Genotyping of *Chlamydia trachomatis* detected in South African pregnant women

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PREFACE

One of the main sexually transmitted infections (STIs) amongst pregnant women is caused by the bacterial species *Chlamydia trachomatis* (*C. trachomatis*). Serovars D-K are responsible for this infection amongst pregnant women. The different serovars amongst pregnant South African women, are hindered by the paucity of epidemiological research of *C. trachomatis* infections in this population. The detection of *C. trachomatis* variations within serovars is made easier and more accurate by genotyping techniques targeting the outer membrane protein gene (*omp1*) of this pathogen. The current study was conducted to identify circulating serovars of *C. trachomatis* by restriction analysis of the *omp1* and to link clinical factors to serovars in a South African population of pregnant women. This study provided a backbone to future research on the serovars and prevalence of *C. trachomatis* in our local setting. The experimental work described in this dissertation was conducted at the Nelson R. Mandela School of Medicine, University of KwaZulu-Natal (UKZN), Durban, South Africa. All work was conducted under the supervision of Professor Nathlee Abbai and co-supervised by Doctor Nonkululeko Gladness Mabaso.

PLAGIARISM DECLARATION

I, **Caitlin Ramnarain** declare that

(i) The research reported in this dissertation, except where otherwise indicated, is my original work.

(ii) This dissertation has not been submitted for any degree or examination at any other university.

(iii) This dissertation does not contain other persons' data, pictures, graphs, or other information, unless specifically acknowledged as being sourced from other persons.

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Signed:  Date: **23/11/2022**

PERMISSION TO SUBMIT

As the candidate's supervisors, we have read the dissertation and have given approval for submission for examination.

[redacted]

Supervisor: **Prof. Nathlee Abbai**

Date: **23/11/2022**

[redacted]

Co-supervisor: **Dr. Nonkululeko Gladness Mabaso**

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RESEARCH OUTPUTS

Accepted Manuscripts

Manuscript 1:

Caitlin Ramnarain, Rowen Govender, Nonkululeko Mabaso & Nathlee Abbai. Mini Review: The impact of *Chlamydia trachomatis* infection on pregnancy and neonatal outcomes. This manuscript was accepted by the Journal of Medical Laboratory Science & Technology of South Africa (Reference number: 135).

Manuscript 2:

Caitlin Ramnarain, Nonkululeko Mabaso, Bongekile Ngobese & Nathlee Abbai. Genotyping of *Chlamydia trachomatis* from vaginal swabs by restriction analysis of the outer membrane protein gene. This manuscript was accepted by the Journal of Medical Laboratory Science & Technology of South Africa (Reference number: 130).

DEDICATION

This dissertation is dedicated to God Almighty, my supportive parents, kind sister and loving fiancé.

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Throughout my Master's research project, I have received a great deal of support and assistance.

Firstly, I would like to thank God for everything that I have been blessed with. Thank you for giving me the strength and perseverance to complete my Master's degree.

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LIST OF SYMBOLS

&	And
x^2	Chi square
°C	Degrees Celsius
=	Equal to
>	Greater-than
<	Less-than
μL	Microlitre
N	Number
%	Percent
P	Probability
X	Times

LIST OF ACRONYMS

ANC	Antenatal clinic
Bp	Base pair(s)
BREC	Biomedical Research Ethics Committee
CDC	Centres for Disease Control and Prevention
CT	*Chlamydia trachomatis*
DNA	Deoxyribonucleic acid
EBs	Elementary bodies
ECDC	European Centre for Disease Control and Prevention
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
IQR	Interquartile range
Mg	Milligrams
MTCT	Mother-to-child transmission
Nm	Nanometres
NG-MLST	Next generation-multi locus sequence typing
NAATs	Nucleic acid amplification tests
Omp1	Outer membrane protein gene
PAMPs	Pathogen-associated molecular patterns
PID	Pelvic inflammatory disease
PCR	Polymerase chain reaction
PCR-RFLP	Polymerase chain reaction-restriction fragment length polymorphism
PROM	Premature rupture of membranes
RFLP	Restriction fragment length polymorphism
RBs	Reticulate bodies
STD	Sexually transmitted disease
STI	Sexually transmitted infection
SSA	Sub-Saharan Africa
TLRs	Toll-like receptors
USA	United States of America
UKZN	University of KwaZulu-Natal
WHO	World Health Organization

LIST OF APPENDICES

ABSTRACT

Background

Chlamydia trachomatis (*C. trachomatis*) is a common cause of bacterial sexually transmitted infections (STIs). The genetic characterisation of *C. trachomatis* serovars reveals significant genetic diversity in this organism. Untreated *C. trachomatis* infection in pregnant women has been linked to miscarriage, low birth weight babies, premature rupture of membranes, postpartum endometritis, and transmission to the new-born babies. Currently, there is limited data and analyses on the serovars of *C. trachomatis* circulating in South African pregnant women. In this study, the prevalence of *C. trachomatis* infection was determined, and the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the outer membrane protein gene (*omp1*) was performed in order to identify the different serovars circulating in the population of pregnant women.

Methods

In this study, 385 vaginal swab samples were analysed for the presence of *C. trachomatis*. The swabs were collected from human immunodeficiency virus (HIV)-positive pregnant women at the King Edward VIII hospital in Durban, South Africa. *Chlamydia trachomatis* was detected using commercial primers and probes (TaqMan Assay, assay ID Ba04646249_s1) which targets the gene encoding the translocated actin-recruiting phosphoprotein of *C. trachomatis*. Genotyping of *C. trachomatis* positive samples was performed by an *omp1* semi-nested polymerase chain reaction (PCR) assay followed by restriction fragment length polymorphism (RFLP) analysis. The *omp1* from *C. trachomatis* was amplified with gene-specific primers in the first round PCR to yield a 1033 base pair (bp) fragment. 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 978 bp *omp1* amplicons were digested with *AluI, DdeI* and *HinfI*, and the banding patterns were compared across the three digests for assignment of serovars. Associations between categorical variables was assessed using chi square (x^2) tests. All statistical analysis was conducted using RStudio, version 3.6.3. All *p*-values were considered significant at < 0.05.

Results

The actin-recruiting phosphoprotein of *C. trachomatis* was detected in 47/385 swab samples using the TaqMan Assay. The prevalence of *C. trachomatis* in the study population was 12.2%. All negative no-template controls did not produce any amplification. Factors associated with testing positive for *C. trachomatis* included, having a low level of education, being unemployed, being unmarried, not cohabitating with sex partner, early age of first sex, high number of lifetime sex

partners, partner having other partners, lack of condom use, lacking symptoms of STIs, lacking treatment for STIs and women having a perceived risk of getting STIs. Serovar E (20/43) - 46.5% was the most frequent serovar in our study population, followed by serovar F (9/43) - 20.9%, G (6/43) - 14.0%, D (5/43) - 11.6%, and the least frequent serovar I (2/43) - 4.7% which was detected in two samples. From the five women that carried serovar D, 20.0% (1/5) reported past treatment of STIs. From the 20 women that carried serovar E, 20.0% (4/20) reported having abnormal vaginal discharge. Of the women with serovar E, 20.0% (4/20) reported past treatment of STIs. From the nine women that carried serovar F, 11.1% (1/9) reported having abnormal vaginal discharge and 22.2% (2/9) reported past treatment of STIs. From the six women that carried serovar G, 16.7% (1/6) reported past treatment of STIs. From the two women that carried serovar I, 50.0% (1/2) reported having abnormal vaginal discharge.

Conclusion

This study detected an overall 12.2% prevalence rate for *C. trachomatis* in the pregnant women. The identification of factors associated with infection provided evidence on the importance of antenatal clinics to screen women during their routine check-ups for vaginal infections and provide continuous risk reduction counselling to this vulnerable population. Five different serovars were observed in the studied population with serovars E and F being the most prevalent. The observed diversity of serovars reported within specific populations can be challenging for future vaccine design and development for chlamydia. However, many of the South African serovars detected correlated with serovars found in studies conducted throughout the world. This suggests the possibility of conserved *C. trachomatis* strains from various geographical areas, which may offer some hope for future vaccine development and diagnostic research aimed at the entire *C. trachomatis* population.

Keywords: *Chlamydia trachomatis*, sexually transmitted infections, pregnant women, serovar, South Africa

CHAPTER 1

1.1 Dissertation Overview

1.1.1 Dissertation structure

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

Chapter 1: This chapter comprises an introduction to the study, and it describes the problem statement, research questions, rationale, aim, objectives, and hypothesis of the study.

Chapter 2: This chapter is a review of the literature on *C. trachomatis*. The literature review titled **"Mini Review: The impact of *Chlamydia trachomatis* infection on pregnancy and neonatal outcomes"** is presented in the format of a manuscript that was accepted by the Journal of Medical Laboratory Science & Technology of South Africa. This manuscript is authored by Caitlin Ramnarain, Rowen Govender, Nonkululeko Mabaso & Nathlee Abbai.

Submission URL:
https://jmlstsa.smltsa.org.za/index.php/JMLSTSA/authorDashboard/submission/135

Chapter 3: This chapter is entitled: **"Genotyping of *Chlamydia trachomatis* from vaginal swabs by restriction analysis of the outer membrane protein gene".** This manuscript was accepted by the Journal of Medical Laboratory Science & Technology of South Africa. The manuscript is authored by Caitlin Ramnarain, Nonkululeko Mabaso, Bongekile Ngobese & Nathlee Abbai. This article details the prevalence of *C. trachomatis* infection among pregnant women from Durban, South Africa as well as the serovars and clinical factors associated with the infection.

Submission URL:
https://jmlstsa.smltsa.org.za/index.php/JMLSTSA/authorDashboard/submission/130

Chapter 4: In this final chapter the relevant findings of the project in context with literature are discussed. The limitations and strengths are discussed and the conclusions emanating from this study are also presented.

1.1.2 Study design and Methodology

Briefly, this was a cross-sectional study which included 385 HIV-positive pregnant women recruited from the antenatal clinic (ANC) at the King Edward VIII hospital in Durban, South Africa. Enrolled women provided socio-demographic, behavioural, clinical data, and self-collected vaginal swabs for the detection of vaginal infections. Deoxyribonucleic acid (DNA) was extracted from the vaginal swabs using the PureLink™ Microbiome Purification Kit (Thermo-Fisher Scientific, United States of America). *Chlamydia trachomatis* was detected using the Applied Biosystems™ TaqMan® Assays. Molecular genotyping of *C. trachomatis* positive samples was performed by an *omp1* semi-nested PCR followed by RFLP analysis. All statistical analyses were conducted using RStudio, version 3.6.3. All *p*-values were considered significant at < 0.05. This study was approved by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (BREC/00004226/2022).

The study design and methodology involved in this study are detailed in the respective manuscripts within the dissertation.

1.2 Introduction

Reproductive tract infections rank amongst the most significant public health issues in both developed and developing nations, including South Africa [1]. *Chlamydia trachomatis* (*C. trachomatis*) is an obligatory intracellular, gram-negative bacteria that can only replicate inside a host cell. Globally, it is the leading cause of bacterial sexually transmitted infections (STIs) amongst young women. Asymptomatic *C. trachomatis* infections can occur in up to 80% of female patients [2]. According to data from the World Health Organization (WHO), *C. trachomatis* is one of the most prevalent bacterial STI pathogens, causing an estimated 127 million new infections each year [3]. In a study conducted in Cape Town, South Africa, the prevalence of *C. trachomatis* among pregnant women was approximately 20% (49/242) [4]. The number of chlamydial infections reported in the United States of America (USA) in 2014 was over 1.44 million, which is the highest number since cases were first identified in 1984. Between 2004 and 2014, the number of reported cases of chlamydial infection rose from 316.5 to 456.1 per 100 000 individuals. The actual number of new infections is probably much higher since many infections are asymptomatic and go unreported [3]. In pregnancy, *C. trachomatis* infection can lead to, ectopic pregnancy, early membrane rupture, preterm delivery, spontaneous abortion, and perinatal death [1].

Nucleic acid amplification tests (NAATs), nucleic acid hybridisation tests, direct immunofluorescence, and enzyme immunoassays are amongst the tests used to diagnose urogenital *C. trachomatis* infections. The NAATs are the most precise and sensitive assays for identifying *C. trachomatis* infections and have become the gold standard for diagnosis and screening [5]. Whilst the host's genetics may play a role in disease severity [6], this pathogen must adapt to living in the harsh host environment. To this end, *C. trachomatis* has evolved a multitude of adaptations to fit into the intracellular niches of its hosts [6]. Prokaryotes acquire new genetic features that are beneficial to them through a variety of ways. Point mutations that arise randomly in genomes code for effectors with better functional qualities [7]. Horizontal gene transfer is enabled by transduction, transformation, or conjugation. Gene duplication creates new gene families with distinct functions and expression patterns. All these factors have a significant role in bacterial gene variability [8]. *Chlamydia trachomatis* genomes are highly syntenic, and the sequence variation between genomes can occasionally be as low as 20 single nucleotide polymorphisms. Recombination is thought to be the mechanism that creates diversity, according to recent indications from partial genome analysis [9].

Cervicitis can be caused by urethral infection of chlamydia [10]. Cervicitis can be asymptomatic or cause mucopurulent vaginal discharge and postcoital haemorrhage in women. Pelvic

inflammatory disease (PID) or salpingitis is frequently asymptomatic. More than 15% of women with past episodes of PID may experience chronic pelvic pain associated with the existence of peritoneal adhesions [10]. Infants born to mothers who have chlamydial infections can become infected shortly after birth. Infected vaginal fluids have a high transmission rate (50%-70%). Conjunctivitis affects 30%-50% of infants born to infected mothers, 5-10 days after delivery. Nasopharyngeal infection affects as least half of all new-borns with conjunctivitis [11]. After 2-3 weeks of incubation, 30% of these new-borns develop chlamydial pneumonia. Untreated infections acquired at birth can last for months or years if not managed appropriately [12, 13].

Genotyping is a method for investigating small genetic anomalies that can lead to large phenotypic alterations, such as physical characteristics that distinguish individuals from each another and pathological changes that cause disease [14]. Genotyping is a popular method for bacterial strain typing and permits the separation of bacterial strains based on their genetic makeup due to its high resolution. A serovar is a collection of bacteria or viruses based on the antigens on their cell surfaces. Serovars enable sub-species level classification of organisms, which is essential in epidemiology [14].

Cervicitis is a widespread condition caused by *C. trachomatis* serovars D, E, F, G, H, I, J and K, whereas lymphogranuloma venereum is associated with serovars L1, L2 and L3. According to reports, in industrialized nations, serovars D, E and F are more frequently linked to cervical, vaginal, and urethral infections [15]. *Chlamydia trachomatis* serovars D through K are mostly responsible for causing urogenital infections and of these, serovars E, F and D account for up to 60%-70% of these infections. Genomic methods, targeting the outer membrane protein gene (*omp1*), are more sensitive and accurate than immunotyping in detecting *C. trachomatis* variations within serovars as well as potential recombinants between serovars [14].

There are a limited number of studies that have investigated the prevalence of circulating serovars of *C. trachomatis* in pregnant women from South Africa, particularly those from KwaZulu-Natal. In this study, the prevalence of circulating serovars of *C. trachomatis* were investigated in a cohort of human immunodeficiency virus (HIV)-positive pregnant women. Future studies on the genetic variation in *C. trachomatis* from pregnant South African women will be supported by the findings of this study.

1.3 Problem statement

Early and late miscarriage, increased risk of post-abortal infections, histopathological chorioamnionitis, premature rupture of membranes, postpartum endometritis and preterm birth are all linked to imbalances in the vaginal microbiota during pregnancy [16]. The prevalence of

C. trachomatis amongst pregnant women in Durban, South Africa was 11% as reported by Mabaso et al. (2022) [17]. The infection rates in South Africa are higher when compared to other parts of the world such as 3.5% in the USA, 10% in Peru, and 6.4% in Australia [18]. Untreated *C. trachomatis* infection in pregnant women has been linked to miscarriage, low birth weight babies, premature membrane rupture, postpartum endometritis, and transmission to the new-born babies [18]. The documented diversity of serovars of *C. trachomatis* and clinical factors associated with *C. trachomatis* is challenging for upcoming chlamydia vaccine design and development [15].

1.4 Research questions

The research questions for this study were, what is the prevalence of *C. trachomatis* detected in the population of pregnant women and which serovars of *C. trachomatis* are circulating in the infected pregnant women?

1.5 Rationale

This study is important since *C. trachomatis* infection is the common STI amongst pregnant women. Identifying factors associated with infection will aid in developing targeted interventions to reduce the prevalence of this infection. Genotyping of *C. trachomatis* positive samples is important for identifying the level of genetic variation in this pathogen within a targeted population since the levels of variation within a pathogen significantly impacts future vaccine design strategies.

1.6 Aim of the study

To investigate the prevalence and serovars of *C. trachomatis* in a population of South African pregnant women.

1.7 Objectives of the study

- To determine the prevalence of *C. trachomatis* in the study population by a nucleic acid amplification test.
- To identify circulating serovars of *C. trachomatis* by restriction analysis.
- To link the different serovars with clinical factors (abnormal vaginal discharge and past treatment of STIs).

CHAPTER 2

Literature Review

The literature review is presented in the format of a manuscript that is currently under review in the **Journal of Medical Laboratory Science & Technology of South Africa** (Reference number: 135) and has been formatted according to the journal's guidelines for authors. See Appendix D.

Mini Review: The impact of *Chlamydia trachomatis* infection on pregnancy and neonatal outcomes

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Abstract

Chlamydia trachomatis (*C. trachomatis*) is an obligate intracellular bacterium whose only natural host is humans. Although presenting as asymptomatic in most women, genital tract chlamydial infections are a leading cause of pelvic inflammatory disease (PID), premature rupture of membranes (PROM), tubal factor infertility, and ectopic pregnancy. *Chlamydia trachomatis* is one of the most common bacterial sexually transmitted infection (STI) pathogens, resulting in an estimated 127 million new infections each year. Although *C. trachomatis* infections are prevalent in pregnant women and their neonates on the African continent, there are no published review articles from South Africa that focus on these infections. This review article aims to fill this gap in the literature. The biology, risk factors, co-infections, clinical features, and implications of this infection on South African pregnant women and their neonates were identified and extracted using an electronic search of online databases. Unmarried status, low socioeconomic level and a high number of sexual partners are all established risk factors for chlamydial infection amongst pregnant South African women. Infection with *C. trachomatis* has been linked to a variety of clinical symptoms in neonates, including low birth weight, nasopharyngitis, conjunctivitis and pneumonia. It is essential to screen sexually active young women and high-risk patients more frequently and raise awareness on the risks of *C. trachomatis* in developing countries such as South Africa. In addition, continuous initiatives to create effective vaccines that are safe to be administered to pregnant women against this pathogen are encouraged.

Keywords: chlamydia, pregnant women, neonates, South African, infection

Introduction

The physiological changes that occur during pregnancy induces a weakened immune system in pregnant women, which renders them more susceptible to infections and ailments.[1] Reproductive tract infections rank amongst the most significant public health concerns in both developed and developing nations, including South Africa.[2] *Chlamydia trachomatis* (*C. trachomatis*), the sexually transmitted infection (STI) responsible for causing the sexually transmitted disease (STD) chlamydia, is a gram-negative, obligate intracellular bacterium that can only replicate inside a host cell. It is the most frequent bacterial STI amongst young women worldwide.[3] The prevalence of *C. trachomatis* infection is rising worldwide.[1] The World Health Organization (WHO) has highlighted the increase in STI cases in recent years, with *C. trachomatis* being one of the most prevalent bacterial STI pathogens, causing an estimated 127 million new infections annually on a global scale.[3]

The WHO estimates that 50 million women globally have contracted *C. trachomatis* for the first time, with 34 million cases occurring in Sub-Saharan Africa (SSA) and south to southeast Asia.[4] The prevalence of *C. trachomatis* is thought to be high in SSA, with more than 10 million new infections reported annually.[4] In South Africa, it was projected that 6.0% of men and 14.7% of women had *C. trachomatis* in 2017.[5] Amongst South African pregnant women, the prevalence of *C. trachomatis* ranges from 6.5%-36.8%.[6] Up to 80% of *C. trachomatis* infections in women are asymptomatic.[2] Therefore, the true number of new infections is probably much higher since many infections are asymptomatic and go unreported.[3] Unmarried status, low socioeconomic level, a high number of sexual partners and inconsistent condom use are all established risk factors for chlamydial infection amongst pregnant women.[7]

Problems caused by *C. trachomatis* colonization in the reproductive tract of pregnant women include, infertility, chronic pelvic pain, ectopic pregnancy, early membrane rupture, preterm delivery, spontaneous abortion, and perinatal death. Furthermore, new-borns can develop lung and eye infections if exposed to *C. trachomatis* during the gestation period.[1] Approximately, 10%-20% of infants born to mothers with untreated chlamydial infections may develop pneumonia.[8] Additionally, it has been demonstrated that *C. trachomatis* vaginal infections increases the risk of cervical cancer caused by human papillomavirus (HPV) and human immunodeficiency virus (HIV) by a factor of three to four fold.[9] Chlamydia during pregnancy can be harmful to both the mother and the unborn child. The prevalence of *C. trachomatis* infections amongst South African pregnant women is high and pregnant women are more susceptible to acquiring *C. trachomatis* and co-infections such as HIV. This review article highlights the impact of *C. trachomatis* infections on South African pregnant women and their neonates.

C. trachomatis biology and pathogenesis

Chlamydia trachomatis is a bacterium that affects the genital and non-genital areas, such as the lungs, eyes, and the columnar epithelium of the cervix, urethra, and rectum, respectively.[10] *Chlamydia trachomatis* is an obligate, aerobic, intracellular pathogen, of eukaryotic cells (Figure 1). It is a rod-shaped, coccoid Gram-negative bacterium. *Chlamydia trachomatis* is categorized as a Gram-negative bacterium since it shares the same cytoplasmic membrane and outer membrane, however it lacks peptidoglycan cell walls.[11] Since *C. trachomatis* cannot produce its own adenosine triphosphate, it needs developing cells to survive and cannot thrive on its own without a host organism.[11] The 1 042 519 nucleotide base pairs that make up the genome of *C. trachomatis*, contain about 894 probable protein coding sequences. At 1.04 mega-bases, this bacterium genome is smaller than the genomes of many other bacteria and encodes about 900 genes. It is possible that the *C. trachomatis* genome scavenges several crucial metabolic processes from the host cell since they are not encoded in the organism.[12]

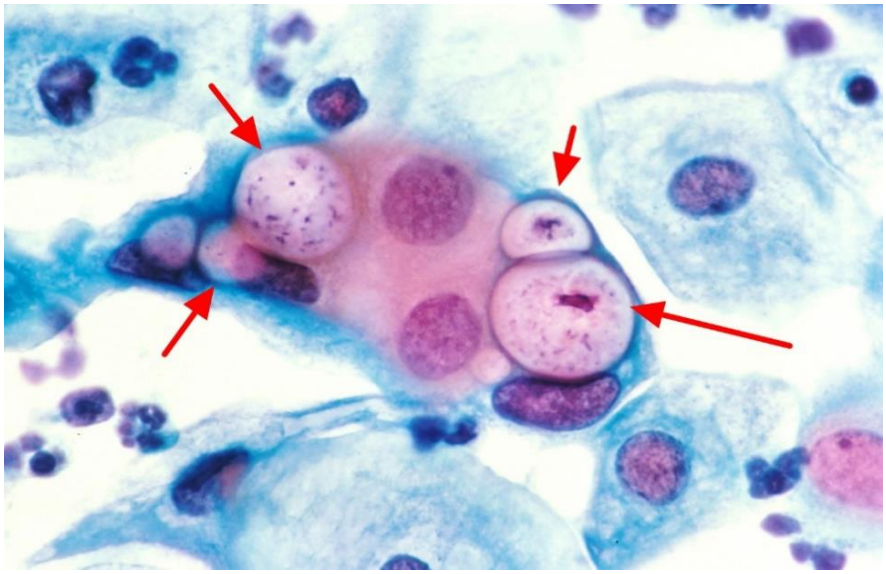

Figure 1. Human Papanicolaou smear showing eukaryotic cells infected with *C. trachomatis* at 500x magnification, stained with haematoxylin and eosin. [Adapted from Britannica *et al.*, 2017][13]

Chlamydia trachomatis has a life cycle that consists of two physically different forms, similar to other *Chlamydia* species. In order to live outside of a host cell, elementary bodies (EBs), which range in size from 200 to 400 nanometres (nm), are enclosed by a stiff cell wall. Upon contact with a vulnerable host cell, this form has the ability to start a new infection.[10] Reticulate bodies

(RBs) are only present inside host cells and range in size from 600 to 1500 nm. Both EBs and RBs are immobile. Initially, the EB of *C. trachomatis*, which resembles a little spore, attaches to a new host cell. The inclusion occurs when the EB enters the host cell surrounded by a host vacuole. The RB, a more substantial and metabolically active type of *C. trachomatis*, develops inside the inclusion. The inclusion is significantly altered by the RB, which creates a more favourable environment for the bacteria's fast multiplication during the next 30 to 72 hours.[14] The vast majority of internal bacteria then change back to resistant EBs, rupturing the cell and dispersing into the surrounding space. The fresh elementary organisms shed in the semen or that are released from the female vaginal tract's epithelial cells then bind to fresh host cells[15] (Figure 2).[16]

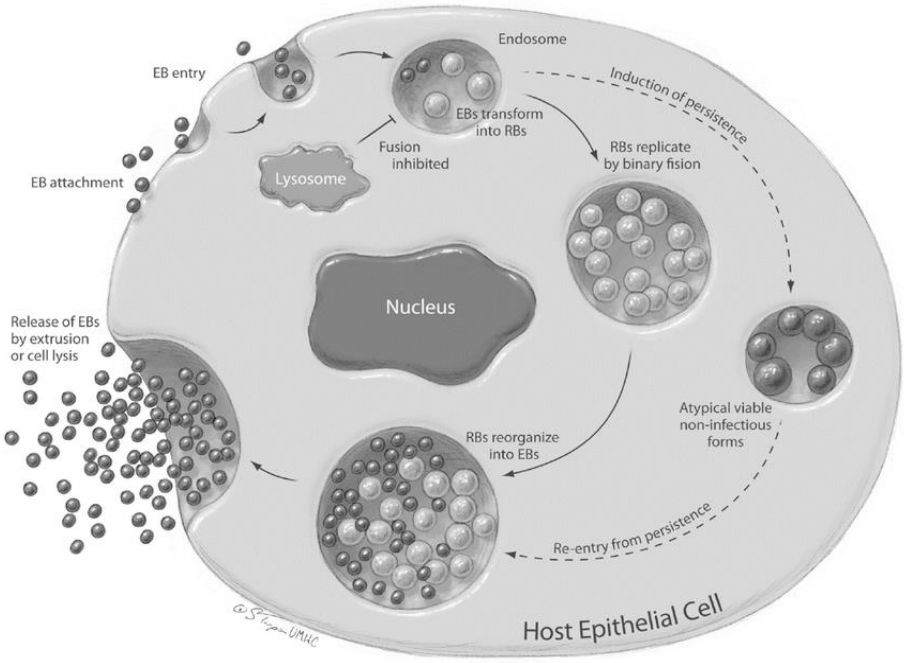

Figure 2. The life cycle of *C. trachomatis*. [Adapted from Schust *et al*., 2012][16]

Components of the innate immune system are capable of quickly detecting the presence of chlamydial EBs in the extracellular environment (Figure 3).[17] Pathogen-associated molecular patterns (PAMPs) on the surface of EBs bind to Toll-like receptors (TLRs) on all innate immune system cells, including phagocytic cells and epithelial cells, in particular TLR 2 and TLR 4. This triggers the release of proinflammatory cytokines and chemokines, which draw immune cells to the site of infection.[18] Pathogen-associated molecular patterns on the newly integrated EB are also identified by the cytoplasmic pattern recognition receptor, nucleotide-binding oligomerization

domain protein 1, once it invades the host cell cytoplasm, leading to additional pro-inflammatory gene activation.[19] Chlamydial antigen-specific, cell-mediated, and humoral immunity are produced as a result of the phagocytosis of *C. trachomatis* and the subsequent production of distinct antigens on the cell surface.[20] In some infected women, the organism migrates to the uterus and fallopian tubes, leading to chronic infection.[20]

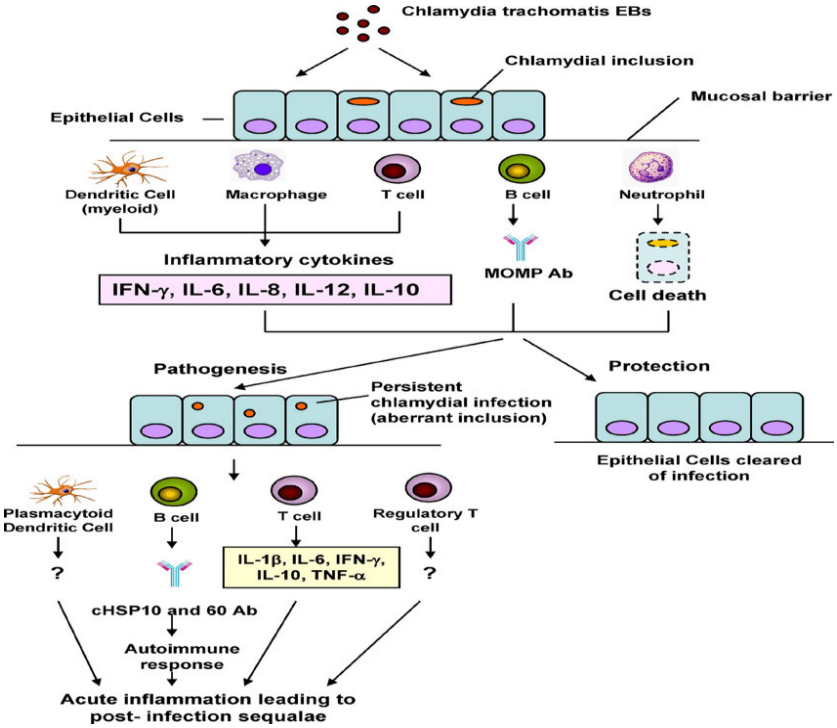

Figure 3. Mechanism of the immune response to *C. trachomatis*. [Adapted from Agrawal *et al*., 2009][17]

C. trachomatis infection during pregnancy

The cervix is the most frequently infected region in pregnant women, and the urethra may be impacted in a smaller percentage of women.[21] These cervical infections have the potential to progress and result in PID, which can eventually cause infertility, persistent discomfort, and ectopic pregnancy. Most women with cervical infections will not exhibit any symptoms or warning indications of the infection. The typical cervicitis features, such as mucopurulent endocervical discharge, endocervical friability, or oedematous ectopy of the cervix, are evident when clinical indications of the condition are present.[22] The impact of chlamydia on pregnancy is a topic of debate, since this disease is asymptomatic in majority of patients. It can be difficult to estimate the infection date for asymptomatic pregnant women since chlamydia can be carried for several months or even a year. Asymptomatic chlamydial infection may cause early pregnancy

loss or recurrent pregnancy loss by activating the immune system. Chlamydia is strongly associated with ectopic pregnancy and tubal factor infertility (Figure 4).[23] *Chlamydia trachomatis* infection rates amongst pregnant women range from 2%-35%.[24] Pregnant women infected with chlamydia are more likely to experience negative pregnancy outcomes and postpartum PID. There have been reported consequences such as stillbirth, low birth weight, neonatal death, shorter gestational periods, preterm delivery and PROM.[25] According to a study conducted in the past, women with a history of PID experienced chronic pelvic pain in 18% of cases, ectopic pregnancy in 9% of cases, and tubal scarring in 8% of cases.[26] If symptoms do exist, they are typically associated with a urinary tract infection, including frequent urination and dysuria.[27]

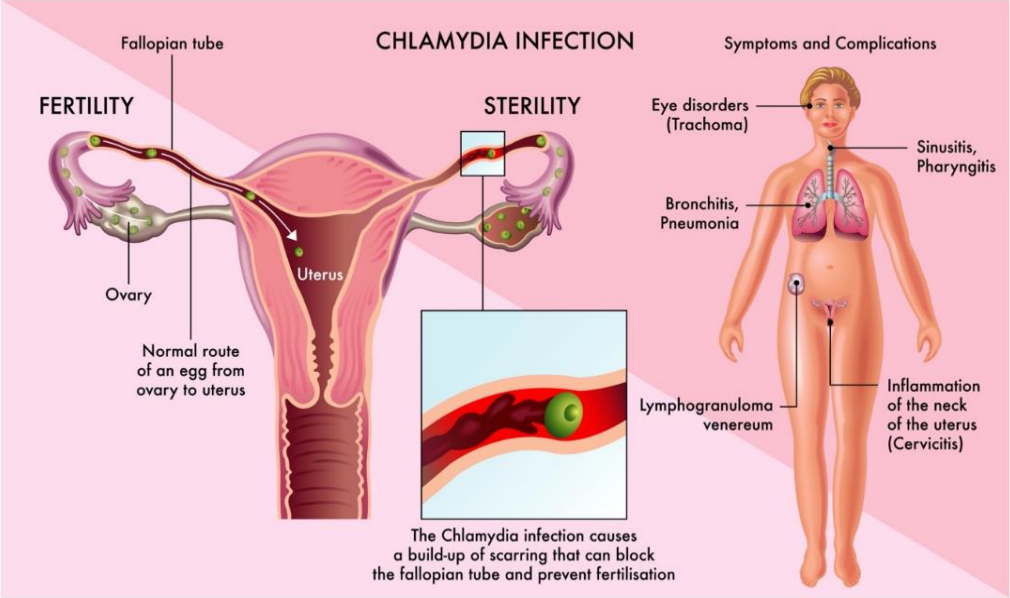

Figure 4. *C. trachomatis* infection. Symptoms and possible impact on fertility. [Smolarczyk *et al*., 2021][28]

Unmarried status, low socioeconomic level and a high number of sexual partners are all established risk factors for chlamydial infection amongst pregnant women.[7] The usage of oral contraceptives has also been linked to cervical chlamydial infections.[29] New sexual partners, having partners who previously had a chlamydial infection or another STD and inconsistent condom use are all sexual risk factors for chlamydial infection amongst pregnant women (some of which are more common in younger women).[30] Family planning clinics (8%), prenatal clinics (7.2%), national employment training programs for women (11.4%), and detention centres (14.5%) have the greatest rate of chlamydia infections amongst pregnant women.[31] Additionally,

the Centres for Disease Control and Prevention (CDC) advises pregnant women at continued risk for infection and those who tested positive at the initial prenatal visit to undergo retesting in the third trimester.[21,22,32] Whilst the age range of 14-25 years continues to be a significant risk factor for chlamydia infection, women who have recently started having sex and pregnant women who have a history of STIs are at a higher risk of infection.[33]

According to several studies, HIV is a co-infection of chlamydial genital infections amongst pregnant women around the world.[34,35,36] The mixed epidemiology of this infection may in part be explained by the shared sexual and behavioural risk factors shared by STIs such as *C. trachomatis* and HIV. These pathogens have a link with one another.[37] The vaginal epithelial layer is susceptible to damage from the invasive intracellular pathogenesis of *C. trachomatis*, which may make it easier for HIV to infect the host.[34] Immunological alterations brought on by HIV may increase the rate of chlamydial infections.[34] Additionally, those who are infected with HIV may experience more severe clinical features such as PID. To reduce the risk of HIV and its clinical effects, it is crucial to diagnose and treat chlamydial infections early.[38,39] Furthermore, there is a concern that STIs may raise the risk of HIV mother-to-child transmission (MTCT), as preliminary research suggests that genital infections caused by *C. trachomatis* may increase the amount of HIV that is shed in the cervicovaginal region and cause chorioamnionitis.[38,39] However, only a small number of published studies have examined the potential impact of STIs such *C. trachomatis* during pregnancy on HIV MTCT. In a HIV prevention trial conducted in 2015 of 1373 HIV infected pregnant women, the rates of HIV MTCT among women infected with *C. trachomatis* (27/249, 10.7%), was higher than those in the uninfected women (8/98, 8.1%).[40] Upon further analysis, this study suggested a possible correlation between chlamydial infection and increased HIV MTCT in pregnant women.[40]

Perinatal health care providers need to be informed of patients infected with *C. trachomatis* in order for them to take the appropriate steps to stop the spread of this organism. Studies suggest that advanced practice nurses should follow the CDC guidelines when screening and treating pregnant women for chlamydial infections that have been confirmed or suspected.[41] Patient education is essential as the expectant mother has to understand that despite receiving treatment, she is still susceptible to reinfection from a sexual partner. Advanced practice nurses need to promote healthy sexual behaviour and if necessary, make a treatment offer to the woman's sexual partner.[42,43] It is essential to obtain a thorough sexual history along with a health history.[42] There is proof that prenatal screening results in better outcomes. Therefore, it is crucial to screen pregnant South African women for chlamydia. Studies suggest that nurses should ensure that pregnant patients receive safe medical care. The mother and her unborn child will receive safer

medical treatment if pregnant women are aware of the perinatal implications and undergo routine testing for chlamydia.[44]

Neonatal implications of *C. trachomatis* infection

Infection with *C. trachomatis* has been linked to a variety of clinical symptoms in new-borns, including low birth weight, nasopharyngitis, conjunctivitis (inflammation of the outer eye) (Figure 5) and pneumonia.[45] Infants infected with *C. trachomatis* have a conjunctivitis risk of 20%-50% and a pneumonia risk of 5%-30%.[45] During vaginal delivery, the new-born is exposed to untreated maternal cervical chlamydial infection. However, transmission can also occur after a caesarean section when membranes are ruptured.[45] The infection rate in neonates of infected mothers range from 23%-70%.[46] The most often infected anatomical site in new-borns is the nasopharynx, which causes nasal stuffiness. After birth, conjunctivitis can start a few days to a few weeks later. Approximately, 35%-50% of new-borns of untreated mothers develop inclusion conjunctivitis, and 11%-20% of these new-borns develop pneumonia.[47] Infants may contract pneumonia anywhere between 2 and 19 weeks after birth. Without a temperature, it typically manifests as a staccato cough, tachypnoea, and rales. Chest radiographs may show hyperinflation and infiltrates. The new-born may show symptoms such as sneezing, vomiting, cyanosis, nasal blockage, and discharge. Chest films may show diffuse interstitial or patchy infiltrates and hyperinflation.[48] Other respiratory conditions associated with *C. trachomatis* include new-born gastroenteritis, otitis media, bronchiolitis, rhinitis and pharyngitis.[48] *Chlamydia trachomatis* can cause genitourinary tract infections or invasive lymphogranuloma venereum in older children and adults.[45] A type of conjunctivitis that affects new-born infants after delivery is known as neonatal conjunctivitis (ophthalmia neonatorum).[49] To prevent ophthalmia neonatorum which is caused by *C. trachomatis*, antibiotic ointment is typically applied to a new-born within an hour of birth to the eyes.[46] All parents and caregivers should follow this recommendation for their neonates. Most hospitals are legally compelled to apply eye drops or ointment as soon as possible after birth in order to prevent this infection. If untreated, neonatal conjunctivitis can result in scarring and blindness (Figure 5).[47,50] Once the baby is born, the labour and delivery nurses should apply erythromycin eye ointment to the baby's eyes. Until proven differently, every neonatal conjunctivitis should be considered contagious.[51]

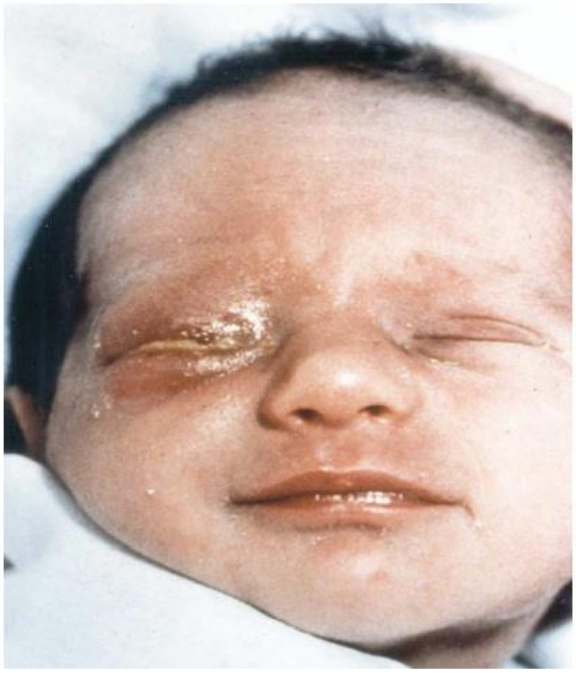

Figure 5. An infant with chlamydial conjunctivitis. [Adapted from Pickering & Prober, 2003][52]

Treatment of *C. trachomatis* infection

Treatment for *C. trachomatis* is determined by infection site, patient age and presence of other infections. It is possible to simultaneously have a *C. trachomatis* infection and one or more other STDs such as HIV. To avoid reinfection, treatment is frequently administered to both partners at the same time. Several antibiotics, including tetracycline, erythromycin, ofloxacin, azithromycin and doxycycline can be used to treat *C. trachomatis*.[44] The CDC recommends treating women with 500 milligrams (mg) tetracycline, orally four times per day.[53,54] Damage to the fallopian tubes of women occur if the infection has spread, ascending the reproductive tract, and developing PID. It is advised to treat the mother of a new-born with conjunctivitis, which can progress to pneumonia.[42] The CDC recommends taking 500 mg of erythromycin, orally four times a day for seven days during pregnancy.[53,54] Alternatives include azithromycin and amoxicillin. Levofloxacin is a different potential option that has been demonstrated in human research to be low risk during pregnancy; however, animal studies raise concerns about neonatal cartilage damage with this antibiotic. Taking doxycycline during the second and third trimesters of pregnancy is not advised.[23] It is possible to retest while pregnant three weeks after treatment. Throughout the course of the pregnancy, screening may be performed if the risk of reinfection is significant.[51] The CDC advises treating *C. trachomatis* infected new-borns with oral erythromycin (50 mg), per day divided into four daily doses for 14 days if they have conjunctivitis or

pneumonia. Conjunctivitis topical therapy has a high failure rate, is ineffective, and does not completely remove nasopharyngeal carriage. Infants should be regularly monitored, if symptoms persist, they might require additional treatment (in about 20% of cases).[45]

Prevention of *C. trachomatis* infection

The prevention of STIs is a public health priority, and during the past ten years HIV co-transmission has grown in developing countries such as India & South Africa, therefore the significance of *C. trachomatis* has become more apparent.[42] The CDC guidelines for the prevention and control of STDs are based on five major concepts: (i) Persons at risk should receive education and counselling on safer sexual behaviour. (ii) Immunization before exposure to the disease which can be prevented by vaccination. (iii) Identification of infected people who are asymptomatic and of symptomatic people who are unlikely to seek diagnostic and therapeutic services. (iv) Effective identification and care for affected individuals. (v) Examining, treating, and counselling sex partners of infected patients.[55] The CDC strongly advises that pregnant women who are sexually active (25 years old) and those who are at higher risk of infection undergo routine testing for chlamydia. However, the majority of pregnant women are uneducated and fear the testing process.[41] Primary, secondary, and tertiary prevention strategies for *C. trachomatis* infection are all possible. Since treatment of *C. trachomatis* can still result in re-infection, lifestyle counselling and health education are better means of prevention.[56] Inquiring about risk-taking sexual behaviour, encouraging screening tests for those who are at risk, ensuring that partners are assessed and treated and providing counselling for safe sex practices are all crucial roles played by clinicians. Adolescents should participate in effective school-based health programs. Sadly, primary STI prevention has not gained much attention, particularly amongst pregnant women in developing countries such as South Africa.[57] In order to avoid the severe effects of chlamydial infection, secondary prevention refers to the early discovery of disease by screening. Since chlamydial infections are widespread, linked with significant morbidity, detectable, and curable, they fulfil the general prerequisite for disease prevention by screening.[44] Recent developments such as non-invasive specimen testing and azithromycin single dose therapy may improve attempts to prevent chlamydial infection, but majority of pregnant South African women are uneducated and may not approve of these methods. The main concern is that tubal damage has already occurred amongst South African women and by the time the patient develops symptoms, tertiary prevention of acute and chronic chlamydial infection of the upper genital tract has generally failed and therefore requires further research.[56]

C. trachomatis vaccines

In comparison to other biomedical therapies, vaccination may be significantly more efficient at containing chlamydia infection epidemics in Africa. Despite many attempts to create awareness on chlamydia by making visual posters at clinics and by educating people on the dangers of STIs. The best public health measure is to speed up the detection and treatment of pregnant women, which is difficult to do since majority of South African women are uneducated and do not understand the importance of screening. Even with 100% coverage, administering a preventive vaccine to women before their first sexual experience could result in a considerable decline in prevalence that could not be achieved by screening.[58] Despite numerous attempts to develop a vaccine, there are currently no fully or partially effective preventative vaccines. The development of effective delivery systems and adjuvants to boost immune effectors to achieve long-term protective immunity have been major challenges in the quest for an effective human chlamydial vaccine. These challenges include defining the components of protective immunity to facilitate vaccine evaluation and selecting appropriate vaccine candidates that have stable antigenic and immunologic properties.[42] The genital tract's immunological features and chlamydia's affinity for mucosal epithelial cells highlight the need for a *C. trachomatis* vaccine to elicit both mucosal and systemic protective responses especially amongst pregnant women.[41] The fundamental immunologic paradigms for vaccine selection and evaluation, including the unavoidable requirement for a vaccine to generate T-helper type 1 immune response that regulates chlamydia, have been established by advancements in the functional immunobiology of chlamydia.[41] However, progress must be made in the creation and development of creative and efficient delivery mechanisms, such as vectors and adjuvants.[59] Despite the many achievements of vaccine research to date, there are still a number of problems that limit our capacity to use immunological and biological knowledge in the development of vaccines. To find innovative immunogen candidates and delivery systems that are secure, immunogenic and produce the necessary protective immunity, more research must be done.[60]

Conclusion

It has been established that *C. trachomatis* is a significant STI pathogen, particularly amongst pregnant South African women and their new-borns. Infantile pneumonia and new-born inclusion conjunctivitis both have well-established clinical characteristics. There is currently ongoing research into the connection between maternal *C. trachomatis* infection and preterm and perinatal mortality. However, it is necessary to conduct more research, which should look into the pathological function of *C. trachomatis* during the perinatal period and co-infections such as HIV amongst pregnant women. Despite the current antibiotic treatment being effective when used, the majority of women with *C. trachomatis* exhibit no symptoms, making it difficult to diagnose and

treat them. Therefore, it is necessary to screen high-risk individuals more frequently and create awareness in third world countries such as South Africa to educate women on the dangers of *C. trachomatis*. Researchers have already established the viability and advantages of screening sexually active young women, particularly pregnant women. These approaches should be implemented by public health professionals along with ongoing efforts to develop effective vaccinations that are safe to be administered to pregnant women.

Acronyms

CDC - Centres for Disease Control and Prevention, EBs - Elementary bodies, HIV - Human immunodeficiency virus, HPV - Human papillomavirus, Mg - Milligrams, MTCT - Mother-to-child transmission, Nm - Nanometres, PAMPs - Pathogen-associated molecular patterns, PID - Pelvic inflammatory disease, PROM - Premature rupture of membranes, RBs - Reticulate bodies, STD - Sexually transmitted disease, STI - Sexually transmitted infection, SSA - Sub-Saharan Africa, TLRs - Toll-like receptors, WHO - World Health Organization

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Conflict of interest

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References

1. Azami M, Badfar G, Mansouri A et al. Prevalence of *Chlamydia trachomatis* in pregnant Iranian women: a systematic review and meta-analysis. *International Journal of Fertility and Sterility* 2018; 12(3):191-199. doi: 10.22074/ijfs.2018.5191

2. Balle C, Lennard K, Dabee S et al. Endocervical and vaginal microbiota in South African adolescents with asymptomatic *Chlamydia trachomatis* infection. *Scientific Reports* 2018; 8(1):11109. doi: 10.1038/s41598-018-29320-x

3. Huai P, Li F, Chu T et al. Prevalence of genital *Chlamydia trachomatis* infection in the general population: a meta-analysis. *BMC Infectious Diseases* 2020; 20, 589. doi: 10.1186/s12879-020-05307-w

4. Hussen S, Wachamo D, Yohannes Z et al. Prevalence of *Chlamydia trachomatis* infection among reproductive age women in sub-Saharan Africa: a systematic review and meta-analysis. *BMC Infectious Diseases* 2018; 18:1-8. doi: 10.1186/s12879-018-3477-y

5. Kularatne R.S, Niit R, Rowley J et al. Adult gonorrhoea, chlamydia and syphilis prevalence, incidence, treatment, and syndromic case reporting in South Africa: Estimates using the Spectrum-STI model, 1990-2017. *PLoS ONE* 2018; 13:e0205863. doi: 10.1371/journal.pone.0205863

6. Ngobese B & Abbai N.S. Sexually transmitted infections in pregnant women from Sub-Saharan Africa. *Southern African Journal of Infectious Diseases* 2021; 36(1). doi: 10.4102/sajid.v36i1.312

7. Novak M & Novak D. Risk factors for *Chlamydia trachomatis* infection among users of an internet-based testing service in Sweeden. *Sexual and Reproductive Healthcare* 2013; 4:23-27. doi: 10.1016/j.srhc.2012.11.004

8. Gottlieb S.L, Low N, Newman L.M et al. Toward global prevention of sexually transmitted infections (STIs): The need for STI vaccines. *Vaccine* 2014; 32:1527-1535. doi: 10.1016/j.vaccine.2013.07.087

9. Foschi C, Salvo M, Cevenini R et al. *Chlamydia trachomatis* antimicrobial susceptibility in colorectal and endocervical cells. *Journal of Antimicrobial Chemotherapy* 2018; 73:409-413. doi: 10.1093/jac/dkx392

10. Elwell C, Mirrashidi K & Engel J. Chlamydia cell biology and pathogenesis. *Nature Reviews Microbiology* 2016; 14(6):385-400. doi: 10.1038/nrmicro.2016.30

11. Byrne G.I. Chlamydia uncloaked. *Proceedings of the National Academy of Sciences* 2003; 100(14):8040-8042. doi: 10.1073/pnas.1533181100

12. Stephens R.S, Kalman S, Lammel C et al. Genome sequence of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*. *Science* 1998; 282(5389):754-759. doi: 10.1126/science.282.5389.754

13. Britannica T et al. *Chlamydia*. *Encyclopedia Britannica* 2017.

14. Nunes A & Gomes J.P. Evolution, phylogeny, and molecular epidemiology of chlamydia. *Infection, Genetics and Evolution* 2014; 23:49-64. doi: 10.1016/j.meegid.2014.01.029

15. Witkin S.S, Minis E, Athanasiou A et al. *Chlamydia trachomatis*: the persistent pathogen. *Clinical and Vaccine Immunology* 2017; 24(10). doi:10.1128/cvi.00203-17

16. Schust J.D, Ibana J.A, Buckner L.R et al. Potential mechanisms for increased HIV-1 transmission across the endocervical epithelium during *C. trachomatis* infection. *Current HIV Research* 2012; 10(3):218-227. doi: 10.2174/157016212800618093

17. Agrawal T, Vats V, Salkan S et al. The mucosal immune response to *Chlamydia trachomatis* infection of the reproductive tract in women. *Journal of Reproductive Immunology* 2009; 83:173-178. doi: 10.1016/j.jri.2009.07.013

18. Bulut Y, Faure E, Thomas L et al. Chlamydial heat shock protein 60 activates macrophages and endothelial cells through Toll-like receptor 4 and MD2 in a MyD88-dependent pathway. The *Journal of Immunology* 2002; 168:1435-1440. doi: 10.4049/jimmunol.168.3.1435

19. Welter S.L, Ojcius D.M, Viala J et al. Stimulation of the cytosolic receptor for peptidoglycan, Nod1, by infection with *Chlamydia trachomatis* or *Chlamydia muridarum*. *Cellular Microbiology* 2006; 8:1047-1057. doi: 10.1111/j.1462-5822.2006.00686.x

20. Vasilevsky S, Greub G, Nardelli H.D et al. Genital *Chlamydia trachomatis*: understanding the roles of innate and adaptive immunity in vaccine research. *Clinical Microbiology Reviews* 2014; 27:346 -370. doi: 10.1128/CMR.00105-13

21. U.S. Preventive Services Task Force. Screening for chlamydial infection: U.S. Preventive Services Task Force recommendation statement. *Annals of Internal Medicine* 2007; 147(2):12-34. doi: 10.7326/0003-4819-147-2-200707170-00172

22. Centres for Disease Control and Prevention. Sexually transmitted disease surveillance 2015. *STDs in adolescents and young adults* 2016. URL: https://www.cdc.gov/std/stats

23. Johnson R.E, Newhall W.J, Papp J.R et al. Screening test to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections. *Centres for Disease Control and Prevention: MMWR* 2002; 51:1-22. URL: http://www.cdc.gov/mmwr/PDF/rr/rr5115.pdf

24. Black C.M. Current methods of laboratory diagnosis of *Chlamydia trachomatis* infection. *Clinical Microbiology Reviews* 1997; 10:160-184. doi: 10.1128/cmr.10.1.160

25. Ward M.E & Ridgway G. Chlamydia. *Topley and Wilsons microbiology and microbial infection* 1999; 9:1331-1336.

26. Weström L, Joesoef R, Reynolds G et al. Pelvic inflammatory disease and infertility: a cohort study of 1844 women with laparoscopically verified disease and 657 control women with normal laparoscopy. *Sexually Transmitted Diseases* 1992; 19(4):185-192.

27. Stamm W.E. *Chlamydia trachomatis* infections of the adult. *Sexually Transmitted Diseases* 2008; 575-594.

28. Smolarczyk K, Mlynarczyk B.B, Rudnicka E et al. The impact of selected bacterial sexually transmitted diseases on pregnancy and female fertility. *International Journal of Molecular Sciences* 2021; 22(4):2170. doi: 10.3390/ijms22042170

29. Stamm W.E & Batteiger B.E. *Chlamydia trachomatis* (trachoma, perinatal infections, lymphogranuloma venereum and other genital infections). 2010; 7. doi: 10.1016/B978-0-443-06839-3.00180-6

30. Workowski K.A & Bolan G.A. Sexually transmitted diseases treatment guidelines, 2015. *Recommendation and Reports: Morbidity and mortality weekly report* 2015; 64(33):924. URL: https://www.ncbi.nlm.nih.gov/pubmed/26042815

31. Centres for Disease Control and Prevention. Sexually transmitted disease surveillance 2010, Atlanta. *U.S. Department of Health and Human Services* 2011. URL: https://www.cdc.gov/std/statistics/2019

32. Final Recommendation Statement. Chlamydia and gonorrhoea: screening. *U.S. Preventive Services Task Force* 2016. URL: https://www.uspreventiveservicestaskforce.org/uspstf/document/RecommendationStatementFinal/chlamydia-and-gonorrhea-screening

33. LeFevre M.L & Kurth A. Screening for chlamydia and gonorrhoea: U.S. Preventive Services Task Force recommendation statement. *Annals of Internal Medicine* 2014; 161(12):902-910.

URL: https://nyuscholars.nyu.edu/en/publications/screening-for-chlamydia-and-gonorrhea-us-preventative-services-ta

34. Miller E.K. Diagnosis and treatment of *Chlamydia trachomatis* infection. *American Family Physician* 2006; 73:1411-1416. URL: https://pubmed.ncbi.nlm.nih.gov/16669564/

35. Fleming D.T & Wasserheit J.N. From epidemiological synergy to public health policy and practice: the contribution of sexually transmitted diseases to sexual transmission of HIV infection. *Sexually Transmitted Infections* 1999; 75:3-17. doi: 10.1136/sti.75.1.3

36. Paavonen J. Immunopathogenesis of pelvic inflammatory disease and infertility - what do we know, what shall we do. *Journal of the British Fertility Society* 1996; 1:42-45.

37. Joyee A.G, Thyagarajan S.P, Sowmya B et al. Need for specific and routine strategy for the diagnosis of genital chlamydial infection among patients with sexually transmitted disease in India. *The Indian Journal of Medical Research* 2003; 118:152-157.

38. Chi B.H, Mudenda V, Levy J et al. Acute and chronic chorioamnionitis and the risk of perinatal human immunodeficiency virus-1 transmission. *American Journal of Obstetrics & Gynecology* 2006; 194(1):174-181. doi: 10.1016/j.ajog.2005.06.081

39. Wabwire F.M, Gray R.H, Mmiro F.A et al. Placental membrane inflammation and risks of maternal-to-child transmission of HIV-1 in Uganda. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology* 1999; 22(4):379-385. doi: 10.1097/00126334-199912010-00009

40. Adachi K, Klausner J.D, Bristow C.C et al. Chlamydia and gonorrhoea in HIV-infected pregnant women and infant HIV transmission. *Sexually Transmitted Diseases* 2015; 42(10):554-565. doi: 10.1097/OLQ.0000000000000340

41. Mestecky J, Moldoveanu Z & Russell M.W. Immunologic uniqueness of the genital tract: challenge for vaccine development. *American Journal of Reproductive Immunology* 2005; 53:208-214. doi: 10.1111/j.1600-0897.2005.00267.x

42. Malhotra M, Sood S, Mukherjee A et al. Genital *Chlamydia trachomatis*: an update. *The Indian Journal of Medical Research* 2013; 138(3):303-316. URL: https://pubmed.ncbi.nlm.nih.gov/24135174/

43. Lorch R, Hocking J, Guy R et al. Do Australian general practitioners believe practice nurses can take a role in chlamydia testing? A qualitative study of attitudes and opinions. *BMC Infectious Diseases* 2015; 15(1). doi: 10.1186/s12879-015-0757-7

44. Nelson H.D & Helfand M. Screening for chlamydial infection. *American Journal of Preventive Medicine* 2001; 20(3):95-107. doi: 10.1016/s0749-3797(01)00253-7

45. American Academy of Paediatrics. *Chlamydia trachomatis*. *Report of The Committee on Infectious Diseases* 2018; 276-283.

46. Matejcek A & Goldman R.D. Treatment and prevention of ophthalmia neonatorum. *Canadian Family Physician* 2013; 59(11):1187-1190.

47. Conjunctivitis | Pink Eye | New-borns, 2016. URL: https://www.cdc.gov/conjunctivitis/newborns.html

48. Conjunctivitis, Neonatal: Overview. *Medicine* 2019.

49. Tan A.K. Ophthalmia neonatorum. *New England Journal of Medicine* 2019; 380(2). doi: 10.1056/NEJMicm1808613

50. Curry S.J, Krist A.H, Owens D.K et al. Ocular prophylaxis for gonococcal ophthalmia neonatorum: US preventive services task force reaffirmation recommendation statement. *Journal of the American Medical Association* 2019; 321(4):394-398. doi: 10.1001/jama.2018.21367

51. Tiller C.M. Chlamydia during pregnancy: implications and impact on perinatal and neonatal outcomes. *Journal of Obstetric, Gynecologic & Neonatal Nursing* 2002; 31(1):93-98. doi: 10.1111/j.1552-6909.2002.tb00027.x

52. Pickering S & Prober L.K. Principles and Practice of Paediatric Infectious Diseases. *New York, Churchill-Livingstone* 2003*;* 904.

53. Centres for Disease Control and Prevention. Some facts about Chlamydia. 2001. URL: https://www.cdc.gov/std/chlamydia/stdfact-chlamydia.htm

54. Weingarten C.T. Nurse's drug guide. *Springhouse* 2000. 3rd ed.

55. Workowski K.A, Bachmann L.H, Chan P.A et al. Sexually transmitted infections treatment guidelines, 2021. *Morbidity & Mortality Weekly Report - Recommendations & Reports* 2021; 70(4):1-187. doi: 10.15585/mmwr.rr7004a1

56. Horner P. The case for further treatment studies of uncomplicated genital *Chlamydia trachomatis* infection. *Sexually Transmitted Infections* 2006; 82:340-343. doi: 10.1136/sti.2005.019158

57. Kufa-Chakezha T. Preventing sexually transmitted infections: Why South Africa isn't winning. *National Institute for Communicable Diseases* 2019. URL:

https://www.nicd.ac.za/preventing-sexually-transmitted-infections-why-south-africa-isnt-winning/

58. Gray R.T, Beagley K.W, Timms P et al. Modelling the impact of potential vaccines on epidemics of sexually transmitted *Chlamydia trachomatis* infection. *The Journal of Infectious Diseases* 2009; 199:1680-1688. doi: 10.1086/598983

59. Crane D.D, Carlson J.H, Fischer E.R et al. *Chlamydia trachomatis* polymorphic membrane protein D is a species-common pan-neutralizing antigen. *Proceedings of the National Academy of Sciences of the United States of America* 2006; 103(6):1894-1899. doi: 10.1073/pnas.0508983103

60. Kis Z, Shattock R, Shah N et al. Emerging technologies for low-cost, rapid vaccine manufacture. *Biotechnology Journal* 2019; 14(1):1800376. doi: 10.1002/biot.201800376

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Chapter 2 illustrating the literature review revealed that South African women have a high prevalence rate for *C. trachomatis* infection. Several socio-demographic, behavioural and clinical factors such as unmarried status, low socioeconomic level and a high number of sexual partners have been shown to be associated with *C. trachomatis* infection. The manuscript in Chapter 3 adds to the growing body of literature on the prevalence and factors associated with *C. trachomatis* infection in pregnant women from Durban, South Africa. It also identifies circulating serovars present in the study population and links the different serovars with clinical factors (abnormal vaginal discharge and past treatment of STIs).

CHAPTER 3

This manuscript was accepted by the **Journal of Medical Laboratory Science & Technology of South Africa** (Reference number: 130) and has been formatted according to the journal's guidelines for authors. See Appendix D.

Genotyping of *Chlamydia trachomatis* from vaginal swabs by restriction analysis of the outer membrane protein gene

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Abstract

Background:

Chlamydia trachomatis (*C. trachomatis*) is a common cause of bacterial sexually transmitted infections (STIs). The genetic characterisation of *C. trachomatis* serovars reveals significant genetic diversity in this organism. This study investigated the diversity of *C. trachomatis* serovars in human immunodeficiency virus (HIV) infected pregnant women in South Africa.

Methods:

For this study, 385 vaginal swab samples were tested for the presence of *C. trachomatis*. The swabs were collected from HIV infected pregnant women at the King Edward VIII hospital in Durban, South Africa. The outer membrane protein gene (*omp1*) from *C. trachomatis* was amplified and positive amplicons were digested with restriction enzymes *AluI, DdeI* and *HinfI* for the assignment of serovars.

Results:

The prevalence of *C. trachomatis* in the study population was 12.2% (47/385). Serovar E (20/43) - 46.5% was the most frequent serovar in our study population, followed by serovars F (9/43) - 20.9%, G (6/43) - 14.0% and D (5/43) - 11.6%. Serovar I (2/43) - 4.7%, which was detected in two samples, was the least frequent. Risk factors for *C. trachomatis* included having a low level of education, being unemployed, being unmarried, not cohabitating, early age of first sex, high number of lifetime sex partners, a partner having other partners, lack of condom use, lacking symptoms of STIs and lacking treatment for STIs.

Conclusion:

Five different serovars were observed among the participants. The high genetic diversity observed in this study contributes to the challenges regarding future vaccine design and the development of antigen-based rapid diagnostic tests for chlamydia.

Keywords: chlamydia, sexually transmitted infections, pregnant women, serovars, South Africa

Introduction

In both industrialised and developing countries, reproductive tract infections are one of the most important public health challenges.[1] *Chlamydia trachomatis* (*C. trachomatis*) is a Gram- negative, obligate intracellular bacterium that can replicate only within a host cell. It is the most common cause of bacterial sexually transmitted infections (STIs) in young women globally. Up to 80% of *C. trachomatis* infections in women are asymptomatic.[2] Infection with *C. trachomatis* is becoming more common throughout the world.[1]

According to the World Health Organization (WHO) reports, STIs have risen globally in recent years, with *C. trachomatis* being one of the most common bacterial STI pathogens, producing an estimated 106 million new infections each year.[3] Infertility, persistent pelvic pain, ectopic pregnancy, early rupture of membranes, spontaneous preterm, abortion and perinatal death are all problems of *C. trachomatis* colonisation in the reproductive tract of pregnant women.[1] In 2014, approximately 1.44 million chlamydial infections were reported in the United States of America (USA), being the most significant number since cases were first recorded in 1984. The rate of reported chlamydial infections showed a world-wide increase from 316.5 to 456.1 occurrences per 100 000 people between 2004 and 2014.[3] According to data from the European Centre for Disease Control and Prevention (ECDC), the number of registered *C. trachomatis* infections in Europe has increased from 191 000 in 2004 to 385 000 in 2013, equating to a rise in incidence of 162.8 to 181.8 infections per 100 000 people.[3]

The WHO estimates that 50 million women globally have contracted *C. trachomatis* for the first time, with 34 million cases occurring in Sub-Saharan Africa (SSA) and south to southeast Asia.[4] The prevalence of *C. trachomatis* is considered to be high in SSA, with more than 10 million new infections reported annually.[4] In South Africa, it was reported that 6.0% of men and 14.7% of women had *C. trachomatis* in 2017.[5] In a study published in 2019, the prevalence of *C. trachomatis* was 20% (49/242) in a population of pregnant South African women.[6] Since many infections are asymptomatic and go unnoticed, the actual number of new infections is likely to be substantially greater.[3]

Genotyping is a technique used to detect minor genetic differences that can contribute to significant phenotypic changes, such as pathological changes that underpin disease.[7] Owing to its high resolution, genotyping allows the differentiation of bacterial strains based on their genetic content. Recently, this has become frequently employed for chlamydial strain typing.[7] A grouping of bacteria or viruses based on cell surface antigens is known as a serovar. Serovars allow organisms to be classified down to the sub-species level, which is crucial in epidemiology.[8]

Chlamydia trachomatis is divided into 15 serovars namely, A, B, Ba, C, D, E, F, G, H, I, J, K, L1, L2 and L3, based on the structure of the outer membrane protein gene (*omp1*).[8]

Sexually transmitted serovars of *C. trachomatis*- (i.e. D, E, F, G, H, I, J and K) cause cervicitis in patients in numerous countries, while L1, L2 and L3 are linked to lymphogranuloma venereum. It has been reported that serovars D, E and F are more typically related to cervical, vaginal and urethral infections in industrialised countries.[9] Urogenital infections are caused mostly by *C. trachomatis* serovars D to K. Of these, serovars D, E and F account for up to 60%-70% of these infections. Genomic techniques, particularly *omp1* genotyping, is more sensitive and precise than serotyping or immunotyping.[7] The variable domains of the *omp1* nucleotide sequences exhibit significant variations in different serovars and have become widely used for genotyping *C. trachomatis* isolates. The most common serovars among pregnant women infected with *C. trachomatis* are unknown in most African nations, including South Africa.[9]

Currently, there are limited data on the serovars of *C. trachomatis* detected in pregnant women in South Africa. This study determined the prevalence and factors associated with *C. trachomatis* infection and the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the *omp1* was performed in order to identify the different serovars present in the population of pregnant women. This study will form the basis for subsequent research on the genetic diversity of *C. trachomatis* in South African pregnant women.

Methods

Ethical approval

This study was approved by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (UKZN) (BREC/00004226/2022).

Study setting, population and sample collection

This was a cross-sectional study of pregnant women attending the antenatal clinic (ANC) at the King Edward VIII hospital in Durban, South Africa. Recruitment for this study took place between October 2020 and April 2021. Women were enrolled in this study if they were HIV-positive, 18 years and older, willing to provide written informed consent, willing to provide vaginal swab samples, and willing to provide socio-demographic, behavioural and clinical data. Participants were provided with instructions on proper sample collection and each participant provided self-collected vaginal swabs (dry swabs) for the detection of vaginal infections. The participants also completed a questionnaire on socio-demographic, behavioural and clinical factors. A total of 385 vaginal swab samples were collected and tested for the presence of *C. trachomatis*.

DNA isolation from vaginal swabs

Deoxyribonucleic acid (DNA) was extracted from the 385 vaginal swabs using the PureLink™ Microbiome DNA Purification Kit (Thermo-Fisher Scientific, USA). This kit is designed to extract DNA using bead beating technology. The DNA extractions included a bead beating step for mechanical lysis of cells. DNA was purified using a column-based protocol that was performed as per the manufacturer's instructions. DNA concentration and quality were determined using a NanoDrop® spectrophotometer (Thermo-Fisher Scientific, USA).

Detection of C. trachomatis

Chlamydia trachomatis was detected using the Applied Biosystems™ TaqMan® Assays. Commercial primers and probes (Assay ID Ba04646249_s1) which targets the gene encoding 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, USA). Briefly, 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 (Ba04646249_s1, Thermo-Fisher Scientific, USA), 1.5 μL template DNA and 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 3 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 *omp1* semi-nested PCR followed by restriction fragment length polymorphism (RFLP) analysis. The first product of 1033 base pairs (bp) was amplified using the following paired primers: forward (SERO1A) (5'-ATGAAAAAACTCTGAAATCGG-3') and reverse (SERO2A) (5'-TTTCTAGATCTTCATTCTTGTT-3').[7] The reaction was performed in a final volume of 50 μL containing 16 μL of nuclease-free PCR water, 25 μL of the DreamTaq PCR Master Mix (Thermo-Fisher Scientific, USA), 2 μL of each primer and 5 μL template DNA. PCR was performed with the following cycling conditions: initial denaturation 94ºC for 7 minutes, followed by 40 cycles of denaturation at 95ºC for 1 minute, annealing at 45ºC for 3 minutes and extension at 72ºC for 3 minutes. An additional 7-minute extension at 72ºC was performed at the end of the 40 cycles.

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 and with the following primers: reverse primer (SERO2A) from the first round and PCTM3 (5'-TCCTTGCAAGCTCTGCCTGTGGGGAATCCT-3').[7] After the PCR step, the amplified product was digested with either *AluI*, *DdeI* or *HinfI* restriction enzymes and visualised after electrophoresis on a 2% agarose gel. *Chlamydia trachomatis* serovar identification was made by analysis of the specific restriction pattern.

Statistical data analysis

Prevalence was calculated using the following equation: Prevalence = $^{\text{number of cases}}/_{\text{population size}}$ Associations between categorical variables was assessed using chi-square (x^2) tests. All statistical analyses were conducted using RStudio, version 3.6.3. All *p*-values were considered significant at < 0.05.

Results

Detection of C. trachomatis from DNA extracted from vaginal swabs

The actin-recruiting phosphoprotein of *C. trachomatis* was detected in 47/385 swab samples using the Applied Biosystems™ TaqMan® Assays. The prevalence of *C. trachomatis* in the study population was 12.2%. All no-template controls did not produce any amplicons.

Characteristics of participants infected with C. trachomatis

The socio-demographic, behavioural and clinical characteristics of the participants who tested positive for *C. trachomatis* infection are shown in Table I.

The median age of the participants who tested positive for *C. trachomatis* was 26 years old. Of the participants who tested positive for *C. trachomatis*, (42/47) - 89.4% had a secondary educational level, while (4/47) - 8.5% attended university ($p < 0.001$). There was also a significant association between employment status and *C. trachomatis* infection. The majority of participants who tested positive were unemployed (40/47) - 85.1% compared to being employed (7/47) - 14.9%. Nearly all participants who tested positive for *C. trachomatis* were unmarried (46/47) - 97.9% compared to being married (1/47) - 2.1%, ($p < 0.001$).

Approximately, (42/47) - 89.0% of the participants who tested positive had a regular sex partner, compared to (5/47) - 10.6% who did not have a regular sex partner ($p < 0.001$). Of the 47 positive participants, there was a significant association between having a circumcised partner and *C. trachomatis* infection ($p < 0.001$). Most participants who tested positive had partners who were circumcised (38/47) - 80.9% compared to participants who had uncircumcised partners (9/47) - 19.1%.

Of the participants who tested positive, (34/47) - 72.3% were not living with their partners while (13/47) - 27.7% lived with their partners ($p = 0.002$). There was a significant association between lifetime sex partners and testing positive for *C. trachomatis* ($p = 0.006$). Of the participants who tested positive, (26/47) - 55.3% reported having had two to four lifetime sex partners while (11/47) - 23.4% reported having had only 1 lifetime sex partner. Age of first sex was significantly associated with testing positive for *C. trachomatis* infection ($p < 0.001$). The majority of the positive participants (36/47) - 76.6% had their first sexual intercourse around 15-20 years of age compared to participants who had their first sexual intercourse over 21 years of age (6/47) - 12.8%.

From the 47 participants, (26/47) - 55.3% did not know whether their partner had other partners while (10/47) - 21.3% knew that their partner had other partners. There was a significant association between not knowing if their partner had other partners and testing positive for *C. trachomatis* ($p = 0.006$). There was also a significant association between not using a condom during sex and testing positive for *C. trachomatis* ($p = 0.006$). Of the participants who tested positive, only (14/47) - 29.8% had used a condom during their last sexual activity and (33/47) - 70.2% did not. Among participants who tested positive for *C. trachomatis*, (38/47) - 80.9% reported that their partners did not have symptoms of STIs while (9/47) - 19.1% had partners with STI symptoms. There was a significant association between the participants' partners having STI symptoms and testing positive for *C. trachomatis* ($p < 0.001$).

Previous STI treatment was significantly associated with *C. trachomatis* infection. A high proportion of participants who tested positive (38/47) - 80.9% had not previously been treated for STIs while (9/47) - 19.1% of the participants had been treated for STIs ($p < 0.001$). Only (16/47) - 34.0% of the participants showed current symptoms of STIs (abnormal vaginal discharge) while (31/47) - 66.0% did not show current symptoms of STIs ($p = 0.029$). Of the 47 participants who tested positive, (45/47) - 95.7% did not engage in intravaginal practices while (2/47) - 4.3% of women who engaged in such practices ($p < 0.001$). There was a significant association between participants having a perceived risk of acquiring STIs and testing positive for *C. trachomatis* ($p = 0.013$).

Table I: Socio-demographic, behavioural and clinical characteristics of participants who tested positive for *C. trachomatis* ($n = 47$)

Variable	Response	*n* (%)	*p*-value
Age	Median (IQR)	26.0 (10.5)	0.011
Educational level	Primary school and below	1 (2.1)	< 0.001

Variable	Response	*n* (%)	*p*-value
	High school	42 (89.4)	
	College, University	4 (8.5)	
Employed	No	40 (85.1)	< 0.001
	Yes	7 (14.9)	
Married	No	46 (97.9)	< 0.001
	Yes	1 (2.1)	
Regular sex partner	No	5 (10.6)	< 0.001
	Yes	42 (89.4)	
HIV status of partner(s)	Negative	21 (44.7)	0.144
	Positive	16 (34.0)	
	Does not know	10 (21.3)	
Cohabiting	No	34 (72.3)	0.002
	Yes	13 (27.7)	
Age of first sex	< 15 years	5 (10.6)	< 0.001
	15-20 years	36 (76.6)	
	21+ years	6 (12.8)	
Lifetime sex partners	1	11 (23.4)	0.006
	2-4	26 (55.3)	
	> 4	10 (21.3)	
Partner has other partners	No	11 (23.4)	0.006
	Yes	10 (21.3)	
	Does not know	26 (55.3)	
Condom used during last sex	No	33 (70.2)	0.006
	Yes	14 (29.8)	
Partner circumcised	No	9 (19.1)	< 0.001
	Yes	38 (80.9)	
Partner STI symptoms	No	38 (80.9)	< 0.001
	Yes	9 (19.1)	
Previously treated for STIs	No	38 (80.9)	< 0.001
	Yes	9 (19.1)	
Current symptoms of STIs	No	31 (66.0)	0.029
	Yes	16 (34.0)	
Intravaginal practices	No	45 (95.7)	< 0.001

Variable	Response	*n* (%)	*p*-value
	Yes	2 (4.3)	
Perceived risk of getting STIs	No	15 (31.9)	0.013
	Yes	32 (68.1)	

HIV – human immunodeficiency virus, IQR – interquartile range

Amplification of the outer omp1 of C. trachomatis

Of the 47 *C. trachomatis*-positive samples, 43 had produced positive amplicons for the *omp1* gene. The expected fragment size of 1033 bp was observed by agarose gel electrophoresis in the 43 positive samples (Figure 1, Supplementary Figure 1, Supplementary Figure 2, Supplementary Figure 3).

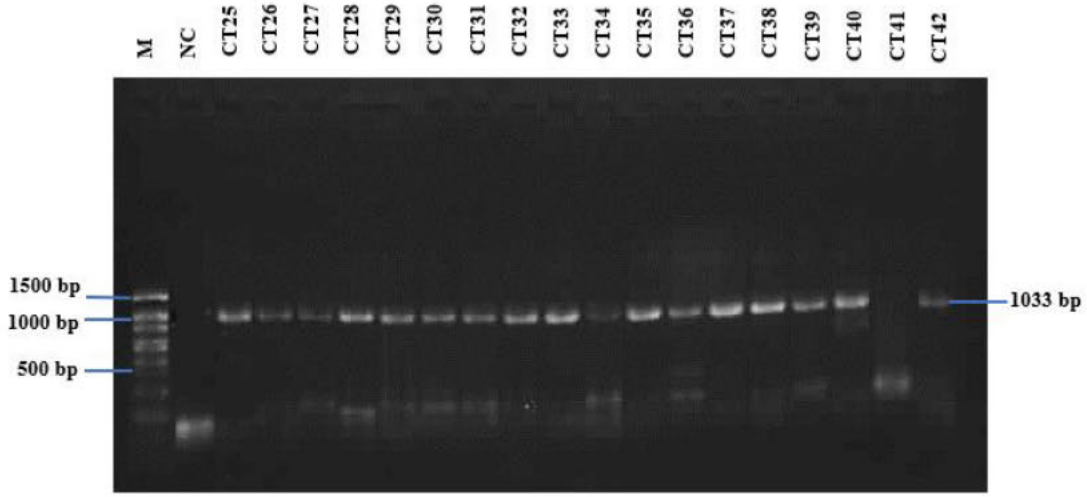

Figure 1: The 1.5% agarose gel showing 17 positive amplicons generated for the outer *omp1*. The expected fragment size of 1033 bp was observed. M - 100 bp DNA molecular ladder showing 500 bp, 1000 bp and 1500 bp (Thermo-Fisher Scientific, USA), NC - negative control (no template DNA added) and a subset of 18 out of the 47 amplified clinical samples. A product size of 1033 bp of the outer *omp1* was only present in 43 out of 47 samples tested.

Genotyping analysis

AluI profile

A total of 42 samples produced banding patterns for *AluI*. Multiple bands were observed for this digest which had previously not been published. Five different serovars were detected with the *AluI* enzyme, the serovars identified were serovars D, E, F, G and I. Band sizes of 207 bp and 256

bp were observed for sample *C. trachomatis* (CT) 22 and this pattern showed the presence of serovar F (Figure 2, Table II, Supplementary Figure 4, Supplementary Figure 5).

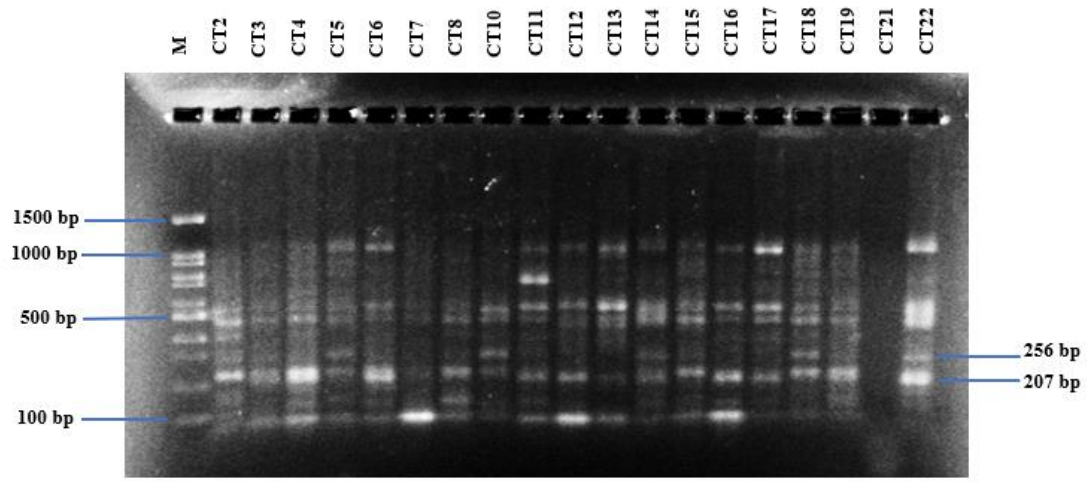

Figure 2: The *AluI* RFLP pattern of the digested *omp1* amplicon resolved on a 2% agarose gel. M - 100 bp DNA molecular ladder showing 100 bp, 500 bp, 1000 bp and 1500 bp (Thermo-Fisher Scientific, USA) and banding profiles for a subset of 19 out of the 43 clinical samples that generated positive amplicons. Size fragments of 207 bp and 256 bp were observed for sample CT 22. Sample CT 21 was the only positive amplicon that showed no restriction banding profile for *AluI*.

DdeI profile

A total of 42 samples produced banding patterns for *DdeI*. Different banding patterns were observed across the samples (Figure 3, Supplementary Figure 6, Supplementary Figure 7), indicating banding patterns for a subset of 17 out of 43 clinical samples that generated positive amplicons. Serovar I was detected using the *DdeI* enzyme. The expected band sizes for serovar I included: 110 bp, 183 bp, 285 bp and 323 bp (Table II, CT 2 and CT 17).

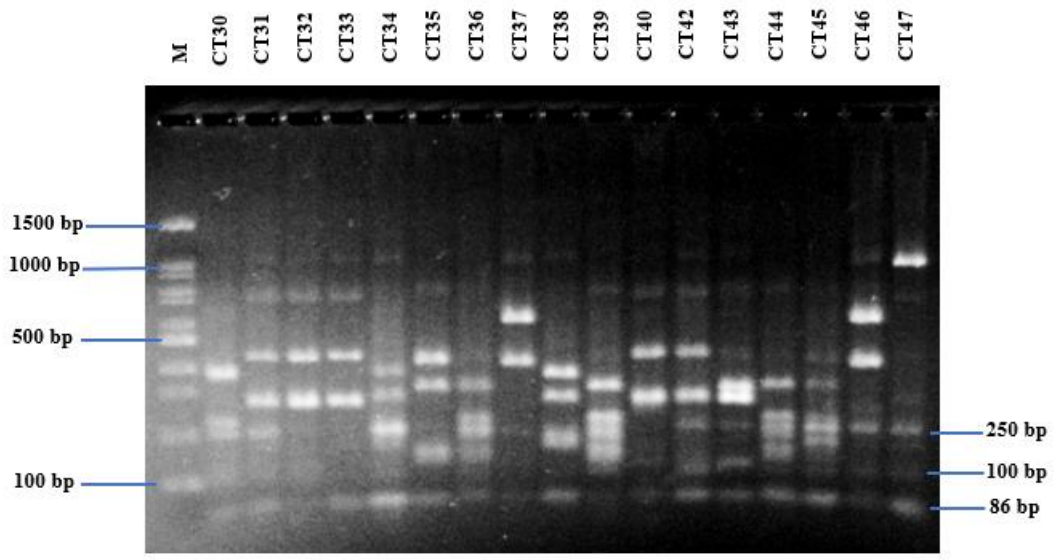

Figure 3: The *DdeI* RFLP pattern of the digested *omp1* amplicon resolved on a 2% agarose gel. M - 100 bp DNA molecular ladder showing 100 bp, 500 bp, 1000 bp and 1500 bp (Thermo-Fisher Scientific, USA) and banding profiles for a subset of 17 out of 43 clinical samples that generated positive amplicons. Size fragments of 86 bp, 100 bp and 250 bp were observed for sample CT 47. Sample CT 21 was the only positive amplicon that showed no restriction banding profile for *DdeI*.

HinfI profile

A total of 42 samples produced banding patterns for *HinfI*. Different banding patterns were observed across the samples, (Figure 4, Supplementary Figure 8, Supplementary Figure 9), indicating banding patterns for a subset of 19 out of the 43 clinical samples that generated positive amplicons. Serovar J was not detected with the *HinfI* enzyme. The expected band size for serovar J included: 540 bp; however, this band size was not detected throughout the *Hinf* profile.

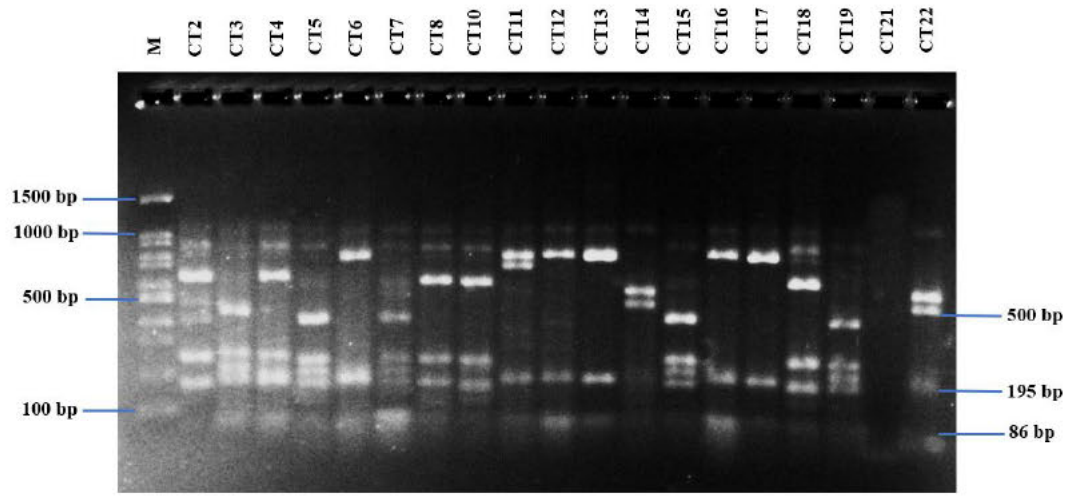

Figure 4: The *HinfI* RFLP pattern of the digested *omp1* amplicon resolved on a 2% agarose gel. M - 100 bp DNA molecular ladder showing 100 bp, 500 bp, 1000 bp and 1500 bp (Thermo-Fisher Scientific, USA) and banding profiles for a subset of 19 out of the 43 clinical samples that generated positive amplicons. Size fragments of 86 bp, 195 bp and 500 bp were observed for sample CT 22. Sample CT 21 was the only positive amplicon that showed no restriction banding profile for *HinfI*.

Frequency of serovars

Table II describes the different serovars obtained due to combining the banding patterns across the three enzyme profiles. All the band sizes in red indicate the published band sizes used to determine the specific serovar for each sample with a positive amplicon; the band sizes in black indicate additional bands that were observed.[7] A total of 42 out of 43 samples were assigned serovars. The prevalence of the individual serovars were as follows: serovar E (20/43) - 46.5%, followed by serovar F (9/43) - 20.9%, serovar G (6/43) - 14.0%, serovar D (5/43) - 11.6% and serovar I (2/43) - 4.7%. Sample CT 21 did not produce any bands during digestion; therefore, the serovar was not identified and not included in the analysis.

Table II: Fragment sizes obtained after digestion of the *omp1* with the restriction enzymes *AluI, DdeI* or *HinfI* as well as the assignment of the *C. trachomatis* serovars based on combining the patterns across the three enzyme profiles (expected band sizes are shown in red according to previous publications)

Sample name	*AluI* fragment sizes (bp)	*DdeI* fragment sizes (bp)	*HinfI* fragment sizes (bp)	Serovar
CT 2	225, 302, 400, 458, 550	86, 183, 285, 323	195, 225, 450, 495, 675	**I**

Sample name	*AluI* fragment sizes (bp)	*DdeI* fragment sizes (bp)	*HinfI* fragment sizes (bp)	Serovar
CT 3	95, 225, 486	86, 225, 250, 300, 350, 486, 800	86, 200, 250, 295, 475	**E**
CT 4	96, 125, 195, 225, 486, 550	86, 225, 250, 300, 350, 400, 800	86, 225, 295, 675	**D**
CT 5	95, 225, 241, 486, 550	86, 225, 250, 300, 350, 486, 800	195, 250, 275, 450, 895	**E**
CT 6	95, 132, 225, 486, 550	86, 225, 250, 295, 400, 695, 700	86, 200, 850	**E**
CT 7	102, 225, 486, 550	96, 225, 300, 486, 800	86, 200, 250, 295, 475	**E**
CT 8	95, 132, 225, 486, 550	86, 150, 225, 300, 400, 600	86, 195, 275, 695, 875	**E**
CT 10	95, 132, 225, 300, 486, 550	86, 295, 450, 650	86, 195, 275, 695, 875	**E**
CT 11	95, 132, 225, 486, 575, 700	86, 200, 275, 300, 400, 650	86, 200, 700, 800	**E**
CT 12	102, 225, 486, 575	86, 200, 295, 425, 650	86, 200, 800	**E**
CT 13	95, 225, 486, 575	86, 450, 700	86, 195, 800	**E**
CT 14	95, 225, 300, 486, 575	86, 250, 395, 450, 800	500, 600	**E**
CT 15	95, 241, 486, 575	86, 275, 395, 500, 750	195, 250, 275, 450	**E**
CT 16	102, 225, 486, 575	86, 150, 250, 275, 400, 695	86, 200, 875	**E**
CT 17	225, 458, 575	96, 250, 285, 486, 695	86, 200, 875	**I**
CT 18	256, 321, 486, 575	96, 150, 195, 225, 300, 400	86, 195, 275, 675, 850	**F**

Sample name	*AluI* fragment sizes (bp)	*DdeI* fragment sizes (bp)	*HinfI* fragment sizes (bp)	Serovar
CT 19	256, 486, 575	96, 150, 225, 300, 486, 900	195, 250, 275, 450	**F**
CT 22	207, 256, 486, 575	195, 200, 225, 275, 300, 395, 495, 600, 700, 900	86, 195, 500, 600	**F**
CT 23	195, 225, 486, 575	125, 195, 200, 425, 650	86, 195, 850	**D**
CT 24	95, 225, 486, 575	86, 125, 207, 430, 675	86, 195, 850	**E**
CT 25	80, 256, 486, 575, 800	86, 295, 450, 700	100, 195, 200, 250, 450, 600, 850	**F**
CT 26	132, 225, 400, 486, 575	100, 250, 400	150, 200, 850	**E**
CT 27	225, 241, 486, 575	86, 200, 250, 300	96, 200, 495, 550, 800	**D**
CT 28	80, 195, 207, 375, 486, 575	86, 125, 195, 250, 400	96, 195, 200, 850	**G**
CT 29	95, 225	86, 100, 195, 207, 295, 486	86, 195, 200, 250, 400, 900	**E**
CT 30	80, 150, 207, 486, 575	86, 200, 225, 400	200, 895	**F**
CT 31	80, 150, 195, 207, 225, 486, 575, 750	86, 200, 275, 486, 750	86, 195, 200, 250, 400, 500, 595, 650, 850	**G**
CT 32	132, 225, 486, 575, 750	295, 486, 750	86, 195, 200, 250, 450, 650, 900	**E**
CT 33	95, 225, 486, 550, 750	86, 295, 486, 750	86, 195, 200, 250, 450, 900	**E**
CT 34	80, 207, 225, 256, 486, 550	86, 225, 300, 400	86, 200, 275, 650, 900	**F**
CT 35	80, 256, 486, 575	86, 175, 300, 450	86, 500, 600	**F**

Sample name	*AluI* fragment sizes (bp)	*DdeI* fragment sizes (bp)	*HinfI* fragment sizes (bp)	Serovar
CT 36	80, 101, 175, 207, 225, 256, 400, 486, 550	86, 175, 200, 250, 300	90, 200, 400, 500, 600	**F**
CT 37	95, 225, 486, 550, 700	86, 450, 695	96, 200, 895	**E**
CT 38	95, 225, 486, 550	86, 195, 295, 395	200, 250, 650	**E**
CT 39	80, 125, 175, 207, 225, 256, 486, 575	195, 200, 225, 300, 750	195, 500, 600	**F**
CT 40	80, 125, 195, 207, 225, 486, 550, 700	86, 295, 450, 750	200, 225, 250, 450, 750	**G**
CT 42	80, 125, 195, 207, 225, 486, 550	86, 100, 200, 295, 450, 750	96, 195, 200, 250, 450	**G**
CT 43	86, 195, 225, 486, 550, 700	86, 100, 200, 295, 350	200, 250, 450, 650	**D**
CT 44	95, 102, 195, 225	86, 195, 200, 225, 325	96, 225, 500, 600	**E**
CT 45	80, 195, 207, 550	86, 125, 195, 200, 295, 400	96, 195, 200, 500, 600	**G**
CT 46	80, 107, 195, 225, 400, 486, 575	86, 100, 200, 425, 650	96, 200, 895	**G**
CT 47	86, 195, 225, 400, 486	86, 100, 200, 250	96, 200, 225, 250, 450	**D**

bp – base pair(s), CT – *Chlamydia trachomatis*

Distribution of serovars in relation to clinical factors

The serovars linked to clinical factors of the participants are showed in (Table III).

Of the five participants who carried serovar D, 100.0% (5/5) reported not having abnormal vaginal discharge, and 20.0% (1/5) reported past treatment of STIs while 80.0% (4/5) did not report past treatment of STIs. Of the 20 participants who carried serovar E, 20.0% (4/20) reported having abnormal vaginal discharge while 80.0% (16/20) reported not having abnormal vaginal discharge. Also, 20.0% (4/20) reported past treatment of STIs and 80.0% (16/20) did not report past treatment of STIs. Of the nine participants who carried serovar F, 11.1% (1/9) reported having

abnormal vaginal discharge while 88.9% (8/9) reported not having abnormal vaginal discharge. Also, 22.2% (2/9) reported past treatment of STIs and 77.8% (7/9) did not report past treatment of STIs. Of the six participants who carried serovar G, 100.0% (6/6) reported not having abnormal vaginal discharge, 16.7% (1/6) reported past treatment of STIs and 83.3% (5/6) did not report past treatment of STIs. Of the two participants who carried serovar I, 50.0% (1/2) reported having abnormal vaginal discharge while 50.0% (1/2) reported not having abnormal vaginal discharge, and 100.0% (2/2) did not report past treatment of STIs.

Table III: Serovars linked to clinical factors of infection

Clinical factors	**Serovars**				
	D	E	F	G	I
Current abnormal vaginal discharge	**0.0%**	**20.0%**	**11.1%**	**0.0%**	**50.0%**
No	5/5	16/20	8/9	6/6	½
Yes	0/5	4/20	1/9	0/6	½
Past treatment of STIs	**20.0%**	**20.0%**	**22.2%**	**16.7%**	**0.0%**
No	4/5	16/20	7/9	5/6	2/2
Yes	1/5	4/20	2/9	1/6	0/2

Discussion

Chlamydia trachomatis infection is one of the most prevalent curable STIs worldwide. However, if left untreated, this Gram-negative intracellular bacterium can cause pathology in the reproductive system. This pathogen affects (uro)genital epithelial cells, escaping the host immune system and causing cervicitis, pelvic inflammatory illness and tubal factor infertility.[10] *Chlamydia trachomatis* infections of the genitourinary tract is a leading source of morbidity in sexually active people,[11] and women bear most of the disease's burden.[12] The infection increases the chance of negative pregnancy outcomes such as premature delivery, low birth weight and postpartum sepsis. In SSA, almost 5.1 million (2.6%) women were infected with *C. trachomatis* in 2012.[13] However, in 60%-80% of patients, *C. trachomatis* infection does not manifest and remains asymptomatic.[14] *Chlamydia trachomatis* infections in pregnant women globally have only been explored in a few studies. Nonetheless, infection rates have been observed to range from 6.1% to 16.8%, with some cases reaching as high as 23% to 37%.[15,16] In this study, the prevalence rate of *C. trachomatis* in

pregnant women was 12.2% which is similar to the prevalence rate of 12.5% among a study population in Tanzania.[17]

This study also correlates with individual studies of pregnant women in SSA (Sudan, Cameroon, Democratic Republic of Congo, Gabon, Nigeria, Kenya, Uganda, Malawi, Zambia, Botswana, Mozambique and South Africa) which suggests prevalence rates of 0%-31.1%.[16,18-31-38] Pooled prevalence rates in east and southern Africa are 6.9% and 6.1%, respectively.[16,18-31-38] In a study conducted using urine samples, the prevalence rate was higher than the present study with 15% of the study population infected with *C. trachomatis*.[39] In Brazilian studies, the rates of *C. trachomatis* infection in urine samples from pregnant women ranges from 6.9% to 18%.[40] Other countries report prevalence rates of 6.4%-18.1%.[41] The current study showed a higher prevalence rate compared to studies conducted among pregnant women from regions such as Iran (8.74%),[1] China (10.1%),[42] and Saudi Arabia (10.5%).[43] However, the prevalence rate reported in the current study is lower than a study conducted in India, which indicated that 35% of women were infected with *C. trachomatis*.[44]

Regarding the characteristics of infected women, this study had shown that the median age of infected pregnant women was 26 years of age. Therefore, this infection is far more common in young sexually active women.[45] Similar studies have shown that *C. trachomatis* infection was found predominantly in pregnant women around 25 years of age.[46-48] Another study indicated that the average age of infected women was 24 years old, showing that young women are at higher risk of acquiring this infection.[49] Education has been considered a protective factor against engagement in high-risk sexual behaviour and STI diagnosis.[50] Studies have shown that the highest rates of *C. trachomatis* infection are found in developing countries, among the poorer, unemployed and uneducated women.[46-49,51,52] This finding is consistent with the current study as there was a significant association between women who tested positive for *C. trachomatis* and having low levels of education and being unemployed. Other studies have shown that the prevalence of infection tends to be higher in single women and in those with multiple partners.[46,48,53] This is similar to the current study as there was a significant association between being married and testing positive for infection. Chlamydial infection is a major burden in Africa,[54] because of the asymptomatic nature of the infection. The current study also showed a significant association between being asymptomatic and testing positive for infection.

The prevalence of *C. trachomatis* serovars has been determined for several countries; however, limited data is available for South Africa. Genotyping approaches, using the *omp1*, are more precise than previous techniques such as immunotyping,[55] which has many limitations in determining *C. trachomatis* variations within serovars.[55] Serovars D to K are chiefly responsible

for urogenital infections.[56] Currently, the PCR-RFLP technique based on the *omp1* amplification provides a sensitive and reliable method for typing of *C. trachomatis* isolates.[29] In this study, five different serovars of *C. trachomatis* were identified by PCR-RFLP of the *omp1*. The clinical samples could have been discriminated by restriction endonuclease digestion with *AluI* alone. However, digestion with *DdeI* and *HinfI* were also performed to further aid in discriminating among *C. trachomatis* serovars in this study. The most frequent serovar in the study population was serovar E (20/43) - 46.5%, followed by serovar F (9/43) - 20.9%, serovar G (6/43) - 14.0%, serovar D (5/43) - 11.6% and serovar I (2/43) - 4.7%.

The current study correlates with other European and American studies conducted among pregnant women, which show serovars E and F as the predominant urogenital serovars, accounting for up to 60%-70% of cases.[57-63] These serovars have been reported to be the globally dominant strains.[59,60,64] In Taiwan, the top three serovars among pregnant women were E, D and F.[65] Similar results were found in Japan and Korea.[65] In Thailand, serovar F (25%-60%) was the most prevalent serovar among pregnant women, followed by E (9%-20%) and D (7%-23%).[66] A study conducted among pregnant Japanese women reported the most prevalent serovars to be D (12/40) - 30%, F (5/40) - 12.5% and E (3/40) - 7.5%.[57] In a previous study of *C. trachomatis* in Mexico, serovar F was most frequent (13/24 - 54.2%), followed by serovars E, G and K (2/24 - 8.3% each). Serovars D and I were detected at a frequency of (1/24 - 4.2% each).[9] In a study conducted in India, serovar D (135/280 - 48%) was found to be the most prevalent serovar, followed by serovars E (96/280 - 34%), F (34/280 - 12%) and I (17/280 - 6%) from urogenital samples.[7] Similar, to the study conducted in India,[7] the current study did not identify serovars H, J and K of *C. trachomatis* in pregnant women which have been reported by studies conducted in other countries.[56,66,67] This indicates that different serovar frequencies among pregnant women are found in different study populations in different geographical regions.

In the present study, the majority of women who harboured serovar D (100.0%), E (80.0%), F (88.9%), G (100.0%) and I (50.0%) did not report having abnormal vaginal discharge. Therefore, they were characterised as being asymptomatic. The study correlates with other studies conducted both in South Africa and across the globe which shows that the true number of new *C. trachomatis* infections is likely to be substantially greater since many infections are asymptomatic and go unnoticed.[2,3] It is possible that the genetic differences in the pathogen may contribute to the clinical manifestations associated with it. However, this will need to be confirmed by further investigations. In the current study, 20.0% of the women who carried serovar E reported experiencing abnormal vaginal discharge. This is similar to another study suggesting that serovar E is mostly associated with abnormal vaginal discharge which is green in colour.[68] In another

study, it was observed that *C. trachomatis* serovar F has also been associated with reduced mucopurulent endocervical discharge.[69]

Serovars D, E, F, G and I were present in small proportions of women who were treated for STIs in the past. Therefore, the majority of women positive for *C. trachomatis* were not treated for STIs in the past. Individuals with *C. trachomatis* should be treated to avoid damaging effects on their reproductive system and ongoing sexual transmission.[70] Additionally, treating their sex partners helps prevent reinfection and infection of other partners. Treating pregnant women reduces *C. trachomatis* from being transmitted to new-borns at birth. All individuals with chlamydial infection should receive treatment as soon as possible since treatment delays among women have been linked to problems such as pelvic inflammatory disease.[55] Most studies investigating the association between *C. trachomatis* serovars and clinical factors of infection show contradictory results. This can be explained by geographical variations and differences in study size and population composition.[55,70]

Study limitations

In this study, the *omp1* was not shown to be present in all the swab samples which tested *C. trachomatis* positive by the TaqMan® Assays. Other *C. trachomatis* genotyping studies which have been based on the *omp1* were conducted on pure isolates rather than the primary vaginal swab samples. The use of the primary sample to infer serovars may not be the most appropriate method as evidenced by the lack of amplification of certain samples. An alternate method which may be more useful for investigating the genetic variation in *C. trachomatis* from non-cultured vaginal swabs would be the next generation-multi locus sequence typing (NG-MLST) technique. However, due to budget constraints, the NG-MLST method was not used in the present study. Despite these limitations, this study was able to provide data on the serovars of *C. trachomatis* that is circulating in our setting, a previously under-researched area.

Conclusion

This study showed that genotyping could be achieved from uncultured vaginal swabs. The observed diversity of serovars and the different clinical factors reported within specific populations can be challenging for future vaccine design and development for chlamydia. However, many of the South African serovars detected correlated with serovars found in studies conducted worldwide. This holds some promise that there may be conserved *C. trachomatis* strains from different geographical regions and could lend some hope for future vaccine design and diagnostic studies focused on the entire *C. trachomatis* population. Future studies emanating from this study would be to investigate the association of the serovars with patterns of drug susceptibility in this pathogen. In addition, it would be useful to compare the distribution of the

serovars in pregnant and non-pregnant populations as well as to further investigate the serovars in connection with the presence or absence of clinical symptoms, possibly by investigation of virulence genes in these specific serovars.

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Conflict of interest

The authors declare no conflict of interest.

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Ethical approval

This study was approved by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (UKZN) (BREC/00004226/2022).

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References

1. Azami M, Badfar G, Mansouri A et al. Prevalence of *Chlamydia trachomatis* in pregnant Iranian women: a systematic review and meta-analysis. *International Journal of Fertility & Sterility* 2018; 12(3):191-199. doi: 10.22074%2Fijfs.2018.5191

2. Balle C, Lennard K, Dabee S et al. Endocervical and vaginal microbiota in South African adolescents with asymptomatic *Chlamydia trachomatis* infection. *Scientific Reports* 2018; 8(1):11109. doi: 10.1038/s41598-018-29320-x

3. Meyer T. Diagnostic procedures to detect *Chlamydia trachomatis* infections. *Microorganisms* 2016; 4(3):25. doi: 10.3390/microorganisms4030025

4. Hussen S, Wachamo D, Yohannes Z et al. Prevalence of *Chlamydia trachomatis* infection among reproductive age women in Sub-Saharan Africa: a systematic review and meta-analysis. *BMC Infectious Diseases* 2018; 18:1-8. doi: 10.1186/s12879-018-3477-y

5. Kularatne R.S, Niit R, Rowley J et al. Adult gonorrhoea, chlamydia and syphilis prevalence, incidence, treatment, and syndromic case reporting in South Africa: Estimates using the Spectrum-STI model, 1990-2017. *PLoS ONE* 2018; 13:e0205863. doi: 10.1371/journal.pone.0205863

6. Joseph D.D.L, Nyemba D.C, Gomba Y et al. Prevalence and correlates of sexually transmitted infections in pregnancy in HIV infected and- uninfected women in Cape Town, South Africa. *PLoS ONE* 2019; 14(7):e0218349. doi: 10.1371/journal.pone.0218349

7. Singh V, Salhan S, Das B.C et al. Predominance of *Chlamydia trachomatis* serovars associated with urogenital infections in females in New Delhi, India. *Journal of Clinical Microbiology* 2003; 41(6):2700-2702. doi: 10.1128/jcm.41.6.2700-2702.2003

8. Chen Y, Chen J, Yang L et al. Distribution of *Chlamydia trachomatis* genotypes in infective diseases of the female lower genital tract. *Medical Science Monitor* 2017; 23:4477-4481. doi: 10.12659/MSM.902756

9. Cruz M.J.D.H, Rodriguez I.D, Guerra M.R.E et al. Genotyping of *Chlamydia trachomatis* from endocervical specimens of infertile Mexican women. *Infectious Diseases and Clinical Microbiology* 2011; 29(2):100-108. doi: 10.1016/j.eimc.2010.08.014

10. McGregor J.A, French J.I & Seo K. Premature rupture of membranes and bacterial vaginosis. *American Journal of Obstetrics and Gynecology* 1993; 169:463-466. doi: https://doi.org/10.1016/0002-9378(93)90342-G

11. Mahony J.B, Luinstra K.E, Tyndall M et al. Multiplex PCR for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in genitourinary specimens. *Journal of Clinical Microbiology* 1995; 33:3049-3053. doi: 10.1128/jcm.33.11.3049-3053.1995

12. Malhotra M, Sood S, Mukherjee A et al. Genital *Chlamydia trachomatis*: An update. *The Indian Journal of Medical Research* 2013; 138:303-316. URL: https://pubmed.ncbi.nlm.nih.gov/24135174

13. World Health Organization. Global incidence and prevalence of selected curable sexually transmitted infections - 2008. *World Health Organization: Geneva, Switzerland* 2012. URL: https://apps.who.int/iris/handle/10665/75181

14. World Health Organization. Guidelines for the Treatment of *Chlamydia trachomatis*. *World Health Organization: Geneva, Switzerland* 2016. URL: https://www.ncbi.nlm.nih.gov/books/NBK379707

15. Khurana C.M, Deddish P.A & Delmundo F. Prevalence of *Chlamydia trachomatis* in the pregnant cervix. *Obstetrics and Gynecology* 1985; 66:241-243. URL: https://www.ncbi.nlm.nih.gov/pubmed/4022486

16. Janse van Rensburg H.J & Odendaal H.J. The prevalence of potential pathogenic micro-organisms in the endocervix of pregnant women at Tygerberg Hospital. *South African Medical Journal* 1992; 81(3):156-157.

17. Juliana N.C.A, Deb S, Ouburg S et al. The prevalence of *Chlamydia trachomatis* and three other non-viral sexually transmitted infections among pregnant women in Pemba island, Tanzania. *Pathogens* 2020; 9(8):625. doi: 10.3390/pathogens9080625

18. Abdelaziz Z.A, Ibrahim M.E, Bilal N.E et al. Vaginal infections among pregnant women at Omdurman Maternity Hospital in Khartoum, Sudan. *Journal of Infection in Developing Countries* 2014; 8(4):490-497. doi: 10.3855/jidc.3197

19. Ngassa P.C & Egbe J.A. Maternal genital *Chlamydia trachomatis* infection and the risk of preterm labor. *International Journal of Gynecology and Obstetrics* 1994*;* 47(3):241-246. doi: 10.1016/0020-7292%2894%2990568-1

20. Kinoshita R.M, Smith J.S, Atibu J et al. Low prevalence of HIV and other selected sexually transmitted infections in 2004 in pregnant women from Kinshasa, the Democratic Republic of the Congo. *Epidemiology and Infection* 2008; 136(9):1290-1296. doi: 10.1017/S0950268807009818

21. Beaujean G & Willems I. Prevalence of *Chlamydia trachomatis* infection in pregnant women in Zaire. *Genitourinary Medicine* 1990; 66(2):124-125. doi: 10.1136/sti.66.2.124-a

22. Vuylsteke B, Laga M, Alary M et al. Clinical algorithms for the screening of women for gonococcal and chlamydial infection: evaluation of pregnant women and prostitutes in Zaire. *Clinical Infectious Diseases* 1993; 17(1):82-88. URL: http://lib.itg.be/pdf/itg/1993/1993cidi0082.pdf

23. Laga M, Nzanze H, Brunham R.C et al. Epidemiology of ophthalmia neonatorum in Kenya. *The Lancet* 1986; 328(8516):1145-1149. doi:10.1016/s0140-6736(86)90544-1

24. Marx G, John G.S, Bosire R et al. Diagnosis of sexually transmitted infections and bacterial vaginosis among HIV-1-infected pregnant women in Nairobi. *International Journal of STD & AIDS* 2010; 21(8):549-552. doi: 10.1258/ijsa.2010.010005

25. Aboud S, Msamanga G, Read J.S et al. Genital tract infections among HIV-infected pregnant women in Malawi, Tanzania and Zambia. *International Journal of STD & AIDS* 2008; 19(12):824-832. doi: 10.1258/ijsa.2008.008067

26. Mayaud P, Ka G.G, Cornelissen J et al. Validation of a WHO algorithm with risk assessment for the clinical management of vaginal discharge in Mwanza, Tanzania. *Sexually Transmitted Infections* 1998; 74(1):S7-S84. URL: http://lib.itg.be/pdf/itg/1998/1998stin0S77.pdf

27. Chiduo M, Theilgaard Z.P, Bakari V et al. Prevalence of sexually transmitted infections among women attending antenatal clinics in Tanga, north-eastern Tanzania. *International Journal of STD and AIDS* 2012; 23(5):325-329. doi:10.1258/ijsa.2011.011312

28. Menéndez C, Castellsagué X, Renom M et al. Prevalence and risk factors of sexually transmitted infections and cervical neoplasia in women from a rural area of southern Mozambique. *Infectious Diseases in Obstetrics and Gynecology* 2010; 2010:609315. doi: 10.1155/2010%2F609315

29. Govender S, Theron G.B, Odendaal H.J et al. Prevalence of genital mycoplasmas, ureaplasmas and chlamydia in pregnancy. *Journal of Obstetrics and Gynaecology* 2009; 29(8):698-701. doi: 10.3109/01443610903184033

30. De Jongh M.D, Lekalakala M.R, Roux M.L et al. Risk of having a sexually transmitted infection in women presenting at a termination of pregnancy clinic in Pretoria, South Africa. *Journal of Obstetrics and Gynaecology* 2010; 30(5):480-483. doi: 10.3109/01443611003797687

31. Donders G.G.G, Desmyter J, De Wet D.H.D et al. The association of gonorrhoea and syphilis with premature birth and low birthweight. *Genitourinary Medicine* 1993; 69(2):98-101. doi: 10.1136/sti.69.2.98

32. Moodley D, Moodley P, Sebitloane M et al. High prevalence and incidence of asymptomatic sexually transmitted infections during pregnancy and postdelivery in KwaZulu Natal, South Africa. *Sexually Transmitted Diseases* 2015; 42(1):43-47. doi: 10.1097/OLQ.0000000000000219

33. Adachi K, Klausner J.D, Bristow C.C et al. Chlamydia and gonorrhoea in HIV-infected pregnant women and infant HIV transmission. *Sexually Transmitted Diseases* 2015; 42(10):554-565. doi: 10.1097/OLQ.0000000000000340

34. Romoren M, Rahman M, Sundby J et al. Chlamydia and gonorrhoea in pregnancy: effectiveness of diagnosis and treatment in Botswana. *Sexually Transmitted Infections* 2004; 80(5):395-400. doi: 10.1136/sti.2003.007757

35. Gray R.H, Wabwire F.M, Kigozi G et al. Randomized trial of presumptive sexually transmitted disease therapy during pregnancy in Rakai, Uganda. *American Journal of Obstetrics and Gynaecology* 2001; 185(5):1209-1217. doi: 10.1067/mob.2001.118158

36. Agholor K, Omo L.A & Okonofua F. Association of anti-Chlamydia antibodies with ectopic pregnancy in Benin city, Nigeria: a case-control study. *African Health Sciences* 2013; 13(2):430-440. doi: 10.4314/ahs.v13i2.33

37. Bourgeois A, Henzel D, Malonga G.M et al. Clinical algorithms for the screening of pregnant women for STDs in Libreville, Gabon: Which alternatives? *Sexually Transmitted Infections* 1998; 74(1):35-39. doi: 10.1136/sti.74.1.35

38. Chico R.M, Mayaud P, Mabey C.A.D et al. Prevalence of malaria and sexually transmitted and reproductive tract infections in pregnancy in Sub-Saharan Africa: A systematic review. *The Journal of the American Medical Association* 2012; 307(19):2079-2086. doi: 10.1001/jama.2012.3428

39. Azevedo M.J.N, Nunes S.S, Oliveira F.G et al. High prevalence of *Chlamydia trachomatis* in pregnant women attended at Primary Health Care services in Amazon, Brazil. *Revista Do Instituto de Medicina Tropical de São Paulo* 2019; 61:e6. doi: 10.1590/s1678-9946201961006

40. Santos L.M, Souza I.R, Holanda L.H et al. High incidence of urogenital infection by *Chlamydia trachomatis* in parturient women from Belém, Pará State, Brazil. *Pan-Amazonian Health Magazine* 2016; 7:101-106. doi: 10.5123/s2176-62232016000400012

41. Mafokwane T.M & Samie A. Prevalence of chlamydia among HIV positive and HIV negative patients in the Vhembe District as detected by real time PCR from urine samples. *BMC Research Notes* 2016; 9(102). doi: 10.1186/s13104-016-1887-8

42. Chen X.S, Yin Y.P, Chen L.P et al. Sexually transmitted infections among pregnant women attending an antenatal clinic in Fuzhou, China. *Sexually Transmitted Diseases* 2006; 33(5):296-301. doi: 10.1097/01.olq.0000194580.02116.6b

43. Alzahrani A.J, Obeid O.E, Hassan M.I et al. Screening of pregnant women attending the antenatal care clinic of a tertiary hospital in eastern Saudi Arabia for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections. *Indian Journal of Sexually Transmitted Diseases and AIDS* 2010; 31:81-86. doi: 10.4103/0253-7184.74976

44. Sharma M, Sethi S, Daftari S et al. Evidence of chlamydial infection in infertile women with fallopian tube obstruction. *Indian Journal of Pathology and Microbiology* 2003; 46(4):680-683. URL: http://scholarbank.nus.edu.sg/handle/10635/146026

45. Wilson J.S, Honey E, Templeton A et al. A systematic review of the prevalence of *Chlamydia trachomatis* among European women. *Human Reproduction Update* 2002; 8(4):385-394. doi: 10.1093/humupd/8.4.385

46. Pinto V.M, Szwarcwald C.L, Baroni C et al. *Chlamydia trachomatis* prevalence and risk behaviours in parturient women aged 15 to 24 in Brazil. *Sexually Transmitted Diseases* 2011; 38:957-961.

47. Silveira M.F, Sclowitz I.K, Entiauspe L.G et al. *Chlamydia trachomatis* infection in young pregnant women in Southern Brazil: a cross-sectional study. *Cadernos de Saude Publica* 2017; 33:e00067415. doi: 10.1590/0102-311X00067415

48. Schmidt R, Muniz R.R, Cola E et al. Maternal *Chlamydia trachomatis* infections and preterm births in a university hospital in Vitoria, Brazil. *Plos One* 2015; 10:e0141367. doi: 10.1371/journal.pone.0141367

49. Pánuco C.A.B, Rodríguez I.D, Méndez J.T.H et al. Detection of *Chlamydia trachomatis* in pregnant women by the papanicolaou technique, enzyme immunoassay and polymerase chain reaction. *Acta Cytologica* 2000; 44(2):114-123. doi: 10.1159/000326347

50. Annang L, Walsemann K.M, Maitra D et al. Does education matter? Examining racial differences in the association between education and STI diagnosis among black and white young adult females in the U.S. *Public Health Reports* 2010; 125(4):110-121. doi: 10.1177/00333549101250S415

51. Shilling H.S, Garland S.M, Costa A.M et al. *Chlamydia trachomatis* and *Mycoplasma genitalium* prevalence and associated factors among women presenting to a pregnancy termination and contraception clinic, 2009-2019. *Sexually Transmitted Infections* 2021; 2020-054695. doi:10.1136/sextrans-2020-054695

52. Newman L, Rowley J, Vander H.S et al. Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. *PLoS One* 2015; 10:e0143304. doi: 10.1371/journal.pone.0143304

53. Rocha D.A, Filho R.A, Mariño J.M et al. Hidden sexually transmitted infections among women in primary care health services, Amazonas, Brazil. *International Journal of STD and AIDS* 2014; 25:878-886. doi: 10.1177/0956462414523742

54. Low N, Cassell J.A, Spencer B et al. Chlamydia control activities in Europe: cross-sectional survey. *European Journal of Public Health* 2011; 22(4):556-561. URL: http://edoc.unibas.ch/dok/A6094098

55. Sayada C, Denamur E, Orfila J et al. Rapid genotyping of the *C. trachomatis* major outer membrane protein by the PCR. *FEMS Microbiology Letters* 1991; 83:73-78. doi: 10.1016/0378-1097(91)90447-i

56. Bandea C.I, Kubota K, Brown T.M et al. Typing of *C. trachomatis* strains from urine samples by amplification and sequencing the major outer membrane protein gene (*omp 1*). *Sexually Transmitted Infections* 2001; 77:419-422. doi: 10.1136/sti.77.6.419

57. Yamazaki T, Matsumoto M, Matsuo J et al. Frequency of *Chlamydia trachomatis* in *Ureaplasma*-positive healthy women attending their first prenatal visit in a community hospital in Sapporo, Japan. *BMC Infectious Diseases* 2012; 12(82). doi: 10.1186/1471-2334-12-82

58. Jurstrand M, Falk L, Fredlund H et al. Characterization of *Chlamydia trachomatis omp1* genotypes among sexually transmitted disease patients in Sweden. *Journal of Clinical Microbiology* 2001; 39:3915-3919. doi: 10.1128/JCM.39.11.3915-3919.2001

59. Dean D, Bruno W.J, Wan R et al. Predicting phenotype and emerging strains among *Chlamydia trachomatis* infections. *Emerging Infectous Diseases* 2009; 15:1385-1394. doi: 10.3201/eid1509.090272

60. Smelov V, Vrbanac A, Ess E.F et al. *Chlamydia trachomatis* strain types have diversified regionally and globally with evidence for recombination across geographic divides. *Frontiers in Microbiology* 2017; 13(2195). doi: 10.3389/fmicb.2017.02195

61. Labiran C, Rowen D, Clarke I.N et al. Detailed molecular epidemiology of *Chlamydia trachomatis* in the population of Southampton attending the genitourinary medicine clinic in 2012-2013, reveals the presence of long-established genotypes and transitory sexual networks. *PLoS One* 2017; 12:e0185059. doi: 10.1371/journal.pone.0185059

62. Lysen M, Oserlund A, Rubin C et al. Characterization of *ompA* genotypes by sequence analysis of DNA from all detected cases of *Chlamydia trachomatis* infections during 1 year of contact tracing in a Swedish county. *Journal of Clinical Microbiology* 2004; 42:1641-1647. doi: 10.1128/jcm.42.4.1641-1647.2004

63. Niemi S, Hiltunen B.E & Puolakkainen M. *Chlamydia trachomatis* genotypes and the Swedish new variant among urogenital *Chlamydia trachomatis* strains in Finland. *Infectious Diseases in Obsetrics & Gynecology* 2011; 2011:481890. doi: 10.1155/2011/481890

64. Hadfield J, Harris S.R, Seth S.H.M.B et al. Comprehensive global genome dynamics of *Chlamydia trachomatis* show ancient diversification followed by contemporary mixing and recent lineage expansion. *Genome Research* 2017; 27:1220-1229. doi: 10.1101%2Fgr.212647.116

65. Hsu M.C, Tsai P.Y, Chen K.T et al. Genotyping of *Chlamydia trachomatis* from clinical specimens in Taiwan. *Journal of Medical Microbiology* 2006; 55(3):301-308. doi: 10.1099/jmm.0.46262-0

66. Borrego M.J, Gomes J.P, Lefebvre J.F et al. Genotyping of Portuguese *Chlamydia trachomatis* urogenital isolates. *Genitourinary Medicine* 1997; 73:561-563 doi: 10.1136/sti.73.6.561

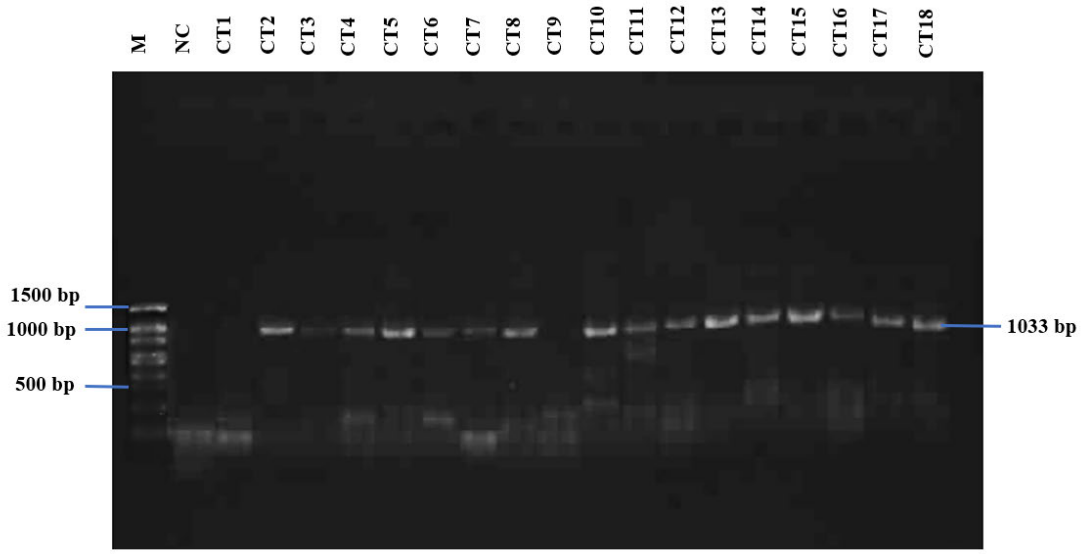
67. Moore S.A, Moes R & Valkengoed I. Genotyping of *Chlamydia trachomatis* in urine specimens will facilitate large epidemiological studies. *Journal of Clinical Microbiology* 1998; 36:3077-3078.

68. Casillas V.N, Morfín O.R, García S et al. Frequency and genotypes of *Chlamydia trachomatis* in patients attending the obstetrics and gynaecology clinics in Jalisco, Mexico and correlation with sociodemographic, behavioural and biological factors. *BMC Women's Health* 2017; 17(1). doi: 10.1186/s12905-017-0428-5

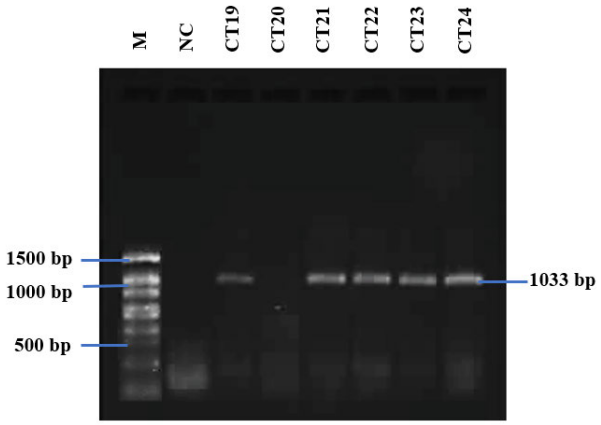
69. Workowski K.A, Stevens C.E, Suchland R.J et al. Clinical manifestations of genital infection due to *Chlamydia trachomatis* in women: Differences related to serovar. *Clinical Infectious Diseases* 1994; 19:756-760.

70. Barnes R.C, Wang S.P, Kuo C.C et al. Rapid immunotyping of *Chlamydia trachomatis* with monoclonal antibodies in a solid-phase enzyme immunoassay. *Journal of Clinical Microbiology* 1985; 22(4):609-613. doi: 10.1128/jcm.22.4.609-613.1985

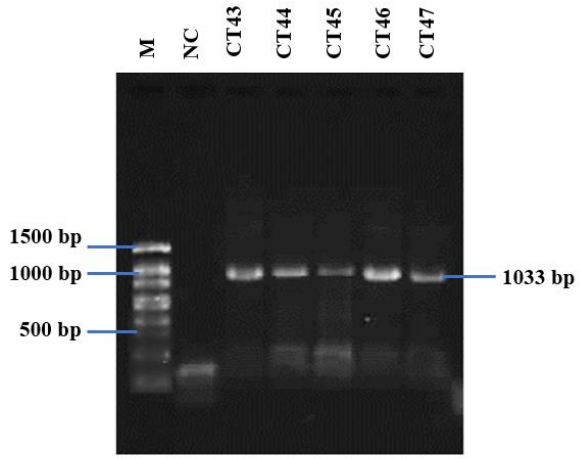
Supplementary material



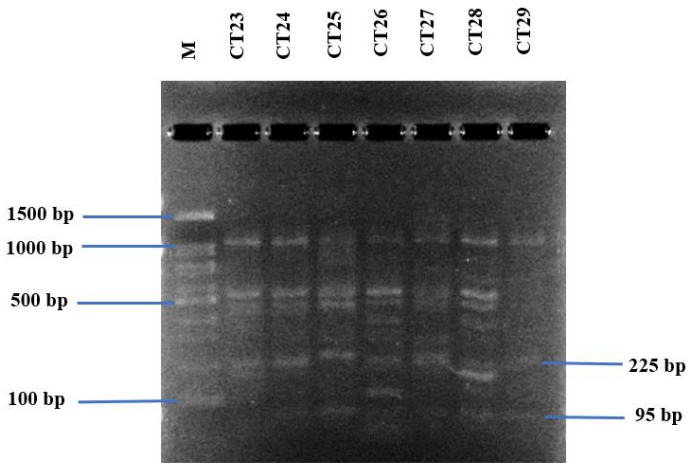
Supplementary Figure 1: The 1.5% agarose gel showing 16 positive amplicons generated for the outer *omp1*. The expected fragment size of 1033 bp was observed. M - 100 bp DNA molecular ladder showing 500 bp, 1000 bp and 1500 bp (Thermo-Fisher Scientific, USA), NC - negative control (no template DNA added) and a subset of 18 out of the 47 amplified clinical samples. A product size of 1033 bp of the outer *omp1* was only present in 43 out of 47 samples tested.



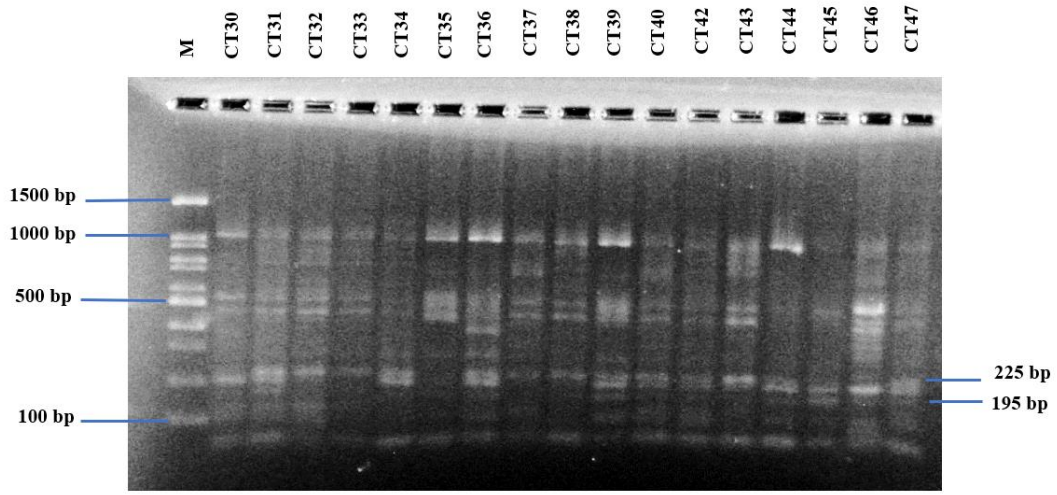
Supplementary Figure 2: The 1% agarose gel showing 5 positive amplicons generated for the outer *omp1*. The expected fragment size of 1033 bp was observed. M - 100 bp DNA molecular ladder showing 500 bp, 1000 bp and 1500 bp (Thermo-Fisher Scientific, USA), NC - negative control (no template DNA added) and a subset of 6 out of the 47 amplified clinical samples. A product size of 1033 bp of the outer *omp1* was only present in 43 out of 47 samples tested.



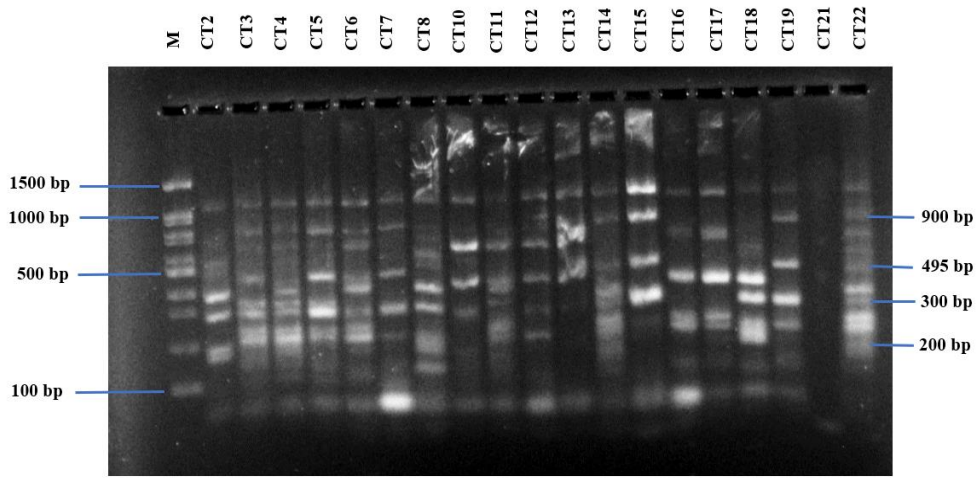
Supplementary Figure 3: The 1% agarose gel showing 5 positive amplicons generated for the outer *omp1*. The expected fragment size of 1033 bp was observed. M - 100 bp DNA molecular ladder showing 500 bp, 1000 bp and 1500 bp (Thermo-Fisher Scientific, USA), NC - negative control (no template DNA added) and a subset of 5 out of the 47 amplified clinical samples. A product size of 1033 bp of the outer *omp1* was only present in 43 out of 47 samples tested.



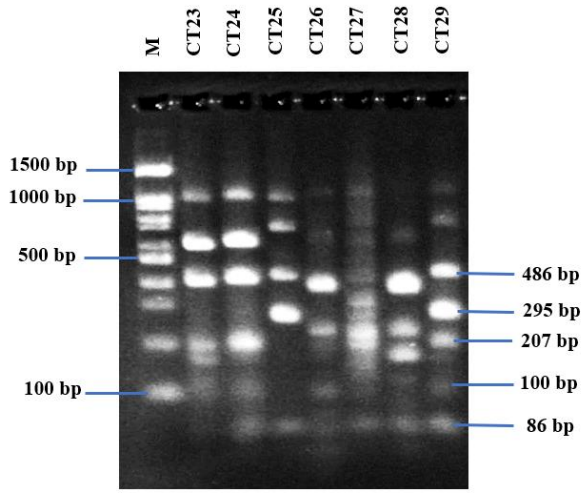
Supplementary Figure 4: The *AluI* RFLP pattern of the digested *omp1* amplicon resolved on a 1.5% agarose gel. M - 100 bp DNA molecular ladder showing 100 bp, 500 bp, 1000 bp and 1500 bp (Thermo-Fisher Scientific, USA) and banding profiles for a subset of seven out of the 43 clinical samples that generated positive amplicons. Size fragments of 95 bp and 225 bp were observed for sample CT 29. Sample CT 21 was the only positive amplicon that showed no restriction banding profile for *AluI.*



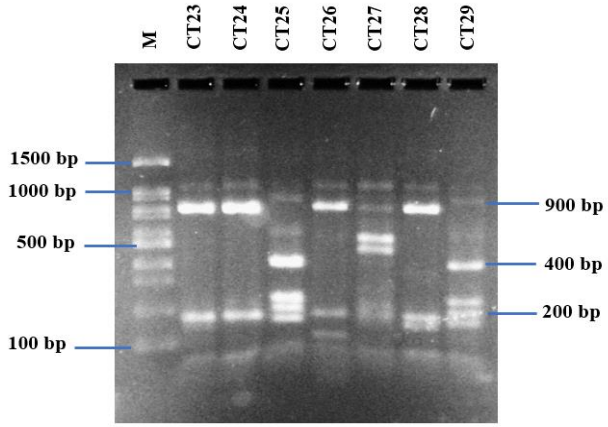
Supplementary Figure 5: The *AluI* RFLP pattern of the digested *omp1* amplicon resolved on a 2% agarose gel. M - 100 bp DNA molecular ladder showing 100 bp, 500 bp, 1000 bp and 1500 bp (Thermo-Fisher Scientific, USA) and banding profiles for a subset of 17 out of the 43 clinical samples that generated positive amplicons. Size fragments of 195 bp and 225 bp were observed for sample CT 47. Sample CT 21 was the only positive amplicon that showed no restriction banding profile for *AluI*.



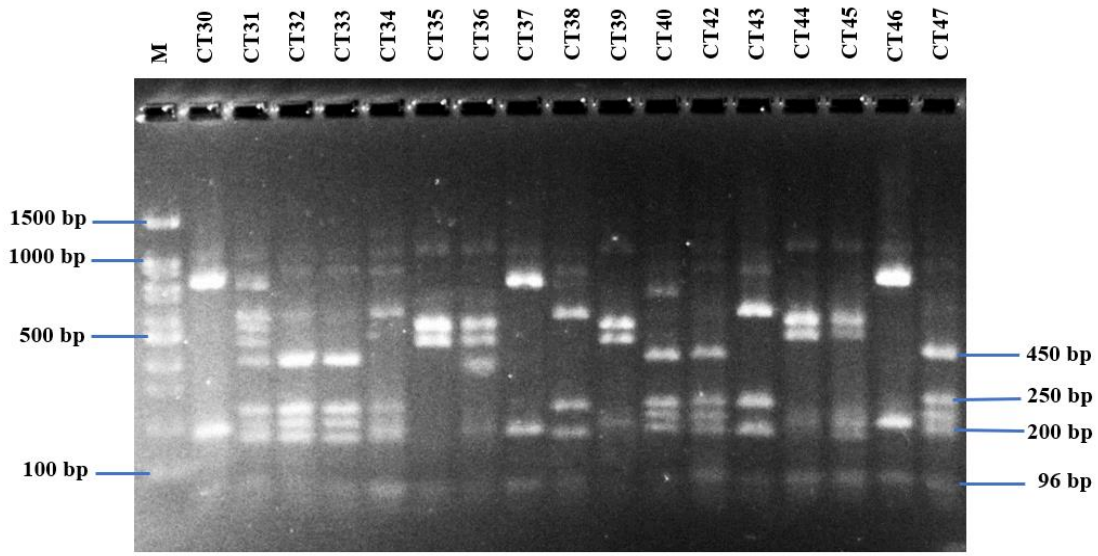
Supplementary Figure 6: The *DdeI* RFLP pattern of the digested *omp1* amplicon resolved on a 2% agarose gel. M - 100 bp DNA molecular ladder showing 100 bp, 500 bp, 1000 bp and 1500 bp (Thermo-Fisher Scientific, USA) and banding profiles for a subset of 19 out of 43 clinical samples that generated positive amplicons. Size fragments of 200 bp, 300 bp, 495 bp and 900 bp were observed for sample CT 22. Sample CT 21 was the only positive amplicon that showed no restriction banding profile for *DdeI*.



Supplementary Figure 7: The *DdeI* RFLP pattern of the digested *omp1* amplicon resolved on a 1.5% agarose gel. M - 100 bp DNA molecular ladder showing 100 bp, 500 bp, 1000 bp and 1500 bp (Thermo-Fisher Scientific, USA) and banding profiles for a subset of 7 out of 43 clinical samples that generated positive amplicons. Size fragments of 86 bp, 100 bp, 207 bp, 295 bp and 486 bp were observed for sample CT 29. Sample CT 21 was the only positive amplicon that showed no restriction banding profile for *DdeI*.



Supplementary Figure 8: The *HinfI* RFLP pattern of the digested *omp1* amplicon resolved on a 1.5% agarose gel. M - 100 bp DNA molecular ladder showing 100 bp, 500 bp, 1000 bp and 1500 bp (Thermo-Fisher Scientific, USA) and banding profiles for a subset of 7 out of the 43 clinical samples that generated positive amplicons. Size fragments of 200 bp, 400 bp and 900 bp were observed for sample CT 29. Sample CT 21 was the only positive amplicon that showed no restriction banding profile for *HinfI*.



Supplementary Figure 9: The *HinfI* RFLP pattern of the digested *omp1* amplicon resolved on a 2% agarose gel. M - 100 bp DNA molecular ladder showing 100 bp, 500 bp, 1000 bp and 1500 bp (Thermo-Fisher Scientific, USA) and banding profiles for a subset of 17 out of the 43 clinical samples that generated positive amplicons. Size fragments of 96 bp, 200 bp, 250 bp and 450 bp were observed for sample CT 47. Sample CT 21 was the only positive amplicon that showed no restriction banding profile for *HinfI*.

CHAPTER 4

Discussion

Chlamydia trachomatis is one of the most frequent sexually transmitted infections (STIs) that can cause significant complications in pregnant women and their new-borns. Despite the fact that *C. trachomatis* can result in pelvic inflammatory disease (PID), infertility, ectopic pregnancy, preterm labour, early membrane rupture and low birth weight babies, the clinical course is typically asymptomatic, and the microbe is infrequently found in women who do not show any obvious signs of infection [19]. In women, up to 80% of *C. trachomatis* infections are asymptomatic [2]. Pregnant women with *C. trachomatis* have the risk of transmitting this infection during labour through the vaginal canal to their new-borns, causing conjunctivitis and pneumonia [20]. Prior to this study, limited data existed on the prevalence and circulating serovars of *C. trachomatis* in pregnant South African women.

With a total of 34 million cases occurring in Sub-Saharan Africa (SSA) and south to southeast Asia, the World Health Organization (WHO) estimates that 50 million women worldwide have contracted *C. trachomatis* for the first time [21]. Sub-Saharan Africa is thought to have a high prevalence of *C. trachomatis*, with approximately 10 million new infections recorded each year [21]. The prevalence of *C. trachomatis* in pregnant South African women ranges from 6.5%-36.8% [22]. In the current study which was conducted in the KwaZulu-Natal province of South Africa, the prevalence rate of *C. trachomatis* in pregnant women was (47/385) - 12.2% which is similar to another African study population in Tanzania, with a prevalence rate of 12.5% [23]. Previous studies revealed global variations in the prevalence of *C. trachomatis* among pregnant women. In a study conducted in southern Iran, the data revealed that 15.5% of pregnant women were infected with C. *trachomatis* [24] and this is higher than the prevalence of the current study. According to studies conducted in 2021, the estimated prevalence of *C. trachomatis* infection amongst pregnant women in the USA (7.4%) [25] and Guangdong; China (6.7%) [26] was lower than the prevalence of the current study. The current South African study correlates with the estimated global prevalence in Africa, which is between 5.2%-18.6% [27]. The current study is also within the global prevalence ranges in Asia (9%-14.9%), Oceania (10.9%-30.6%) [27] and Europe (1.6%-16.4%) [27, 28].

According to the findings of the current study, the median age of *C. trachomatis* infected pregnant women was 26 years of age. A study conducted in Australia reported young pregnant women ranging from 16-25 years old to be infected with *C. trachomatis* [29]. Majority of studies have shown that *C. trachomatis* infection was found predominantly reported in pregnant women up to 25 years old [30-33]. However, a study conducted in Tanzania showed that pregnant women

infected with *C. trachomatis* had a median age of 28 years old [23]. These findings show that young, sexually active women are at a high risk of acquiring *C. trachomatis*. Furthermore, these findings suggest the need for targeted testing of young, sexually active women in the development of *C. trachomatis* prevention strategies.

The factors associated with *C. trachomatis* infection in the current study were low levels of education, being unemployed, not being married, not cohabiting, having first sex at a young age, having numerous lifetime partners, not knowing whether their partner has other partners, not using condoms, not showing symptoms of STIs, and not having received STI treatment in the past. In a study conducted in Brazil, the main risk factor for *C. trachomatis* infection among women was having many lifetime partners, with a prevalence of 44.4% [34]. The current study correlates with other studies showing greater infection rates of *C. trachomatis* in unmarried women and women who have multiple partners [30, 32, 35]. A protective factor against engaging in high-risk sexual behaviour and STI diagnosis has been education [36, 37]. Studies have demonstrated that the poorest and least educated individuals in developing countries, are those with the highest prevalence of *C. trachomatis* infection, and our results are consistent with these findings [27, 30, 38].

The correlation between low income, lack of education and positive chlamydia infection, may be explained by inadequate health literacy and inability to pay for healthcare or screening due to financial restrictions. Women visiting antenatal clinics may not have previously sought treatment for *C. trachomatis* due to socioeconomic factors, and they may be receiving care for the first time at those clinics for health issues [39]. In the current study, the majority of the women (66.0%) did not report having abnormal vaginal discharge and were therefore asymptomatic for *C. trachomatis* infection. This is consistent with research from South Africa and international studies suggesting that the actual incidence of *C. trachomatis* infections is much higher since many infections are asymptomatic and go unreported [2, 3].

In the current study, the majority of the women who tested positive for *C. trachomatis* did not receive STI treatment in the past. Pregnant women who receive treatment have a lower risk of passing on *C. trachomatis* to their new-borns at birth. Furthermore, treating their sex partners prevents reinfection and infection of other partners. A study conducted in Guangdong; China reported a prevalence of 85% for asymptomatic *C. trachomatis* infections [26] and this is consistent with the findings of the current study which showed a high prevalence of asymptomatic *C. trachomatis* infections. In the current study, having a circumcised partner and having a regular sex partner were associated with testing positive for *C. trachomatis*. This correlates with a systematic review conducted by Morris et al. (2019), reporting that partner medical circumcision

reduces a women's risk for *Trichomonas vaginalis* infection, however, this is not always the case for *C. trachomatis* [40].

The prevalence of *C. trachomatis* serovars has been determined for several countries, however, this data is lacking for South Africa. The *Omp1* genotyping is more accurate and sensitive than immunotyping in revealing *C. trachomatis* variations within serovars as well as in possible recombinants between serovars [41-43]. Urogenital infections are mostly caused by serovars D to K. Of these, serovars E, F and D are responsible for up to 60%-70% of these infections [44-47]. In this study, five distinct serovars of *C. trachomatis* were identified by digestion of the *omp1*. By digesting with *AluI* alone, the clinical samples were distinguished. However, digestion with *DdeI* and *HinfI* were also carried out to help with further *C. trachomatis* serovar differentiation in this study. Serovar E (46.5%) was the most prevalent serovar in the study population, followed by serovars F (20.9%), G (14.0%), D (11.6%) and I (4.7%).

This study correlates with studies conducted in other countries showing serovars E and F as the dominant types [48-50]. In a study conducted in Brazil that included 141 women, the most frequent serovar detected was E (39.7%), followed by F (17.7%) [51]. Serovar E was also the most prevalent (31%), followed by F (21%), in a Costa Rican study that included 806 *C. trachomatis*-positive samples [52]. Serovar F was most prevalent (54.2%) in a previous study conducted in Mexico [15]. In addition, both of the above-mentioned studies, showed serovar D as the third most common serovar [51, 52]. This differs from the current study which showed serovar G as the third most common serovar in the studied population. Another study detected serovar F as the more frequent type in pregnant women [53]. Finally, a study conducted in India showed serovar D (48%) as the most prevalent serovar, followed by serovars E (34%), F (12%) and I (6%) in the urogenital samples [14]. Sociodemographic characteristics, the various sensitivity levels of the diagnostic techniques utilized, and specimen types collected could all be used to explain the differences in serovars amongst pregnant women throughout the globe [54, 55].

Potential correlations between *C. trachomatis* serovars and certain clinical factors have not been fully described amongst South African pregnant women. In the current study, 20% of pregnant women who harboured serovar E showed symptoms of abnormal vaginal discharge. This study correlates with another study conducted in Mexico which showed that serovar E was associated with green abnormal vaginal discharge [56]. The current study showed that 11.1% of pregnant women who harboured serovar F had symptoms of abnormal vaginal discharge. Another study revealed that serovar F has been associated with reduced mucopurulent endocervical discharge

[53]. In a previous study, *C. trachomatis* serovar K was associated with abnormal vaginal discharge [57].

Conclusion

In this study, *C. trachomatis* was present in vaginal samples from pregnant women in South Africa at a prevalence rate of 12.2%. Low levels of education, being unemployed, not being married, not cohabiting, having first sex at a young age, having numerous lifetime partners, not knowing whether their partner has other partners, not using condoms, not showing symptoms of STIs, and not having received STI treatment in the past were factors significantly associated with *C. trachomatis* infection. These factors demonstrate the value of prenatal clinics in screening women for vaginal infections during routine check-ups and providing ongoing risk reduction counselling to this high-risk population.

In this study, five different serovars of *C. trachomatis* were identified, with serovars E and F being the most prevalent. This study also provided evidence for the genetic diversity of *C. trachomatis*. This study demonstrated that genotyping using uncultured vaginal swabs is possible. For upcoming chlamydia vaccine design and development, the documented diversity of serovars and the many clinical variables described within certain populations can be challenging. However, several of the prevalent serovars identified in South Africa correlated with serovars identified in other parts of the world. Given the possibility of conserved *C. trachomatis* strains from various geographic locations, this offers some promise and may offer some encouragement for future vaccine development and diagnostic research that focuses on the entire *C. trachomatis* population. Future research resulting from this study should examine the relationship between this pathogen's serovars and patterns of drug susceptibility to azithromycin. The distribution of the serovars in pregnant and non-pregnant populations should also be compared, and the serovars should be further investigated in relation to the presence or absence of clinical symptoms, perhaps by investigating the virulence genes in these particular serovars.

Limitations of the study:

• Participants in the study self-reported their clinical symptoms, which could have introduced biases in the reporting process.

• This study was cross-sectional, therefore, the effects of infection on pregnancy and neonatal outcomes could not be determined.

• Participants in the study were chosen from one hospital, the King Edward VIII hospital and is not representative of the Durban population. For future studies on genotyping in Durban or KwaZulu-Natal, a wider population will need to be sampled from different clinics or hospitals.

• Lastly, since the study only analysed samples from pregnant women, it is not a representation of the overall population.

Strengths of the study:

• This study addresses a gap in the literature about the prevalence and the risk factors associated with *C. trachomatis* infection amongst pregnant South African women.

• The study also provides data on the serovars present in our study population, an under-studied area in South Africa.

REFERENCES

1. Azami M, Badfar G, Mansouri A et al. Prevalence of *Chlamydia trachomatis* in pregnant Iranian women: a systematic review and meta-analysis. *International Journal of Fertility & Sterility* 2018; 12(3):191-199. doi: 10.22074/ijfs.2018.5191

2. Balle C, Lennard K, Dabee S et al. Endocervical and vaginal microbiota in South African adolescents with asymptomatic *Chlamydia trachomatis* infection. *Scientific Reports* 2018; 8(1):11109. doi: 10.1038/s41598-018-29320-x

3. Huai P, Li F, Chu T et al. Prevalence of genital *Chlamydia trachomatis* infection in the general population: a meta-analysis. *BMC Infectious Diseases* 2020; 20, 589. doi: 10.1186/s12879-020-05307-w

4. Joseph Davey D.L, Nyemba D.C, Gomba Y et al. Prevalence and correlates of sexually transmitted infections in pregnancy in HIV-infected and- uninfected women in Cape Town, South Africa. *PLoS One* 2019; 14(7):e0218349. doi: 10.1371/journal.pone.0218349

5. Keegan M.B, Diedrich J.T & Peipert J.F. *Chlamydia trachomatis* infection: Screening and management. *Journal of Clinical Outcomes Management* 2014; 21:30-8. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4279217/

6. Mozzato C.N, Mahdi O.S, Jallow O et al. Polymorphisms in candidate genes and risk of scarring trachoma in a *Chlamydia trachomatis*-endemic population. *The Journal of Infectious Diseases* 2000; 182(5):1545-1548. doi: 10.1086/315891

7. Tokuriki N, Stricher F, Serrano L et al. How protein stability and new functions trade off. *PLoS Computational Biology* 2008; 4(2):e1000002. doi: 10.1371/journal.pcbi.1000002

8. Didelot X & Maiden MC. Impact of recombination on bacterial evolution. *Trends in Microbiology* 2010; 18(7):315-322. doi: 10.1016/j.tim.2010.04.002

9. Clarke I.N. Evolution of *Chlamydia trachomatis*. *Annals of the New York Academy of Sciences* 2011; 1230(1), E11-E18. doi: 10.1111/j.1749-6632.2011.06194.x

10. Rogstad K. Complications in the female and their management. *International handbook of Chlamydia* 2008; 3:111-121.

11. Peipert J.F. 2003. Clinical practice: genital chlamydial infections. *The New England Journal of Medicine* 2003; 349:2424-2430. doi: 10.1056/nejmcp030542

12. Hammerschlag M.R. *Chlamydia trachomatis* in children. *Pediatric Annals* 1994; 23:349-353. doi: 10.3928/0090-4481-19940701-08

13. Stenberg K & Mardh P.A. Persistent neonatal chlamydial infection in a 6-year-old girl. *Lancet* 1986; 2:1278-1279. doi: 10.1016/s0140-6736(86)92702-9

14. Singh V, Salhan S, Das B.C et al. Predominance of *Chlamydia trachomatis* serovars associated with urogenital infections in females in New Delhi, India. *Journal of Clinical Microbiology* 2003; 41(6):2700-2702. doi: 10.1128/jcm.41.6.2700-2702.2003

15. Cruz M.J.D.H, Rodriguez I.D, Guerra M.R.E et al. Genotyping of *Chlamydia trachomatis* from endocervical specimens of infertile Mexican women. *Infectious Diseases and Clinical Microbiology* 2011; 29(2):100-108. doi: 10.1016/j.eimc.2010.08.014

16. Freitas A.C, Chaban B, Bocking A et al. The vaginal microbiome of pregnant women is less rich and diverse, with lower prevalence of Mollicutes, compared to non-pregnant women. *Scientific Reports* 2017; 7:1-16.

17. Mabaso N, Ngobese B, Tinarwo P et al. Prevalence of *Chlamydia trachomatis* infection in pregnant women from Durban, South Africa. *International Journal of STD & AIDS* 2022; 33(10):920-927. doi: 10.1177/09564624221118721

18. Kiguen A.X, Marrama M, Ruiz S et al. Prevalence, risk factors and molecular characterization of *Chlamydia trachomatis* in pregnant women from Córdoba, Argentina: A prospective study. *PLoS One* 2019; 14:e0217245. doi: 10.1371/journal.pone.0217245

19. Mascellino M.T, Boccia P & Oliva A. Immunopathogenesis in *Chlamydia trachomatis* infected women. *ISRN Obstetrics and Gynecol*ogy 2011; 436936:1-9. doi: 10.5402/2011/436936

20. Andrews W.W, Goldenberg R.L, Mercer B et al. The Preterm Prediction Study: association of second-trimester genitourinary chlamydia infection with subsequent spontaneous preterm birth. *American Journal of Obstetrics and Gynecology* 2000; 183:662-668. doi: 10.1067/mob.2000.106556

21. Hussen S, Wachamo D, Yohannes Z et al. Prevalence of *Chlamydia trachomatis* infection among reproductive age women in Sub-Saharan Africa: a systematic review and meta-analysis. *BMC Infectious Diseases* 2018; 18:1-8. doi: 10.1186/s12879-018-3477-y

22. Ngobese B & Abbai N.S. Sexually transmitted infections in pregnant women from Sub-Saharan Africa. *Southern African Journal of Infectious Diseases* 2021; 36(1). doi: 10.4102/sajid.v36i1.312

23. Juliana N.C.A, Deb S, Ouburg S et al. The prevalence of *Chlamydia trachomatis* and three other non-viral sexually transmitted infections among pregnant women in Pemba island Tanzania. *Pathogens* 2020; 9(8):625. doi: 10.3390/pathogens9080625

24. Pourabbas B, Rezaei Z, Mardaneh J et al. Prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections among pregnant women and eye colonization of their neonates at birth time, Shiraz, Southern Iran. *BMC Infectious Diseases* 2018; 477. doi: 10.1186/s12879-018-3382-4

25. Simons J.L, McKenzie J.S, Wright N.C et al. Chlamydia prevalence by age and correlates of infection among pregnant women. *Sexually Transmitted Diseases* 2021; 48:37-41. doi: 10.1097/olq.0000000000001261

26. Li C, Tang W, Ho H.C et al. Prevalence of *Chlamydia trachomatis* among pregnant women, gynecology clinic attendees, and sub-fertile women in Guangdong, China: A cross-sectional survey. *Open Forum Infectious Diseases* 2021; 8(6). doi: 10.1093/ofid/ofab206

27. Newman L, Rowley J, Vander H.S et al. Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. *PLoS One* 2015; 10:e0143304. doi: 10.1371/journal.pone.0143304

28. Goulet V, de Barbeyrac B, Raherison S et al. Prevalence of *Chlamydia trachomatis*: results from the first national population-based survey in France. *Sexually Transmitted Infections* 2010; 86(4):263-270. doi: 10.1136/sti.2009.038752

29. Ong J.J, Chen M, Hocking J et al. Chlamydia screening for pregnant women aged 16-25 years attending an antenatal service: a cost-effectiveness study. *BJOG: An International Journal of Obstetrics and Gynaecology* 2016; 123:1194-1202. doi: 10.1111/1471-0528.13567

30. Pinto V.M, Szwarcwald C.L, Baroni C et al. *C. trachomatis* prevalence and risk behaviours in parturient women aged 15 to 24 in Brazil. *Sexually Transmitted Diseases* 2011; 38:957-961. doi: 10.1097/OLQ.0b013e31822037fc

31. Silveira M.F, Sclowitz I.K, Entiauspe L.G et al. *Chlamydia trachomatis* infection in young pregnant women in Southern Brazil: a cross-sectional study. *Cadernos de Saude Publica* 2017; 33:e00067415. doi: 10.1590/0102-311X00067415

32. Schmidt R, Muniz R.R, Cola E et al. Maternal *Chlamydia trachomatis* infections and preterm births in a university hospital in Vitoria, Brazil. *Plos One* 2015; 10:e0141367. doi: 10.1371/journal.pone.0141367

33. Jalil E.M, Pinto V.M, Benzaken A.S et al. Prevalence of chlamydia and gonococcus infection in pregnant women in six Brazilian cities. *Brazilian Journal of Gynecology and Obstetrics* 2008; 30:614-619. doi: 10.1590/S0100-72032008001200005

34. Azevedo M.J.N, Nunes S.S, Oliveira F.G et al. High prevalence of *Chlamydia trachomatis* in pregnant women attended at Primary Health Care services in Amazon, Brazil. *Revista Do Instituto de Medicina Tropical de São Paulo* 2019; 61:e6. doi: 10.1590/s1678-9946201961006

35. Rocha D.A, Filho R.A, Mariño J.M et al. Hidden sexually transmitted infections among women in primary care health services, Amazonas, Brazil. *International Journal of STD and Aids* 2014; 25:878-886. doi: 10.1177/0956462414523742

36. Annang L, Walsemann K.M, Maitra D et al. Does education matter? Examining racial differences in the association between education and STI diagnosis among black and white young adult females in the U.S. *Public Health Reports* 2010; 125:110-121. doi: 10.1177/00333549101250S415

37. Halpern C.T, Joyner K, Udry J.R et al. Smart teens don't have sex (or kiss much either). *The Journal of Adolescent Health* 2000; 26:213-225. doi: 10.1016/S1054-139X(99)00061-0

38. Santos L.M, Souza I.R, Holanda L.H et al. High incidence of urogenital infection by *Chlamydia trachomatis* in parturient women from Belém, Pará State, Brazil. *Pan-Amazonian Health Magazine* 2016; 7:101-106. doi: 10.5123/s2176-62232016000400012

39. Garba B.F, Abdulsalami M.I & Egbe N.E. Seroprevalence of *Chlamydia trachomatis* infection among pregnant women attending antenatal clinics within Kaduna metropolis, North-Central, Nigeria. *Journal of Public Health and Epidemiology* 2018; 10(9):320-325. doi: 10.5897/JPHE2018.1036

40. Morris B.J, Hankins C.A, Banerjee J et al. Does male circumcision reduce women's risk of sexually transmitted infections, cervical cancer, and associated conditions? *Frontiers in Public Health* 2019; 7, 4. doi: 10.3389/fpubh.2019.00004.

41. Ossewaarde J.M, Rieffe M, Rozenberg A.M et al. Development and clinical evaluation of a polymerase chain reaction test for detection of *Chlamydia trachomatis*. *Journal of Clinical Microbiology* 1992; 30:2122-2128. doi: 10.1128/jcm.30.8.2122-2128.1992

42. Sayada C, Denamur E, Orfila J et al. Rapid genotyping of the *C. trachomatis* major outer membrane protein by the PCR. *FEMS Microbiology Letters* 1991; 83:73-78. doi: 10.1016/0378-1097(91)90447-i

43. Stephens R.S, Wagar E.A & Schoolnik G.K. High resolution mapping of serovar-specific and common antigenic determinants of the major outer membrane protein of *Chlamydia trachomatis.* *Journal of Experimental Medicine* 1988; 167:817-831. doi: 10.1084/jem.167.3.817

44. Bandea C.I, Kubota K, Brown T.M et al. Typing of *Chlamydia trachomatis* strains from urine samples by amplification and sequencing the major outer membrane protein gene (*omp 1*). *Sexually Transmitted Infections* 2001; 77:419-422. doi: 10.1136/sti.77.6.419

45. Gaydos C.A, Bobo L, Welsh E.W.L. Gene typing of *Chlamydia trachomatis* by polymerase chain reaction and restriction endonuclease digestion. *Sexually Transmitted Diseases* 1992; 19:303-308. URL: http://www.jstor.org/stable/44971152.

46. Rodriguez P, de Barbeyrac B.K, Persson B et al. Evaluation of molecular typing for epidemiology study of *Chlamydia trachomatis* genital infections. *Journal of Clinical Microbiology* 1993; 31:2238-2240. doi: 10.1128/jcm.31.8.2238-2240.1993

47. Wagenvoort J.H.T, Suchland R.J & Stamm W.E et al. Serovar distribution of urogenital *C. trachomatis* strains in the Netherlands. *Genitourinary Medicine* 1988; 64:159-161. doi: 10.1136/sti.64.3.159

48. Morr S.A, Ossewaarde J.M, Savelkoul P.H et al. Analysis of genetic heterogeneity in *Chlamydia trachomatis* clinical isolates of serovars D, E and F by amplified fragment length polymorphism. *Journal of Clinical Microbiology* 2000; 38:3463-3466. doi: 10.1128/jcm.38.9.3463-3466.2000

49. Gao X, Chen X.S, Yin Y.P et al. Distribution study of *Chlamydia trachomatis* serovars among high-risk women in China performed using PCR-restriction fragment length polymorphism genotyping. *Journal of Clinical Microbiology* 2007; 45:1185-1189. doi: 10.1128/JCM.02076-06

50. Geisler W.M, Suchland R.J, Stamm W.E. Association of *Chlamydia trachomatis* serovar Ia infection with black race in a sexually transmitted diseases clinic patient population in Birmingham, Alabama. *Sexually Transmitted Diseases* 2006; 33:621-624. doi: 10.1097/01.olq.0000216027.71144.74

51. Machado A.C, Bandea C.I, Alves M.F et al. Distribution of *Chlamydia trachomatis* genovars among youths and adults in Brazil. *Journal of Medical Microbiology* 2011; 60:472-476. doi: 10.1099/jmm.0.026476-0

52. Quint K, Porras C, Safaeian M et al. Evaluation of a novel PCR-based assay for detection and identification of *Chlamydia trachomatis* serovars in cervical specimens. *Journal of Clinical Microbiology* 2007; 45:3986-3991. doi: 10.1128/JCM.01155-07

53. Workowski K.A, Stevens C.E, Suchland R.J et al. Clinical manifestations of genital infection due to *Chlamydia trachomatis* in women: differences related to serovar. *Clinical Infectious Diseases* 1994; 19(4):756-760. doi: 10.1093/clinids/19.4.756.

54. Romoren M, Sundby J, Velauthapillai M et al. Chlamydia and gonorrhoea in pregnant Batswana women: time to discard the syndromic approach? *BMC Infectious Diseases* 2007; 7:27. doi: 10.1186/1471-2334-7-27

55. Harrison H.R, Alexander E.R, Weinstein L et al. Cervical *Chlamydia trachomatis* and mycoplasmal infections in pregnancy. Epidemiology and outcomes. *The Journal of the American Medical Association* 1983; 250(13):1721-1727.

56. Casillas V.N, Morfín O.R, García S et al. Frequency and genotypes of *Chlamydia trachomatis* in patients attending the obstetrics and gynaecology clinics in Jalisco, Mexico, and correlation with sociodemographic, behavioural, and biological factors. *BMC Women's Health* 2017; 17(1): 83. doi: 10.1186/s12905-017-0428-5

57. Morr S.A, Rozendaal L, van Valkengoed I.G et al. Urogenital *Chlamydia trachomatis* serovars in men and women with a symptomatic or asymptomatic infection: an association with clinical manifestations? *Journal of Clinical Microbiology* 2000; 38:2292-2296. doi: 10.1128/jcm.38.6.2292-2296.2000

APPENDICES

APPENDIX A: Protocol approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

COLLEGE OF HEALTH SCIENCES

13 May 2022

Prof NS Abbai
School of Clinical Medicine

Dear Prof Abbai

MMEDSCI PROTOCOL: "Genotyping of Chlamydia trachomatis detected in South African pregnant women"
Student: Ms C Ramnarain **Student Number:** 217003421 **(Department of Medicine)**

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 Ms C Ramnarain
Dr NG Mabaso

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 **Facsimile:** +27 (0) 31 260 4723 **Email:** konar@ukzn.ac.za **Website:** www.ukzn.ac.za

APPENDIX B: Biomedical Research Ethics Committee approval (UKZN)

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

28 June 2022

Miss Caitlin Ramnarain (217003421)
School of Clinical Medicine
Medical School

Dear Miss Ramnarain,

Protocol reference number: BREC/00004226/2022
Project title: Genotyping of Chlamydia trachomatis detected in South African pregnant women
Degree: MMedSc

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 28 June 2022. Please ensure that any outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is valid for one year from **28 June 2022**. 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 12 July 2022.

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

APPENDIX C: Informed consent form

INFORMED CONSENT DOCUMENT

Investigating the burden of sexually transmitted infections in HIV infected pregnant women from Durban KwaZulu-Natal

Version 1.0

PRINCIPAL INVESTIGATOR: Ms. Bongekile Ngobese
PHONE: 031 260 4439

INFORMED CONSENT

You are being invited to take part in a study called: **Investigating the burden of sexually transmitted infections in HIV infected pregnant women from Durban KwaZulu-Natal.** My name is Bongekile Ngobese, I am a Doctoral student from the University of KwaZulu-Natal and this is my research study. This study is for HIV positive pregnant women, 18 years and older. Approximately 385 women will be in this study. Before you decide if you want to join this study, we want you to learn about the study. I will talk with you about the study and answer your questions. You may decide not to join or to leave the study at any time.

YOUR PARTICIPATION IS VOLUNTARY

This consent form gives information about the study procedures that will be discussed with you. Your participation is voluntary; you do not have to have the procedures if you do not want to participate in this study. Once you understand the study tests, and if you agree to take part, you will be asked to sign your name on this form.

PURPOSE OF THE STUDY

HIV positive pregnant women have higher number of sexually transmitted infections (STIs) compared to HIV negative pregnant women. HIV positive pregnant women can transmit the STIs to their partners and new-borns. In this study, we want to test you to see if you have any of these infections. We will also like you to answer a few quick questions about yourself. You can fill in the answers to these questions on your own. We will just like to know if you understand the risks of STIs. This study will not provide any treatment. When you see the doctor and if you have any of the symptoms that we will be telling you about, please let the doctor know so that she/he can treat you.
For this study, you will need to provide 3 vaginal swab samples. You can collect the samples by yourself. We will tell you how to properly collect the samples.
Once the study is over, your samples will be stored and used to test for other infections that may be passed through sexual activity. Your samples will not be used for any commercial use that makes any money. We will not send your samples to any other lab for testing.
If you agree to have your samples used for testing in another study please indicate below:

I agree to have my samples stored and used for future testing

Yes ☐ No ☐

_____

Participant Name (print) | Participant Signature | Date

WHAT DO I HAVE TO DO IF I DECIDE TO TAKE PART IN THE STUDY?

If you decide to be in this study, we will be able to start the procedures today. Today's study procedures will take approximately **15-20 mins**.
You will be asked to:

- Confirm you are able to join the study and that you understand the study requirements.
- You will be asked questions about your yourself and medical history
- You will be asked to provide 3 vaginal swabs.
- You will be asked to complete a 5 min questionnaire about yourself

RISKS AND/OR DISCOMFORTS

Risks of sample collection: You may feel discomfort during the swab sample collection. We will ensure that we give you proper instructions on how to collect the swabs.

Other Possible Risks: You may become embarrassed or worried when discussing your sexual behaviour. We will make every effort to make you feel comfortable and protect your privacy and confidentiality whilst you are part of this study. Your visits will take place in private.

CONFIDENTIALITY

We will keep your information confidential. Your personal information may 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.

PROBLEMS OR QUESTIONS

The Biomedical Research Ethics Committee of the University of KwaZulu-Natal has approved this study.

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

SIGNATURES

If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to participate, please sign your name or make your mark below.

_____	_____	_____
Participant Name (print)	Participant Signature	Date
_____	_____	_____
Study Staff Conducting Consent Discussion (print)	Study Staff Signature	Date
_____	_____	_____
*Witness Name (print)	*Witness Signature	*Date

* Witness name, signature and date are required on this consent form only when the consenting participant is not able to read (illiterate)

APPENDIX D: Journal guidelines

Submission Preparation Checklist

As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.

- ✔ The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in Comments to the Editor).
- ✔ The submission file is in Microsoft Word document file format.
- ✔ Where available, URLs for the references have been provided.
- ✔ The text is single-spaced; uses a 12-point font; employs italics, rather than underlining (except with URL addresses); and all illustrations, figures, and tables are placed within the text at the appropriate points, rather than at the end. Illustrations, figures and tables should also be submitted as supplementary files.
- ✔ The text adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines.

Author Guidelines

ABOUT THE JOURNAL

Publication frequency: The Journal of Medical Laboratory Science and Technology of South Africa (*JMLSTSA*) is published three times annually..

Open access policy – *JMLSTSA* provides immediate open access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge.

Abbreviation – The correct abbreviation for abstracting and indexing purposes is *JMLSTSA.*

ISSN – The international standard serial number (ISSN) for *The Journal of Medical Laboratory Science and Technology of South Africa is* ISSN 2664-2549

SUBMISSION POLICIES

Original research articles, review articles, technical reports, book reviews, case reports and letters to the editor are invited and should be submitted online at https://jmlstsa.smltsa.org.za/index.php/JMLSTSA.

Manuscripts are submitted on the understanding that they have not and will not be published elsewhere. The Editor retains the right, to modify the style and length of the submitted manuscript and to decide the time of publication. The *JMLSTSA* publishes all material on the understanding that the design of the work has been approved by a relevant ethics committee and/or it conforms to the professional standards and legislation currently applied in the country of origin.

Authors are requested to read the following guidelines carefully and to adhere to the requirements herein. Failure to comply may lead to delay in publication. Copyright on all published material belongs to the Society of Medical Laboratory Technology of South Africa (SMLTSA). It is understood that all proprietary rights other than copyright are reserved to the authors, as well as the right to reproduce original figures and tables from this item in their future works, provided full credit is given to the original publication Journal of medical Science and Technology of South Africa ISSN 2664-2549.

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ETHICS POLICY

Manuscripts reporting on research that involve human participants or animals must include a statement confirming that the investigations were carried out according to internationally recognised standards which include **Declaration of Helsinki**; **US Federal Policy for the Protection of Human Subjects**; or **European Medicines Agency Guidelines for Good Clinical Practice**.

All articles published in the JMLSTSA must have been approved by a recognised institutional ethics committee and the approval number should be included in the text. In studies which have recruited human participants, informed consent must have been obtained, and this must be stated in the manuscript. Confidentiality and anonymity of participants must be maintained and a brief description of how this was achieved must be included

CONTENT TYPES

An original research article

The article should be no longer than 4000 words and the layout should follow the following order: title page, abstract and keywords, main text, discussion and conclusion, acknowledgements and references. Ethics committee approval must be included in the methods section of the article.

The title page should include a short title, authors' names including initials, qualifications, address, telephone and the email address of the corresponding author to whom the proofs and requests would be directed. The title should not contain any abbreviations. The title page should also include, the ORCID ID of each author, and a declaration of conflict of interest and sources of funding.

The abstract, comprising a summary of not more than 250 words, should highlight the design and objective of the investigation/research, results and conclusions. The abstract should be followed by a list of not more than five (5) keywords, which should not appear in the main title.

Review articles

Suitable review articles may be submitted to the Editor for consideration. Articles should be no longer than 5000 words, contain no more than 60 references, six figures/tables and include a non-structured summary of up to 250 words.

Case Reports –

Case reports should consist of a title page, a short abstract and keywords, the case report and a brief discussion of the relevant literature. Case reports should not exceed 2000 words with not more than ten (10) references. Figures and images must be included in the text.

Technical Reports –

The title page should include details of author/s as above. The text should include an introduction, main points in the report, concluding remarks, references, and acknowledgements. Not more than two images/diagrams to be included in the report.

Correspondence –

Letters to The Editor intended for publication are encouraged, particularly letters referring to a paper already published and correspondence on pertinent original topics. The style of references is the same as manuscripts. Figures should be included only when absolutely necessary. Anonymous letters will not be accepted without the name and address of the author, however this will remain confidential and will not be published.

Book Reviews –

Book reviews relevant to the profession of medical laboratory science and technology, including education, are encouraged. Reviews intended for publication should be in the following sequence: reviewer's name (with initials, affiliations, departmental address, telephone and, e-mail address), title of book, edition, publisher, place, date, ISBN number, price of the book (if available) and total number of pages. The review should clearly cover the main theme of the book, mentioning the layout, quality of print and its suitability for medical laboratory technologists /scientists and other medical laboratory professionals.

REFERENCES

References cited in the text should be given as superscript numbers in order of citation (e.g. [1] or [4-7]. References cited more than once should retain the first number throughout the text.

References should be listed in numerical order at the end of the article using the following format:

1. Moreno I, Martin G, Bolufer P et al. Incidence and prognostic value of FLT3 internal tandem duplication and D835mutations in acute myeloid leukaemia. *Haematologica* 2003; 88(1):19-24.

2. World Health Organisation, 2005. URL: http://www.who.int/mediacentre/factsheets/fs297/en/index.html

3. Schnittger S, Schoch C, Kern W, Hiddemann W, Haferlach T. FLT3 length mutations as marker for follow-up studies in acute myeloid leukaemia. *Acta Haematol*. 2004; 112(1-2):68–78. doi:10.1159/000077561

The doi numbers if available, as cited in example 3, must be included at the end of each reference.

It is the responsibility of the author(s) to ensure that references quoted in the text are included in the list of references and vice versa. The accuracy of the references is the responsibility of the author(s).

FIGURES, TABLES AND ILLUSTRATIONS

Contributors are responsible for obtaining permission to publish any figures or illustrations that are protected by copyright, including figures published elsewhere and pictures taken by professional

photographers. *Images* cannot be published if downloaded from the Internet without appropriate permission. All images downloaded from the Internet must conform to size and resolution specifications.

Tables –

Each table must be numbered with the legend placed above the table. Column headings should be brief with units of measurements in parenthesis.

PREPARING THE MANUSCRIPT FOR ONLINE SUBMISSION

Papers must be in English and spelling should conform to South African or United Kingdom English. Material for potential publication must be submitted in MS WORD format and, tables, graphs and illustrations/images must be included in the document.

When quoting specific materials or drugs, authors should give their approved names with proprietary names in parenthesis and the name of the manufacturer. Abbreviations should be unambiguous and the full name/words written out when they are first introduced. Measurements of height, length, mass (weight) and volume should be given in metric units (metre, kilogram, litre) or their decimal multiples. Temperatures should be given in degrees Celsius and blood pressure in mmHg. Other measurements including laboratory measurements should be reported in the metric system in terms of the International System of Units (SI).

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All manuscripts will undergo a similarity check before being accepted for publication.

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Manuscripts should be sent to us via our online submission system https://jmlstsa.smltsa.org.za/index.php/JMLSTSA. Registration and logins are required to submit items online and to check the status of current submissions. Once registered, the system will guide authors through the easy submission process.

Authors can upload manuscript files and check on the status of manuscripts during the review process.

The manuscript should be submitted fully blinded, i.e. with no identifying information.

The title page with all the author information should be uploaded as a supplementary file with the following information included:

Authors' details, including qualifications, department, current place of work, email addresses and ORCID IDs of ALL authors as well as the declarations – conflict of interest statement, funding source, ethics committee approval information.