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Passive surveillance of STI pathogens in Cape Town, South Africa: A six-month molecular epidemiology study

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DISSERTATION IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE MASTER OF PUBLIC HEALTH

FACULTY OF HEALTH SCIENCES

UNIVERSITY OF CAPE TOWN

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2023

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PART A: Research Protocol

Passive surveillance of STI pathogens in Cape Town, South Africa: A six-month molecular epidemiology study

1. Background

Sexually transmitted infections (STI) are some of the most commonly occurring infections globally, with countries in sub-Saharan Africa, including South Africa, exhibiting disproportionately higher prevalence rates[1, 2]. Populations considered to be of higher-risk such as HIV-positive individuals, young women, pregnant women, men-who-have-sex-with-men (MSM), and commercial sex workers have increased incidences of STIs, with socio-demographic factors such as stigma, gender discrimination, and gender inequality further compounding the increased rates[2]. STIs commonly present as male urethral discharge syndrome (MUDS), vaginal discharge syndrome (VDS), or genital ulcer disease[3]. They may also cause pelvic inflammatory disease (PID), complications in pregnancy, infertility, arthritis, and encephalitis[4]. Some STI pathogens are also known to increase the risk of HIV-acquisition and infectivity[5].

The most commonly occurring STI pathogens are *Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis,* and *Mycoplasma genitalium*[6], with 127.2 million annual infections, 86.9 million annual infections, and 156.0 million annual infections of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis*, respectively, having some of the highest incidence rates worldwide[1, 7-10]. Genital ulcer disease, commonly associated with herpes simplex virus 2 (HSV-2), *Treponema pallidum*, and *Haemophilus ducreyi*, is also frequently reported[3].

The emergence and spread of drug-resistant bacteria is of global public health concern, leading the World Health Organisation (WHO), in 2017, to compile the first list of 12 bacterial priority pathogens which exhibited high levels of drug resistance to current antibiotics, rendering them difficult to treat, or even untreatable in some instances[11]. Pathogens listed as critical "Priority Level 1" includes

Acinetobacter baumannii, *Pseudomonas aeruginosa*, and Enterobacterales resistant to carbapenem antibiotics, considered the last line of therapy for most complicated infections, and those which produced extended spectrum β-lactamases (ESBL)[12]. The "Priority Level 2" list of high risk pathogens includes *Neisseria gonorrhoeae*, a sexually transmitted infection (STI), resistant to cephalosporins and/or fluoroquinolones [12].

Neisseria gonorrhoeae is a Gram-negative, oxidase-positive, aerobic, nutritionally fastidious, intracellular diplococcus, residing in neutrophils. The fastidious nature of the organism makes routine culture challenging, requiring specialised transport and culture media, as well as specific incubation conditions to limit growth of commensals. The use of molecular methods such as PCR offers a significant improvement in detection rates with high specificity and sensitivity levels. Infection with *N. gonorrhoeae* leads to gonorrhea or gonococcal disease, the STI reported most frequently after Chlamydia in the USA[13]. The WHO reported 555,608 cases of gonorrhea in the US in 2017, an increase of 18.6% over the previous report, and 75.2% higher than the lowest rate reported in 2009 with 106 million new cases globally in 2008[14, 15]. A 2018 study, modelling STI prevalence rates in South Africa over 30-years, indicated an adjusted *N. gonorrhoeae* prevalence estimate of 6.6% for women and 3.5% for men[16]. Gonococcal disease results in cervicitis in women, and urethritis, pharyngitis, and proctitis in men, often presenting with a mucopurulent endocervical or urethral exudate on physical examination. Gonococcal disease is usually symptomatic in infected men, however, up to half of infected women may not show signs of infection, leading to inadvertent spread[17].

Infections in women, if untreated, may lead to PID, chronic pelvic pain, ectopic pregnancy, and tubal infertility, while men may develop epididymitis, prostatitis, and urethral stricture[18]. The organism may also colonise the rectal and oropharyngeal mucosa, which may result in asymptomatic carriage, increasing the risk of inadvertent transmission and the acquisition of resistance determinants from resident or transient commensal biota[19]. New-borns may also be infected during delivery if born to an infected mother. They subsequently develop ophthalmia neonatorum (conjunctivitis), and this

may lead to blindness if untreated. These rates are extremely low with modern maternal testing during pregnancy, and prophylaxis to infants *intra partum*[20].

Chlamydia trachomatis is a Gram negative, obligate intracellular pathogen which only infects humans. *C. trachomatis* is the most reported STI pathogen in the USA, with the number of incident cases increasing yearly, likely due to increased spread and testing in high-risk populations such as MSM, coupled with the increased sensitivity of molecular diagnostic tests[21]. Adolescents and young adults accounted for up to 61% of new cases with women disproportionately affected[21]. There are 12 serovariants of *C. trachomatis* which are further divided into two biovars. The first includes all variants which cause trachoma of the eye (A, B, Ba, and C) and the genital tract (D, E, F, G, H, I, J, and K); and the second includes those which cause invasive lymphogranuloma venereum (L1, L2, and L3)[22].

Diagnosis of chlamydia is complicated by the intracellular nature of the pathogen, requiring cell culture and antigen tests for direct pathogen detection, or the use of molecular testing such as PCR[23]. Most infected women remain asymptomatic, and the organism is able to evade the host immune system and migrate to the genital tract to cause infection. Infection leads to tissue damage and disruption of fallopian tube integrity over time, which may in turn lead to infertility, ectopic pregnancy, or premature delivery if untreated[24].

Mycoplasma genitalium, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Ureaplasma parvum* are commonly referred to as genital mycoplasmas, which belong to the order Mollicutes, and the family *Mycoplasmataceae*[25, 26]. *M. genitalium*, first identified in 1981 in two men with non-gonococcal urethritis, is the smallest known bacterium which is able to self-replicate, and is believed to have evolved as a consequence of genome reduction from Gram positive bacteria[27, 28]. It lacks a cell wall and is therefore not detected by Gram stain, is able to evade the host immune system by antigenic changes to the surface proteins, and is intrinsically resistant to β-lactam antibiotics[26, 28]. It has several additional virulence factors which contribute to its increased pathogenicity including a tip organelle for adherence to host epithelial cells, as well as the secretion of specific enzymes for adhesion and cell

penetration[29]. Due to its fastidious nature and slow growth rate, culture is rarely used as a diagnostic tool, with the majority of studies reporting detection using molecular assays[29]. This has also hindered the association of this organism with disease and the mechanisms used to induce inflammation, cause cell damage, and persist in the host[28].

M. genitalium infection is often asymptomatic and may occur as a prolonged chronic infection[30]. It has been identified in up to 15-25% of men presenting with non-gonococcal urethritis, and up to 10-13% of women presenting with PID[30]. Genital mycoplasmas have been shown to colonise up to 80% of pregnant and non-pregnant women[31]. The role of this organism in male urogenital inflammatory disease has been described, however the role in women remains uncertain[26]. Inflammation following infection is believed to be due to the hosts response, however studies have indicated the organism is able to cause damage to fallopian cilia[30]. Testing for this organism is only indicated in patients with specific indicator syndromes such as men with non-gonococcal urethritis, women with PID, and persons with known exposure to the organism, among others[30].

The role of *M. hominis* and *U. urealyticum* as genital tract pathogens is similarly contentious, but are considered to be opportunistic pathogens which may cause pregnancy complications and are implicated in urogenital infections such as nongonococcal urethritis, dysuria, urgency, and urethral discharge in men, and PID in women[32, 33]. Even though some studies indicated that *M. hominis* is not a pathogen, recent studies characterise it as a pathogen due to its association with *Trichomonas vaginalis*. There are no studies which definitively confirm it as a true pathogen, and as such, treating mono-infections with this organism remains controversial. Laboratory testing often relies on light microscopy for detecting the Mycoplasma's, although this has poor sensitivity due to the subjectivity of microscopy. The use immunochromatographic tests which detect the presence of antibodies, and nucleic acid amplification tests (NAATs) provide an alternative with increased specificity and sensitivity of detection.

A study investigating the prevalence of genital mycoplasmas in pregnant women in Gauteng, South Africa indicated high rates of infection with the common agents. *U. parvum* was the most prevalent at 72.4%, followed by *M. hominis* at 50.7%, *M. genitalium* at 14.5%, and *U. urealyticum* at 2.3%. *M. hominis* and *U. parvum* were also identified in 75% of all HIV positive samples[31]. Other studies have implicated the colonisation with *M. hominis* and *U. urealyticum* with spontaneous abortions and low birth weights, and early interventions may improve these outcomes[33]. Since mycoplasmas lack a cell wall, they are intrinsically resistant to β-lactam antibiotics and vancomycin, and with increasing rates of resistance the efficacy of tetracyclines, macrolides, and quinolones in treating these infections is reduced[33]. There are few studies investigating the occurrence of the less common STI associated organisms such as *U. parvum*, and some contention exists on their occurrence and the association with true infection, and subsequently whether or not to treat. There is no clear evidence that these organisms cause infection independently of confirmed STI pathogens, or whether they are co-factors for infection, or simply vaginal commensals, though their presence has been linked to complications during pregnancy[25, 34].

Trichomoniasis, caused by the protozoan *Trichomonas vaginalis*, is reported to be the most commonly occurring STI globally, with an estimated 276.4 million cases reported in 2008, with up to 90% of these occurring in resources limited settings, and up to 30 million cases reported in Sub-Saharan Africa annually[35-37]. *T. vaginalis* is an extracellular, flagellated protozoan parasite, with a distinctive axostyle and undulating membrane[35, 36]. It is an obligated parasite relying on carbohydrates obtained from phagocytosed vaginal epithelial cells, bacteria, and red blood cells, for survival[35, 36]. It infects the genital epithelium of humans, causing asymptomatic infection in most men and women, which may persist for a few days or even years[36]. Symptomatic cases in men usually present with dysuria and urethral discharge, and similarly in women who may also present with valvar itching and abdominal pain[35, 36]. Research has indicated an association between *T. vaginalis* and other STI pathogens like *N. gonorrhoeae*, *C. trachomatis*, Herpes Simplex Virus, and syphilis[38], as well as with an increased risk of HIV infection in *T. vaginalis* infected women, likely due to the resultant local inflammation and

recruitment of target cells for HIV infection, and increased shedding of the virus[39]. Laboratory diagnosis is based on light microscopy, though the use of antigen tests and PCR may offer significant improvements in detection, due the increase in sensitivity and specificity.

With a global estimate of up to 1 million new cases of STIs reported daily, and the associated burden on medical and socio-economic resources, rapid and accurate diagnosis of these infections is critical to limit spread and guide appropriate targeted therapy[40]. Current WHO guidelines recommend syndromic management of STIs, including *N. gonorrhoeae*[41]. These guidelines make provision for clinicians to presumptively treat STI, based solely on clinical presentation, and without confirmatory laboratory testing, by administering treatment which covers the most likely causes. This approach has added value in certain settings, such as low resourced countries where routine testing or surveillance may not be feasible.

Numerous reports have subsequently emerged indicating the poor performance of this approach to accurately detect and correctly treat these infections empirically[16, 42-44]. These reports indicated the need for better guidelines and criteria to ensure syndromic management is more informed in the absence of laboratory testing. The use of syndromic management guidelines has also led to a paucity in the available data on national and global STI prevalence and incidence rates, relying instead on sporadic surveillance studies, which may not present current trends or unnoticed outbreaks, which, in turn, may drive spread and resistance rates[2]. One of the key points in the current WHO Global Health Sector Strategy was the improvement of STI case management[2].

Traditional laboratory testing relies on routine culture for *N. gonorrhoeae*, cell culture for *C. trachomatis*, and light microscopy for the Mycoplasma's and genital parasites. These methods report poor sensitivity due to the fastidious nature of these organisms, as well as the need for specialised culture methods, media, expertise, and the subjectivity of microscopy. The use of multiplexed tests such as immunochromatographic tests which detect the presence of antibodies, and especially nucleic acid amplification tests (NAATs) which detect the presence of target nucleic acids, as an alternative to

current laboratory testing, offers several advantages for the detection of STIs. Multiplexed NAATs are able to test for several different targets simultaneously which reduce the number of tests and healthcare visits required, thereby reducing patient anxiety and the associated costs of multiple tests[45]. Since the risk factors for the acquisition of several STI pathogens overlap, persons at risk often acquire multiple infections simultaneously and certain multiplexed assays are able to detect these in a single reaction[40].

The efficacy of multiplexed NAATs for the diagnosis of STIs have reported a 95% sensitivity and specificity of detection in a large number of currently available tests, with some assays reporting up to 100% sensitivity and specificity over a range of STI pathogens[40]. One such assay, the Allplex STI Essential Assay, reported consistently high diagnostic performance (100% sensitivity) and high specificity rates for seven STI pathogens included in the assay, with high concordance to other assays[46-48]. This assays offers the added advantage of rapid, even one day turn-around-times, reductions in the associated testing costs providing results for multiple pathogens in a single assay, allowing for rapid and targeted therapy, improving patient outcomes, and providing informing on current trends which allow for tailored public health interventions to limit spread and curb resistance rates, although no antimicrobial susceptibility data is provided[40, 46, 47].

2. Aim

This prospective passive surveillance study aims to determine, from urogenital swabs submitted to the National Health Laboratory Service at Groote Schuur Hospital, the six-month period prevalence of seven commonly occurring STI pathogens.

3. Research question (CoCoPop)

Condition	Seven commonly occurring STI pathogens
Context	National Health Laboratory Service Diagnostic Laboratory at Groote Schuur Hospital
Population	All patients submitting urogenital swabs for routine evaluation

What is the prevalence of seven commonly occurring STI organisms among patients submitting urogenital swabs to the National Health Laboratory Service Diagnostic Laboratory at Groote Schuur Hospital?

4. Objectives

For urogenital swabs submitted to Groote Schuur Hospital, over a period of 6 months, for routine microbiological investigations, we seek to:

- Extract DNA for screening for the presence of seven commonly occurring STI pathogens, using a commercial multiplex Allplex STI Essential PCR assay
- Characterize the distribution of pathogens isolated including prevalence of co-infection
- Correlate infection rates and patient clinical or demographic information with organisms detected
- Determine the diagnostic test accuracy of the commercial PCR assay against routine microbiological investigations and an in-house PCR assay

5. Methods

5.1. Sample collection and routine testing

The NHLS laboratory at Groote Schuur Hospital provides diagnostic services to multiple facilities in the Cape Metro. Routine microbiological testing is performed on patients exhibiting recurrent symptomatic infections, as well as those for antenatal Group B Streptococcal screening, minors who may have had sexual non-accidental injury, and patients with other symptoms which may be associated with urogenital infection. Urogenital samples are collected by trained medical staff.

Based on the local laboratory standard operating procedure (SOP), genital swabs (excluding GBS) will be inoculated onto New York City media containing vancomycin, colistin, nystatin and trimethoprim lactate[49] to inhibit growth of commensals which may out compete *N. gonorrhoeae*, and chocolate agar to detect the presence of viable *N. gonorrhoeae*. These samples are incubated at 5% CO_2 for 24 – 48 hours. Growth of grey, oxidase-positive colonies, indicative of *N. gonorrhoeae* on chocolate agar, are submitted to the Vitek 2 (BioMérieux, Marcy-l'Etoile, France) automated identification system using an NH card and following manufacturer instructions to confirm identification.

No routine laboratory testing for *C. trachomatis* or any of the other pathogens included in the commercial PCR assay is conducted at this laboratory. Once all routine diagnostic tests have been performed, the residual urogenital swabs will be collected and stored at -20°C until DNA is extracted for batched PCR. Cultured and confirmed *N. gonorrhoeae* isolates will also be collected for further molecular testing.

5.2. Research ethics approval

Since human derived samples, as well as demographic and clinical results will be used for this study, approval from the University of Cape Town Human Research Ethics Committee will be obtained, prior to commencement of the study.

5.3. DNA extraction

To detect the presence of the seven commonly occurring STI pathogens, purified nucleic acids will be extracted from all collected samples. The Quick-DNA Fungal/Bacterial Miniprep Kit will be used to extract DNA from the collected urogenital swabs and bacterial isolates, according to the manufacturer's recommendations. Briefly, once routine microbiological testing is complete, the urogenital swab will be cut into the kit provided lysis buffer and bashing beads, and placed in the Tissue Lyser (QIAGEN) for mechanical lysis. This will allow for maximal nucleic acid extraction. The homogenate will then be purified as per kit instructions. Cultured bacterial isolates will be placed directly into the lysis buffer and bashing beads, and processed as per kit instructions.

5.4. PCR screening

All samples collected for this study will be screened for the presence of seven commonly occurring STI pathogens (*N. gonorrhoeae*, *C. trachomatis*, *M. hominis*, *M. genitalium*, *T. vaginalis*, *U. urealyticum*, *U. parvum*) using the Allplex STI Essential Assay (Inqaba Biotech), according to the manufacturer's instructions. The purified DNA extracted for each sample will be added to the prepared master mix and run on an CFX-96™ DX real time PCR thermocycler, with CFX Manager™ DX Software, Version 3.1. The results of each run will be exported and analysed using the manufacturers Seegene Viewer online software tool, and the interpreted results exported to Excel for further analysis.

The utility of a previously validated in-house multiplex real time PCR assay for *N. gonorrhoeae* and *C. trachomatis* will also be evaluated in this study. This PCR is widely used with excellent reported sensitivity and specificity for the intended targets. The primers and probes (Table 1) will be supplied by Integrated DNA Technologies (Whitehead Scientific), with concentrations optimized for our setting[6, 8]. A previously validated internal amplification control will be included to monitor the impact of inhibiting substances which may be present. The performance of this assay will be compared to the commercial assay. This PCR assay will serve as a comparative to highlight differences in detection

rates using different assays, and since both assays are fully validated, no third reference test is required.

Table 1: In-house primers, probes, and PCR conditions to detect *N. gonorrhoeae* and *C. trachomatis*.

Name	Sequence (5' – 3')	Concentration	Fluorophore	Target
NGF	GGATACGACGTAACCTTGACTATGG	200 nM		*N. gonorrhoeae* cytosine-specific DNA-methyltransferase gene[6, 8]
NGR	CCGATGTAGAAGACCCTTTTGC	200 nM		
NGP	CAACGCCAAAGACTACGGTGTAGCACAG	200 nM	FAM	
CTF	GGATTGACTCCGACAACGTATTC	200 nM		Cryptic plasmid of *C. trachomatis*[6, 8]
CTR	ATCATTGCCATTAGAAAGGGCATT	200 nM		
CTP	TTACGTGTAGGCGGTTTAGAAAGCGG	300 nM	HEX	
GFPF	CCTGTCCTTTTACCAGACAACCA	400 nM		Green fluorescent protein of *Aequorea Victoria*[50]
GFPR	GGTCTCTCTTTTCGTTGGGATCT	400 nM		
GFPP	TACCTGTCCACACAATCTGCCCTTTCG	50 nM	Cy5	
PCR program				
• **95°C – 5 min (Polymerase Activation), followed by 40 cycles of:** . **95°C – 20 sec (Denaturation)** . **60°C – 60 sec (Primer annealing and amplification)** . **Acquire to Green (FAM), Yellow (HEX), and Red (Cy5)**				

5.5. Data analysis

We will document the six-month period prevalence of each of the seven STI pathogens included in the Allplex STI Essential assay. Pearson's chi-squared test, Fisher's exact test, and logistic regression will be used to determine associations between the PCR results and the clinical and demographic data collected, and Student's t test with unequal variance will be used to compare the difference between mean Ct values of the two different PCR assays used. Statistical analyses will be performed using STATA v16.0, and GraphPad Prism v9.0.

5.6. Impact

Results of this study will provide current data on local STI prevalence rates which are not available. Furthermore, the results will inform the rationale for a molecular assay at the NHLS GSH to improve pathogen detection sensitivity, range, and result turn-around-time. Should introduced, the assay will aid with local disease tracking to limit spread and resistance, thereby reducing attributable morbidity and mortality.

6. References cited:

[1] **Rowley J, Vander Hoorn S, Korenromp E, Low N, Unemo M, Abu-Raddad LJ*, et al.*** 2019. Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. Bulletin of the World Health Organization **97:**548-62p.

[2] **Peters RPH, Garrett N, Chandiwana N, Kularatne R, Brink AJ, Cohen K*, et al.*** 2022. Southern African HIV Clinicians Society 2022 guideline for the management of sexually transmitted infections: Moving towards best practice. 2022 **23**.

[3] **Ahmed J, Rawre J, Dhawan N, Dudani P, Khanna N, Dhawan B.** 2022. Genital ulcer disease: A review. J Family Med Prim Care **11:**4255-62.

[4] **Unemo M, Bradshaw CS, Hocking JS, de Vries HJC, Francis SC, Mabey D*, et al.*** 2017. Sexually transmitted infections: challenges ahead. Lancet Infect Dis **17:**e235-e79.

[5] **Cohen MS, Council OD, Chen JS.** 2019. Sexually transmitted infections and HIV in the era of antiretroviral treatment and prevention: the biologic basis for epidemiologic synergy. J Int AIDS Soc **22 Suppl 6:**e25355.

[6] **Mhlongo S, Magooa P, Müller EE, Nel N, Radebe F, Wasserman E*, et al.*** 2010. Etiology and STI/HIV coinfections among patients with urethral and vaginal discharge syndromes in South Africa. Sexually transmitted diseases **37:**566-70.

[7] **Newman L, Rowley J, Vander Hoorn S, Wijesooriya NS, Unemo M, Low N*, et al.*** 2015. Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. PloS one **10:**e0143304.

[8] **Venter JM, Mahlangu PM, Müller EE, Lewis DA, Rebe K, Struthers H*, et al.*** 2019. Comparison of an in-house real-time duplex PCR assay with commercial HOLOGIC® APTIMA assays for the detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in urine and extra-genital specimens. BMC infectious diseases **19:**1-7.

[9] **Ohnishi M, Golparian D, Shimuta K, Saika T, Hoshina S, Iwasaku K*, et al.*** 2011. Is *Neisseria gonorrhoeae* initiating a future era of untreatable gonorrhea?: detailed characterization of the first strain with high-level resistance to ceftriaxone. Antimicrob Agents Chemother **55:**3538-45.

[10] **Costa-Lourenço A, Barros Dos Santos KT, Moreira BM, Fracalanzza SEL, Bonelli RR.** 2017. Antimicrobial resistance in *Neisseria gonorrhoeae*: history, molecular mechanisms and epidemiological aspects of an emerging global threat. Braz J Microbiol **48:**617-28.

[11] **World Health Organisation W.** 2017. WHO publishes list of bacteria for which new antibiotics are urgently needed, p. http://www.who.int/mediacentre/news/releases/2017/bacteria-antibioticsneeded/en/. *In* Release P (ed.).

[12] **Asokan GV, Ramadhan T, Ahmed E, Sanad H.** 2019. WHO Global Priority Pathogens List: A Bibliometric Analysis of Medline-PubMed for Knowledge Mobilization to Infection Prevention and Control Practices in Bahrain. Oman Med J **34:**184-93.

[13] **Costa-Lourenço APRd, Barros Dos Santos KT, Moreira BM, Fracalanzza SEL, Bonelli RR.** 2017. Antimicrobial resistance in Neisseria gonorrhoeae: history, molecular mechanisms and epidemiological aspects of an emerging global threat. Braz J Microbiol **48:**617-28.

[14] **World Health Organisation W.** 2018. Sexually Transmitted Disease Surveillance 2017 - National Profile Overview, Centers for Disease Control and Prevention. https://www.cdc.gov/std/stats17/gonorrhea.htm, Online Publication.

[15] **Unemo M, Shafer WM.** 2014. Antimicrobial Resistance in *Neisseria gonorrhoeae* in the 21st Century: Past, Evolution, and Future. Clinical Microbiology Reviews **27:**587-613.

[16] **Kularatne RS, Niit R, Rowley J, Kufa-Chakezha T, Peters RPH, Taylor MM*, et al.*** 2018. Adult gonorrhea, chlamydia and syphilis prevalence, incidence, treatment and syndromic case reporting in South Africa: Estimates using the Spectrum-STI model, 1990-2017. PLOS ONE **13:**e0205863.

[17] **Martín-Sánchez M, Fairley CK, Ong JJ, Maddaford K, Chen MY, Williamson DA*, et al.*** 2020. Clinical presentation of asymptomatic and symptomatic women who tested positive for genital gonorrhoea at a sexual health service in Melbourne, Australia. Epidemiol Infect **148:**e240.

[18] **Ng L-K, Martin IE.** 2005. The laboratory diagnosis of *Neisseria gonorrhoeae*. Can J Infect Dis Med Microbiol **16:**15-25.

[19] **Chan PA, Robinette A, Montgomery M, Almonte A, Cu-Uvin S, Lonks JR*, et al.*** 2016. Extragenital Infections Caused by *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: A Review of the Literature. Infectious Diseases in Obstetrics and Gynecology **2016:**5758387.

[20] **Matejcek A, Goldman RD.** 2013. Treatment and prevention of ophthalmia neonatorum. Can Fam Physician **59:**1187-90.
[21] **Prevention CfDCa.** 2019. Sexually transmitted disease surveillance 2018. .
[22] **Faris R, Andersen SE, McCullough A, Gourronc F, Klingelhutz AJ, Weber MM.** 2019. Chlamydia trachomatis Serovars Drive Differential Production of Proinflammatory Cytokines and Chemokines Depending on the Type of Cell Infected. Front Cell Infect Microbiol **9:**399.
[23] **Meyer T.** 2016. Diagnostic Procedures to Detect Chlamydia trachomatis Infections. Microorganisms **4**.
[24] **Witkin SS, Minis E, Athanasiou A, Leizer J, Linhares IM.** 2017. Chlamydia trachomatis: the Persistent Pathogen. Clinical and vaccine immunology : CVI **24**.
[25] **Patel MA, Nyirjesy P.** 2010. Role of Mycoplasma and ureaplasma species in female lower genital tract infections. Curr Infect Dis Rep **12:**417-22.
[26] **Sethi S, Singh G, Samanta P, Sharma M.** 2012. Mycoplasma genitalium: an emerging sexually transmitted pathogen. Indian J Med Res **136:**942-55.
[27] **Taylor-Robinson D, Jensen JS.** 2011. Mycoplasma genitalium: from Chrysalis to multicolored butterfly. Clin Microbiol Rev **24:**498-514.
[28] **McGowin CL, Totten PA.** 2017. The Unique Microbiology and Molecular Pathogenesis of Mycoplasma genitalium. J Infect Dis **216:**S382-s8.
[29] **le Roux MC, Hoosen AA.** 2010. Mycoplasma genitalium: a brief review. Southern African Journal of Epidemiology and Infection **25:**7-10.
[30] **Pinto-Sander N, Soni S.** 2019. Mycoplasma genitalium infection. BMJ **367:**l5820.
[31] **Redelinghuys MJ, Ehlers MM, Dreyer AW, Lombaard H, Kock MM.** 2013. P3.035 Prevalence of Genital Mycoplasmas and Bacterial Vaginosis in Pregnant Women in Gauteng, South Africa. Sexually Transmitted Infections **89:**A159-A.
[32] **Naicker M, Dessai F, Singh R, Mitchev N, Tinarwo P, Abbai NS.** 2021. 'Mycoplasma hominis does not share common risk factors with other genital pathogens': Findings from a South African pregnant cohort. S Afr J Infect Dis **36:**207.
[33] **Bayraktar MR, Ozerol IH, Gucluer N, Celik O.** 2010. Prevalence and antibiotic susceptibility of Mycoplasma hominis and Ureaplasma urealyticum in pregnant women. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases **14:**e90-5.
[34] **Taylor-Robinson D, Lamont R.** 2011. Mycoplasmas in pregnancy. BJOG: An International Journal of Obstetrics & Gynaecology **118:**164-74.
[35] **Kissinger P.** 2015. Trichomonas vaginalis: a review of epidemiologic, clinical and treatment issues. BMC infectious diseases **15:**307.
[36] **Kissinger PJ, Gaydos CA, Seña AC, Scott McClelland R, Soper D, Secor WE*, et al.*** 2022. Diagnosis and Management of Trichomonas vaginalis: Summary of Evidence Reviewed for the 2021 Centers for Disease Control and Prevention Sexually Transmitted Infections Treatment Guidelines. Clinical Infectious Diseases **74:**S152-S61.
[37] **Mabaso N, Abbai NS.** 2021. A review on Trichomonas vaginalis infections in women from Africa. S Afr J Infect Dis **36:**254.
[38] **Allsworth JE, Ratner JA, Peipert JF.** 2009. Trichomoniasis and other sexually transmitted infections: results from the 2001-2004 National Health and Nutrition Examination Surveys. Sex Transm Dis **36:**738-44.
[39] **Kissinger P, Adamski A.** 2013. Trichomoniasis and HIV interactions: a review. Sex Transm Infect **89:**426-33.
[40] **Karellis A, Naeem F, Nair S, Mallya SD, Routy JP, Gahagan J*, et al.*** 2022. Multiplexed rapid technologies for sexually transmitted infections: a systematic review. Lancet Microbe **3:**e303-e15.
[41] **World Health Organisation W.** 2021. Guidelines for the management of symptomatic sexually transmitted infections. WHO **https://www.who.int/publications/i/item/9789240024168**.
[42] **Vallely LM, Toliman P, Ryan C, Rai G, Wapling J, Gabuzzi J*, et al.*** 2017. Performance of syndromic management for the detection and treatment of genital Chlamydia trachomatis, Neisseria gonorrhoeae and Trichomonas vaginalis among women attending antenatal, well woman and sexual health clinics in Papua New Guinea: a cross-sectional study. BMJ open **7:**e018630-e.
[43] **Ndowa FJ, Francis JM, Machiha A, Faye-Kette H, Fonkoua MC.** 2013. Gonococcal antimicrobial resistance: perspectives from the African region. Sex Transm Infect **89 Suppl 4:**iv11-5.

[44] **van der Eem L, Dubbink JH, Struthers HE, McIntyre JA, Ouburg S, Morré SA*, et al.*** 2016. Evaluation of syndromic management guidelines for treatment of sexually transmitted infections in South African women. Tropical Medicine & International Health **21:**1138-46.

[45] **Pant Pai N, Daher J.** 2015. Multiplexed testing for HIV and related bacterial and viral co-infections at the point-of-care: quo vadis? Expert Rev Mol Diagn **15:**463-9.

[46] **Berçot B, Amarsy R, Goubard A, Aparicio C, Loeung HU, Segouin C*, et al.*** 2015. Assessment of coinfection of sexually transmitted pathogen microbes by use of the anyplex II STI-7 molecular kit. J Clin Microbiol **53:**991-3.

[47] **Choe HS, Lee DS, Lee SJ, Hong SH, Park DC, Lee MK*, et al.*** 2013. Performance of Anyplex™ II multiplex real-time PCR for the diagnosis of seven sexually transmitted infections: comparison with currently available methods. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases **17:**e1134-40.

[48] **de Salazar A, Espadafor B, Fuentes-López A, Barrientos-Durán A, Salvador L, Álvarez M*, et al.*** 2019. Comparison between Aptima Assays (Hologic) and the Allplex STI Essential Assay (Seegene) for the diagnosis of Sexually transmitted infections. PLoS One **14:**e0222439.

[49] **Young H.** 1978. Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. Sexually Transmitted Infections **54:**36-40.

[50] **Murphy NM, McLauchlin J, Ohai C, Grant KA.** 2007. Construction and evaluation of a microbiological positive process internal control for PCR-based examination of food samples for *Listeria monocytogenes* and S*almonella enterica*. International Journal of Food Microbiology **120:**110-9.

PART B: Journal Manuscript (BMC ID)

Passive surveillance of STI pathogens in Cape Town, South Africa: A six-month molecular epidemiology study

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1. Abstract

Background: Sexually transmitted infections are among the most commonly occurring infections globally, with countries in sub-Saharan Africa exhibiting disproportionately higher prevalence rates. Reports indicate the need for accurate detection, epidemiological characterisation, and appropriate management of these infections. This prospective passive surveillance study sought to document local STI prevalence and, in addition, to evaluate the potential of a molecular assay as a surveillance tool in our setting.

Methods: Urogenital swabs, submitted to Groote Schuur Hospital over a period of 6 months, for routine microbiological investigations, were subjected to a commercial multiplex PCR assay to determine the distribution of STI pathogens. Correlations between detected organisms and clinical and demographic information were determined using Stata® software.

Results: A total of 148 urogenital swabs were collected and tested, with the majority from women. Up to 83.79% of the samples tested positive for one or more pathogen, with all seven assayed pathogens detected in one or more sample. *Ureaplasma parvum* was the most prevalent pathogen detected overall, with a 6-month period prevalence of 42.57%, followed by *N. gonorrhoeae* (37.84%), *M. hominis* (34.46%), *U. urealyticum* (23.65%), *T. vaginalis* (11.49%), *C. trachomatis* (10.14%), with *M. genitalium* (1.35%) the least prevalent. There were several different combinations of co-infections with multiple pathogens, with one sample testing positive for five organisms. *M. hominis* and *T. vaginalis* were only detected in co-infection with other pathogens. Persons aged 17-30- and 31-40-years old were 51-times and 16-times, respectively, more likely to test PCR-positive for one or more STI pathogen. Samples submitted with non-urogenital specific indications were 11.82 times more likely to test positive for *C. trachomatis*. There was an association between samples submitted for GBS screening and PCR-positivity for any of the pathogens tested, which were 3.03 times more likely to test positive for *U. parvum*. Routine microbiological investigations only detected three infections.

Conclusions: There is a significantly higher than expected rate and difference in organism distribution of STI prevalence in Cape Town, South Africa as compared with global and regional estimates. The use of molecular testing methods may improve detection, providing rapid results, which may allow for tailored guidelines and interventions to limit spread and resistance.

(350/350 words)

2. Key words

Sexually transmitted infections, prevalence, molecular diagnostics, surveillance, syndromic management

(9/10 words)

3. Introduction

Sexually transmitted infections are one of the most commonly occurring infections globally, with countries in sub-Saharan Africa exhibiting disproportionately higher prevalence rates[1, 2]. Populations considered higher-risk such as HIV-positive individuals, young women, pregnant women, men-who-have-sex-with-men (MSM), and commercial sex workers have increased incidences of STIs, with socio-demographic factors such as stigma, gender discrimination, and gender inequality further compounding the increased rates[2].

The most commonly occurring STI pathogens are *Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis, Mycoplasma genitalium* and *Gardnerella vaginalis*[3], with 127.2 million annual infections, 86.9 million annual infections, and 156.0 million annual infections of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis*, respectively, having some of the highest incidence rates worldwide[1, 4-7].

Neisseria gonorrhoeae, a Gram-negative, oxidase-positive, aerobic, nutritionally fastidious, intracellular diplococcus, residing in neutrophils, is a sexually transmitted infection (STI), and those resistant to cephalosporins and/or fluoroquinolones are included in the WHO "Priority Level 2", list of high risk pathogens[8]. Infection with *N. gonorrhoeae* leads to gonorrhea or gonococcal disease, the STI reported most frequently after *C. trachomatis* in the USA[9]. The WHO reported 555,608 cases of gonorrhea in the US in 2017, an increase of 18.6% over the previous report, and 75.2% higher than the lowest rate reported in 2009[10, 11].

C. trachomatis is the most reported STI pathogen in the USA, with the number of incident cases increasing yearly, likely due to increased spread and testing in high-risk populations such as MSM, coupled with the increased sensitivity of molecular diagnostic tests[12]. Adolescents and young adults accounted for up to 61% of new cases with women disproportionately affected[12].

M. genitalium infection is often asymptomatic and may occur as a prolonged chronic infection[13]. It has been identified in up to 15-25% of men presenting with non-gonococcal urethritis, and up to 10-13% of women presenting with PID[13]. Genital mycoplasmas have been shown to colonise up to 80% of pregnant and non-pregnant women[14]. The role of this organism in male urogenital inflammatory disease has been described, however the role in women remains uncertain, although inflammation following infection is believed to be due to the hosts response, however studies have indicated the organism is able to cause damage to fallopian cilia[13, 15]. There are few studies investigating the occurrence of the less common STI associated organisms such as *U. parvum*, and some contention exists on their occurrence and the association with true infection, and subsequently whether or not to treat. There is no clear evidence that these organisms cause infection independently of confirmed STI pathogens, or whether they are co-factors for infection, or simply vaginal commensals, though their presence has been linked to complications during pregnancy[16, 17].

The role of *Mycoplasma hominis* and *Ureaplasma urealyticum* as genital tract pathogens is similarly contentious, but are considered to be opportunistic pathogens which may cause pregnancy complications and are implicated in urogenital infections[18, 19]. A study investigating the prevalence of genital mycoplasmas in pregnant women in Gauteng, South Africa indicated high rates of infection with the common agents. *Ureaplasma parvum* was the most prevalent at 72.4%, followed by *M. hominis* at 50.7%, *M. genitalium* at 14.5%, and *U. urealyticum* at 2.3%. *Mycoplasma hominis* and *U. parvum* were also identified in 75% of all HIV positive samples[14].

Trichomoniasis, caused by the protozoan *Trichomonas vaginalis*, is reported to be the most commonly occurring STI globally, with an estimated 276.4 million cases reported in 2008, with up to 90% of these occurring in resources limited settings, with up to 30 million cases reported in Sub-Saharan Africa annually[20-22]. Research has indicated an association between *T. vaginalis* and other STI pathogens like *N. gonorrhoeae*, *C. trachomatis*, Herpes Simplex Virus, and syphilis[23], as well as with an increased risk

of HIV infection in *T. vaginalis* infected women, likely due to the resultant local inflammation and recruitment of target cells for HIV infection, and increased shedding of the virus[24].

With a global estimate of up to one million new cases of STIs reported daily, and the associated strain on medical and socio-economic resources, rapid and accurate diagnosis of these infections is critical to limit spread and guide appropriate targeted therapy[25]. In the latest WHO Global Health Sector Strategy one of the key points is the improvement of STI case management[2]. Syndromic management is still the standard of care in resource limited settings.

Current WHO guidelines recommend syndromic management of STIs, including *N. gonorrhoeae*[26]. These guidelines make provision for clinicians to presumptively treat *N. gonorrhoeae* infections, based solely on clinical presentation, and without laboratory confirmatory testing, by administering treatment which covers this most likely causes. Numerous reports have subsequently emerged indicating the poor performance of this approach to accurately detect and correctly treat these infections empirically[27-30]. These reports indicated the need for better guidelines and criteria to ensure syndromic management is more informed in the absence of laboratory testing.

Traditional laboratory testing relies on routine culture for *N. gonorrhoeae*, cell culture for *C. trachomatis*, and light microscopy for the Mycoplasma's and genital parasites. These methods report poor sensitivity due to the fastidious nature of these organisms, as well as the need for specialised culture methods, media, expertise, and the subjectivity of microscopy.

Multiplexed NAATs are able to test for several different targets simultaneously which reduce the number of tests and healthcare visits required, thereby reducing patient anxiety and the associated costs of multiple tests[31]. Since the risk factors for the acquisition of several STI pathogens overlap, persons at risk often acquire multiple infections simultaneously and a multiplexed assay is able to detect these in a single reaction[25]. Multiplex NAATs for the diagnosis of STIs have demonstrated a 95% sensitivity and specificity of detection in a large number of currently available tests, with some assays reporting up to 100% sensitivity and specificity over a range of STI pathogens[25]. One such assay,

147 the Allplex STI Essential Assay, reported consistently high diagnostic performance (100% sensitivity)
148 and high specificity rates for seven STI pathogens included in the assay[32, 33].

149 Currently, no such multiplex molecular panel is used in our setting to detect multiple STI pathogens
150 concurrently, and the introduction of such an assay may significantly improve detection rates, allowing
151 for targeted therapy, thereby limiting spread and resistance. This study therefore aimed to determine
152 the local prevalence of selected STI pathogens, using a validated commercial multiplex PCR assay.

153

154 **4. Methods**

155 Urogenital samples submitted to the National Health Laboratory Service (NHLS) at Groote Schuur
156 Hospital, Cape Town, South Africa, for routine diagnostic testing, from 04 August 2021 – 03 February
157 2022, were included in this study. Urogenital swabs were collected using Amies agar gel transport
158 swabs (ThermoFisher), or sterile dry swabs, by trained medical staff. The investigating laboratory, one
159 of two public-sector National Health Laboratory Service laboratories in Cape Town, serves 65
160 government healthcare facilities (58 primary healthcare facilities, three district, two regional, and two
161 tertiary hospitals) with a referral area of 754.8 km2 and estimated population of 1.85 million.

162 Routine diagnostic testing was performed using local standard operating procedures, which included
163 Gram-staining, vaginal wet mount, and routine culture on selective media (NYC agar containing
164 vancomycin, colistin, nystatin and trimethoprim lactate), and on chocolate agar.

165 Swabs collected from patients for antenatal Group B streptococcal (GBS) screening were processed as
166 per routine, by placing the swab directly into Todd-Hewitt Broth (THB) for enrichment. No STI testing
167 is performed for these samples. For these samples, a new sterile dry swab was placed into the residual
168 gel transport medium for PCR testing. Routine testing for *C. trachomatis* and other STI pathogens is
169 not available at this facility.

DNA was extracted from all swabs using the Zymo Research Quick DNA Fungal/Bacterial Miniprep kit (Inqaba Biotech), and swabs were cut directly into the lysis tubes and extracted, according to the manufacturer's instructions.

PCR was performed on each extracted sample using the Allplex STI Essential Assay (Inqaba Biotech), according to the manufacturer's instructions. This assay has been clinically validated and is CE and IVD certified for diagnostic use. A previously validated in-house PCR assay for *N. gonorrhoeae* and *C. trachomatis* was also evaluated, using primers and probes supplied by Integrated DNA Technologies, with concentrations optimized for our setting[3, 5]. This PCR assay will serve as a comparative to highlight differences in detection rates using different assays, and since both assays are fully validated, no third reference test is required.

Pearson's chi-squared test, Fisher's exact test, and logistic regression were used to determine associations between variables, and Student's t test with unequal variance was used to compare the difference between mean Ct values of the different PCR assays. Statistical analyses were performed using STATA v16.0 and GraphPad Prism v9.0.

Patient demographic information including age, sex, sample type, and clinical diagnostic test results, ward, and clinical indications were recorded. Research ethics approval, and a waiver of informed consent was obtained from the University of Cape Town Human Research Ethics Committee (HREC REF 408/2021).

5. Results

Of the 148 vaginal and urethral swabs collected during the 6-month study period the majority of samples (91.2%) were obtained from women (Table 1). The median age of men (31 y/o) and women (28 y/o) were not significantly different (p=0.6493) (Figure 1 left).

Table 1. Participant demographics and associations.

	Number (n=148)	*Percentage*	*OR (PCR-positivity)*	*p-value*	*95% CI*
SEX				**Logistic regression**	
Male	13	8.8	1.6	0.486	0.4 - 6.4
Female	135	91.2			
AGE CATEGORY (yrs)				**Logistic regression**	
0-10	26	17.6	10.0	0.064	0.9 – 114.8
11-16	6	4.1	6.0	0.214	0.4 – 101.6
17-30	54	36.5	**51.0**	**0.002**	4.0 – 650.0
31-40	45	30.4	**16.3**	**0.023**	1.5 – 180.0
41-50	7	4.7	7.5	0.158	0.5 – 122.7
51-60	4	2.7	9.0	0.178	0.4 – 220.9
61-70	4	2.7	1.0		
71-80 (reference category)	2	1.4	1.0		
CLINICAL INDICATION				**Fischer's exact test**	
Group B Streptococcal Screening	25	16.9		**0.014**	1.5 - .
Non-specific Indication	8	5.4		0.355	0.4 - .
Not Stated	52	35.1		0.489	0.3 – 2.0
Sexual Non-Accidental Injury	5	3.4		0.185	0.03 - 3.5
STI syndrome	56	37.8		0.250	0.2 - 1.5
Surgery	2	1.4		1.000	0.1 - .
INDICATION by PATHOGEN	**Indication (nr)**	**Indication (%)**		**Logistic regression**	
Non-specific Indication - CT	8	5.4	**11.8**	**0.010**	1.8 – 77.9
GBS Screening - UP	25	16.9	**3.0**	**0.029**	1.1 – 8.2
STI-syndrome - UU	56	37.8	**0.3**	**0.023**	0.1 – 0.9
STI-syndrome - TV	56	37.8	0.3	0.090	0.07 – 1.2
ASSAY PERFORMANCE	**Nr Positive**	**Mean Ct**	**Ct difference**	**t-test unequal var**	**95% CI**
Allplex N. gonorrhoeae	56	31.3	-3.4	**0.0014**	-5.4 - 1.4
In-house N. gonorrhoeae	78	34.7			
Allplex C. trachomatis	15	24.2	-4.3	0.0580	-8.7 - 0.2
In-house C. trachomatis	19	28.5			

*OR – Odds Ratio relative to PCR-positivity, p-values and 95% confidence intervals are reflective of each category compared.

Using the Allplex STI essential assay, all seven STI pathogens included in the panel were detected in one or more of the samples tested, with 83.79% of samples testing positive for one or more pathogen.

198 *Ureaplasma parvum* was the most prevalent pathogen detected overall, with a 6-month period
199 prevalence of 63/148 (42.57%), followed by *N. gonorrhoeae* 56/148 (37.84%), *M. hominis* 51/148
200 (34.46%), *U. urealyticum* 35/148 (23.65%), *T. vaginalis* 17/148 (11.49%), *C. trachomatis* 15/148
201 (10.14%), with *M. genitalium* 2/148 (1.35%) the least prevalent (Figure 1 right).

202

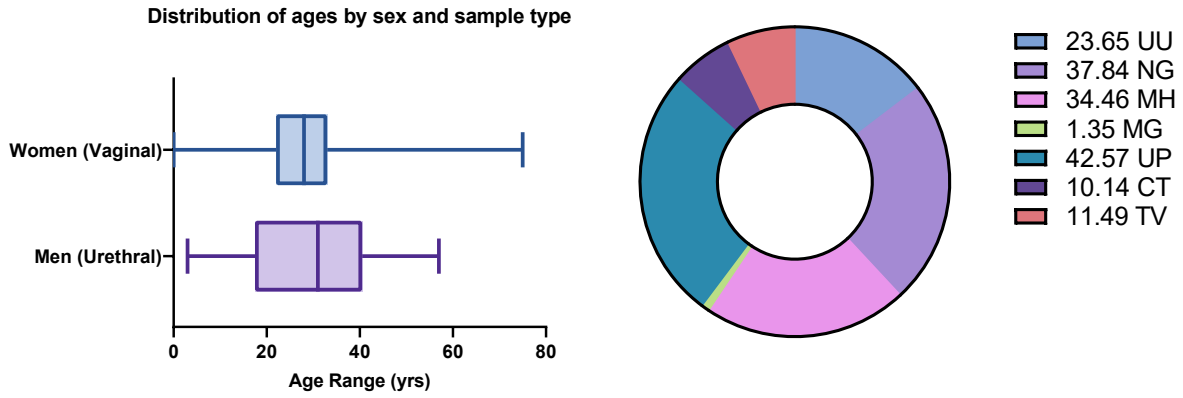
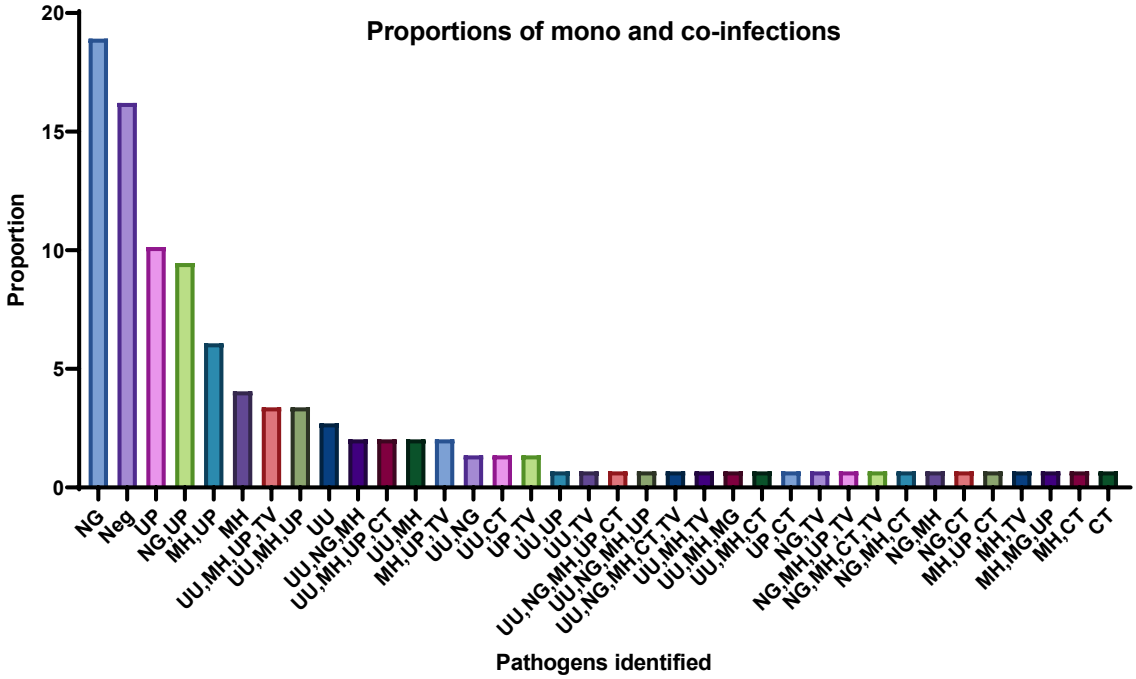


Figure 1. (Left) Box plot of the age distribution of all samples stratified by sex and sample type. (Right) Pie chart indicating the proportion of positive samples for each target tested. *Yrs, years; U. urealyticum; NG, N. gonorrhoeae; MH, M. hominis; MG, M. genitalium; UP, U. parvum; CT, C. trachomatis; TV, T. vaginalis.*

203 Based on the clinical indications for testing, greater than a third of samples submitted were for
204 suspected STI-syndromes, followed by Group B Streptococcal screening (GBS), non-urogenital specific
205 indications, sexual non-accidental injuries, surgical, and 35.14% had no clinical indication recorded
206 (Table 1). *M. genitalium* was detected in only two samples, both submitted for GBS screening, and
207 was in co-infection with both *M. hominis*, and either *U. urealyticum*, or *U. parvum* (Figure 2).

208 Of all the samples tested, 36.49% were positive for a single organism, where *N. gonorrhoeae* was the
209 most commonly occurring pathogen in mono-infection (18.92%), followed by *U. parvum* (10.14%), *M.*
210 *hominis* (4.05%), *U. urealyticum* (2.70%), *C. trachomatis* (0.68%). 16.21% of samples tested negative
211 for all assayed pathogens (Figure 2).



212

213 ***Figure 2. Proportions of mono and co-infections***

214 *U. urealyticum; NG, N. gonorrhoeae; MH, M. hominis; MG, M. genitalium; UP, U. parvum; CT, C.*
215 *trachomatis; TV, T. vaginalis.*

216

217 There were a number of combinations of co-infections with multiple pathogens (Figure 2). 27.03% of
218 samples were positive for two pathogens, while 11.49% tested positive for three pathogens, and
219 1.35% with five pathogens. *M. hominis* and *U. parvum* were the most commonly occurring pathogens
220 in co-infections (36.29% each), followed by *U. urealyticum* (25.0%) and *N. gonorrhoeae* (22.58%), with
221 each of the seven assayed pathogens present in at least one co-infection combination (Figure 2). *M.*
222 *hominis* and *T. vaginalis* were only detected in co-infection with other pathogens.

223 All samples submitted for GBS screening, non-urogenital specific indications, and surgery were
224 positive for at least one pathogen, and 17.86% of samples submitted for STI syndromes were negative
225 for all pathogens (Figure 3). Of the SNAI samples submitted, 60% (3/5) were positive with 1, 2, and 5
226 STI-pathogens, respectively. Samples submitted for non-urogenital specific indications were 11.82
227 times more likely to test positive for *C. trachomatis* (p=0.010) (Table 1). There was also an association

between samples submitted for GBS screening and PCR-positivity for any of the pathogens tested (p=0.014), where all 25 samples were positive for at least one pathogen, and samples submitted for GBS screening were 3.03 times more likely to test positive for *U. parvum* than any other pathogen (p=0.029) (Table 1).

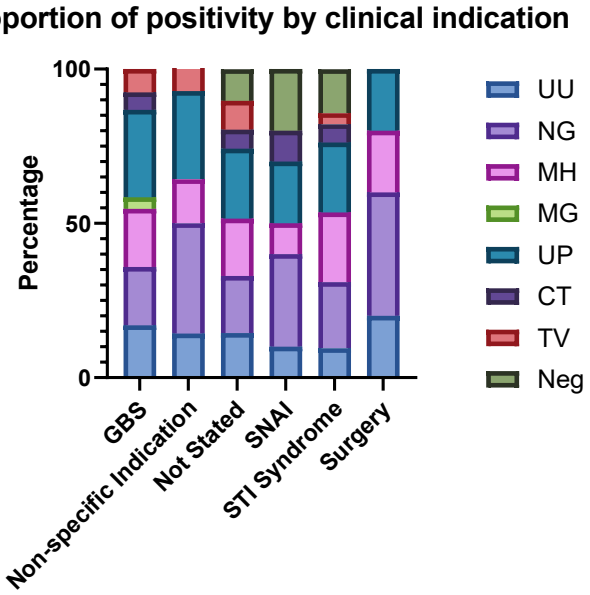


Figure 3. Proportions of samples submitted based on clinical indication recorded.

GBS, Group B Streptococcal Screening; SNAI, sexual non-accidental injury; U. urealyticum; NG, N. gonorrhoeae; MH, M. hominis; MG, M. genitalium; UP, U. parvum; CT, C. trachomatis; TV, T. vaginalis.

Most samples received were in the 0-10-, 17-30- and 31-40-year-old age categories (Figure 4). Multivariate logistic regression analysis indicated that, compared to persons 71-80-years-old, persons aged 17-30- and 31-40-years old were 51-times (95% CI: 4.0 – 650; p=0.002) and 16-times (95% CI: 1.47 – 179.95; p=0.023), respectively, more likely to test PCR-positive for one or more STI pathogen (Table 1).

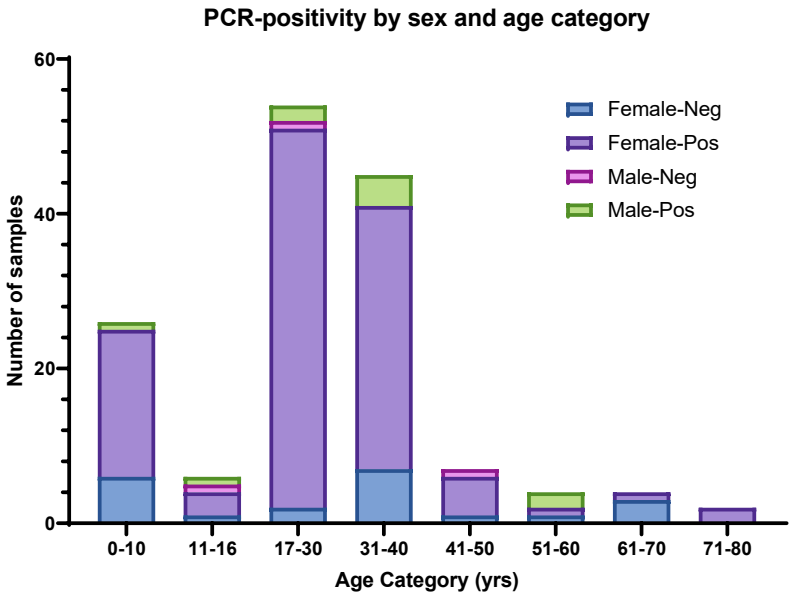


Figure 4. Distribution of PCR-positive results in each age category stratified by sex.

All samples submitted to Groote Schuur Hospital were tested with standard selective microbiological culture and Gram stain procedures. Of the 148 samples selected for this study 56 (37.84%) were positive for *N. gonorrhoeae* mono or co-infection using the Allplex STI Essential Assay. Of these, 3/56 (5.36%) were culture-positive for *N. gonorrhoeae*, and only 1/3 was also concurrently positive for Gram negative diplococci using microscopy. An additional sample was positive for Gram negative diplococci on microscopy, but this sample was culture negative for *N. gonorrhoeae*, and was PCR-positive for *U. parvum* alone (Supplementary Figure S1). Of the 17 *T. vaginalis* PCR-positive samples, only one was positive on wet mount microscopy. Sex, Gram stain, and routine culture were not associated with the likelihood of testing PCR-positive, but this cannot be ruled out due to the small sample size.

The results of this PCR assay for detecting *N. gonorrhoeae* and *C. trachomatis* were compared to an in-house multiplex PCR assay for *N. gonorrhoeae* and *C. trachomatis*[5]. The in-house PCR assay produced higher six-month period prevalence rates compared to those using the Allplex assay with a 52.70% *N. gonorrhoeae* prevalence, and 12.84% *C. trachomatis* (Table 1). Using the estimated population prevalence for *N. gonorrhoeae* (10.4%) described by Jongen *et al*., the positive percent

agreement between the two assays was only 13.44% (10.33% - 17.29%), and negative percent agreement was 92.45% (89.24% - 94.75%), with an accuracy of 54.22% (45.84% - 62.43%)[34]. For *C. trachomatis* (33.70% reported prevalence) the positive percent agreement was much better but still poor at 45.80% (21.76% - 71.98%), and negative percent agreement was 68.39% (62.50% - 73.74%), with an accuracy of 65.06% (56.80% - 72.71%)[34].

When investigating the CT values of the positive results using both assays the results indicated that the mean CT value for the Allplex assay (31.28) was significantly lower (p=0.0014) than that of the in-house assay (34.67) for detecting *N. gonorrhoeae* (Table 1), with a difference in means greater than a log-fold decrease in sensitivity. This was not reflected in the results for *C. trachomatis* where even though the mean difference was larger, it was not significantly different (Table 1).

6. Discussion

This study aimed to determine the six-month period prevalence of 7 commonly occurring bacterial STI pathogens at Groote Schuur Hospital, using a commercial multiplex PCR assay. Most of the swabs tested were positive for at least one of the seven STI-pathogens included in the PCR assay. *Ureaplasma parvum* was the most prevalent pathogen detected overall, followed by *N. gonorrhoeae*, *M. hominis*, *U. urealyticum*, *T. vaginalis*, *C. trachomatis*, with *M. genitalium* the least prevalent. This was alarming considering there were no study specific *a priori* selection criteria for high-risk populations. However, since the swabs were submitted for routine urogenital investigations, this may have presented a high pre-test probability for an STI.

With the introduction of the WHO and CDC guidelines for the management of STIs[35, 36], the available data for laboratory confirmed infections and antimicrobial susceptibility testing are sparce, making comparisons of estimated prevalence rates complex, relying primarily on national surveillance

estimates. Most studies focused on high-risk populations or young women, since they are disproportionately at a higher risk for STI[2, 12].

According to the World Health Organization (WHO) in 2012 the global prevalence rates in women aged 15-49 years old for *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, and *T. pallidum* were 4.2%, 0.8%, 5.0%, and 0.5%, respectively, and Africa was noted to account for approximately 40% of the global occurrence of STIs[4, 37].

Almost all swabs submitted for testing in this study were received from women, and the median age correlated well with international studies where STI-prevalence was highest[4, 29]. National and regional surveillance studies describing STI prevalence rates are limited and vary significantly due to demographic, socio-economic, and geographic factors, as well as national policies for prevention and treatment. A 2018 study, modelling STI prevalence rates in South Africa over 30-years, indicated an adjusted *N. gonorrhoeae* prevalence estimate of 6.6% for women and 3.5% for men, and *C. trachomatis* prevalence of 14.7% and 6.0% for women and men respectively, which were among the highest reported globally[29].

The *N. gonorrhoeae* prevalence estimates in this study represents an alarming increase compared to the global and previous local estimates[3, 4, 37]. This may be due to an increase in transmission as asymptomatic infections may be inadvertently spread, and previous studies have indicated that the risk for STI in high HIV-burdened regions are increased and is associated with lower education levels, several sex partners, drug and alcohol use, and early sexual debut[38]. This was not as marked for *C. trachomatis* where the estimated prevalence rate was higher than the global estimate, but comparable to other local estimates[4, 29].

There are few studies investigating the prevalence of the less common STI pathogens, and some contention exists on the occurrence of these organisms and their association with true infection, and whether or not to treat. There is no clear evidence that these organisms cause infection

independently of other STI pathogens, or whether they are co-factors for infection, or simply vaginal commensals, though they have been linked to complications during pregnancy[17, 39].

The period prevalence of *U. parvum*, *U. urealyticum*, *M. hominis*, and *M. genitalium* in this study were all markedly higher than those determined in an Italian study on women with vaginitis or pregnancy complications, which used the same PCR assay[40]. This is further highlighted in this study where although *U. parvum*, *M. hominis*, and *U. urealyticum* were identified in mono-infection, albeit at lower rates, *M. hominis*, *U. urealyticum*, and *U. parvum* were the most commonly occurring pathogens in co-infections, with *M. hominis* and *T. vaginalis* only detected in coinfection with other pathogens. Compared to a 2013 prevalence estimate of genital mycoplasmas in the North of South Africa, the current *M. genitalium*, *M. hominis*, and *U. parvum* prevalence rates are dramatically lower, but the rates for *U. urealyticum* reported here are more than ten-fold higher[14].

The prevalence estimate of *T. vaginalis* in this study was greater than double the global estimate, yet only one of the PCR positive samples was also positive using wet mount microscopy, indicating the poor performance of this method for detection[4, 37]. Similar to previous reports, *T. vaginalis* was only detected in co-infection with other STI pathogens in this study[23]. Since no HIV data was collected in this study, it is not possible to correlate this to an increased risk of HIV acquisition or virus shedding[24].

Most samples submitted for testing in this study were for suspected STI-syndromes, GBS screening, or where no clinical indication was recorded. Interestingly, about one fifth of the samples submitted for suspected STI-syndromes were negative for all pathogens tested. Considering most samples were obtained from women, vaginal candidiasis, or bacterial vaginosis, which may also present with discharge, may have accounted for a proportion of these results. This may reflect a large proportion of patients where unnecessary antibiotic therapy may have been initiated based on the current syndromic testing guidelines.

Samples submitted for GBS screening are routinely collected from pregnant women in the final stages of pregnancy to determine whether antibiotic prophylaxis should be administered during delivery to

prevent vertical GBS infection in the new-born infant, which may cause severe morbidity and even mortality, according to the CDC guidelines[41, 42]. Interestingly, only two GBS screening samples were culture positive for GBS, whereas they all tested positive for one or more STI pathogens, and these samples were three times more likely to harbour *U. parvum* than any of the other organisms tested. Further, *M. genitalium* was only detected in GBS screening samples, and was in co-infection with both *M. hominis* and either *U. urealyticum* or *U. parvum*. Nyemba *et al*., reported a 40% STI prevalence in pregnant women living with HIV and 27% in women living without HIV, and that baseline infection was associated with younger age, and the need for antenatal STI screening[43]. These findings highlighted a potential high-risk population group which may need to be considered for more comprehensive STI screening in our setting, as studies have indicated an association between STI infections and pre-term birth, transmission to the foetus or new-born baby, or even stillbirth[44].

The association between samples non-urogenital specific indications and testing positive for *C. trachomatis* may also represent a cohort of participants at risk for STI. These participants may have presented with non-descript or mild symptoms not indicative of STI, likely the result of *C. trachomatis* infection, which is often asymptomatic, especially in women.

Most alarmingly, more than half of the samples submitted from suspected child abuse or sexual non-accidental injury were positive for an STI, with one sample testing positive for five different pathogens, likely a result of high risk unprotected sexually activity during sexual misconduct. The use of a broad range molecular test would provide rapid results for early interventions, increase detection sensitivity, and provide reliable results for further legal proceedings.

When compared to microbiological investigations, selective culture, Gram stain, and wet mount proved extremely poor, with only a few samples testing positive for *N. gonorroheae* or *T. vaginalis*. This is not surprising considering the fastidious nature of *N. gonorroheae* and that low level infections may be below the detection limit for these methods.

Using the in-house PCR assay, several discordant results were obtained compared to the commercial assay. Even though the in-house assay produced higher prevalence rates, reproducibility was poor, as reflected by the accuracy values. Care must be taken when selecting diagnostic assays for STIs, which are known to cross react with other species and may be impacted by interfering substances. The use of certified assay, with excellent assay performance criteria should be favoured.

Limitations of this study includes (1) a small and heterogenous sample size, as the associations described may not hold true at a population level. Thus, a population surveillance study, especially with respect to the higher risk groups identified in this study, is thus recommended. (2) There is a paucity in the current local and National prevalence data, making comparisons of these findings challenging. (3) Finally, the lack of complete clinical data, including HIV data may have allowed for the determination of additional associations.

7. Conclusions

This study highlights alarming prevalence estimates of common STIs locally at a regional level. High risk populations such as pregnant women and cases of SNAI may benefit from routine screening. Continued surveillance or routine testing should be considered, especially in resource limited settings where transmission rates may be higher and syndromic treatment guidelines may not reflect the true local scenario.

Word count = 4057

8. Declarations

8.1. Ethics approval and consent to participate

This was a prospective laboratory study approved by the University of Cape Town Human Research Ethics Committee (HREC REF 408/2021).

8.2. Consent for publication

Not applicable.

8.3. Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

8.4. Competing interests

The authors declare that they have no competing interests.

8.5. Funding

This study was funded by internal research funding.

8.6. Authors' contributions

CM conceptualised the study, conducted laboratory testing, data collection and analyses, and prepared the manuscript. HT provided clinical guidance and expertise in result interpretation, and assisted with manuscript preparation. IA assisted with DNA extractions, in-house PCR, and manuscript preparation. ME provided guidance throughout the study including protocol preparation, result interpretation, and manuscript preparation.

8.7. Acknowledgements

None.

9. References cited:

[1] **Rowley J, Vander Hoorn S, Korenromp E, Low N, Unemo M, Abu-Raddad LJ*, et al.*** 2019. Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. Bulletin of the World Health Organization **97:**548-62p.

[2] **Peters RPH, Garrett N, Chandiwana N, Kularatne R, Brink AJ, Cohen K*, et al.*** 2022. Southern African HIV Clinicians Society 2022 guideline for the management of sexually transmitted infections: Moving towards best practice. 2022 **23**.

[3] **Mhlongo S, Magooa P, Müller EE, Nel N, Radebe F, Wasserman E*, et al.*** 2010. Etiology and STI/HIV coinfections among patients with urethral and vaginal discharge syndromes in South Africa. Sexually transmitted diseases **37:**566-70.

[4] **Newman L, Rowley J, Vander Hoorn S, Wijesooriya NS, Unemo M, Low N*, et al.*** 2015. Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. PloS one **10:**e0143304.

[5] **Venter JM, Mahlangu PM, Müller EE, Lewis DA, Rebe K, Struthers H*, et al.*** 2019. Comparison of an in-house real-time duplex PCR assay with commercial HOLOGIC® APTIMA assays for the detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in urine and extra-genital specimens. BMC infectious diseases **19:**1-7.

[6] **Ohnishi M, Golparian D, Shimuta K, Saika T, Hoshina S, Iwasaku K*, et al.*** 2011. Is *Neisseria gonorrhoeae* initiating a future era of untreatable gonorrhea?: detailed characterization of the first strain with high-level resistance to ceftriaxone. Antimicrob Agents Chemother **55:**3538-45.

[7] **Costa-Lourenço A, Barros Dos Santos KT, Moreira BM, Fracalanzza SEL, Bonelli RR.** 2017. Antimicrobial resistance in *Neisseria gonorrhoeae*: history, molecular mechanisms and epidemiological aspects of an emerging global threat. Braz J Microbiol **48:**617-28.

[8] **Asokan GV, Ramadhan T, Ahmed E, Sanad H.** 2019. WHO Global Priority Pathogens List: A Bibliometric Analysis of Medline-PubMed for Knowledge Mobilization to Infection Prevention and Control Practices in Bahrain. Oman Med J **34:**184-93.

[9] **Costa-Lourenço APRd, Barros Dos Santos KT, Moreira BM, Fracalanzza SEL, Bonelli RR.** 2017. Antimicrobial resistance in Neisseria gonorrhoeae: history, molecular mechanisms and epidemiological aspects of an emerging global threat. Braz J Microbiol **48:**617-28.

[10] **World Health Organisation W.** 2018. Sexually Transmitted Disease Surveillance 2017 - National Profile Overview, Centers for Disease Control and Prevention. https://www.cdc.gov/std/stats17/gonorrhea.htm, Online Publication.

[11] **Unemo M, Shafer WM.** 2014. Antimicrobial Resistance in *Neisseria gonorrhoeae* in the 21st Century: Past, Evolution, and Future. Clinical Microbiology Reviews **27:**587-613.

[12] **Prevention CfDCa.** 2019. Sexually transmitted disease surveillance 2018. .

[13] **Pinto-Sander N, Soni S.** 2019. Mycoplasma genitalium infection. BMJ **367:**l5820.

[14] **Redelinghuys MJ, Ehlers MM, Dreyer AW, Lombaard H, Kock MM.** 2013. P3.035 Prevalence of Genital Mycoplasmas and Bacterial Vaginosis in Pregnant Women in Gauteng, South Africa. Sexually Transmitted Infections **89:**A159-A.

[15] **Sethi S, Singh G, Samanta P, Sharma M.** 2012. Mycoplasma genitalium: an emerging sexually transmitted pathogen. Indian J Med Res **136:**942-55.

[16] **Patel MA, Nyirjesy P.** 2010. Role of Mycoplasma and ureaplasma species in female lower genital tract infections. Curr Infect Dis Rep **12:**417-22.

[17] **Taylor-Robinson D, Lamont R.** 2011. Mycoplasmas in pregnancy. BJOG: An International Journal of Obstetrics & Gynaecology **118:**164-74.

[18] **Naicker M, Dessai F, Singh R, Mitchev N, Tinarwo P, Abbai NS.** 2021. 'Mycoplasma hominis does not share common risk factors with other genital pathogens': Findings from a South African pregnant cohort. S Afr J Infect Dis **36:**207.

[19] **Bayraktar MR, Ozerol IH, Gucluer N, Celik O.** 2010. Prevalence and antibiotic susceptibility of Mycoplasma hominis and Ureaplasma urealyticum in pregnant women. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases **14:**e90-5.

[20] **Kissinger P.** 2015. Trichomonas vaginalis: a review of epidemiologic, clinical and treatment issues. BMC infectious diseases **15:**307.

[21] **Kissinger PJ, Gaydos CA, Seña AC, Scott McClelland R, Soper D, Secor WE*, et al.*** 2022. Diagnosis and Management of Trichomonas vaginalis: Summary of Evidence Reviewed for the 2021 Centers for Disease Control and Prevention Sexually Transmitted Infections Treatment Guidelines. Clinical Infectious Diseases **74:**S152-S61.

[22] **Mabaso N, Abbai NS.** 2021. A review on Trichomonas vaginalis infections in women from Africa. S Afr J Infect Dis **36:**254.

[23] **Allsworth JE, Ratner JA, Peipert JF.** 2009. Trichomoniasis and other sexually transmitted infections: results from the 2001-2004 National Health and Nutrition Examination Surveys. Sex Transm Dis **36:**738-44.

[24] **Kissinger P, Adamski A.** 2013. Trichomoniasis and HIV interactions: a review. Sex Transm Infect **89:**426-33.

[25] **Karellis A, Naeem F, Nair S, Mallya SD, Routy JP, Gahagan J*, et al.*** 2022. Multiplexed rapid technologies for sexually transmitted infections: a systematic review. Lancet Microbe **3:**e303-e15.

[26] **World Health Organisation W.** 2021. Guidelines for the management of symptomatic sexually transmitted infections. WHO **https://www.who.int/publications/i/item/9789240024168**.

[27] **Vallely LM, Toliman P, Ryan C, Rai G, Wapling J, Gabuzzi J*, et al.*** 2017. Performance of syndromic management for the detection and treatment of genital Chlamydia trachomatis, Neisseria gonorrhoeae and Trichomonas vaginalis among women attending antenatal, well woman and sexual health clinics in Papua New Guinea: a cross-sectional study. BMJ open **7:**e018630-e.

[28] **Ndowa FJ, Francis JM, Machiha A, Faye-Kette H, Fonkoua MC.** 2013. Gonococcal antimicrobial resistance: perspectives from the African region. Sex Transm Infect **89 Suppl 4:**iv11-5.

[29] **Kularatne RS, Niit R, Rowley J, Kufa-Chakezha T, Peters RPH, Taylor MM*, et al.*** 2018. Adult gonorrhea, chlamydia and syphilis prevalence, incidence, treatment and syndromic case reporting in South Africa: Estimates using the Spectrum-STI model, 1990-2017. PLOS ONE **13:**e0205863.

[30] **van der Eem L, Dubbink JH, Struthers HE, McIntyre JA, Ouburg S, Morré SA*, et al.*** 2016. Evaluation of syndromic management guidelines for treatment of sexually transmitted infections in South African women. Tropical Medicine & International Health **21:**1138-46.

[31] **Pant Pai N, Daher J.** 2015. Multiplexed testing for HIV and related bacterial and viral co-infections at the point-of-care: quo vadis? Expert Rev Mol Diagn **15:**463-9.

[32] **Berçot B, Amarsy R, Goubard A, Aparicio C, Loeung HU, Segouin C*, et al.*** 2015. Assessment of coinfection of sexually transmitted pathogen microbes by use of the anyplex II STI-7 molecular kit. J Clin Microbiol **53:**991-3.

[33] **Choe HS, Lee DS, Lee SJ, Hong SH, Park DC, Lee MK*, et al.*** 2013. Performance of Anyplex™ II multiplex real-time PCR for the diagnosis of seven sexually transmitted infections: comparison with currently available methods. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases **17:**e1134-40.

[34] **Jongen VW, Schim van der Loeff MF, Botha MH, Sudenga SL, Abrahamsen ME, Giuliano AR.** 2021. Incidence and risk factors of C. trachomatis and N. gonorrhoeae among young women from the Western Cape, South Africa: The EVRI study. PLOS ONE **16:**e0250871.

[35] **World Health O.** 2021. Guidelines for the management of symptomatic sexually transmitted infections. World Health Organization, Geneva.

[36] **Workowski KA, Bachmann LH, Chan PA, Johnston CM, Muzny CA, Park I*, et al.*** 2021. Sexually Transmitted Infections Treatment Guidelines, 2021. MMWR Recomm Rep **70:**1-187.

[37] **Organization WH.** 2016. Global Health Sector Strategy on Sexually Transmitted Infections, 2016–2021. https://www.who.int/publications/i/item/WHO-RHR-16.09.

[38] **Semwogerere M, Dear N, Tunnage J, Reed D, Kibuuka H, Kiweewa F*, et al.*** 2021. Factors associated with sexually transmitted infections among care-seeking adults in the African Cohort Study. BMC Public Health **21:**738.

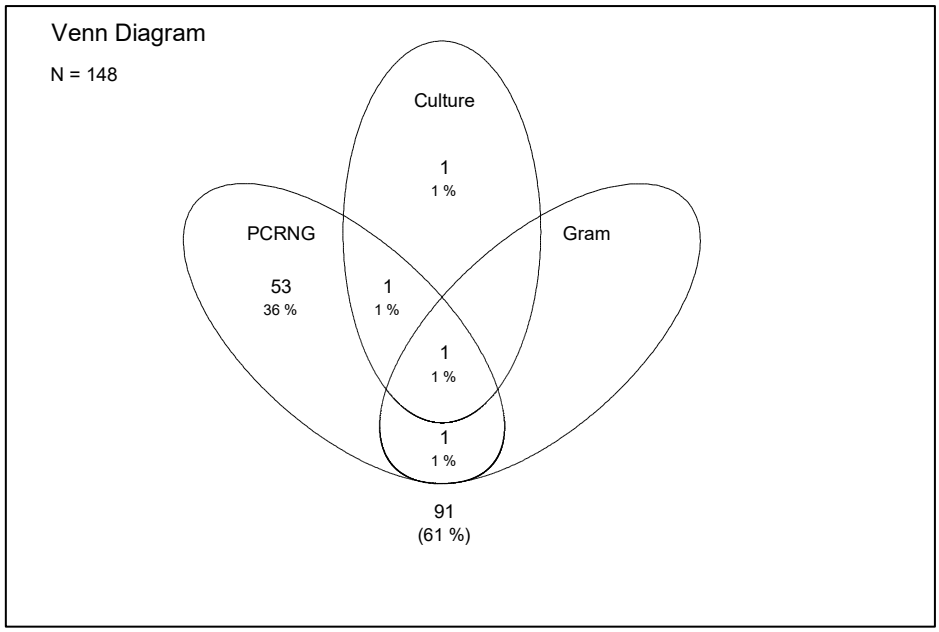
[39] **Patel MA, Nyirjesy P.** 2010. Role of Mycoplasma and Ureaplasma Species in Female Lower Genital Tract Infections. Current Infectious Disease Reports **12:**417-22.

[40] **Leli C, Mencacci A, Latino MA, Clerici P, Rassu M, Perito S*, et al.*** 2018. Prevalence of cervical colonization by Ureaplasma parvum, Ureaplasma urealyticum, Mycoplasma hominis and Mycoplasma genitalium in childbearing age women by a commercially available multiplex real-time PCR: An Italian observational multicentre study. Journal of Microbiology, Immunology and Infection **51:**220-5.

[41] **Ahmadzia HK, Heine RP.** 2014. Diagnosis and management of group B streptococcus in pregnancy. Obstet Gynecol Clin North Am **41:**629-47.

[42] **Verani JR, McGee L, Schrag SJ.** 2010. Prevention of perinatal group B streptococcal disease--revised guidelines from CDC, 2010. MMWR Recomm Rep **59:**1-36.
[43] **Nyemba DC, Medina-Marino A, Peters RPH, Klausner JD, Ngwepe P, Myer L*, et al.*** 2021. Prevalence, incidence and associated risk factors of STIs during pregnancy in South Africa. Sex Transm Infect **97:**375-81.
[44] **Gao R, Liu B, Yang W, Wu Y, Wang B, Santillan MK*, et al.*** 2021. Association of Maternal Sexually Transmitted Infections With Risk of Preterm Birth in the United States. JAMA Network Open **4:**e2133413-e.

10. Supplementary data



Supplementary Figure S1. Venn diagram of PCR-positive samples, culture, and Gram stain.

PART C: Appendices

Determining the six-month period prevalence of STI pathogens at Groote Schuur Hospital using the Allplex STI Essential PCR assay

1. Ethics approval letter

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-submissions@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

06 July 2021

HREC REF: 408/2021

Dr C Moodley
Division of Medical Microbiology
Falmouth Building
Email: c.moodley@uct.ac.za
Student: amnima001@myuct.ac.za

Dear Dr Moodley

PROJECT TITLE: DETERMINING THE PERIOD PREVALENCE OF NEISSERIA GONORRHOEAE AND CHLAMYDIA TRACHOMATIS FROM UROGENITAL AND RESPIRATORY SPECIMENS SUBMITTED TO GROOTE SCHUUR HOSPITAL USING PCR-B. MEDSCI CANDIDATE-MS IMAAN AMIEN

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020 & 06 July 2020.

Approval is granted for one year until the 30 July 2022.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Ms Imaan Amien will also be involved in this study.

Please quote the HREC REF 408/2021 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2020), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC/REF408/2021sa

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mentioned in the figure legend. Corresponding uncropped full-length gels and blot must be included in the supplementary files. These uncropped images should indicate where they were cropped, be labelled as in the main text and placed in a single supplementary figure. The manuscript's figure legends should state that 'Full-length blots/gels are presented in Supplementary Figure X'. Further information can be found under 'Digital image integrity' which is detailed on our Standards of Reporting page.

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The title page should:

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The Abstract should not exceed 350 words. Please minimize the use of abbreviations and do not cite references in the abstract. Reports of randomized controlled trials should follow the CONSORT extension for abstracts. The abstract must include the following separate sections:

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- **Methods: how the study was performed and statistical tests used**
- **Results: the main findings**
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Three to ten keywords representing the main content of the article.

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Methods

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- **the aim, design and setting of the study**
- **the characteristics of participants or description of materials**

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This should include the findings of the study including, if appropriate, results of statistical analysis which must be included either in the text or as tables and figures.

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Funding

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