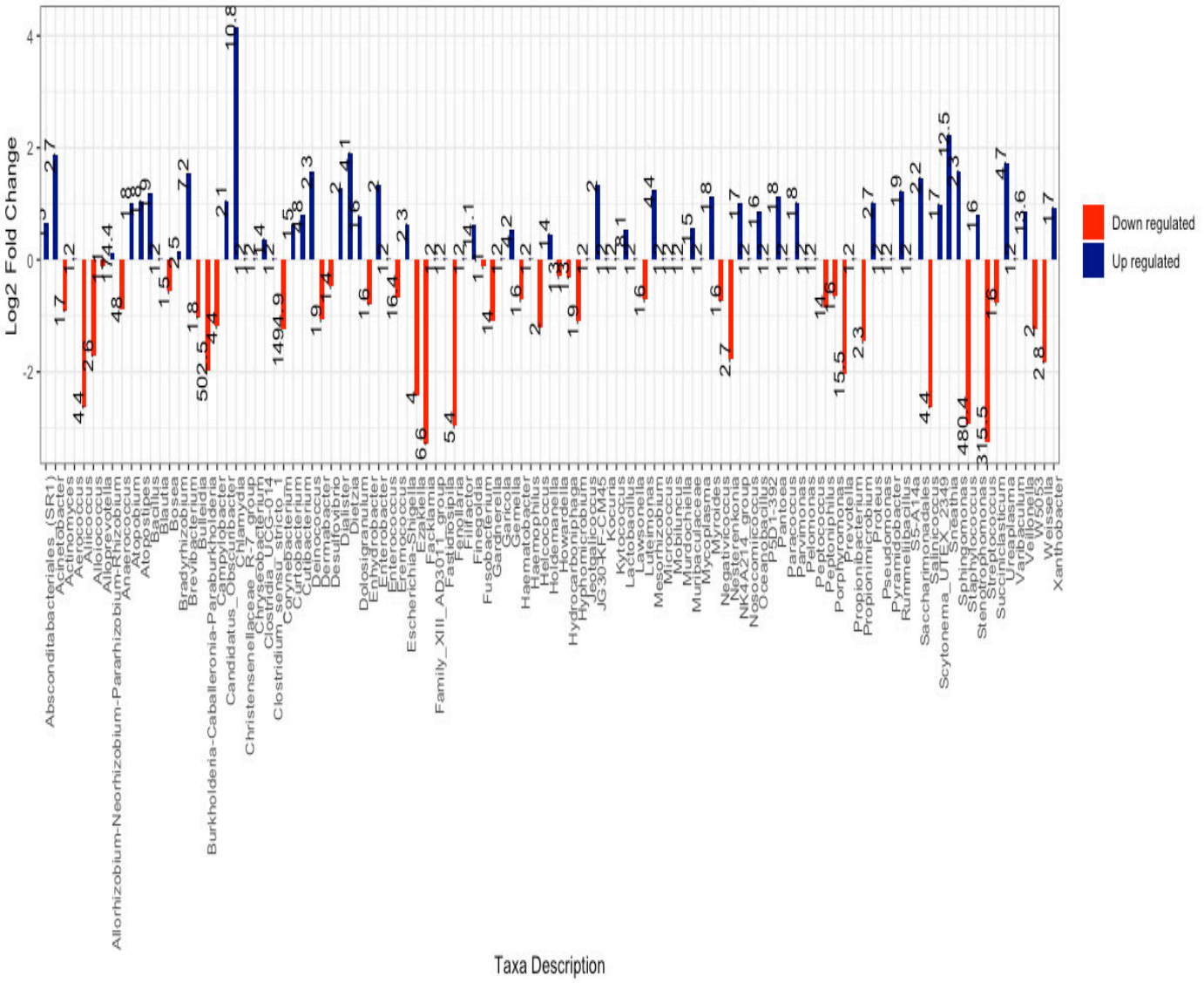

Figure 6: Log2 fold change in abundance of other bacterial taxa identified in urine samples

Discussion

In this study, bacterial taxa were identified and classified to the genus level. The analysis of taxonomic classification at the phylum level showed that most samples were typically composed of five major phyla. Sequences corresponding to five bacterial phyla including *Firmicutes, Actinobacteriota, Proteobacteria, Bacteroidota* and *Fusobacteriota* were frequently detected, whereas sequences corresponding to *Verrucomicrobiota* were less abundant.

Chlamydia trachomatis, a common sexually transmitted infection (STI), is the most reported bacterial STI in the world. It is considered a public health problem due to the high occurrence of active infection in women and men, including MSM [55-57]. Currently, around 131 million people worldwide are infected by *C. trachomatis* each year [58]. To the best of our knowledge, there is a dearth of information in South Africa on the bacterial microbiota in urine from MSM with and without *C. trachomatis*. In this study, the urinary microbiome in the MSM with and without *C. trachomatis* was investigated.

The male genital microbiota has not been as thoroughly researched as the cervicovaginal microbiota. A few of these studies have used urine samples, which may be more reflective of the urethral microbiota [59]. Some studies have also characterized using next-generation sequencing (NGS), the urine and urethral microbiota in asymptomatic men [60-62] and reported a diverse bacterial microbiota which were highly variable from person to person. Similarly, our study revealed a high diversity, as study participants harboured diverse taxons. This is in consonance with findings from a previous study, which reported that a large diversity of microbial species were present in the urinary microbiota of MSM [27]. Another study found that the male urethral microbiota contains a very diverse composition of bacteria, both in patients and in healthy controls [63]. In contrast, a study by Dong et al. [62] found fewer genera. Dong et al. found 88 genera in samples from 10 men positive for *C. trachomatis*, *N. gonorrhoeae* or *T. vaginalis*, and 131 genera in the 22 STI-negative men [62]. Our finding also supports prior studies indicating that the male urinary tract harbours a complex bacterial community that is distinct from other body microenvironments [64-66].

A study by Nelson and colleagues on urine microbiomes and asymptomatic STIs in men identified the following bacterial phyla: *Firmicutes* (52.6%), *Actinobacteria* (18.7%), *Fusobacteria* (10.0%), *Proteobacteria* (9.4%) and *Bacteroidetes* (7.4%). The authors of this study also reported that urine from sexually active men often contains complex microbial communities and that the composition of these urine communities is relevant to STIs [7]. In another published study that characterized the urinary microbiome of 10 healthy men using the 16S rRNA sequencing-based methods, bacterial DNA sequences taken from the urine of study participants were predominantly *Firmicutes* (65%). Other predominant phyla included *Actinobacteria* (15%), *Bacteroidetes* (10%) and *Proteobacteria* (8%) [67]. Another study found that *Bacteroidetes*, *Firmicutes,* and *Proteobacteria* were the most abundant phyla

in three types of urine samples from a population of men [68]. The observed differences in phyla distribution from this current study support the results from a previous study, which indicated that there is substantial intra-individual variation in urine microbiomes at the phylum level [7].

The analysis of the urinary microbiota composition at the genus level showed a higher abundance of *Streptococcus, Corynebacterium* and *Staphylococcus* in both the *C. trachomatis* infected and uninfected groups. In addition, *Prevotella*, *Gardnerella*, and *Lactobacillus* were detected in the urethral microbiota of MSM in this study. Other identified genera included *Sneathia* and *Veillonella*, which were less abundant. Consistent with our findings, Sawhney et al. also recovered similar organisms in a cohort of asymptomatic MSM: *Gardnerella, Streptococcus, Staphylococcus,* and *Corynebacterium* [27]. In a prior study involving males visiting a STI clinic, *Lactobacillus*, *Corynebacteria*, and *Streptococcus* were among the most frequently detected genera. That same study also showed that STIs were associated with organisms not usually related to the male urinary tract, such as *Sneathia* and *Prevotella* [7]. Other studies have also detected similar genera in urine samples from their population of men, including *Prevotella*, *Veillonella* [62, 68], *Lactobacillus,* and *Sneathia* [62]. Findings from our study support that commonly recovered genera in the female urinary microbiota are similar to those detected in MSM [27] and that the urogenital microbiota overlap to a larger extent with vaginal and other mucosal-associated organisms as well as skin-associated microorganisms [35, 69].

Urotypes such as those dominated by *Prevotella* [70], and *Lactobacillus* [43, 64, 66, 67] in the female urinary microbiota have been described as members of the male urinary microbiota [43, 64, 66, 70, 71]. Previous studies have consistently reported that the proportion of *Lactobacillus* in the male urinary microbiota is lower when compared to females [43, 64, 67]. Our results support this observation as only 5 of the 23 urine samples in our investigation had *Lactobacillus* detected. Similarly, a study that assessed the composition of the urinary microbiota of MSM reported that *Lactobacillus* was recovered in only 2 of 129 urine samples [27].

Changes in the urinary microbiota of MSM may result from exposure to unique microenvironments, such as the gut and oral microbiota, through anal and oral sex [27]. Similarly, it has been demonstrated that the rectal microbiota of MSM who engage in condomless receptive anal intercourse, is enriched for the family *Prevotellaceae* [41]. The gut microenvironment has been the subject of the majority of MSM microbiome research to date. These studies have shown that the gut microbiome of MSM is enriched in *Prevotella* when compared to non-MSM populations [27, 40-42]. In the present study, we detected *Prevotella* from urine samples in both the MSM with and without *C. trachomatis* groups. This finding is in agreement with that of Sawhney et al. who also found that *Prevotella* was one of the most frequently recovered genera from urine samples in MSM. Their finding also suggested a potential gut

reservoir as the source of *Prevotella* in the urinary microbiota, with the recovery of *Prevotella* in 59% of MSM who reported recent insertive anal sex [27]. Nelson et al. [7] have demonstrated that the presence of *Prevotella* in urine samples positively correlates with STIs in men and that men with asymptomatic STIs such as *C. trachomatis* were more likely to have urine microbiota dominated by fastidious, anaerobic, and uncultivated bacteria than those without STIs [7].

Our study also identified other genera commonly found in healthy men (*Streptococcus*, *Staphylococcus*, *Gardnerella* and *Corynebacterium*), including those (*Sneathia* and *Veillonella*) observed in the vaginal microbial communities of women with bacterial vaginosis (BV) [72, 73]. Similar to our findings, these genera including *Veillonella*, were among the microorganisms recovered in a study involving a cohort of healthy MSM [27]. Manhart et al in their study, found *Sneathia spp*., which is commonly present in BV to be associated with male idiopathic urethritis [74]. Another study, which examined urine in males visiting a STI clinic without symptoms of urethritis, found that *Sneathia spp*. were the dominant components of the urine microbiome [7]. Several studies have also shown that the male urinary microbiota is dominated by bacteria such as *Corynebacterium* [75], *Gardnerella* [27, 64], *Staphylococcus* [76, 77] and *Streptococcus* [27, 64, 67].

Corynebacterium was one of the commonly detected bacteria in MSM with and without *C. trachomatis* in our study. *Corynebacterium* is frequently recovered from male urine as well as urethral samples [43 61, 63, 78]. *Corynebacterium* is generally considered a commensal of the male genital microbiota [79] and has been associated with positive health outcomes [80]. However, since *Corynebacterium* is a diverse genus, it is possible that specific *Corynebacterium spp*. account for a small number of urethritis cases [80]. A couple of case reports have associated specific species of *Corynebacterium* with urethritis [81-83]. Therefore, further studies on the role of *Corynebacterium spp*. in infection may be relevant to understanding a variety of urogenital disorders in male populations and MSM as well.

Gardnerella has been shown to be present in the vaginal microbiome, with certain species thought to play a key role in BV [84]. However, this genus has been associated with urethritis in men [85, 86]. *Gardnerella* was recovered in this current study. A study conducted among men with and without idiopathic urethritis showed that 28% of MSM who reported not having a female sexual partner three months prior to enrolment had *Gardnerella* present in their urethral microbiota [80]. Other studies have reported that BV-associated bacteria were detected in the genital microbiome of male partners of women without BV [87, 88]. Hence, it is plausible that BV-associated bacteria including *Gardnerella*, are not entirely acquired from the vagina but may form part of the autochthonous male urethral microbiota or may be present in the rectum or mouth [80].

A limitation of this study is that DNA libraries generated for the target metagenomics for this study did not generate adequate sequencing data due to the low number of sequences. Also, the sample size used in the study was small. Future studies with a larger sample size will provide more clarity on the role of urinary microbiomes in MSM sexual health and non-gonococcal urethritis (NGU).

Conclusions

In conclusion, this study adds to the growing literature highlighting the urinary microbiome in MSM. Urine samples from MSM with and without *C. trachomatis* infection was shown to contain diverse microbiota. However, there was no obvious difference between the two groups. We identified several specific bacterial taxa associated with this key population and also showed that BV-associated genera, such as *Gardnerella* and *Sneathia*, were components of the urethral microbiota of MSM. It is clear from our study that unique microbial communities, especially those associated with the female urinary tract, are present in MSM. Therefore, continued research into the role of the urinary microbiota in urinary health of this understudied population is required. This will provide clarity as to whether these microorganisms impact risk for STIs.

Data Availability

Data will be made available upon request.

Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary Material

Supplementary Figure 1. Rarefaction curve showing species richness in association with sample sequences. Supplementary Table S1. Denoising statistics. Supplementary Table S2. Phylogenetic classification at phylum level. Supplementary Table S3. Relative abundance at the genus level.

Supplementary Table 1. Denoising statistics

Supplementary Table 2. Relative abundance at genus level.

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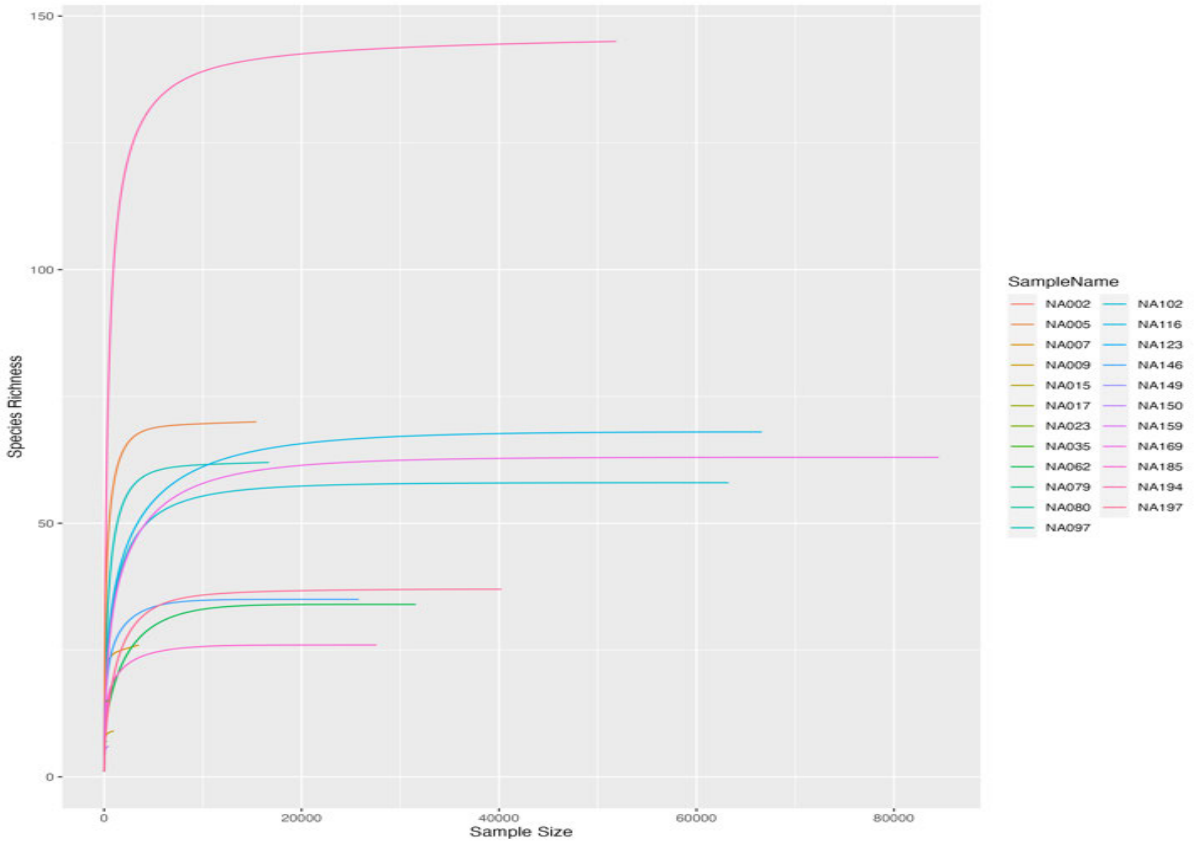
Supplementary Material



Supplementary Figure 1: Rarefaction curve showing species richness in association with samples sequences.

Supplementary Table 1: Denoising statistics

Sample ID	Input	Filtered	Percentage of input passed filter	Denoised	Merged	Percentage of input merged	Non-chimeric	Percentage of input non-chimeric
NA007	31062	6235	20,07	5871	4505	14,5	3534	11,38
NA009	3202	1377	43	1263	1155	36,07	993	31,01
NA015	440	138	31,36	78	65	14,77	65	14,77
NA023	701	174	24,82	111	66	9,42	66	9,42
NA062	123523	53229	43,09	52132	50079	40,54	36411	29,48
NA097	75117	29496	39,27	28693	24808	33,03	16746	22,29
NA116	236828	111792	47,2	109735	102399	43,24	71133	30,04
NA102	177416	86875	48,97	86183	80201	45,21	63484	35,78
NA123	291	83	28,52	23	8	2,75	8	2,75
NA149	396	124	31,31	75	66	16,67	66	16,67
NA185	80447	34353	42,7	33927	32996	41,02	27990	34,79
NA194	249759	81488	32,63	79998	67310	26,95	52073	20,85
NA002	1133	383	33,8	306	272	24,01	272	24,01
NA005	238257	61638	25,87	57537	51358	21,56	41400	17,38
NA017	11939	3763	31,52	3385	2316	19,4	2154	18,04
NA035	3145	855	27,19	706	510	16,22	497	15,8
NA079	1526	438	28,7	362	276	18,09	276	18,09
NA080	299	66	22,07	21	5	1,67	5	1,67
NA146	131610	41913	31,85	41282	37972	28,85	25918	19,69
NA150	2898	751	25,91	689	576	19,88	501	17,29
NA159	730	253	34,66	142	45	6,16	45	6,16
NA169	223944	114472	51,12	113078	104819	46,81	86943	38,82
NA197	129169	52048	40,29	51586	47893	37,08	40418	31,29

Supplementary Table 2: Relatvie abundance at genus level

Supplementary Table 2: Relative abundance at genus level

							Negative												Positive										
ASV	**Kingdom**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**NA007**	**NA009**	**NA015**	**NA023**	**NA062**	**NA097**	**NA116**	**NA102**	**NA123**	**NA149**	**NA185**	**NA194**	**NA002**	**NA005**	**NA017**	**NA035**	**NA079**	**NA080**	**NA146**	**NA150**	**NA159**	**NA169**	**NA197**
b7129eeffa4	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	0,22718169	0,85599194	0	0	0	0,81216694	0,62228261	0,86671096	1	1	0,77728277	0,00094697	0,02205882	0,14713702	0,45098039	0,10261569	0,48188406	0	0,39509188	0	0	0,00026029	0,00099367
9a50d4fcde8	Bacteria	Fusobacteriota	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Sneathia	0	0	0,56923077	0	0,00041202	0	0,00025522	0	0	0	0	0	0,44117647	0,00058362	0,25490196	0	0	0	0,11964023	0,0244898	0,51111111	0	0
bb48d4fe64C	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	0	0	0,23076923	0	0,00019016	0	0,05278645	0	0	0	0	0,00063776	0	0,00038908	0,29411765	0	0	0	0	0,9122449	0	0	0,00136629
3678d26eb8	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales	Finegoldia	0,16366237	0,02114804	0	0,22727273	0	0,00113766	0,00015013	0,00023728	0	0	0,00021732	0,0122333	0	0,02295571	0	0,03822938	0	1	0,00426456	0	0	0,00011831	0
d4e740bc5c2	Bacteria	Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium	0,05865522	0,01913394	0	0	0,10465264	0,14681756	0,03540115	0,0616616	0	0	0,01648013	0,17721861	0	0,34336295	0	0,01006036	0,2826087	0	0,00034892	0	0	0,01777052	0,03788349
5f8b99d96b!	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia-Caballeronia-Paraburkholderia	0	0	0	0	0	0,00658643	0,0711326	0,00294229	0	0	0,1064508	0,30577458	0	0	0	0	0	0	0,00399318	0	0	0,01948605	0,6746988
c5ccea21b43	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	0	0	0,2	0,27272727	0	0,00041914	0,04203699	0	0	0	0	0,00401979	0,15073529	0	0	0,43460765	0	0	0	0	0	0	0
94e5c42670!	Bacteria	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus	0,00286123	0,02618328	0	0	0,59603195	0,00197593	0,06158419	0,03734814	0	0	0,05219313	0,00831014	0	0,04643019	0	0,02012072	0,06521739	0	0	0,01020408	0	0,02794539	0,00228543
8124cdacda3	Bacteria	Firmicutes	Bacilli	Mycoplasmatales	Mycoplasmataceae	Ureaplasma	0	0	0	0	0	0	0,00016515	0,01855543	0	0	0	0	0,03676471	0	0	0	0	0	0,00189967	0	0	0,80439412	0
1e9b089e9e	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Veillonella	0	0	0	0	0	0	0,00135119	0	0	0	0,00572277	0	0,09191176	0	0	0,25352113	0	0	0,31801969	0	0	0	0
c64d0b19442	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0	0	0	0	0	0,00742471	0,09826147	0,00352759	0	0	0,04085624	0,19780071	0	0	0	0	0	0	0,00345041	0	0	0,0182556	0,27658676
4f17105bf00	Bacteria	Actinobacteriota	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Gardnerella	0,40343348	0	0	0	0	0	0,00277744	0	0	0	0	0	0	0	0	0,09054326	0	0	0,10075987	0,04693878	0	0	0
4f8285827d6	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales	Peptoniphilus	0,03147353	0	0	0,15151515	0,00044371	0,00065864	0,00016515	0,00012655	0	0	0	0,08621289	0	0,00408534	0	0,02816901	0	0	0,00213228	0	0,17777778	0	0
26672648de	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Morganellaceae	Proteus	0	0	0	0,09090909	0,29376902	0	0	0	0	0	0	0	0	0	0	0	0,06884058	0	0	0,00612245	0	0	0
ead034c20ec	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales	Anaerococcus	0,07496423	0,06747231	0	0,12121212	0,00031694	0,00958026	7,51E-05	0,00098076	0	0	0,0003622	0,04174397	0	0,07593541	0	0	0	0	0,00585407	0	0	0,00054424	0,00196249
9ef5a20469c	Bacteria	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	0	0	0	0	0	0	0	0	0	0	0	0	0	0,15193567	0	0	0	0	0	0	0	0	0
44536e7d85	Bacteria	Firmicutes	Bacilli	Staphylococcales	Gemellaceae	Gemella	0	0,01007049	0	0	0	0	0,00019517	0	0	0	0	0	0,02205882	0,0047338	0	0	0,10144928	0	0	0	0	0	0
5b14d8715d	Bacteria	Campilobacterota	Campylobacteria	Campylobacterales	Campylobacteraceae	Campylobacter	0	0	0	0,13636364	0,00038032	0	0	0	0	0	0	0,00057978	0	0,00090785	0	0	0	0	0	0	0	0	0
945ca6f18e0	Bacteria	Patescibacteria	Gracilibacteria	Absconditabacteriales_(SR1)	Absconditabacteriales_(SR1)	Absconditabacteriales_(SR1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,13333333	0	0
baefe60379e	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Prevotellaceae	Alloprevotella	0	0	0	0	0	0	0	0	0	0	0	0	0,11397059	0	0	0	0	0	0	0	0	0	0
689221951c	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Muribaculaceae	Muribaculaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,11111111	0	0
b75cef6143b	Bacteria	Verrucomicrobiota	Chlamydiae	Chlamydiales	Chlamydiaceae	Chlamydia	0	0	0	0	0	0	0	0	0	0	0	0	0	0,01089424	0	0	0	0	0,00050399	0	0	0,07810984	0,00034778
e30a5fdf3ff	Bacteria	Fusobacteriota	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	0	0	0	0	0	0	0	0	0	0	0	0	0,08088235	0	0	0	0	0	0	0	0	0	0
983417319c	Bacteria	Firmicutes	Clostridia	Clostridia_UCG-014	Clostridia_UCG-014	Clostridia_UCG-014	0,01287554	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,06666667	0	0
b02a8d33d0	Bacteria	Actinobacteriota	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Cutibacterium	0	0	0	0	0	0	0,00076567	0,00023728	0	0	0	0,00017393	0	0,04753259	0	0	0	0	0,00038769	0	0	0	0,0008943
e1a1353ae2	Bacteria	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Jeotgalicoccus	0	0	0	0	0	0	0	0,00064857	0	0	0	0	0	0,03774074	0	0	0	0	0	0	0	0,00360853	0
a8a626b83b	Bacteria	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Salinicoccus	0	0	0	0	0	0	0	0,0010124	0	0	0	0,0397534	0	0	0	0	0	0	0	0	0	0	0
5ff1bb6103d	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales	Parvimonas	0	0	0	0	0	0	0	0	0	0	0	0	0,04044118	0	0	0	0	0	0	0	0	0	0
8dcb73f7b68	Bacteria	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Aerococcus	0	0	0	0	0	0	0,00165145	0	0	0	0	0	0	0	0	0	0	0	0,03768318	0	0	0	0
ff040254126	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Mesorhizobium	0	0	0	0	0,00047541	0	0	0	0	0	0	0	0	0,03164516	0	0	0	0	0	0	0	0,0003786	0,00039747
a91983be7e	Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae	Nesterenkonia	0	0	0	0	0	0	0	0,00047456	0	0	0	0,02906617	0	0	0	0	0	0	0	0	0	0	0
025b3aa69a	Bacteria	Actinobacteriota	Coriobacteriia	Coriobacteriales	Atopobiaceae	Atopobium	0	0	0	0	0	0	0,00507447	0	0	0	0	0	0	0	0	0,0221328	0	0	0,00166705	0	0	0	0
8f5861dd05f	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales	Fenollaria	0,0148784	0	0	0	0	0,00185618	0	0	0	0	0	0,01001082	0	0	0	0	0	0	0	0	0	0	0
c5d4496f5bc	Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Brevibacteriaceae	Brevibacterium	0	0	0	0	0	0,00305371	0,00018016	0,00023728	0	0	0	0	0	0,01219117	0	0	0	0	0	0	0	0,00110031	0
cf015ff505ec	Bacteria	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Nosocomiicoccus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,01675303	0
44eb603ac1	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales	Ezakiella	0,00572246	0	0	0	0,00107759	0	0	0	0	0	0	0,00970161	0	0	0	0	0	0	0	0	0	0	0
05666c6c867	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	0	0	0	0	0,00022186	0	0	0	0	0	0	0,01553803	0	0	0	0	0	0	0	0	0	0	0
c83512ec28c	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Luteimonas	0	0	0	0	0	0	0,00012011	0	0	0	0	0	0	0,01407172	0	0	0	0	0	0	0	0	0
9ee06c2152	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales	Murdochiella	0	0	0	0	0	0	0	0	0	0	0	0,00981756	0	0	0	0	0	0	0	0	0	0	0
9ae4a24f8af	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Haematobacter	0	0	0	0	0	0	0,00012011	0	0	0	0	0	0	0,00966215	0	0	0	0	0	0	0	0	0
45a84b99b3	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Alloiococcus	0	0	0	0	0	0	0	0,00044293	0	0	0	0,00873531	0	0	0	0	0	0	0	0	0	0,00030761	0
b306b15627	Bacteria	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Eremococcus	0	0	0	0	0	0,00173642	0,00012011	0	0	0	0	0,00318878	0	0,00272356	0	0	0	0	0	0	0	0,00040226	0,00081977
b316544909	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Negativicoccus	0,00228898	0	0	0	0	0	0	0	0	0	0	0,00606834	0	0	0	0	0	0	0	0	0	0	0
c5897998e1	Bacteria	Actinobacteriota	Actinobacteria	Corynebacteriales	Dietziaceae	Dietzia	0	0	0	0	0	0,00035926	0	0	0	0	0	0,00150742	0	0,0058362	0	0	0	0	0	0	0	0,00061522	0
bb4adb1b72	Bacteria	Firmicutes	Bacilli	Staphylococcales	Staphylococcaceae	Aliicoccus	0	0	0	0	0	0	0	0,0010124	0	0	0	0,00344001	0	0	0	0	0	0	0	0	0	0,00311162	0
bc3ad1f27cb	Bacteria	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Facklamia	0	0	0	0	0,00022186	0,00329322	0	0,00166097	0	0	0	0,00092764	0	0	0	0	0	0	0	0	0	0,00049691	0
48609b1df1t	Bacteria	Actinobacteriota	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Propionimicrobium	0,00200286	0	0	0	0	0	0	0	0	0	0	0,00028989	0	0	0	0	0	0	0,00294642	0	0	0	0
3b6ddd8d31	Bacteria	Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Lawsonella	0	0	0	0	0	0	0	0	0	0	0	0,00131416	0	0,0038908	0	0	0	0	0	0	0	0	0
dd8c8d60bfc	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	0	0	0	0	0	0	0,00108095	0	0	0	0	0	0	0,00356657	0	0	0	0	0	0	0	0	0,00054652
51707d8b45	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales	Helcococcus	0	0	0	0	0	0,00023951	0	0,0015186	0	0	0,00043464	0,00243506	0	0	0	0	0	0	0	0	0	0,00033127	0
8cb24777cb4	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00380966	0,00034778
cf6d3714c3d	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcales-Tissierellales	W5053	0	0	0	0	0	0	0	0,00026892	0	0	0	0,00378788	0	0	0	0	0	0	0	0	0	0	0
cc69d24aaf9	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	0	0	0	0	0	0	0,00046541	0	0	0	0	0	0	0,00311264	0	0	0	0	0	0	0	0,00035494	0
e01ccd7499	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	0	0	0	0	0,00098251	0	0	0	0	0	0	0	0	0,00278841	0	0	0	0	0	0	0	0	0
7337431bc3	Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae	Kocuria	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00337203	0	0	0	0	0	0	0	0	0
1d0dc7756fd	Bacteria	Firmicutes	Clostridia	Clostridia	Hungateiclostridiaceae	Fastidiosipila	0	0	0	0	0	0	0	0	0	0	0	0,0032081	0	0	0	0	0	0	0	0	0	0	0
2c4d2cdac1e	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Anaerovoracaceae	S5-A14a	0	0	0	0	0	0	0	0	0	0	0	0,00278293	0	0	0	0	0	0	0	0	0	0	0
879cc9fdee8	Bacteria	Firmicutes	Bacilli	Lactobacillales	P5D1-392	P5D1-392	0	0	0	0	0	0,00269445	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
630860d80a	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00259387	0	0	0	0	0	0	0	0	0
c16ee82d45	Bacteria	Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Veillonellaceae	Dialister	0	0	0	0	0	0	0	0	0	0	0	0,00158472	0	0	0	0	0	0	0,0006203	0	0	0	0
615094ae2e	Bacteria	Actinobacteriota	Actinobacteria	Actinomycetales	Actinomycetaceae	Varibaculum	0	0	0	0	0	0	0	0	0	0	0	0,00200989	0	0	0	0	0	0	0	0	0	0	0
a2885e32b8	Bacteria	Firmicutes	Clostridia	Peptococcales	Peptococcaceae	Peptococcus	0	0	0	0	0	0	0	0	0	0	0	0,00177798	0	0	0	0	0	0	0	0	0	0	0
0300f34d01	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Bosea	0	0	0	0	0	0	9,01E-05	0	0	0	0	0	0	0,00136178	0	0	0	0	0	0	0	0	0
119b3ba9fec	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	Saccharimonadales	Saccharimonadales	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00129693	0	0	0	0	0	0	0	0	0
c17b57af304	Bacteria	Actinobacteriota	Actinobacteria	Actinomycetales	Actinomycetaceae	Mobiluncus	0	0	0	0	0	0	0	0	0	0	0	0,00123686	0	0	0	0	0	0	0	0	0	0	0
de8892eef1	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Filifactor	0	0	0	0	0	0	0	0	0	0	0	0,00123686	0	0	0	0	0	0	0	0	0	0	0
ffc36e27c82	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Escherichia-Shigella	0	0	0	0	0	0	0	0	0	0	0	0,00013528	0	0,00103755	0	0	0	0	0	0	0	0	0
9db2817f5c4	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium	0	0	0	0	0	0	0	0	0	0	0	0,00025124	0	0,00090785	0	0	0	0	0	0	0	0	0
dc78602ea0	Bacteria	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	JG30-KF-CM45	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00110239	0	0	0	0	0	0	0	0	0
2bf739b12f6	Bacteria	Cyanobacteria	Vampirivibrionia	Obscuribacterales	Obscuribacteraceae	Candidatus_Obscuribacter	0	0	0	0	0	0	3,00E-05	0	0	0	0	0	0	0,00103755	0	0	0	0	0	0	0	0	0
594db979ce	Bacteria	Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Anaerovoracaceae	Family_XIII_AD3011_group	0	0	0	0	0	0	0	0	0	0	0	0,00102427	0	0	0	0	0	0	0	0	0	0	0
e6adc4e5ae	Bacteria	Deinococcota	Deinococci	Deinococcales	Deinococcaceae	Deinococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00051877	0	0	0	0	0	0	0	0	0,00034778
3d2fa2f2822	Bacteria	Firmicutes	Bacilli	Mycoplasmatales	Mycoplasmataceae	Mycoplasma	0	0	0	0	0,00082404	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
b9c56e11ffa	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Atopostipes	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00077816	0	0	0	0	0	0	0	0	0
e2bac22481	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Weeksellaceae	Chryseobacterium	0	0	0	0	0	0	0,00076567	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
688ac51ebal	Bacteria	Cyanobacteria	Cyanobacteriia	Cyanobacteriales	Nostocaceae	Scytonema_UTEX_2349	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00071331	0	0	0	0	0	0	0	0	0
919ef4fa379	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Xanthobacter	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00064847	0	0	0	0	0	0	0	0	0
d94c762eb6	Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae	Garicola	0	0	0	0	0	0	0	0	0	0	0	0,00063776	0	0	0	0	0	0	0	0	0	0	0
9f0dedd638	Bacteria	Actinobacteriota	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Propionibacterium	0	0	0	0	0	0	0	0	0	0	0	0,0005218	0	0	0	0	0	0	0	0	0	0	0
1e49ea0519	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Pelomonas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00052167
a81edf8bb4	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Dolosigranulum	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00051877	0	0	0	0	0	0	0	0	0
659d2dc23a	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Erwiniaceae	Pantoea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00050399	0	0	0	0
df829f8d328	Bacteria	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	0	0	0	0	0	0	0,00036032	0,00012655	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3b1820ffea9	Bacteria	Synergistota	Synergistia	Synergistales	Synergistaceae	Pyramidobacter	0	0	0	0	0	0	0	0	0	0	0	0,0004445	0	0	0	0	0	0	0	0	0	0	0
9ff1b82a831	Bacteria	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	0	0	0	0	0	0	0	0	0	0	0	0,0004445	0	0	0	0	0	0	0	0	0	0	0
0940387043	Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Micrococcaceae	Micrococcus	0	0	0	0	0	0	0,00042037	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
b2cb20fee99	Bacteria	Firmicutes	Clostridia	Christensenellales	Christensenellaceae	Christensenellaceae_R-7_group	0	0	0	0	0	0	0	0	0	0	0	0,00040584	0	0	0	0	0	0	0	0	0	0	0
5481dfdbd4	Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Dermacoccaceae	Kytococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00033127	0
f2cbb29998f	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00031944	0
8f6e2a91e2	Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Blautia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00028395	0
0a119bf9afc	Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Dermabacteraceae	Dermabacter	0	0	0	0	0	0	0	0	0	0	0	0,00027056	0	0	0	0	0	0	0	0	0	0	0
2210979789	Bacteria	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Bulleidia	0	0	0	0	0	0	0	0	0	0	0	0,00025124	0	0	0	0	0	0	0	0	0	0	0
cfa64d02b5e	Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Microbacteriaceae	Curtobacterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00023261	0	0	0	0
97a1f31d64	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium	0	0	0	0	0	0	0	0,00022146	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0615a368fcc	Bacteria	Actinobacteriota	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	0	0	0	0	0	0	0	0	0	0	0	0,00021259	0	0	0	0	0	0	0	0	0	0	0
32ab1812bd	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Enterobacter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00020113	0
52c31c3c252	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Rummeliibacillus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00017747	0
612c7d470fd	Bacteria	Firmicutes	Negativicutes	Acidaminococcales	Acidaminococcaceae	Succiniclasticum	0	0	0	0	0	0	0	0	0	0	0	0,00017393	0	0	0	0	0	0	0	0	0	0	0
d563571a07	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Myroides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00015381	0
0a1f5ed70dc	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Enhydrobacter	0	0	0	0	0	0	0,00013512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64b2267714	Bacteria	Firmicutes	Clostridia	Oscillospirales	Oscillospiraceae	NK4A214_group	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00013014	0
fe25a06245!	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Oceanobacillus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,00010648	0
c3188521b2t	Bacteria	Desulfobacterota	Desulfovibrionia	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	0	0	0	0	0	0	0	0	0	0	0	9,66E-05	0	0	0	0	0	0	0	0	0	0	0
0ca1de0b0d	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9,46E-05	0
a99c0ce6a4f	Bacteria	Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Howardella	0	0	0	0	0	0	0	0	0	0	0	5,80E-05	0	0	0	0	0	0	0	0	0	0	0
e6cb374ae3	Bacteria	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Hydrocarboniphaga	0	0	0	0	0	0	0	4,75E-05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
75b9c1c135c	Bacteria	Firmicutes	Bacilli	Erysipelotrichales	Erysipelotrichaceae	Holdemanella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4,73E-05	0

CHAPTER SIX

Discussion

This study investigated the prevalence of and risk factors associated with *N. gonorrhoeae* and *C. trachomatis*, barriers and facilitators to accessing health care, as well as the genotypic diversity of *C. trachomatis* among South African MSM living in Durban. The study also assessed the bacterial composition of the urinary microbiome in the study population. Several studies on the prevalence of *N. gonorrhoeae* and *C. trachomatis* have been conducted in different geographic regions with variable infection rates depending on the population studied and diagnostic methods employed. Prevalence studies conducted in MSM have reported rates ranging from 0.2% to 16.0% for *N. gonorrhoeae* (Benn et al. 2007; Wong et al. 2013; Ross et al. 2014; Hançali et al. 2019; Ribeiro et al. 2019; Mwaniki et al. 2023) and 2.2% to 26.0% for *C. trachomatis* (Mimiaga et al. 2009; Wong et al. 2013; Ross et al. 2014; Hançali et al. 2019; Adamson et al. 2022; Mashingaidze et al. 2023). Findings from our study fell within this range, for *N. gonorrhoeae*, and that for *C. trachomatis* as well (3% and 6%, respectively).

In this study, a considerable number of MSM who tested positive for *N. gonorrhoeae* and *C. trachomatis* infections were asymptomatic. These findings suggest that syndrome-based STI management is an inadequate approach for reducing the burden of STIs in MSM, as this screening approach can create a false sense of security in asymptomatic patients, favouring the progression of silent STIs towards irreversible complications (Unemo et al. 2017). Of the MSM who reported previous history of STIs, 32% reported that they had been diagnosed with at least one STI in the past. Similarly, findings from previous studies found that asymptomatic gonorrhoea or chlamydia infection was associated with a past exposure with at least one STI (Shafer et al. 2002; Mimiaga et al. 2009; Tongtoyai et al. 2015).

Several risk factors have been reported to be associated with chlamydia and gonorrhoea infections. In our study, a range of factors were found to be associated with *C. trachomatis* and/or *N. gonorrhoeae* infections. We found that sex for cash and drug use were associated with testing positive for gonorrhoea. A similar study reported that inhaled drug use was significantly associated with higher rates of gonorrhoea and chlamydia infections (Ribeiro et al. 2019). Persons who transact sex are not likely to be able to negotiate safe sex due to fear of losing out on transactional benefits (Esser et al. 2017). Other studies have found that MSM having sexual contact with individuals who exchanged sex for money or drugs was significantly associated with a high prevalence of gonorrhoea (Dutt et al. 2015; Rebe et al. 2015).

Prior studies have reported that younger age was associated with having chlamydia and/or gonorrhoea infections (Jin et al. 2007; Peters et al. 2011; Castillo et al. 2015; van Liere et al. 2015; Ribeiro et al.

2019). Our analysis found that being younger was significantly associated with testing positive for *C. trachomatis*. Younger populations are more likely to engage in risky sexual practices, and with a greater number of sexual contacts (Allan-Blitz et al. 2017); this may have contributed to the observed trend. Also being older (30-39 years) reduced the risk of acquiring chlamydia infection (p=0.026) in this study. A study conducted by Cunha et al. in a population of MSM in Brazil showed that for every additional 10 years of age, the prevalence of having at least one STI decreased by 22% (Cunha et al. 2015).

There is convincing evidence to show that male circumcision reduces the risk of HIV and STIs (Bailey et al. 2007; Gray et al. 2007; Sobngwi-Tambekou et al. 2009). In our study, being circumcised was shown to reduce the risk of *C. trachomatis* infection (p=0.01). Yuan et al., in a systematic review and meta-analysis found that circumcision was associated with 23% reduced odds of STIs among MSM in low-and middle-income countries (Yuan et al. 2019). A South African study also reported that circumcised adult males had lower STI prevalence rates when compared with those who were uncircumcised (Iyemosolo et al. 2021). It has been reported that cohabiting and/or married MSM are less likely to use condoms (Abara et al. 2017). Previous research have indicated that MSM in committed relationships may see condoms as limiting sexual intimacy and indicating mistrust of a partner (Adam et al. 2005). Our study found that cohabitation status was significantly associated with testing positive for *N. gonorrhoeae*.

Having multiple sexual contacts has been reported to predispose MSM to multiple concurrent STIs (Machouf et al. 2011; Jones et al. 2019). Findings from our study, revealed that MSM having between 2 to 4 sex partners were at risk of testing positive for *C. trachomatis*. Similar study by Ribeiro et al., reported a significant association between infection and having a higher number of concurrent sex partners (Ribeiro et al. 2019). It has been shown that STIs including chlamydia are frequently transmitted through oral-genital or oral-rectal pathways (Edwards and Carne. 1998). In this study, engaging in anal/oral sex showed a marginal significance with testing positive for *C. trachomatis*. The relationship between group sex participation and STIs have been discussed in previous studies (Rice et al. 2016; van den Boom et al. 2016; Scheidell et al. 2017). Furthermore, other studies have documented that group sex is common among MSM (Mimiaga et al. 2011; Prestage et al. 2011; Goedel et al. 2018). In this study, a high proportion of MSM were involved in group sex. Likewise, group sex was significantly associated with testing positive for *N. gonorrhoeae.* A study by Rice et al. reported that participation in group sex was associated with more than twofold increased prevalence of *N. gonorrhoeae* infection (Rice et al. 2016).

N. gonorrhoeae and *C. trachomatis* can infect multiple anatomical sites, including the urogenital, pharyngeal and anorectal regions of both males and females (Patton et al. 2014; Tongtoyai et al. 2015; van Liere et al. 2017). For MSM, it is recognised that even in the absence of genital infection,

individuals may nevertheless present with rectal and pharyngeal infections (Patton et al. 2014; van Liere et al. 2014).

Extragenital (pharyngeal and rectal) screening for MSM who are sexually active is essential to providing this key population with all-inclusive sexual health services (Marcus et al. 2011). This is due to the fact that extragenital *N. gonorrhoeae* and *C. trachomatis* infections are common among MSM and are often undiagnosed (Gunn et al. 2008). Reported estimates have shown that >70% of extragenital *N. gonorrhoeae* infections and 85% of extragenital *C. trachomatis* infections are missed with urethral screening alone (Patton et al. 2014). Also, several studies have also indicated that the burden of chlamydia and gonorrhea infections in MSM is disproportionately higher in the oropharynx and rectum compared to urogenital sites such as urine (Marcus et al. 2011; Anschuetz et al. 2016; Chan et al. 2016). For this reason, annual urethral and rectal screening for *N. gonorrhoeae*/*C. trachomatis*, and pharyngeal screening for *N. gonorrhoeae* are recommended based on exposure (CDC. 2015).

Our study however, screened for *N. gonorrhoeae* and *C. trachomatis* infections among MSM using urine samples. Rectal screening for *N. gonorrhoeae*/*C. trachomatis* could not be achieved in this study due to participants' refusal to present themselves for rectal sample collection. Feeling uncomfortable was the reason for their refusal as most preferred to provide a urine sample. Other studies have reported fear of taking the swab incorrectly, believing urine testing would identify rectal infections (van der Helm et al. 2009; Dodge et al. 2010), perceived no risk of rectal infection, feeling uncomfortable (Weng et al. 2022), discomfort with taking or providing a detailed sexual risk assessment and with the collection of rectal samples (Lutz. 2015) as barriers to chlamydia and gonorrhoea rectal screening in MSM.

While urine samples may not be the most ideal for identifying *N. gonorrhoeae* and *C. trachomatis* in MSM, they do have certain benefits. Previous studies have demonstrated that non-invasive screening methods, such as urine sample, could eliminate some of the barriers for STI detection (Peeling et al. 1998; Cook et al. 2005). Also, López-Corbeto et al. (2020) in their study, reported that non-invasive methods were preferred by patients, and they further suggested that non-invasive methods substantially increased the convenience and acceptability of screening in a variety of settings (López-Corbeto et al. 2020). Furthermore, PCR techniques have been documented to exhibit high sensitivities and specificities in urine samples (Peeling et al. 1998; Cook et al. 2005).

Notwithstanding the fact that infections caused by *N. gonorrhoeae* and *C. trachomatis* at different anatomical sites have distinct characteristics, including clinical manifestations, varying infection durations, and concerns about drug-resistant pathogens (Chow et al. 2016; Wi et al. 2017), the ability in detecting and treating these infections regardless of site, is what is most important (Hiransuthikul et

al. 2019). Nonetheless, we recognise the significance of STI screening at extragenital sites for MSM, and since bacterial STI burden is higher among MSM in South Africa, screening at these sites should be considered as part of an approach to effectively lower the burden of STIs among South African MSM. A future research direction will be to employ the rectal specimen self-collection method for anorectal gonorrhoea and chlamydia testing, as well as explore the acceptability of this collection method among this key population.

Our study also investigated the barriers and facilitators associated with MSM accessing health care. Lea et al. in their study, reported that some gay men preferred accessing sexual health care via their general practitioner (GP) due to ease of access and concerns about anonymity (Lea et al. 2019). In this study, 62% of MSM reported that they would rather seek care from a GP at a surgery and half of MSM positive for *N. gonorrhoeae* preferred to access care from a GP based in a surgery. Studies have shown that MSM oftentimes experience discrimination particularly from public sector healthcare workers (Lane et al. 2008; Rispel et al. 2011). In our study population, being afraid to visit a health care clinic due to stigma was reported by 47.5% of the study participants. A study conducted among MSM utilizing health services in South Africa, found among other factors, discrimination as a major barrier to accessing health services (Rispel et al. 2011). Discrimination has been reported to negatively affect health-seeking behaviours (Ayala. 2014).

Molecular epidemiological studies are vital for understanding the genetic population structure, and gaining insight into the transmission of *C. trachomatis*. In this study we determined genotypic diversity of *C. trachomatis* in a group of MSM resident in Durban, South Africa. Genotypes E, I, and J were the circulating genotypes identified in our study cohort. A previous study conducted in Shenzhen, China revealed the presence of these 3 genotypes among MSM (Li et al. 2011). Another study detected 8 *ompA* genotypes including genotypes E, I, and J in 203 MSM samples (Christerson et al. 2012).

Although published data from around the world have shown that genotypes G and D are the most prevalent in MSM populations (Klint et al. 2006; Li et al. 2011; Christerson et al. 2012; Bom et al., 2013; Herrmann et al. 2015; Köksal et al. 2016), our study revealed that the most prevalent *C. trachomatis* genotype in the study population was genotype E (60%). This is consistent with the findings from a study in Spain where an infection with genotype E was associated with a large number of clinically asymptomatic MSM (Mejuto et al. 2013). A plausible explanation for this observation is that strains can be shared with MSM populations around the globe through the internationalization of sexual contacts (Klint et al. 2006). Genotype I was detected in this study. While previous studies have reported genotype I in heterosexual male patients as well as women (Niemi et al. 2011; Herrmann et al. 2015; Brasiliense et al. 2016; Kiguen et al. 2019), other studies have identified this genotype in MSM (Li et

al. 2011; Christerson et al. 2012). This study finding would suggest that some study participants identify as bisexual.

The male urogenital microbiome has not been thoroughly researched, including those of MSM (Sawhney et al. 2021; Tuddenham et al. 2021). It has been proposed that bacterial colonization of the male urethra may influence the risk of STIs (Nelson et al. 2010). In this study we assessed the bacterial composition of the urinary microbiome in a population of MSM with and without *C. trachomatis* infection. Our study revealed a high diversity of bacterial microbiota, as study participants harboured diverse taxons. Findings from a previous study reported that a large diversity of microbial species were present in the urinary microbiota of MSM (Sawhney et al. 2021). Our finding also supports prior studies indicating that the male urinary tract harbours a complex bacterial community that is distinct from other body microenvironments (Thomas-White et al. 2018; Morand et al. 2019; Pohl et al. 2020).

Sequences corresponding to five bacterial phyla including *Firmicutes, Actinobacteriota, Proteobacteria, Bacteroidota* and *Fusobacteriota* were frequently detected in this study. Our analysis of the urinary microbiota composition at the genus level revealed a higher abundance of *Streptococcus,* Corynebacterium and Staphylococcus in both the MSM with and without *C. trachomatis* groups. Consistent with our findings, Sawhney et al. also recovered similar organisms in a population of asymptomatic MSM (Sawhney et al. 2021). In addition, *Prevotella* was detected in the urinary microbiota of our study cohorts. Sawhney et al. have suggested a potential gut reservoir as the source of *Prevotella* in the urinary microbiota of MSM engaged in insertive anal sex (Sawhney et al. 2021). It has also been demonstrated that the presence of *Prevotella* in urine samples positively correlates with STIs in men (Nelson et al. 2021). *Gardnerella, Sneathia*, and *Veillonella* were also identified in our study. These genera commonly observed in the vaginal microbial communities of women with bacterial vaginosis (BV) (Fredricks et al. 2005; Marrazzo et al. 2008), have been associated with urethritis in men (Iser et al. 2005; Manhart et al. 2013; Babics et al. 2015; Plummer et al. 2022).

Conclusion

Our study provided evidence of STI prevalence rates, particularly the high rates of *C. trachomatis* infection among MSM resident in Durban. The detection of *N. gonorrhoeae* and *C. trachomatis* in asymptomatic MSM is of major concern and as such indicates the need for routine screening for these infections. Fear and stigma were two main barriers to seeking medical care in this study. The results of this calls for more attention to be paid to younger MSM and healthcare workers' unfavourable attitudes towards this key population. Also, based on study findings, we recommend educating young people about safer sexual practices particularly among young MSM.

The detection of *C. trachomatis* genotype E in our study cohort, and its high prevalence suggest that certain genotypes are common and appear to dominate within an MSM population. This study provided information on the genotypes circulating among South African MSM and may serve as a baseline for further research.

Finally, our study highlighted the diversity of microbial species present in the urinary microbiome of MSM. Several bacterial taxa associated with the study population were identified. An important finding of this study was the identification of *Gardnerella*, *Sneathia*, *Veillonella* (BV-associated genera), and *Prevotella* (gut microbiome) as components of the urinary microbiome of MSM. Some of which may play an integral role in the susceptibility of these men to chlamydial infection. Therefore, more studies need to be conducted to investigate the role of the urinary microbiota in MSM sexual health.

Limitations

- The study did not test for anorectal *C. trachomatis* and *N. gonorrhoeae*. Prevalence may have been substantially higher had the test been included.
- Data on demographic and behavioural characteristics as well as symptoms were self-reported, and as such might be subject to both recall and/or social desirability biases.
- The number of samples used for the genotyping analysis was low. Future studies recruiting a larger population of MSM are needed so that sound conclusions can be made regarding the distribution of genotypes amongst MSM in our local setting.
- This study employed the RFLP typing method in identifying *C. trachomatis* strains; this may not be the most appropriate method as the discriminatory power is low. We therefore are hoping to conduct future research where a more effective alternative for investigating the genetic diversity of *C. trachomatis*, such as the multi locus sequence typing (MLST) scheme would be employed to address this limitation.
- The urine samples used for this study did not generate adequate sequencing data due to low number of sequences.
- The sample size used in the study was small. Future studies with a larger sample size will provide more clarity on the role of urinary microbiomes in MSM sexual health and non-gonococcal urethritis (NGU).

All of the limitations listed above will be put into consideration when designing future studies. The strengths of this study are as follow:

- The strength of this study is that it fills a gap in the literature on the prevalence and risk factors associated with *C. trachomatis* and *N. gonorrhoeae* infection among MSM in South Africa as well as identified barriers to accessing healthcare services.
- This study also adds to the growing body of knowledge, providing baseline data on *C. trachomatis* genotypes circulating in our study population as well as the bacterial composition of the urinary microbiome of MSM.

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APPENDICES

1. College of Health Sciences Protocol Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI
COLLEGE OF HEALTH SCIENCES

18 May 2021

Prof N Abbai
School of Clinical Medicine

Dear Prof Abbai

PHD PROTOCOL: "Sexually Transmitted Infections among Men who have Sex with Men in the Durban Area, South Africa"
Student: Mr. KC Mofolorunsho **Student Number:** 217081975 **(Department of Clinical Medicine Laboratory)**

I am pleased to inform you that the abovementioned protocol has been approved.

The RIG application is also approved for onward submission to Ethics. The student may log in to RIG to track the progress of the application.

Please note:

- The Academic Leader: School Research must review any changes made to this study.
- The study may not begin without the approval of the Biomedical Research Ethics Committee.

May I take this opportunity to wish the student every success with the study.

Yours sincerely

[redacted]

Postgraduate Administrator

CC Mr. KC Mofolorunsho

Biomedical Research Ethics Committee
Westville Campus

Postgraduate, Higher Degrees & Research
School of Clinical Medicine, NRMSM Campus
Postal Address: P/Bag X3, Congella, Durban, 4013, South Africa
Telephone: +27 (0) 31 260 4416 **Facsimilie:** +27 (0) 31 260 4723 **Email:** konar@ukzn.ac.za **Website:** www.ukzn.ac.za

Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

INSPIRING GREATNESS

2. King Edward VIII Hospital Gatekeeper Permission

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

OFFICE OF THE HOSPITAL CEO
KING EDWARD VIII HOSPITAL

Private Bag X02, CONGELLA, 4013
Corner of Rick Turner (Francois Road) & Sydney Road
Tel: 031-3603654, Fax:031-2081457, Email: [redacted]
www.kznhealth.gov.za

Ref.: KE 2/7/1/(06/2021)
Enq.: Ms W.C. Madondo
Research Programming

16 September 2021

The Chief Medical Officer/Administrator / Head of Medical Imaging Department / Head of Obstetrics and Gynaecology / Nursing Manager
Kind Edward VIII Hospital
Durban
4013
ABBAIN@ukzn.ac.za

Dear Mr K.C Mafolorunsho

PROTOCOL REFERENCE NUMBER: BREC/00002798/2021

Project Title: "Sexually Transmitted Infections among men who have sex with men in the Durban Area, South Africa."

Permission to conduct research at King Edward VIII Hospital is provisionally granted, pending approval by the Provincial Health Research Committee, KZN Department of Health.

Kindly note the following:-

- The research will only commence once confirmation from the Provincial Health Research Committee in the KZN Department of Health has been received.
- Signing of an indemnity form at Room 8, CEO Complex before commencement with your study.
- King Edward VIII Hospital received full acknowledgment in the study on all Publications and reports and also kindly present a copy of the publication or report on completion.

The Management of King Edward VIII Hospital reserves the right to terminate the permission for the study should circumstances so dictate.

Yours faithfully

[redacted]

UPPORTED/NOT SUPPORTED

16/9/2021
DATE

DR. N. KHUZWAYO
ACTING SENIOR MEDICAL MANAGER

3. KwaZulu-Natal Department of Health Ethical Approval

KWAZULU-NATAL PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

DIRECTORATE:
Health Research & Knowledge Management Unit

Postal Address: Private Bag X9050
Physical Address: 330 Langalibalele Str; PM Burg; 3201
Tel: 0333953189/3123/2805 Fax: 033-3943782
Email address: hrkm@kznhealth.gov.za
www.kznhealth.gov.za

NHRD Ref: KZ_202109_031

Dear Mr K Mofolorunsho
(UKZN)

Approval of research

1. The research proposal titled **'Sexually Transmitted Infections among Men who have Sex with Men in the Durban Area, South Africa.'** was reviewed by the KwaZulu-Natal Department of Health (KZN-DoH).

   The proposal is hereby **approved** for research to be undertaken at King Edward VIII hospital.

2. You are requested to take note of the following:

   a. *All research conducted in KwaZulu-Natal must comply with government regulations relating to Covid-19. These include but are not limited to: regulations concerning social distancing, the wearing of personal protective equipment, and limitations on meetings and social gatherings.*
   b. *Kindly liaise with the facility manager BEFORE your research begins in order to ensure that conditions in the facility are conducive to the conduct of your research. These include, but are not limited to, an assurance that the numbers of patients attending the facility are sufficient to support your sample size requirements, and that the space and physical infrastructure of the facility can accommodate the research team and any additional equipment required for the research.*
   c. *Please ensure that you provide your letter of ethics re-certification to this unit, when the current approval expires.*
   d. *Provide an interim progress report and final report (electronic and hard copies) when your research is complete to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za*
   e. *Please note that the Department of Health shall not be held liable for any injury that occurs as a result of this study.*

For any additional information please contact Ms G Khumalo on 033-395 3189.

Yours Sincerely

[redacted]

Dr E Lutge
Chairperson, Health Research Committee
Date: 05/10/2021

GROWING KWAZULU-NATAL TOGETHER

4. Biomedical Research Ethics Committee Approval (UKZN)

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

08 October 2021

Mr Kehinde Charles Mofolorunsho (217081975)
School of Clinical Medicine
Medical School

Dear Mr Mofolorunsho,

Protocol reference number: BREC/00002798/2021
Project title: Sexually Transmitted Infections among Men who have Sex with Men in the Durban Area, South Africa
Degree: PhD

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 08 October 2021. Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is subject to national and UKZN lockdown regulations, see (http://research.ukzn.ac.za/Libraries/BREC/BREC_Amended_Lockdown_Level_1_Guidelines.sflb.ashx). Based on feedback from some sites, we urge PIs to show sensitivity and exercise appropriate consideration at sites where personnel and service users appear stressed or overloaded.

This approval is valid for one year from 08 October 2021. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2020) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 09 November 2021.

Yours sincerely,

[redacted]

Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

5. Aurum Institute Gatekeeper Permission

THE AURUM INSTITUTE NPC
Registration No. 1998/009355/08
041-083-NPO

Aurum House
The Ridge, 29 Queens Road
Parktown 2193
South Africa

PostNet Suite300
Private Bag X30500
Houghton 2041
South Africa

Tel: +27 (0) 10 590 1300
Fax: +27 (0) 866 180 268
Website: www.auruminstitute.org

7th March 2022

Subject: Letter of approval recruitment of MSM for PhD research study (Reference: DSGC-00006)

Dear Professor Nathlee Abbai,

Kindly note that your request to collect data for your PHD at Aurum-supported sites within KwaZulu-Natal has been approved by Auurm's data sharing governance committee.

Kindly ensure that all contractual agreements between The Aurum Institute and the applicant, yourself are adhered to, to ensure all parties involved can operate within the same sites and still provide valuable care to all patients.

We wish you all the best with your data collection and studies ahead.

Kind Regards,

Salome Charalambous
Group Chief Scientific Officer

[redacted]

Signature

2022/03/07
Date

6. Biomedical Research Ethics Committee Approval (UKZN) - Amendments

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

05 October 2021

Mr Kehinde Charles Mofolorunsho (217081975)
School of Clinical Medicine
Medical School

Dear Mr Mofolorunsho,

Protocol reference number: BREC/00002798/2021
Project title: Sexually Transmitted Infections among Men who have Sex with Men in the Durban Area, South Africa.
Degree: PhD

We wish to advise you that your application for amendments received on 13 May 2022 to add AURUM Institute as a recruitment site, for the above study has been **noted and approved** by a sub-committee of the Biomedical Research Ethics Committee.

The committee will be advised of the above at its next meeting to be held on 14 June 2022.

Yours sincerely

[redacted]

Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

7. Biomedical Research Ethics Committee Approval (UKZN) - Recertification

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

13 October 2022

Mr Kehinde Charles Mofolorunsho (217081975)
School of Clinical Medicine
Medical School

Dear Mr Mofolorunsho,

Protocol reference number: BREC/00002798/2021
Project title: Sexually Transmitted Infections among Men who have Sex with Men in the Durban Area, South Africa. Degree: PhD

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	**08 October 2022**
Expiration of Ethical Approval:	**07 October 2023**

I wish to advise you that your application for Recertification received on 10 October 2022 for the above protocol has been **noted and approved** by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The committee will be notified of the above approval at its next meeting to be held on 08 November 2022.

Yours sincerely

[redacted]

Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

8. Informed Consent

Information Sheet and Consent to Participate in Research

Date:

Dear Sir,

My name is Kehinde Charles MOFOLORUNSHO, I am a Doctoral student from the School of Clinical Medicine, University of KwaZulu-Natal.

You are being invited to consider participating in a study that involves research on **Sexually transmitted infections among men who have sex with men in the Durban area of South Africa**. The aim and purpose of this research is to investigate the prevalence and identify risk factors for sexually transmitted infections in men who have sex with men (MSM). The study is expected to enrol 200 MSM from the King Edward Hospital in Berea, Durban. It will involve the following procedures; completing a 5-minute questionnaire about yourself, collection of one sample of urine and one throat swab, and sample testing to test for sexually transmitted infections (STIs). The procedures for collection of samples and sample testing are experimental. The duration of your participation if you choose to enrol and remain in the study is expected to be approximately 15-20 minutes. The study is funded by Institut Merieux.

The study may involve the following risks and/or discomforts; feeling of discomforts during the swab sample collection, feeling of worry or embarrassment when discussing your sexual behaviour. There will be no direct benefit from this study. However, from the data obtained, we will be able to provide preliminary data for future STI intervention/prevention studies for this vulnerable population.

This study has been ethically reviewed and approved by the UKZN Biomedical research Ethics Committee (approval number: **BREC/00002798/2021**).

In the event of any problems or concerns/questions you may contact the researcher at:

School of Clinical Medicine
University of KwaZulu-Natal
South Africa
Phone: [redacted]
Email: [redacted]

or the UKZN Biomedical Research Ethics Committee, contact details as follows:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

Research Office, Westville Campus

Govan Mbeki Building

Private Bag X 54001

Durban

4000

KwaZulu-Natal, SOUTH AFRICA

Tel: 27 31 2602486 - Fax: 27 31 2604609

Email: BREC@ukzn.ac.za

Your participation in this study is voluntary. You are free to decline or withdraw from this study at any time. There will be no costs incurred in the course of your participation in the study. You will be asked to be in this study whilst attending your routine clinic visit. Since study is cross-sectional with only one study visit, there will be no need to incur any travel costs for a return visit.

Data collected are anonymous and will not be able to identify the participant. We will keep your information confidential. Your personal information may however be disclosed if required by law.

Your records may be reviewed by:

- Biomedical Research Ethics Committee of the University of KwaZulu-Natal
- Study PI

The researchers will do everything they can to protect your privacy.

DECLARATION OF CONSENT

I_____ have been informed about the study entitled **Sexually transmitted infections among men who have sex with men in the Durban area, South Africa** by Kehinde Charles Mofolorunsho.

I understand the purpose and procedures of the study.

I have been given an opportunity to answer questions about the study and have had answers to my satisfaction.

I declare that my participation in this study is entirely voluntary and that I may withdraw at any time without affecting any treatment or care that I would usually be entitled to.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher at:

School of Clinical Medicine
University of KwaZulu-Natal
South Africa
Phone: [redacted]
Email: [redacted]

If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2602486 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

_____ _____

Signature of Participant **Date**

_____ _____

Signature of Witness **Date**
(Where applicable)

_____ _____

Signature of Translator **Date**
(Where applicable)

9. Informed Consent – IsiZulu Version

Ishidi Lolwazi Kanye Nemvume Yokubamba Iqhaza Kucwaningo

Usuku:

Sawubona Mnumzana,

Igama lami ngingu Kehinde Charles MOFOLORUNSHO, ngingumfundi weziqu zobudokotela ovela e-School of Clinical Medicine, kwi-Nyuvesi yaKwaZulu-Natal.

Uyamenywa ukuba ube ingxenye yocwaningo **lwezifo ezithathelana ngokocansi phakathi kwabantu besilisa abalala nabanye abantu besilisa endaweni yaseThekwini eNingizimu Afrika.** Inhloso nenjongo yalolu cwaningo ukuphenya ukusabalala kwezifo zocansi nokuthola izinto ezinobungozi ekusabalaleni kwalezi izifo kubantu besilisa abalala nabesilisa. Lolu cwaningo kulindeleke ukuthi lubhalise abantu besilisa abazwana nananye abantu besislisa abangu-200 abavela esibhedlela i-King Edward Hospital eBerea, eThekwini. Lolu cwaningo luzobandakanya lezinqubo ezilandelayo; ukuphendula uhlu lwemibuzo ezothatha imizuzu engu-5 mayelana nawe, ukuqoqwa kwesampula elilodwa lomchamo kanye ne-swab eyodwa yomphimbo, nokuhlolwa kwesampula ukuhlola izifo ezithathelana ngokocansi (STIs). Izinqubo zokuqoqwa kanye nokuhlolwa kwamasampula kungokocwaningo kuphela. Isikhathi sokubamba iqhaza kwakho uma ukhetha ukubhalisa futhi uhlala ocwaningweni kulindeleke ukuthi sibe cishe imizuzu engu-15 kuya ku-20. Ucwaningo luxhaswe yi-Institut Merieux.

Ucwaningo lungabandakanya izingozi ezilandelayo kanye / noma ukungakhululeki; umuzwa wokungakhululeki ngesikhathi sokuqoqwa kwesampula ye-swab, ukuzwa ukukhathazeka noma amahloni lapho uxoxa ngokuziphatha kwakho kocansi. Ngeke kube khona nzuzo eqondile kulolu cwaningo. Kodwa-ke, kusukela emininingwaneni ezotholakala, sizokwazi ukuhlinzeka ngemininingwane kanye nolwazi okuzosiza ekuqhamukeni nezindlela zokungenelela kanye nokuvikela abanye abantu kwizifo zocansi.

Lolu cwaningo luye lwabuyekezwa futhi lwavunywa yi-UKZN Biomedical Research Ethics Committee (inombolo yokugunyazwa_____).

Uma kuba nezinkinga noma ukukhathazeka / imibuzo ungaxhumana nomcwaningi ku:

School of Clinical Medicine
Inyuvesi yaKwaZulu-Natali
Iningizimu Afrika
Ucingo: +[redacted]
I-imeyili: [redacted]

noma UKZN Biomedical Research Ethics Committee, imininingwane yokuxhumana ngale ndlela elandelayo:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Ucingo: 27 31 2602486
Ifekisi: 27 31 2604609
I-imeyili: BREC@ukzn.ac.za

Ukubamba iqhaza kwakho kulolu cwaningo kungokuzithandela. Ukhululekile ukwenqaba noma ukuhoxa kulolu cwaningo nganoma yisiphi isikhathi. Ngeke zibe khona izindleko ezitholakele ngenkathi ubamba iqhaza ocwaningweni. Uzocelwa ukuba ube kulolu cwaningo ngenkathi uhambela ukuvakashelwa kwakho okuvamile emtholampilo. Njengoba lolu cwaningo ludinga ukuthi uhambele kanye emtholampilo, ngeke sibe khona isidingo sokuba nezindleko zokuhambela emtholampilo okwesibili.

Imininingwane ezoqoqwa izoba imfihlo futhi ngeke ikwazi ukukhomba obambe iqhaza. Sizogcina imininingwane yakho iyimfihlo. Imininingwane yakho yangasese ingadalulwa uma kudingwa ngumthetho.

Amarekhodi akho angabuyekezwa ngu:
• Biomedical Research Ethics Committee yase-Nyuvesi yakwaKwaZulu-Natal
• Umcwaningi

Abaphenyi bazokwenza konke okusemandleni ukuvikela ubumfihlo bakho.

ISIMEMEZELO SOKUVUMELANA

Mina_____ ngazisiwe ngocwaningo olunesihloko esithi **Izifo ezithathelana ngokocansi phakathi kwabantu besilisa abalala nabesilisa endaweni yaseThekwini, eNingizimu Afrika** oluzobe lwenziwa ngu Kehinde Charles Mofolorunsho.

Ngiyayiqonda inhloso nezinqubo zalolucwaningo.
Nginikezwe ithuba lokuphendula imibuzo mayelana nalolu cwaningo futhi ngaba nezimpendulo zokweneliseka kwami.
Ngiyavuma ukuthi ukuzibandakanya kwami kulolu cwaningo kungokuzithandela ngokuphelele nokuthi ngingahoxa nganoma yisiphi isikhathi ngaphandle kokuthinta noma yikuphi ukwelashwa noma ukunakekelwa ebengingaba nelungelo lokukuthola.

Uma ngineminye imibuzo / ukukhathazeka noma imibuzo ehlobene nesifundo ngiyaqonda ukuthi ngingaxhumana nomcwaningi ku:
School of Clinical Medicine
Inyuvesi yaKwaZulu-Natali
Iningizimu Afrika
Ucingo: +[redacted]
I-imeyili: [redacted]
Uma nginemibuzo noma ukukhathazeka ngamalungelo ami njengomhlanganyeli ocwaningweni, noma uma ngikhathazekile ngengxenye yocwaningo noma ngababaphenyi ngingaxhumana ne:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus
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4000
KwaZulu-Natal, SOUTH AFRICA
Ucingo: 27 31 2602486
Ifekisi: 27 31 2604609
I-imeyili: BREC@ukzn.ac.za

_____ _____

Isiginesha Lomhlanganyeli **Usuku**

_____ _____

Isiginesha Likafakazi **Usuku**

(Lapho kufanele khona)

_____ _____

Isiginesha Lomhumushi **Usuku**

(Lapho kufanele khona)

10. Study Participants Enrolment Form

ENROLLMENT FORM

Participant Identifier: ☐☐☐☐☐☐

Visit Date: ☐☐/☐☐/☐☐☐☐

1. How old are you? _____ years Date of Birth: _____

If younger than 18 years of age, please do not enrol into study……. End of form

2. What is your highest level of education?

Did not attend school	
Primary school	
High school	
College, University	

3. Do you have a job?

Yes	
No	

4. Do you have a regular male partner?

Yes	
No	

5. Do you currently live with your regular partner?

Yes	
No	

6. How many male sexual partners do you estimate you have had sex with in the last 30 days?

None	
1 partner	
2-4 partners	
>4 partners	

7. Does your partner have other male partners?

Yes	
No	
Don't Know	

8. In the last 30 days, what sort of sexual practices did you engage in?

Oral sex	
Anal sex	
Oral and anal sex	

9. How often do you use condoms when engaging in this type of sex (from question 8 above)?

Always	
Frequently	
Sometimes	

10. In the last 30 days, how often did you have group sex involving at least 2 other men?

Once	
A few times	
Every week	
Monthly	

11. In the last 30 days, have you been paid for sex?

Once	
A few times	
Every week	
Monthly	

12. Are you experiencing any of the following symptoms?

Painful urination Yes☐ No☐

Discharge from the penis Yes☐ No☐

Pain during sex Yes☐ No☐

Presence of genital warts Yes☐ No☐

Testicular pain Yes☐ No☐

Genital itching Yes☐ No☐

13. Are you circumcised?

Yes	
No	

14. What is your HIV status?

Negative	
Positive	
Don't Know	

15. When was your last STI screening?

Less than 30 days ago	
Greater or equal to 30 days but less than 90 days ago	
Greater or equal to 90 days ago	

16. Have you been diagnosed with a previous STI other than HIV in the past 90 days?

Yes	
No	

17. What STIs have you been diagnosed with?

Gonorrhoeae	
Chlamydia	
Syphilis	
Hepatitis B	
Hepatitis C	
Herpes	

18. Have you used any drugs/substances during sexual encounters in the last 90 days?

Yes	
No	

19. Which of these substances do you use?

Crystal meth (Methamphetamine)	
Cannabis	
Heroin	

Alcohol	
Others (Specify)	

20. Do you know any sexual health clinic in Durban?

Yes	
No	

21. If yes, are you aware of the available sexual health services rendered and how to access them?

Yes	
No	

22. Do you think these sexual health clinics are able to protect patient's privacy and confidentiality?

Yes	
No	
Don't Know	

23. Will you prefer accessing sexual health services via a general practitioner?

Yes	
No	
Don't Know	

24. Are you comfortable disclosing your sexual identity to your general practitioner?

Yes	
No	

25. Have you experienced any form of internalized stigma due to your sexuality?

Yes	
No	

26. Has the fear of being publicly outed as gay deterred you from accessing sexual health services?

Yes	
No	

27. Will having a trusted and flexible sexual health clinic encourage gay men in accessing care for STIs?

Yes	
No	
Don’t Know	

11. Study Participants Enrolment Form – IsiZulu Version

IFOMU LOKUBHALISA

Inombolo yomhlanganyeli: ☐☐☐☐☐☐

Usuku: ☐☐/☐☐/☐☐☐☐

1. Uneminyaka emingaki? _____ **Usuku lokuzalwa:** _____

Uma uneminyaka engaphansi kuka-18, uyacelwa ukuba ungabi yingxenye yalolucwaningo……. Ukuphela kwefomu

2. Yiliphi izinga lakho eliphezulu lemfundo?

Angiyanga esikoleni	
Isikole samabanga aphansi	
Isikole samabanga aphezulu	
Ikolishi, Inyuvesi	

3. Ingabe uyasebenza?

Yebo	
Cha	

4. Unaye umlingani wesilisa ozwana naye?

Yebo	
Cha	

5. Uhlala naye umlingani wakho?

Yebo	
Cha	

6. Bangaki abalingani besilisa olinganisela ukuthi wenze nabo ucansi ezinsukwini ezingu-30 ezedlule?

Akekho	
Uyedwa	

Babili kuya kwabane	
Bangaphezu kuka-4	

7. Ingabe umlingani wakho unabo abanye abantu akazwana nabo?

Yebo	
Cha	
Angazi	

8. Ezinsukwini ezingu-30 ezedlule, inhloboni yezenzo zocansi oke wazibandakanya kuzo?

Ucansi olwenziwa ngomlomo (oral sex)	
Ucansi lwangemumva (anal sex)	
Ucansi olwenziwa ngomlomo nolwangemumva (Oral and anal sex)	

9. Uwasebenzisa kangaki amakhondomu uma wenza loluhlobo locansi (olukumbuzo wesi-8 phezulu)?

Njalo	
Kuvamile	
Kwesinye isikhathi	

10. Ezinsukwini ezingu-30 ezedlule, uke walwenza kangaki ucansi neqembu okubandakanya okungenani amanye amadoda amabili?

Kanye	
Izikhathi ezimbalwa	
Njalo ngeviki	
Njalo ngenyanga	

11. Ezinsukwini ezingu-30 ezedlule, uke wakhokhelwa ukwenza ucansi?

Kanye	
Izikhathi ezimbalwa	
Njalo ngeviki	
Njalo ngenyanga	

12. Iziphi izimpawu ohlangabezana nazo njengamanje kulezi ezilandelayo?

Ukuchama kabuhlungu Yebo☐ Cha☐

Uketshezi oluphuma esithweni sangasese Yebo☐ Cha☐

Ubuhlungu uma wenza ucansi	Yebo☐ Cha☐
Izinsumpa esithweni sangasese	Yebo☐ Cha☐
Ubuhlungu emasendeni	Yebo☐ Cha☐
Ukulunywa esithweni sangasese	Yebo☐ Cha☐

13. Usokile?

Yebo	
Cha	

14. Sithini isimo sakho se-HIV?

Anginayo	
Nginayo	
Angazi	

15. Wagcina nini ukuhlolelwa izifo ezithathelana ngokocansi (STI)?

Ngaphansi kwezinsuku ezingu-30 ezedlule	
Ngaphezu noma kulingana nezinsuku ezingu-30 kepha ngaphansi kwezinsuku ezingu-90 ezedlule	
Kungaphezu noma kulingana nezinsuku ezingu-90 ezedlule	

16. Uke watholakala unesifo esithathelana ngokocansi ngaphandle kwe-HIV ezinsukwini ezingu-90 ezedlule?

Yebo	
Cha	

17. Isiphi isifo esithathelana ngokocansi oke watholakala unaso?

Gonorrhoeae	
Chlamydia	
Syphilis	
Hepatitis B	
Hepatitis C	
Herpes	

18. Uke wasebenzisa izidakamizwa ngesikhathi uzimbandakanya ngokocansi ezinsukwini ezingu-90 ezedlule?

Yebo	
Cha	

19. Yiziphi izidakamizwa ozisebenzisayo kulezi?

Crystal meth (Methamphetamine)	
Insangu	
Heroin	
Utshwala	
Okunye (cacisa)	

20. Ukhona umtholampilo wezempilo zocansi owaziyo eThekwini?

Yebo	
Cha	

21. Uma kunjalo, ingabe uyazi ngosizo lwezempilo zocansi ezitholakalayo nokuthi ungazifinyelela kanjani?

Yebo	
Cha	

22. Ucabanga ukuth lemitholampilo yezempilo zocansi iyakwazi ukuvikela izimfihlo zeziguli zawo?

Yebo	
Cha	
Angazi	

23. Ungakhetha ukufinyelela ezinsizakalweni zezempilo zocansi ngokusebenzisa udokotela ojwayelekile?

Yebo	
Cha	
Angazi	

24. Ukhululekile ukudalula isimo sakho sezocansi kudokotela wakho?

Yebo	
Cha	

25. Uke wahlangabezana nanoma yiluphi uhlobo lokucwaswa ngenxa yobulili bakho?

Yebo	
Cha	

26. Ingabe ukwesaba ukuhlambalazwa esidlangalaleni ngokuthandana nabanye abantu besilisa kuyakuvimba ekutholeni izinsizakalo zezempilo zocansi?

Yebo	
Cha	

27. Ingabe ukuba nomtholampilo wezempilo zocansi othembekile kuzokhuthaza abantu besilisa abazwana nabantu besilisa ekutholeni ukunakekelwa kwezifo zocansi?

Yebo	
Cha	
Angazi	