Ethinylestradiol (EE2) and Human Reproductive Health: Investigating the Potential Effects of Contraceptives on Human Reproductive Health and Sexually Transmitted Diseases in the Era of HIV and AIDS

BY

Manavhela Murendeni

(11606607)

A Thesis Submitted in Fulfillment of the Requirements for Doctor of Philosophy in Microbiology (PhD)

To The

Department of Biochemistry and Microbiology

The Faculty of Science, Engineering and Agriculture

University of Venda

Private Bag X5050

Thohoyandou

0950

South Africa

Promoter: Prof A. Samie Co-promoter: Prof N.A Traore

March, 2025

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Declaration

I, Manavhela Murendeni, hereby declare that the thesis titled: **Ethinylestradiol (EE2) and Human Reproductive health: Investigating the Potential Effects of Contraceptives on Human Reproductive Health and Sexually Transmitted Diseases in the Era of HIV and AIDS**, hereby submitted by me, has not been submitted previously for a degree at this or any other university, and that this is my own work in design and that all the reference therein has been fully acknowledged.

Signature Murendeni Manavhela Date 25 March 2025

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Acknowledgement

First and foremost, I would like to thank God for enabling me to finish my Ph.D.; this was one of the most difficult journeys in my life and God gave me strength through it all. I am short of words for the blessings, and I am sincerely grateful for the gift of life and family throughout this journey.

I would like to express my sincere gratitude to my supervisor Professor Amidou Samie for his invaluable guidance and support throughout my Ph.D. journey. His knowledge and experience have really been instrumental in the completion of my thesis.

I am also thankful to my co-supervisor Professor Afsatou Ndama Traore for the support and insightful suggestions throughout my Ph.D.

In addition, I am grateful to my fellow-students in the parasitology laboratory, with special thanks to Innocent Moagi and Murunwa Nemaguvhuni. I am very thankful to you guys because without you, this journey would have been an extremely difficult one, thank you very much for helping to make this easier for me.

My appreciation also goes to the National research foundation for the scholarship, without the financial assistance I wouldn't have been able to complete my Ph.D. work.

I am extremely grateful to my husband for pushing me and encouraging me all the way.

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Dedication

I would like to dedicate this Ph.D. to my family.

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Papers emanating from the study

i. A Community-based Survey of the Knowledge, Attitude and Practice of Reproductive Age Women Concerning Contraceptives and their Usage in the Vhembe District of Northern South Africa. *Submitted to Healthcare Journal*.

ii. Detection of *Chlamydia trachomatis, Neisseria gonorrhea and Trichomonas vaginalis* causing non-viral STIs, using non-invasive specimen. *Intended journal (BMC Infectious Diseases).*

iii. Urine metabolomics: A snapshot into metabolic variations and biomarker identification across contraceptive methods.

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Abbreviations

%	Percent
^{0}C	degree Celsius
AIDS	Acquired Immunodeficiency Syndrome
AMR	Antimicrobial Resistance
AUC	Area under plasma concentration
BSA	Bovine Serum Albumin
C. trachomatis	*Chlamydia trachomatis*
CDC	Centers for Disease Control and prevention
CHC	Community Health Centre
cm	Centimeters
Cmax	Plasma concentration
CPA	Cyproterone Acetate
CPY	Cytochrome
CSA	Casual Sex Abroad
CVD	Cardiovascular Disease
DMPA	Depot-medroxy progesterone acetate
DNA	Deoxyribonucleic Acid
DVT	Deep Vein Thrombosis

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EB	Elementary body
EE2	Ethinylestradiol
ELISA	Enzyme-linked Immunoassay
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
g/mol	Grams per mole
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
HSV2	Herpes Simplex Virus 2
ICPD	International Conference of Population & Development
IUD	Intra Uterine Device
KAP	Knowledge, attitude and practices
LARC	Long-Acting Reversible Contraceptives
LGV	Lymphogranuloma venereum
LH	Luteinizing Hormone
LOS	Lipo-oligosaccharide
mg	Milligram
mg/L	Milligram per Liter

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mg/L	Milligrams per liter
ml	Milliliter
N. gonorrhoea	*Neisseria gonorrhoea*
NAATs	Nucleic Acid Amplification Tests
ng	Nanogram
ng/ml	Nanogram per milliliter
NHS	National Health Services
NIH	National Institute of Health
PCR	Polymerase Chain Reaction
pg	Picograms
pg/ml	Pictograms per milliliter
PK	Proteinase K
RB	Reticulate body
rpm	Rounds per minute
SHBG	Sex Hormone Binding Globulin
SPSS	Statistical Package for the Social Sciences
STDs	Sexual Transmitted Diseases
STI	Sexual Transmitted Infection
T. vaginalis	*Trichomonas vaginalis*

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TBG	Thyroid Binding Globulin
Tmax	Maximum time
ug	Microgram
ul	Microliter
µm	micrometer
UN	United Nations
US	United States
USPSIF	U.S. Preventative Services Task Force
WHO	World Health Organization

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Abstract

Background: Reproduction and sexual health are fundamental aspects of human life and are influenced by various hormones, including sex steroids. Sex steroids, estrogen in particular have been implicated in numerous human health issues. The association between estrogens and sexually transmitted diseases (STIs) is not clear as studies are contradictory; other studies suggest that estrogens confer protection against STIs and others report that they facilitate the progression of STIs. Because of the extensive use of Ethinylestradiol (EE2) in birth control, it has now become a major global pollutant due to its presence in wastewater, either through household waste, agricultural waste or through industrial wastes. In the aquatic environments where wastewater is discharged, there is a wide variety of organisms that reside there, and in the end they become victims of the impact the hormones ~~has~~ have on them. It's been reported that exposure of aquatic organisms to estrogens has been linked to endocrine disruption, including feminization, and these changes are correlated with EE2 concentrations. While extensive research has been on the ecological consequences of EE2, there remains a gap in understanding the impact of estrogens in human health, particularly synthetic estrogens. Given the widespread environmental distribution of these hormones, further research is necessary to elucidate their potential effects on human reproductive health, particularly in relation to sexually transmitted infection (STIs).

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Aims and Objectives: The main aim of the study was to further our understanding of the potential impacts of birth control and the tools used particularly ethinylestradiol on sexual health and sexually transmitted infections.

A number of objectives were identified and included:

1. To assess the knowledge, ~~attitude~~attitude, and practices of contraceptives amongst women of reproductive age.
2. To determine the prevalence of contraceptive use amongst women of reproductive age.
3. To describe contraception association with sexually transmitted diseases.
4. To identify microorganisms causing sexually transmitted infections among study participants (*Neisseria gonorrhea, Chlamydia trachomatis and Trichomonas vaginalis*).
5. To determine ethinylestradiol concentration in the study population.
6. To assess the potential association between contraceptive use and UTI.
7. Provide insight into urine metabolomics across study participants using different birth control methods.

Methods: A community based cross-sectional study was conducted in Northern Limpopo, in the Vhembe district. A paper-based questionnaire was used to collect data such as demographic data, knowledge, attitude and practice of women of reproductive age towards contraceptives. Urine samples were collected from the study participants using sterile sample collection containers. In the laboratory, urine samples were cultured for UTI determination. Enzyme-linked immunosorbent assay (ELISA) was used to quantify Ethinylestradiol (EE2) concentration from the samples.

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Polymerase Chain Reaction (PCR) was used for detection of three different microorganisms causing non-viral sexually transmitted infections including *Chlamydia trachomatis, Neisseria gonorrhea and Trichomonas vaginalis*. For metabolite extraction, solid phase extraction (SPE) was used to extract metabolites from urine samples using C18-E silica-based cartridges. Collected data was entered and cleaned on excel spread sheet. SPSS software V27 and Stata V12 were used to analyze the data (descriptive statistics was used to estimate frequencies and chi-square test was used for examine associations). Metabolomics data was analyzed using MetaboAnalyst 6.0 online software.

Results: Of the 364 women who participated in the study, 96.11% were aware of what contraceptives are. Participants acquired knowledge through more than one platform, with 41.67% through media, 41.38% via health care workers and 31.61% through social media networks. Participants had knowledge of at least one method of contraception. In practice, 75.1% were using at least one form of contraceptive and the most common methods were injectables (46.1%), male condoms (23.8%), and pills (17.4%). Most participants (96.9%) had a positive attitude towards contraceptives and 94.4% said they think contraceptives are beneficial, while 79.8% agreed that the main benefit is to avoid unwanted pregnancy.

Chlamydia trachomatis was the most common STI with a prevalence of (17.39%), followed by *Neisseria gonorrhea* with a prevalence of (5.43%) and *Trichomonas vaginalis* with a prevalence of (2.72%). Based on the age group, *N. gonorrhea* had the highest rate of (75.0%), followed by *C. trachomatis* with (59.68%) and then *T. vaginalis* with a prevalence rate of (30.0%) in participants who were aged between 20-30 years. Educational status appeared to have an influence on the prevalence of *N. gonorrhea* showing the highest infection rate of (55.0%) in individuals who studied up to tertiary level as well as *C. trachomatis* (53.33%) and *T. vaginalis* (50.0%) in those

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with high school education. Coinfections with *C. trachomatis* and *N. gonorrhea* were observed in (30.0%) of the participants.

Urinary tract infections (UTIs) were detected in 34.2% of the study population. Education did not seem to reduce the impact of UTIs among the study participants. UTIs were statistically significant among patients who had been using contraceptives for a long time and also among patients who were using Intrauterine devices as contraceptive method particularly (p value less than 0.05. UTIS were also common among participants who had experienced miscarriage although the difference was not statistically significant. The presence of blood, nitrate and ketones in the urine samples were significant markers of UTI among the study participants.

The concentration of ethinylestradiol varied across the samples from low concentration of 11pg/ml to the highest concentration of 804 pg/ml. The concentration of EE2 varied with age, marital status and education level. Individuals with higher educational level also seemed to have higher concentration of EE2 in their urine samples. There was a statistically significant association between ethinylestradiol occurrence and urinary tract infection (UTI) with p value of 0.006. However, there was no significant association with the three STDs tested, although more samples positive for the infection tended to be positive for EE2. Individuals who had experienced miscarriage tended to have higher concentrations of EE2 although the difference was not statistically significant.

Metabolomics study~~ies~~ identified a number of compounds in the urine samples. The metabolites identified were mostly associated with lipid metabolism changes, hormonal and estrogenic activity, inflammation and immune responses. Metabolites with neurological impact were also detected. Some of these metabolites included but are not limited to Equol, Pergolide, Leukotriene and Scopamine.

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Conclusion:

We observed a high rate of awareness towards family planning methods. However, there is still a need for improvement in terms of translating the information into practice. Although, women of reproductive age were the primary focus, regular screening for sexually transmitted infections (STIs) should be expanded to other significant groups such as sex workers and men who have sex with men (MSM). Ethinylestradiol (EE2) can be detected in individuals beyond those actively using EE2-containing contraceptives; however, prolonged use of such contraceptives may contribute to elevated levels of EE2 in urine samples over time.~~Ethinylestradiol (EE2) is not only found in individuals who are using ethinylestradiol-containing contraception, however, long term usage of contraceptive might increase the chances of increased EE2 in the urine samples~~. However, there is need for further studies to understand the origins of the synthetic compound as there are external factors contributing to levels of the synthetic compound in the body. Furthermore, variation of urine metabolomics is dependent on individual physiology and could be used as biomarkers for lifestyle factors, drug response and to monitor diseases.

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Chapter 1: Introduction and rationale

1.1 Background

Reproduction and sexual health are an integral part of our lives. According to the National Health Institute (NIH), ~~reproduction~~ reproductive health is the condition of female and male reproduction systems during all stages of life. Sexual health refers to the state of physical, mental, emotional and social well-being in relation to sexuality, it is not solely about the absence of disease, dysfunction or infirmity (WHO, 2006). Women's reproductive system is multi-organ and includes the hypothalamus, pituitary gland, ovaries, uterus, and the vagina (Clarke and Khosla, 2010). These definitions in themselves showcase how important reproductive health is, and anything that concerns it. There are several factors that affect the reproductive health such as sexually transmitted infections as well as chemicals that women consume, for example, contraceptives.

Contraceptives are defined as drugs, chemicals, surgical procedures, sexual practices or devices that are used to intentionally prevent pregnancy (David *et al*., 2022). Contraception gives freedom

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to couples to have physical relationship unafraid of having unplanned or unwanted pregnancy (Jain and Muralidhar, 2012). Since the introduction of contraceptives, regardless of the side-effects, women now have the ability to control when to have children. This has afforded women with a great opportunity to make a choice when it comes to procreation. Methods of contraception include injectables, pills, patches, sponges, creams, condoms and many others (Blumenstock and Barber, 2023). Contraceptives exist as hormonal contraceptives and non-hormonal contraceptives and are further categorized as long-acting reversible contraceptives (LARC) and non-long acting reversible contraceptives (non-LARC) (Kortsmit *et al*., 2019, Adedini *et al*., 2019). Some of the contraceptives (male and female condoms) can also prevent sexually transmitted infections including human immunodeficiency virus (HIV) (Grubb *et al*., 2020). Contraceptives are widely used, globally 1.1 billion of the 1.9 billion women of reproductive age, who are aged between 15-49 years, require family planning while 842 million women are actually using contraceptive methods (Kantorova *et al*., 2019). As much as they are useful in preventing pregnancy, some of the contraceptives are made of chemicals that have been reported to have long-lasting effects. The ingredients used in contraceptives are synthetic hormones. Amongst the synthetic hormones is 17α- ethinylestradiol (EE2) also referred to as 17α-estradiol, which is an analogue of endogenous estrogen 17β-estradiol (E2) and is used as oral contraceptive combined with other hormones (Wan and O'Brien, 2014).

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Ethinylestradiol is the most effective and most frequently used estrogen in birth control tools. Studies have reported that it shows higher endocrine activity when administered orally, this has allowed its wide use in practically all modern formulations of combined oral contraceptive tablets (Frobenius, 2011, Adedini et al., 2019). Ethinylestradiol (EE2) is produced in its active free form. Since it is an exogenous estrogen, there are studies that have suggested that it may be having

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negative effects on sexual health of both men and women, mainly women because they use it as oral contraceptive and sometimes it is prescribed as treatment for conditions such as endometriosis and other women conditions. The hormone has been implicated in many human health issues such as breast cancer, migraines, deep vein thrombosis, gall bladder diseases, heart diseases, benign uterine fibroids and cervical cancer (Mir et al., 2020, Gemzell-Danielsson et al., 2019).

Because of the extensive use of EE2 in birth control, it has now become a major global pollutant due to its secretion into wastewater, either by birth control users or through industrial wastes. Wastewater treatments are failing to remove this compound, and it has been detected in many water sources (Manavhela et al., unpublished; Abibu *et al*., 2021). EE2 is piling up in the environment because it gets discharged from wastewater treatment plants into rivers and it's not easily degraded;- it takes months for EE2 to be degraded due to its high resistance to degradation. A 1.2 mg of EE2 is reported to have a half-life of +/- 27 hrs (Zhang *et al*., 2017). In the aquatic environments where it is discharged there are a wide variety of organisms that inhabit the environment, and as a result they fall victim to the effects the synthetic hormone (EE2) impacts on them. It's been reported that aquatic organisms are often feminized, and these changes are correlated with EE2 concentrations (Saraf *et al*., 2019).

Other studies have reported that exposure to low concentrations of 17α- ethinylestradiol can have negative effects on aquatic organisms as well as humans. This occurs because steroid hormones act as agonist and/or antagonist and they bind to estrogen receptors in the body and activate, block or modify the synthesis and degradation of natural hormones which results in pseudo lack or abnormal hormones that either encourages or hinder the normal endocrine function (Pironti *et al*., 2021). In addition, other studies have reported on the effects of other hormones on humans showing that they can cause cancer, including EE2 (du Plessis *et al*., 2023). However, these studies

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have not reported more information on the ethinylestradiol effects on the sexual health of women. In addition, sexually transmitted infections also affect the reproductive health of women.

Sexually transmitted infections (STIs) are defined as infections that are transmissible through sexual contact and are caused by more than 30 different viruses, ~~bacteria~~bacteria, and parasite (Workowski *et al*., 2021). They are a major public health concern as they are the leading cause of acute illnesses that lead to serious reproductive health implications, if left untreated (Coorevits *et al*., 2018). It is estimated that 1 million cases of STIs are reported on a daily basis at a global scale. The ~~most~~ leading curable STIs are caused mainly by *Chlamydia trachomatis*, *Neisseria gonorrhea*, *Trichomonas vaginalis* and *Treponema pallidium*. These pathogens are transmitted through sexual intercourse, and majority of sexually active individuals are infected with STIs once in their lifetime (Kim *et al*., 2014). It has been reported that among women of reproductive age, the sub-Saharan Africa constituted about 40% of the burden of sexually transited infections with a prevalence of 20.2% of the curable STIs between 2009 and 2016 (Julian *et al*., 2020). The World Health Organization reported 170 million cases of sexually transmitted infections annually (Dessi *et al*., 2018). Early intervention helps prevent complications that can be caused by untreated sexually transmitted infections such as infertility as well as the transmission of the infections to healthy individuals.

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Women of reproductive age are at higher risk of acquiring STIs by being involved in risky sexual behaviors which include having multiple sexual partners, inconsistent use of condoms, engaging in sexual activities while under the influence of intoxicants as well as having sex in exchange for money or drugs (Scull *et al*., 2018). Also, a report by the U.S. Preventative Services Task Force (USPSTF) in America stipulated that regardless of sexual behaviors nor age, black women are at

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an increased risk of acquiring *Chlamydia trachomatis* or *Neisseria gonorrhea* if they live in areas where the prevalence of sexually transmitted infections is high (Jackson *et al*., 2015). Therefore, reporting on the prevalence of STIs is an essential part of screening and monitoring the infection rate of STIs.

1.2 Rationale

Ethinylestradiol (EE2) is an emerging endocrine-disrupting compound (EDC) with documented adverse effects on both wildlife and human health. As a synthetic estrogen, EE2 can mimic or antagonize the physiological actions of endogenous hormones, thereby disrupting endocrine signaling pathways. Numerous studies have reported the detrimental effects of synthetic hormones on aquatic organisms and laboratory rodents (Zrinyi *et al*., 2017; Thrupp *et al*., 2018; Chen *et al*., 2017; Jackson *et al*., 2019). Although EE2 is primarily used in contraceptives, emerging evidence implicates it in potential adverse human health outcomes. For example, it has been associated with increased risks of liver injury, liver cancer, and exhibits carcinogenic properties (Wan and O'Brien, 2014).

Massawe *et al*. (2018) demonstrated that the combined administration of quinestrol and levonorgestrel in rodents led to a significant reduction (60–80%) in the weight of the testes, epididymis, and seminal vesicles, suggesting profound endocrine disruption. Despite such findings, there remains a lack of comprehensive studies investigating the long-term effects of EE2 on human reproductive health. In a study by Zethraeus *et al*. (2016), it was found that women using levonorgestrel-containing combined oral contraceptives did not experience significant impairments in sexual function, including arousal, desire, orgasm, pleasure, or responsiveness.

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Epidemiological studies, however, have raised concerns regarding the association between hormonal contraceptives and increased susceptibility to HIV acquisition, particularly with depot medroxyprogesterone acetate (DMPA) use (Polis *et al*., 2016). Notably, most prior studies have focused broadly on estrogens or other synthetic hormones, with limited attention specifically on EE2. While estrogens have been implicated in the pathogenesis of breast cancer, there is limited data exploring the potential link between EE2 exposure and the risk of acquiring sexually transmitted infections (STIs).

Given these gaps, further research is warranted to assess the specific effects of EE2 on female reproductive health and its possible role in facilitating or mitigating STI susceptibility. Although some evidence suggests a higher risk of STI acquisition among women using oral contraceptives, it remains unclear whether EE2 contributes directly to infection establishment. Moreover, uncertainties persist regarding the role of estrogens, including EE2, in cancer development—whether they act as promoters of carcinogenesis or confer protective effects in certain ~~Ethinylestradiol (EE2) is an emerging endocrine disrupting compound that negatively impacts both wildlife and human health. As a synthetic hormone, ethinylestradiol can mimic or block the normal effects of endogenous hormones in the human body. There are several studies that have extensively reported on the effects of synthetic hormones in aquatic organisms and rodents (Zrinyi et al., 2017; Thrupp et al., 2018; Chen et al., 2017; Jackson et al., 2019). Although EE2 is used as in contraceptives, research suggests that it is implicated in adverse human health effects. It's been associated with the increase in the risk of liver cancer and injury and exhibit carcinogenic properties (Wan and O'Brien, 2014). A study done by Massawe et al., (2018) indicated that other~~

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~~hormones such as quinostrol combined with levonorgestrel, reduced the weight of rat testes, the epidermis and the seminal vesicles by 60-80%. However, more studies focusing on human beings to evaluate the effects of ethinylestradiol (EE2) on human reproductive health are needed. In a study that was done by Zethraeus and colleagues in 2016, they found that sexual function of women using levonorgestrel-containing combined oral contraceptive was not reduced. In addition, they found that impairments on arousal, sexual desire, orgasm, pleasure and responsiveness was not affected. There are epidemiological studies that have reported that some hormonal contraceptive increases the risk of acquiring HIV in women, especially the depot medroxyprogesterone acetate (DMPA) (Polis et al., 2016).~~

~~Previous studies have not focused on ethinylestradiol, hence the reason why there is a need to conduct such study. Previous studies have been done that reported estrogens to elicit human breast cancer. However, there is dearth of information on whether ethinylestradiol has any potential association with sexually transmitted diseases. Therefore, it is imperative that we study how ethinylestradiol may affect the reproductive health of human beings and try to find out how it correlates with STIs. Although the risk of acquiring sexually transmitted diseases among women who use oral contraceptives seems to be high, more research is needed to understand if the hormones; more specifically ethinylestradiol; influences infection establishment. There are uncertainties on whether estrogens (ethinylestradiol included) cause cancers or confer protection against cancers.~~ contexts. Therefore, this study aims to assess the potential association between contraceptive use—particularly ethinylestradiol-containing formulations—and reproductive health outcomes, as well as to evaluate the correlation between contraceptive use and the risk of sexually transmitted infections.~~Therefore, this study focuses on assessing the potential association~~

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~~between contraception (particularly ethinylestradiol) and reproductive health as well as evaluating correlation between contraceptives and sexually transmitted infections.~~

1.3 Hypothesesis

In the present study we hypothesized the following:

1. High level of knowledge about contraceptives is associated with increased use of contraceptives.
2. Contraceptive use is common among females of reproductive age in the Limpopo Province.
3. Contraceptive use is associated with sexually transmitted diseases in the study community.
4. Sexually transmitted diseases are common among young females in the study area.
5. Ethinylestradiol concentrations are high amongst the study participants and may increase their chances of getting STDs.

1.4 Research questions

1. What is the level of knowledge, attitude, and practice of contraceptives among women of reproductive age in the ~~region~~study community?
2. What is the prevalence and types of contraception used by the study population?
3. Is contraceptive use associated with increased UTI in the study community?
4. Is contraception use associated with sexually transmitted diseases in the study community?
5. Which microorganisms are causing sexual transmitted infections among the study population?
6. What are the levels of ethinylestradiol among the study participants?

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1.5 Objectives

1. To assess the knowledge, ~~attitude~~attitude, and practices of contraceptives amongst women of reproductive age.
2. To determine the prevalence of contraceptive use amongst women of reproductive age.
3. To describe contraception association with sexually transmitted diseases.
4. To identify microorganisms causing sexually transmitted infections among study participants (*Neisseria gonorrhea, Chlamydia trachomatis and Trichomonas vaginalis*).
5. To assess the correlation between use of contraception and urinary tract infection.
6. To determine ethinylestradiol concentration within the study participants and its possible impact on STDs.
7. To determine the metabolomics of urine samples in relation to contraceptive usage.

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Chapter 2: Literature review

2.1 Overview of female reproductive health/reproductive system

The reproductive health concept was first endorsed in 1994 at the United Nations International Conference on Population and Development (ICPD) that was held in Cairo. During the conference representatives from 179 countries came to an agreement that family planning should be made available in the context of comprehensive reproductive health care, that social development beyond family planning should be addressed by population policies, and that addressing the needs of individuals is the best way to address the overall issue of rapid population growth. Furthermore, the ICPD established the objective that all nations should work toward ensuring that reproductive health services are available to all people of suitable age group through the primary healthcare system as soon as feasible and no later than the year 2015 (UN, 1994; Fathalla and Fathalla, 2008). Reproductive health per definition adopted during the ICPD conference suggests that it encompasses all aspects of an individual's physical, mental and social well-being, rather than just the lack of illness or disability, with regards to the reproductive system and all of its operations. Reproductive health suggests that individuals can have fulfilling and safe sexual relationships, reproduce, and have the liberty to choose if, when, and how frequently to do so (WHO, 2017). Reproductive health is essential for the social and economic development of a family, community or nation as well as its key component in an equal society. It is also vital for the empowerment of women and gender equality is reliant on reproductive health (Kalidhasan and Arumugam, 2020).

For women, the reproductive system is an extremely complex biological multi-organ system that is comprised of the brain, pituitary gland, ovaries, uterus and vagina. It is responsible for the

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production, transportation, and storage of eggs and facilitates the meeting between the sperm cell and eggs for fertilization (Young *et al*., 2017). The system is made of internal and external organs. The internal organs include the ovaries, fallopian tubes, uterus, cervix and vagina while the external organs include labia majora, labia minora, mons pubis, clitoris, perineum and vestibule (Andina, 2023).

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2.1.1 Internal organs

The vagina is an elastic fibromuscular canal, 9 to 10 cm long. The vagina extends up and back from the introitus to the bottom of the uterus. The vagina is located between the urethra and the rectum. Four layers make up the vaginal wall: smooth muscle, fibrous connective tissue, mucous membrane layer, and rich connective tissue vascularization. Another organ is the uterus which is a hollow pear-shaped organ that opens into the vagina from the lower end. Just below the fundus, both fallopian tubes bilaterally enter the uterus. Their role is to transport the egg from the area surrounding the ovary to the uterus. The primary female reproductive organ is the ovary, sometimes known as the female gonad. The fundamental function of the ovaries is the produce and release female gametes, or ova, as well as to secrete female sex hormones (Ramírez-González *et al*., 2016; Hoare and Khan, 2020; Ford ~~C~~, 2023).

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2.1.2 External organs

The mons pubis is a layer of fatty tissue located above the symphysis pubis, a junction created by the fusion of the pubic bones. The labia majora, also known as the labium majus, are two cleft-shaped folds of skin that start at the mons pubis and continue rearward into the fourchette. The labia minora (singular: labium minus) are two smaller, thinner, asymmetrical skin folds that create the frenulum and clitoral hood (prepuce) within the labia majora. These folds subsequently split to close the vestibule and converge around the anus to form the fourchette. The hairless, pink, and

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moist labia minora are filled with blood vessels, nerves, and sebaceous glands that exude a bactericidal fluid that lubricates and waterproofs the vulvar skin. The clitoris, which is situated between the labia minora, is an erectile organ with a dense innervation. The vestibule, which is the space where the labia minora are covered, has the exterior vaginal opening. And last but not least is the perineum which is the region between the vaginal and anal openings that has minimal skin, hair, and subcutaneous tissue (Rosner *et al*., 2019; Nguyen and Duong, 2019; Chumduri and Turco, 2021; Andina, 2023).

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2.1.3 Woman's maturation

A woman's first menstrual cycle, or menarche, is indicated by her first menstrual bleeding episode. During puberty, a growth spurt, pubic and axillary hair growth, and breast growth all occur prior to menarche. Numerous primordial follicles in the female ovary continue to grow at the start of each menstrual cycle (Itriyeva, 2022). While the other follicles become atretic and stop developing, one becomes the dominant follicle and keeps growing. Following the development of the dominant follicle into a Graafian follicle, meiosis concludes, and the ovum is no longer in prophase arrest (Ma *et al*., 2023). The Graafian follicle, also known as the corpus luteum, expels the ovum from the surrounding tissue during ovulation. In the event that fertilization is unsuccessful, the egg and secretory endometrial lining are expelled from the uterus as a result of decreasing progesterone levels; this is exhibited as monthly bleeding. In the event that fertilization does take place, the fertilized egg implants in the endometrial wall, and progesterone released (initially) by the corpus luteum maintains the endometrial lining until the placenta takes over (Murray and Orr, 2020).

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2.2 Introduction to contraceptives

Contraception, commonly known as birth control, refers to methods and devices that are used for intentional prevention of pregnancy. It enables people to plan their families based on their circumstances and have control over their reproductive decisions. There are several classifications of contraceptive methods, including hormonal methods, barrier methods, intrauterine devices, and sterilization. Every technique has a unique efficacy, side effect potential, and mechanism of action. In addition, when selecting a method, it's critical to take into account personal preferences, medical history and lifestyle considerations. To make an informed choice, it's also critical to comprehend each method's efficacy, possible drawbacks, and long-term repercussion (Fruhauf and Rankin, 2023).

The most common method of contraception that is used is the birth control pill, which is taken on a daily basis. It's made of hormones that thickens cervical mucus to hinder sperm and prevent ovulation. On the other hand, other individuals prefer non-hormonal approaches, including the copper IUD, a reversible, long-acting contraception that is implanted inside the uterus. For individuals seeking short-term form of contraception, barrier methods such as condoms and diaphragms provide protection against pregnancy and sexually transmitted infections. However, there are also permanent methods such as tubal ligation and vasectomy that offer long-term or permanent sterility and are appropriate for individuals who have reached their preferred family size (Jalalvandi et al., 2021).

2.3 Overview of ethinylestradiol (EE2)

Ethinylestradiol is a synthetic derivative of the natural estrogen estradiol. It is one of two estrogens currently used in oral contraceptive pills. The other, mestranol, is converted to ethinylestradiol

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before it is biologically active. The powder form of ethinylestradiol is finely white to creamy white. The chemical formula of ethinylestradiol is $C_{20}H24O2$ with a molecular weight of 296.4 g/mol. Ethinylestradiol, also referred to as 17alpha-ethynylestradiol is a 3-hydroxy steroid in which an ethinyl group has replaced estradiol at position 17. It is a xenoestrogen that is made from estradiol and has demonstrated strong estrogenic effects when taken orally. It exhibits a xenoestrogen function. It is a terminal acetylenic molecule, a 3-hydroxy steroid, and a 17-hydroxy steroid. It shares functional similarities with both estradiol and 17beta-estradiol (Toran-Allerand *et al*., 2005). Hans Herloff Inhoffen and Walter Hohlweg synthesized ethinylestradiol for the first time in 1938 at Schering. The purpose of its development was to produce an estrogen that would be more orally bioavailable. The substitution of an ethinyl group at carbon 17 of estradiol resulted in these characteristics. In contraceptive pills, ethinylestradiol quickly took the place of mestranol. The FDA approved ethinylestradiol on June 25 in 1943 (Thompson, 1948; Laurenson *et al*., 2014). It is mostly sold under ~~the~~ brand names such as Yasmin, Tri-Cyclen, Alesse, and Triphasil as a combination oral contraceptive. The FDA label has a black box warning which indicates that women who smoke and are over 35 years of age should not take combination oral contraceptives because there is a higher risk of serious cardiovascular side effects (FDA, 2017). It is scantily soluble in ethanol (1 part in 6 parts of ethanol) but has relatively low solubility in water (4.8 mg/L at 20 °C) compared to natural estrogenic steroid. From the physicochemical properties of these synthetic estrogens, it can be noted that EE2 is a kind of nonpolar and hydrophobic organic compound with low volatility and is more resistant to biodegradation, even though EE2 and E2 have a similar chemical structure (Aris *et al*., 2014).

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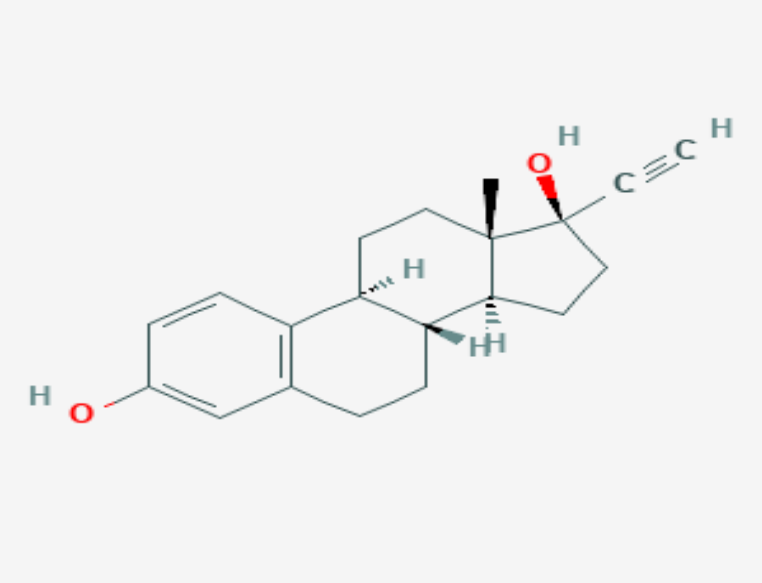


Figure 2.1: Structure of ethinylestradiol (EE2) (Li *et al*., 2008)

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2.4 Uses of ethinylestradiol combined with other drugs

Ethinylestradiol is used in almost all modern formulations of combined oral contraceptive pills and is one of the most commonly used medications (Meyer *et al*., 2019). One common combination of ethinylestradiol, is the combination of EE2 with Cyproterone acetate (CPA) which is used as a treatment for hyperandrogenic symptoms. It is reported to treat moderate to severe acne related to androgen-sensitivity in young women (Blitzer *et al*., 2017). Cyproterone acetate CPA is a derivative of progesterone having some antiandrogenic activity. It is applied as a safe method of contraception in women suffering from systemic lupus erythematosus having elevated risk of venous and arterial thrombosis but does not increase the risk. The association of

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ethinylestradiol with CPA has been indicated to elevate the risk of deep vein thrombosis (DVT) in healthy women. Ethinylestradiol with CPA have been reported to impair glucose tolerance and to alter insulin tolerance in adolescents suffering from polycystic ovarian syndrome (Ruan et al., 2017). There is also another drug combined with ethinylestradiol called drospirenone that is commercially available. It is used to exhibit the progestational and antiandrogen activity and betters the premenstrual syndrome symptoms and light acne in women. It's been suggested that unfavorable results such as DVT in combinations of drospirenone and ethinylestradiol and Cyproterone acetate and ethinylestradiol are due to the component containing ethinylestradiol (Society, 2018).

2.5 Mechanism of action of Ethinylestradiol (EE2) and Progestin based contraceptives

Estrogen-based contraceptives work by suppressing the release of eggs from the ovaries, ethinylestradiol can disrupt the normal menstrual cycle and hinder a woman's ability to conceive. Aside from its effect on ovulation, ethinylestradiol can also lead to changes in cervical mucus and the uterine lining, which may have an impact on the movement and survival of sperm, further reducing the chances of conception (Roe *et al*., 2023).

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Ethinylestradiol is subjected to intensive effect in the cytochrome P450 (CPY) which leads to formation of its metabolites (Hoy and Scott, 2009). Up to 95% of the oral dose containing ethinylestradiol is metabolized before it gets into the system circulation. The half-life of estradiol in plasma is approximately 2.5 hrs, whereas the terminal half-life is approximately 14–17 hrs and depends on enterohepatic circulation and on circulating levels of sulfate and glucuronide

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metabolites. Estradiol is mainly eliminated in the urine; almost 10% is eliminated in feces (Zhang *et al*., 2024).

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Inside women's body, ethinylestradiol increases the hepatic synthesis of sex hormone binding globulin (SHBG), thyroid-binding globulin (TBG), and other serum proteins and suppress follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary. Once these two hormones are suppressed, the egg cell will be released from the ovary because the corpus luteum does not mature into an egg cell (**figure 2.2)** (Sachse, 2006).

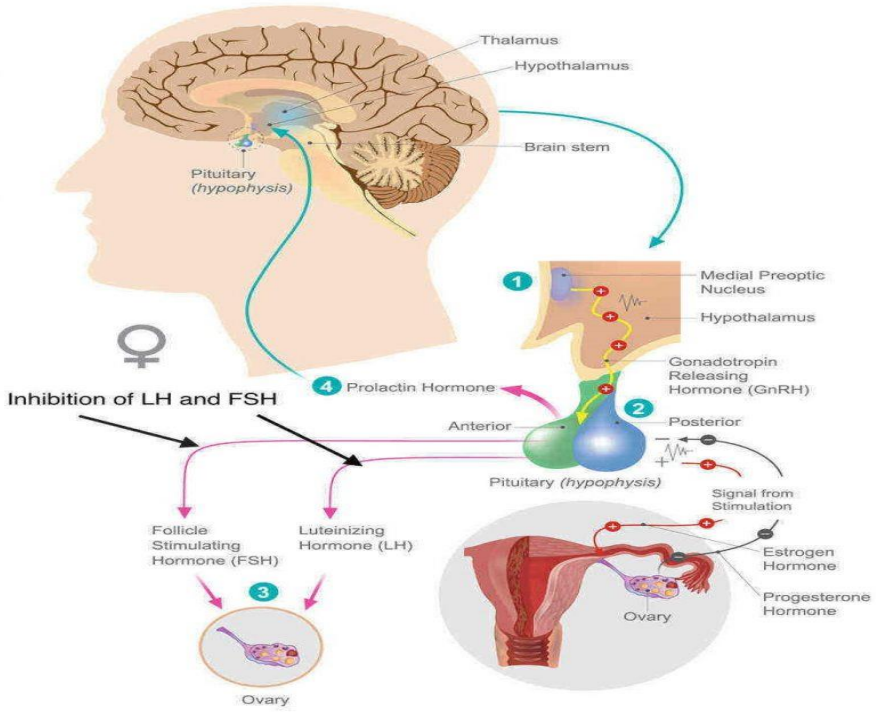


Figure 2.2: Mechanism of action of estrogen-based contraceptives (psychscenehub.com)

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Figure 2.3 below illustrates the mechanism of ethinylestradiol. Within the body ethinylestradiol binds to the estrogen receptor complex and then enters the nucleus, therefore, inducing DNA transcription of genes which plays a role in estrogenic cellular responses. It's been suggested to also inhibit the 5-alpha reductase in the epididymal tissue, which then lowers the levels of testosterone and delays progression of prostatic cancer (Robaire *et al*., 2006). It has been suggested that ethinylestradiol also confer protection against osteoporosis and breast cancer. (Sier *et al*., 2017).

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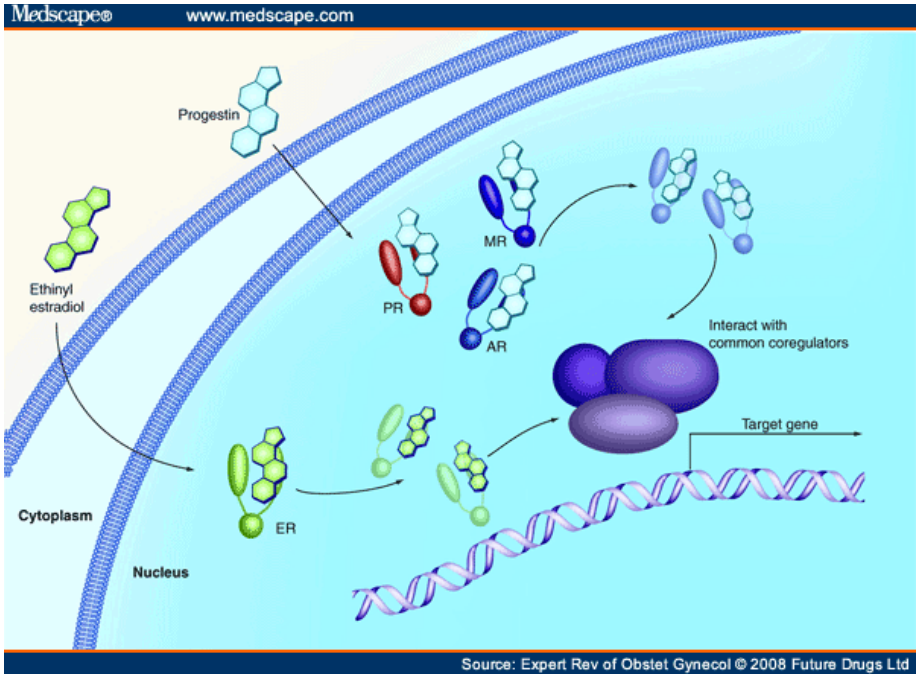


Figure 2.3: Mechanism of action estrogen and progestin-based contraceptives (Ouzounian *et al*., 2008)

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2.6 Classification of contraceptives

There are many different types of birth control that women can choose from. These methods are classified into 5 groups, namely, Long-Acting Reversible Contraception (LARC), Hormonal Methods, Barrier Methods, Emergency Contraception and Sterilization.

2.6.1 Long-Acting Reversible Contraception (LARC)

These are contraceptives that last for a long time, you only take them once and never take them again until they run out of time. These can last from 3-10 years. This type of contraceptive includes intrauterine devices and hormonal implants.

The implant is a 4cm long thin flexible rod which is inserted under the skin of the upper arm. It works by preventing the release of the eggs from the ovaries by constantly releasing small amounts of progestogen into the body. In turn, the progestogen thickens the cervical mucus and also thins the uterine lining. All this make it difficult for the sperms to pass through the cervix and if there is a fertilized egg, it's harder for them to implant since the thin uterine wall is unable to support fertilized eggs. This type of contraception can last up to 3- 5 years (Boog and Cooper, 2021; Averbach and Hofler, 2022). The intrauterine devices (IUD) work in similar ways. The IUD is a small t-shaped plastic and cooper device.

2.6.2 Hormonal methods

This type of contraception is also referred to as the short-acting hormonal methods. These are birth controls that an individual consumes every day, or they are given on a monthly basis or every 3 months. These include pills and injections. The mechanism of action of combined oral contraceptives is the inhibition of ovulation.

i. **Combined oral contraceptive (The pill)**

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Commonly known as the pill, it is made up of a combination of synthetic female hormones i.e. estrogen and progestogen. In the body they mimic the normal functions of endogenous hormones naturally produced in the ovaries. The hormones contained in the pill work by preventing ovulation (stopping ovaries from releasing an egg) or by making it difficult for embryo implantation to occur by thinning the lining of the uterus and also making it difficult for a sperm cell to reach an egg by thickening the neck of the womb (Fryer. 2006). However, apart from pregnancy prevention the pill can also be used for treatment of other health issues faced by women. It can reduce painful periods and heavy periods; help alleviate endometriosis symptoms as well as premenstrual symptoms. If the pill is taken correctly (24 hours' time interval, same time every day) it is 99% effective. When taken correctly only 1 in 100 women can have accidental pregnancy. According to National Health Service (NHS) about 8 women out of 100 fall pregnant when using the pill due to factors such as forgetting to take the pill in the correct time which reduces effectiveness from 99% to 92%.

Generally, one has to take the pill every 21 days and then stop for 7 days during the monthly bleeding period. However, these days contraceptive pills include pills taken every day for 28 days, 21 active pills with estrogens and 7 pills that consists of sugar-only that are taken for 7 days during menstruation. The 7-non-active pills are included in order to bridge the gap of forgetting to take the pills as other women would not take pills at regular times after the 7-day period of stopping.

Minor reported side effects include breast tenderness, mood swings and headaches. Pills have low risk of serious side effects such as blood clots and cervical cancer. The COCs cannot be used by pregnant or breastfeeding women, women who are 35 years and above,

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smokers or those who have stopped smoking in less than a year, overweight women as well as women taking antibiotics for epilepsy, tuberculosis or HIV (Lopez *et al*., 2016; Simmons *et al*., 2018). Also, women who experienced thrombosis, or are suffering from migraine with aura, breast cancer and heart problems.

Advantages of using the pill reported by users includes, no sex interruption, monthly bleeding becomes regular and lighter and less painful. The risk of ovarian, womb and colon cancer is reduced. The disadvantages reported include elevation of blood pressure, no protection against sexually transmitted diseases and at first one can experience headaches, nausea, mood swings and breast tenderness, breakthrough bleeding and spotting as well as increased risk of thrombosis and breast cancer. Apart from skipping days, effectiveness can be reduced by antibiotics such as rifabutin and rifampicin (used to treat Meningitis and tuberculosis).

ii. Injectables

~~This~~ These type~~s~~ of contraceptives consist~~s~~ of: Depo-Provera, Sayana Press and Noristerat and progestin-based contraceptives. Depending on the type used these injections can last up to 8 (Noristerat), 12 (Depo-Provera) and 13 (Sayana Press) weeks (Oluwasanu *et al*., 2021). The mechanism of action is as follows: Immediately after being administered, the injection releases the progestogen hormone steadily into the bloodstream. The constant release of the progestogen prevents the woman from ovulating, and it thickens the mucus from the cervix which makes it difficult for the sperm cells to pass through to the womb to reach the unfertilized egg. It also makes the uterine wall thinner such that it cannot support the fertilized egg. One is immediately protected if they receive~~d~~ the injection during the

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first 5 days of their menstrual cycle, but if it's administered any other day of the cycle, there is no protection for the first 7 days (Abbey, 2022).

2.6.3 Barrier methods

Barrier methods are type of contraceptives that one uses or take every time you have sex. This includes male and female condoms, Diaphragms, contraceptive sponge, cervical caps and spermicidal foams, creams, films, suppositories and jellies. All these barrier methods can readily be found over the counter with the exception of diaphragms and cervical caps (Schickler and Patel, 2020). To increase the efficacy of barrier methods, they can be used simultaneously. For example, external methods such as condoms can be used in dual with cervical caps or diaphragms (Chappell *et al*., 2022).

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2.6.4 Emergency Contraception

Emergency contraception is for contraceptives that are taken immediately after unprotected sexual intercourse. These are said to be effective if taken within 12 hours and some within 120 hours. This type includes Copper IUD and emergency contraceptive pills.

Copper IUD is said to be the most effective form of emergency contraception and can be left in place for up to 10 years. It works by slowly releasing copper in the uterus, which will then change the make-up of the fluids in the uterus and the fallopian tubes, therefore, preventing the survival of sperm cells in the womb. It also prevents the implantation of fertilized eggs. Oral emergency contraception works by delaying ovulation and its effectiveness post ovulation is debatable (Cleland *et al*., 2014; Batur *et al*., 2016).

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2.6.5 Sterilization

This is the type of contraception that prevents pregnancy for the rest of your life. Among these are: female tubal ligation and male vasectomy.

i. Vasectomy

Male vasectomy is a procedure that is performed on men to stop them from impregnating women. It works by preventing the sperm cells from getting into the semen, and as a result when a man ejaculates, the eggs will not be fertilized as the semen contains no sperm cells. The surgical procedure is relatively painless and is quick. During the procedure, the tubes that carry sperm cells from the testicles to the penis are cut, blocked or sealed with heat. It's normally performed with the patient awake.

ii. Tubal ligation

Tubal ligation is also known as female sterilization. This is a permanent form of contraception, as it prevents the women from becoming pregnant. This involves the blocking of the fallopian tubes, meaning the eggs and sperm cells are prevented from meeting, thus preventing conception (Sastre *et al*., 2023). This type of method is one of the most common used methods in the United States, and less so in Africa. Statistics shows that this method in Africa is utilized by 2% of women, which shows a huge gap with the utilization in the United States where it's used by about 24% of contraceptive users (Welch *et al*., 2024). This involves two types of procedures that can be used to achieve sterilization. The first one is a procedure called laparotomy, during this procedure the surgeon makes a small cut in the abdominal wall and inserts a laparoscope to obtain visualization of the inside and they block the tubes by using either clips or by tying and cutting the tubes.

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Another procedure is called salpingectomy, during this procedure the tubes are completely removed.

2.7 Pharmacodynamics and Pharmacokinetics of Ethinylestradiol (EE2).

Pharmacokinetics refers to the study of how a drug is absorbed, distributed, metabolized, and eliminated by the body. In the case of ethinylestradiol, its pharmacokinetics in women have been found to be influenced by several factors including obesity, sex hormones, transporters, and metabolizing enzymes. For example, obesity has been shown to impact the pharmacokinetics of ethinylestradiol. Obese women taking oral contraceptives containing ethinylestradiol and norethisterone have been found to have lower levels of these hormones compared to non-obese women taking the same medications

Ethinylestradiol reduces gonadotrophic hormone to inhibit ovulation and luteinizing hormone to lessen endometrial vascularization. It's duration of action is long, and has a broad therapeutic index because overdosing is typically not associated with major side effects (Regidor, 2018). Combination of oral contraceptives block the release of sperm by thickening cervical mucus, inhibiting the gonadotrophic hormone, and blocking the endometrial alterations necessary for the implantation of a fertilized egg. Because luteinizing hormone is reduced by ethylestradiol, the endometrium becomes less vascularized. Moreover, it raises sex hormone binding globulin levels (Regidor, 2018).

A 30µg oral ethinylestradiol can reach plasma concentration (Cmax) of 74.1±35.6pg/mL with maximum time (Tmax) of 1.5±0.5h and an area under plasma concentration-time (AUC) of 487.4±166.6pg*h/mL. An administered patch dosage of 1.2 mg can reach a Cmax of 28.8±10.3 pg/mL, a Tmax of 86±31 h, and an AUC of 3895±1423 pg*h/mL. A 1.2mg dose applied topically

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has an apparent volume of distribution of 11745.3±15934.8L, while a 30µg oral dose has an apparent volume of distribution of 625.3±228.7L (Zhang et al., 2017).

In serum, 98.3–98.5% of ethinylestradiol is bound to albumin; however, it also shows binding to sex hormone binding globulin. While 2–3% of ethinylestradiol is excreted in feces, while 59.2% is eliminated in urine and bile. Over 90% of the parent drug, ethinylestradiol, is eliminated unchanged (Kuhnz *et al*., 1990). It is important to note that ethinylestradiol is extensively metabolized in the body. The CYP3A4 is one of the main enzymes responsible for the metabolism of ethinylestradiol. Additionally, ethinylestradiol inhibits CYP2C19-dependent omeprazole hydroxylation and weakly inhibits CYP3A4. Pharmacokinetic interactions between ethinylestradiol and co-administered drugs, such as omeprazole and brivaracetam, can occur due to these metabolic pathways (Wang *et al*., 2004).

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2.8 Known negative effects of contraceptives

Contraceptives are widely used around the world, but they also have side effects, from nausea, vomiting, headaches, migraines, breast tenderness, decreased libido, mood swings and others as described by users (David *et al*., 2011). Other studies have demonstrated that women using combined hormonal contraceptives of the nature of estrogen and progestin have a higher risk of having ischemic stroke (Xu *et al*., 2015). These combined hormonal contraceptives contain combined oral contraceptives, combined hormonal patch and combined hormonal ring (Daniels *et al*., 2015). Matlala and Mpolokeng in 2010 reported that men have complains about contraceptives that their partners use and are of opinions that contraception causes weak erections, early death, premature aging, aches and pains due to contraceptives, however these claims have not yet been validated (Matlala and Mpolokeng, 2010).

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Oral contraceptives contain estrogens like mestranol and ethinylestradiol as already mentioned. Nonetheless, these pills make the level of estrogen in the body ~~to~~ rise and prevent ovulation. But, as useful as contraceptives are in preventing unwanted pregnancy their high level or their continued increase of level in the body results in negative effects, including high risk of getting breast cancer, weight gain, headache, blood clotting and hypertension (Coetzee and Ngunyulu, 2015).

2.8.1 Less severe side effects of contraceptives

2.8.1.1 Nausea and Vomiting

Nausea, breast tenderness, mood swings and vomiting are common side effects that some individuals may experience when taking ethinylestradiol. These symptoms can occur within the first few weeks of starting the medication and usually improve with continued use (Basciani and Porcano, 2022).

2.8.1.2. Headaches

Some individuals may experience mild headaches while others may experience more severe migraines. This is caused mainly by hormonal fluctuations, and women who suffers from migraine with aura often experience worsened symptoms and have an increased risk of suffering from stroke as well. It is important to note that if you have a history of migraines or are prone to headaches, taking ethinylestradiol may potentially exacerbate these symptoms. Furthermore, exogenous hormones have the potential to alter the course of migraine by causing de novo headaches, de novo auras, aggravating prior migraine attacks, and improving migraine attacks, especially those associated with menstruation (Sacco *et al*., 2018).

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2.8.1.3. Breast tenderness

Breast tenderness is another common side effect that some individuals may experience while taking combined oral contraceptives. This can manifest as increased sensitivity, soreness, or swelling in the breasts. It is important to note that this side effect is generally temporary and should resolve over time. Wearing a supportive bra and applying warm compresses may help alleviate discomfort (Cooper *et al*., 2017).

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2.8.1.4. Mood Changes

Since the inception of combined hormonal contraceptives, many women have suffered from side effects related to mood changes. This has led to many users discontinuing the use of Combined oral contraceptives (COC). Furthermore, about 10% of users have complained about increased irritability and anxiety as well as depressed mood (Lundin *et al*., 2017). These changes have been reported in some users, and they are clinically relevant and can be a final nail to causing mental health problems deserving psychotropic treatment (Sundström-Poromaa, 2021).

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2.8.2 Concerning side effects of contraceptives

Ethinylestradiol can have various serious side effects. Although not common, these side effects can occur in some individuals. Some of the common serious side effects may include blood clots, stroke, heart attack, and liver problems.

2.8.2.1 Blood Clots

One of the potential side effects associated with ethinylestradiol is an increased risk of blood clots. Blood clots, medically known as venous thromboembolism, refer to the formation of clumps within the blood vessels that can obstruct proper blood flow. Research indicates that taking

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medications containing ethinylestradiol, particularly oral contraceptive pills, may elevate the likelihood of developing blood clots. The estrogen component in ethinylestradiol can affect the clotting factors in the blood, leading to a higher risk. It is important to be aware of this side effect and take necessary precautions to minimize the risk of blood clots. It is reported that combined oral contraceptives increase venous thromboembolism two to four-fold when compared to non-users (Haverinen *et al*., 2022). While blood clots can occur naturally, ethinylestradiol increases the risk of developing them. Blood clots can form in the legs (deep vein thrombosis) or travel to the lungs (pulmonary embolism), which can be life-threatening. Signs of blood clots include swelling, pain, warmth, or redness in the legs, sudden shortness of breath, chest pain, or coughing up blood (Stalas *et al*., 2024).

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2.8.2.2 Changes in Menstrual Cycle

Understanding the changes in the menstrual cycle as a side effect of hormonal contraceptive is of importance for women who are using it. Since the hormonal contraceptives contains ethinylestradiol, it can affect the regularity and symptoms of menstruation. By understanding these changes, women can have better control over their reproductive health and make informed decisions about their birth control options. Additionally, understanding menstrual cycle changes can help healthcare providers accurately diagnose any abnormalities or potential health concerns (Vollmar *et al*., 2025). It is crucial for both women and healthcare providers to be knowledgeable about the impact of ethinylestradiol on the menstrual cycle to ensure the best possible management of its side effects and overall reproductive well-being.

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2.8.2.3 Libido

One of the potential effects of ethinylestradiol on libido is a decrease in sexual desire. This is believed to occur due to the suppression of testosterone levels, as ethinylestradiol can bind to the androgen receptors and inhibit their function. The hormonal changes caused by contraceptives

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containing ethinylestradiol can also lead to vaginal dryness and decreased vaginal lubrication, which may contribute to a decrease in sexual pleasure (Casado-Espada *et al*., 2019).

Several research studies have investigated the impact of ethinylestradiol on libido. A meta-analysis of these studies found that ethinylestradiol-containing oral contraceptives were associated with a small but statistically significant decrease in sexual desire compared to non-hormonal contraceptives. However, it is important to note that individual responses to ethinylestradiol can vary, and not all individuals will experience a decrease in libido. Additionally, some studies have reported conflicting findings, highlighting the need for further research in this area. Future studies should also explore potential differences in libido changes between different formulations and dosages of ethinylestradiol (Jarząbek-Bielecka *et al*., 2018; Haverinen *et al*., 2022).

Several factors can influence the impact of ethinylestradiol on libido. These include individual variations in hormone sensitivity, baseline testosterone levels, and pre-existing sexual function. Women with lower baseline testosterone levels may be more susceptible to experiencing a decrease in libido when taking ethinylestradiol. Additionally, psychological factors such as stress, relationship dynamics, and body image can also play a role in how ethinylestradiol affects libido.

2.8.2.4 Stroke

Another serious side effect of ethinylestradiol is the increased risk of stroke. Stroke occurs when blood flow to the brain is disrupted, either due to a blood clot or a burst blood vessel. Symptoms of ~~a~~ stroke may include sudden numbness or weakness on one side of the body, difficulty speaking or understanding speech, severe headache, dizziness, and loss of balance or coordination. It is reported that the increased risk of stroke is because of the hypercoagulable effects of estrogen (Champaloux *et al*., 2017).

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2.8.2.5 Heart Attack

Ethinylestradiol can also potentially lead to a heart attack, a serious and life-threatening condition. A heart attack occurs when the blood flow to the heart is blocked, usually due to a blood clot. Symptoms of a heart attack include chest pain or discomfort, shortness of breath, nausea, lightheadedness, and pain or discomfort in other areas of the upper body such as the arms, back, neck, or jaw. Ethinylestradiol has hepatic and vascular actions that can lead to dyslipidemia, prothrombotic and proinflammatory effects, and elevated vascular resistance—all of which are factors in the pathophysiology of cardiovascular disease (CVD). Because CHC causes the liver to produce more angiotensinogen, which activates the renin-angiotensin-aldosterone pathway, blood pressure rises. Immediate medical attention is essential if these symptoms arise, as prompt treatment can be crucial in preventing further damage to the heart (Schufelt and LeVee, 2020).

2.8.2.6 Liver Problems

Oral contraceptives can potentially lead to liver problems in some individuals. These problems can range from mild liver dysfunction to more severe conditions such as liver tumors or liver cancer, although the latter are rare. Signs of liver problems may include yellowing of the skin or eyes (jaundice), dark urine, clay-colored stools, persistent abdominal pain, and unexplained fatigue or weakness. Although oral contraceptives have been of great health benefit, its abuse and misuse, mostly in doses not prescribed over a long period of time may alter the integrity and functionality of the liver, hence its toxicity (Odinga *et al*., 2022).

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2.8.2.7 Breast Cancer

Ethinylestradiol has been associated with an increased risk of breast cancer. This relationship between ethinylestradiol and breast cancer has been extensively studied, with various mechanisms

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proposed to explain the potential link. Epidemiological studies have provided evidence suggesting a positive association between ethinylestradiol use and breast cancer incidence. Additionally, potential risk factors and subgroups have been identified that may further increase the risk of developing breast cancer in individuals exposed to ethinylestradiol (Hunter *et al*., 2010,).

The mechanism of action of ethinylestradiol in breast tissue involves its binding to estrogen receptors, resulting in the activation of downstream signaling pathways. By interacting with these receptors, ethinylestradiol promotes cell proliferation and inhibits apoptosis, potentially contributing to the development and progression of breast cancer. It has also been proposed that ethinylestradiol may influence the expression of genes involved in cell cycle regulation and DNA repair, further contributing to its carcinogenic effects in breast tissue (Mir *et al*., 2022).

Numerous epidemiological studies have been conducted to investigate the association between ethinylestradiol use and breast cancer risk. These studies, involving large cohorts of women, have consistently reported an increased risk of breast cancer in individuals exposed to ethinylestradiol. The magnitude of this increased risk varies depending on factors such as duration of use, dose, and age at initiation of ethinylestradiol-containing contraceptives.

Several potential risk factors and subgroups have been identified that may modify the association between ethinylestradiol use and breast cancer risk. Age is an important factor, with younger women at a higher risk compared to older age groups. Other factors such as family history of breast cancer, obesity, and certain genetic mutations may also contribute to an increased susceptibility to the carcinogenic effects of ethinylestradiol (Wan *et al*., 2022). Furthermore, individuals who have used ethinylestradiol-containing contraceptives for a longer duration or at higher doses may face

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an elevated risk. These findings highlight the importance of considering individual characteristics and risk factors when evaluating the association between ethinylestradiol and breast cancer.

2.9 Contraceptives and sexually transmitted diseases

The use of barrier contraceptives such as condoms is essential in halting the spread of sexually transmitted illnesses and diseases (STIs/STDs). They serve as a barrier, keeping bodily fluids that may have been contaminated with STIs from being exchanged. Condoms and other barrier techniques work well to lower the chance of transmission (Mane and Maid, 2021). Hormonal therapies, like birth control tablets, also offer some protection because they control the menstrual cycle and decrease the probability of unintended pregnancies, which raises the possibility of contracting STIs or STDs. IUDs (intrauterine devices) provide long-term protection against some STDs/STIs and unintended pregnancies. It's crucial to remember that although contraceptives can lower the risk, it's not completely eliminated. In order to promote safe sexual practices and stop the transmission of STDs, it is crucial to use contraceptives correctly and consistently (Haddad *et al*., 2014). Other key factors that help in this regard include frequent STD/STI testing and treatment, education and awareness campaigns, and the engagement of healthcare providers. All forms of contraceptives are useful in preventing unwanted pregnancy empowering men and women to have over their ability to have children. While others can protect from sexual transmitted infections such as HIV (Matlala and Mpolokeng, 2010).

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Globally, different forms of hormonal contraceptives such as oral contraceptive pills, hormone containing technologies (including implants, patches, rings and intrauterine) progestin only injectables (including depo-medroxyprogesterone acetate) are used by approximately 150 million women (Baeten, and Heffron, 2015). Contraceptives have been reported to be effective methods of preventing pregnancies, however most of them don't prevent contraction of sexually transmitted

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diseases (Tyler *et al*., 2014). Use of condoms is the most effective method of preventing STDs but have been reported to have a failure of 15 to 17%. Because of this reason, many clinicians recommend using a dual contraceptive method (Peipert *et al*., 2011). Previous studies show that many women of reproductive age do not adhere to those recommendations of using dual contraceptive method, which means using a condom and another type of contraception, either a long last or short reversible method of contraception (McNicholas et al., 2017).

In a study done in Denmark in a shade cohort about STDs in women living with HIV, a low prevalence was observed. Most of the study participants reported using condoms as a contraceptive method more specially to prevent pregnancies. However, more than 10 percent of women at reproductive age were found to be at risk of having unintended pregnancies (Thorsteinsson *et al*., 2016).

Grabowski and colleagues in The Lancet Global Health gave a report that, women who are using Depo- medroxyprogesterone acetate were at a double risk of contracting Herpes simplex virus type 2, when compared to those who are not using any contraception in a study limited to the ones whose partners has HSV2 (Grabowski *et al*., 2015).

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2.10 Perception of contraceptives by man and women

The world's population is increasing every day due to couples wanting to have large families or even small ~~family~~ families but to have children in general, but above that it's also because of unintended/unplanned pregnancy. Uncontrolled population explosion is a burden on resources of many developing countries. More than half of the world's population lives in developing countries where fertility, infant and maternal mortality rates are high. And it's been estimated that the

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world's population will likely increase by 2.5 billion over the next years to ~~be~~ 9.2 billion in 2050 (Page *et al*., 2014)

One in three women gives birth before they reach the age of 20 in developing countries. Death related to pregnancy during childbirth is higher in contrast with women above 20 years of age. There is an estimate of 20 million unsafe abortions per year, and a quarter of these are reported to occur among women between the age of 15 and 24 years (Munakampe *et al*., 2018). Cleland and colleagues in 2006 suggested that using contraceptives would prevent about 90% of abortions, 20% morbidity related to pregnancy and 32.5 % maternal deaths globally (Cleland *et al*., 2006).

World health organization (WHO) defined family planning as a way of thinking and living that is adopted voluntarily upon the basis of knowledge, attitudes and responsible decisions by individuals and couples in order to promote health and welfare of family groups and thus contribute effectively to the social development of a country (Mustafa *et al*., 2008). Man and women knowledge about contraceptives vary, and they have different attitude as well. In a study done at Ga-Sekororo Village, South Africa, male participants of the study showed to have adequate knowledge about contraceptive methods that are available in South Africa (Matlala and Mpolokeng, 2010). In a study done in India it was found that majority of the participants had knowledge about contraceptives, but only 48.5% female and 44.5% of male had positive attitude about contraception (Pegu *et al*., 2014).

In a study done in Uganda among Ugandan university students, they found that students had knowledge. Nonetheless, the amount of knowledge did not correspond to the use of contraceptives as there was a low rate of usage. They also found that many of the students perceived that contraceptives are used by poor people (Nsubuga *et al*., 2016).

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Religion and education have also been reported to play a role in knowledge about contraceptives in some studies. For example, Saelim and colleagues in a study done among Muslim Thai women demonstrated that educated women understood health-related problems more than those who have low education-to-illiterate (Saelim *et al*., 2016).

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2.11 Contraceptive usage and occurrence of hormones in the environment

Contraceptives including fertility drugs as well as steroids are widely and effectively used by humans and are also applied in farm animals. Their misuse by humans, farm animals, and fisheries as well as poultry farms results in their abundance in the environment. Usually, hormones that we excrete everyday always end up in the environment. Contraceptives have been discovered in surface water, groundwater, meats and milk products (Kaur *et al*., 2018).

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The need to have the ability to control number of children that women can have has resulted in the rampant use of contraceptives. And fertility drugs are used more in treating infertility or to increase chances of productivity in humans, animals and fisheries (Elakkanai *et al*., 2015). Therefore, the more we consume foods (such as meat, milk, poultry, water and agricultural products) that are contaminated by contraceptives and fertility drugs it results in disorders like fertility loss and high risk of different types of cancers. A study done by Pasqualotto and colleagues in 2004 discovered that about 15% of sexually active population was not fertile, half of those cases were found to be related to man. Contraceptives drugs are widely available in markets as contraceptive pills and their use is increasing because of their awareness amongst women, particularly teenagers, to use them for preventing unintended pregnancy. They are convenient to use and are easily accessible from health-care facilities.

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For over two decades pharmaceuticals ~~are being~~have been detected in aquatic environment in ng-ug/L range. These compounds have biological activity which has been observed to induce negative effects at very low levels of exposure. In the aquatic environment organisms exposed to ethinylestradiol EE2 have reduced fertility (Capolupo *et al*., 2018).

Steroid hormones found in ~~the~~ aquatic environments have been shown to have negative effects on aquatic organisms and it's been suggested that they influence reproduction and immune systems in humans. Most of the synthetic hormones that are contaminating the environment originate from us humans mainly through contraception use, and from animal feed as well as agricultural run-off, supplements used in husbandry and aquaculture. Also, our domestic effluents are the main contributors to pollution with steroid hormones in the aquatic environments as they discharge indirectly to the aquatic environments (Bouman *et al*., 2005; Noppe *et al*., 2007; Pignotti *et al*., 2017).

Humans and animals need drugs in their life cycle because they play an important role. However, these drugs are also unintentionally causing ill-effects on the environment, especially on humans and animals. Now when the drugs are consumed, they end up in the environment through human and animals' excretion, hence their detection in high levels in the environment. Nonetheless, the use of drugs has escalated in recent years because of more drugs being discovered and disease progression. All this has resulted in accumulation of the drugs in water and soil because even the wastewater treatment plants are not efficient enough to remove them (Kamyota *et al*., 2010; Martella and Coleman, 2015, Kaur *et al*., 2018).

Ideally, the pathway for environmental pollution with hormones/drugs is basically the same as illustrated in figure 1 below (Barbosa *et al*., 2016). All compounds ~~either~~ whether pharmaceuticals,

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steroid hormones, personal care products, industrial compounds and drugs of abuse follow the same pathway. Point of source in the environment is as discussed in paragraph 2, can be through disposal at landfill after use in our homes or public or private places such as hospitals and private surgeries, runoff from agriculture after use as manure, domestic and hospital effluents from our everyday excretion and industrial wastewater. From there, hormones and other compounds contaminate the soil, and ground water as they are sifted through the soil overtime. From wastewater treatment plants and landfill, these lead to contamination of surface water which is usually used in water treatment for dinking purposes, and because our treatment plants are not equipped enough to remove these contaminates, we end up drinking contaminated water from our taps.

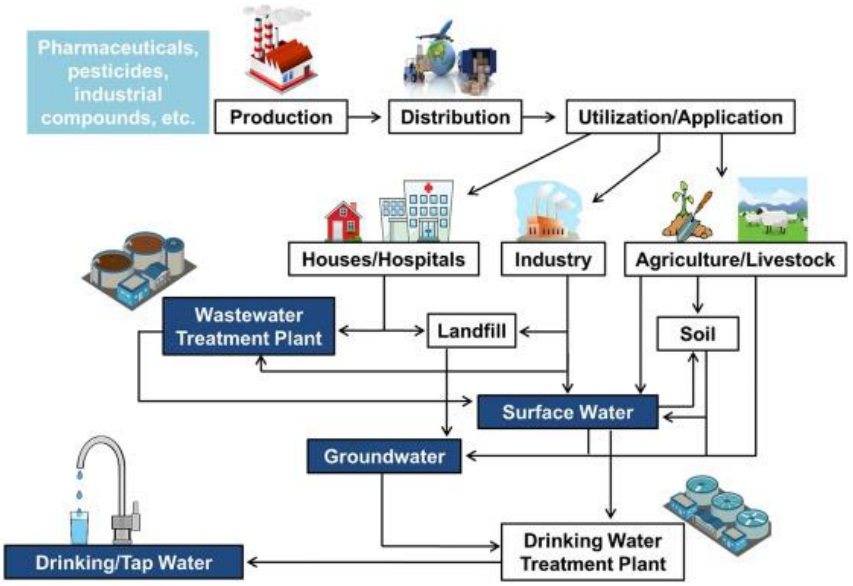


Figure 2.4: Pathways for environmental pollution with hormones/drugs (Barbosa *et al*., 2016)

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2.2 Overview of Sexually transmitted infections/diseases

Sexually transmitted infections (STIs), also known as sexually transmitted diseases (STDs) are a cohort of infections that are transmitted via unprotected sexual intercourse. Amongst these are bacterial, viral, and protozoan infections. Others are curable while others are manageable but lacks cure. The most notable infections are caused by *Chlamydia trachomatis*, *Neisseria gonorrhea*, *Trichomonas vaginalis* (Trichomoniasis) and *Treponema pallidum* (Syphilis), mycoplasma, Herpes virus, hepatitis B virus and Bacterial Vaginosis (vaginosis) (Seidu *et al*., 2021). Jackson and colleagues (2015) reported that women of reproductive age are at higher risk of acquiring STIs by being involved in risky sexual behaviors which includes: having multiple sexual partners, inconsistence use of condoms, engaging in sexual activities while under influence of intoxicants as well as having sex in exchange for money of drugs. In addition, men who have sex with other men are also at high risk of acquiring STIs (Jackson *et al*., 2015; Unerno *et al*., 2017). The world health organization indicated that about 1 million persons are contracting sexually transmitted infections daily, with 357.4 million cases reported annually on a global scale (Unerno *et al*., 2017). On this global burden of STIs, Sub-Saharan Africa constitutes about 40% of the burden (Nodjikouambaye *et al*., 2019).

Infection with STIs is reported to increase the risk of acquiring HIV. Infection with *Chlamydia trachomatis, Neisseria gonorrhea* and *Trichomonas vaginalis* have been said to result in the upregulation of the inflammatory cytokine with accumulation of leukocytes, of which some are target cells for HIV in tissues surrounding the genital tract, which heightens the risk of acquiring HIV (Mudau *et al*., 2017). Human immunodeficiency virus and sexually transmitted diseases are

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reported to share a bidirectional relationship. Studies suggest that for HIV infected ~~persons~~people, high risk behavior confer susceptibility to infection with STIS, and as the HIV infection advances, it leads to treatment failures for STIs (Khaw *et al*., 2018; Davey *et al*., 2019; Kharsany *et al*., 2020).

Although STIs affect ~~persons~~ people of all ages, it is adolescents and young people who are affected disproportionately. Many of the STIs, especially curable ones, do not present symptoms and have consequences if left untreated (Francis *et al*., 2017). For example, in pregnant women they can result in complications like spontaneous abortion, low-birth weight, preterm birth, intrauterine retardation, ectopic pregnancy, stillbirth, postpartum sepsis, congenital infection and an increased risk of mother-to-child transmission. In addition, babies born of mothers infected with *Chlamydia trachomatis* are said to have an increased risk of suffering neonatal pneumonia and infant blindness (Mudau *et al*., 2017; Torrone *et al*., 2018; Masha *et al*., 2017). In non-pregnant women, complications of untreated STIs includes permanent infertility, pelvic chronic pains, cardiovascular diseases, genital malignancies and increases the risk of HIV acquisition (Teasdale *et al*., 2018).

Most sexually transmitted infections are diagnosed using nucleic acid amplification tests (NAATs). The incorporation of microbiological screening is implemented in order to overcome the shortcomings of clinic-based sampling and conventional culture methods since most people are afraid of invasive genital examinations and are also reluctant to attend treatment, thus self-collected and non-invasive samples are ideal for use in NAATs (Nodjikouambaye *et al*., 2019).

Sexually transmitted infections (STIs) are defined as infections that are transmissible through sexual contact (Lautenschlager and Buder, 2022). A major public health concern as they are the leading cause of acute illnesses that leads to serious reproductive health implications if left

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untreated (Coorevits *et al*., 2018). It is estimated that 1 million cases of STIs are reported on a daily basis at a global scale. The ~~most~~ leading curable STIs are caused mainly by *Chlamydia trachomatis*, *Neisseria gonorrhea*, *Trichomonas vaginalis* and *Treponema pallidium*. These pathogens are transmitted through sexual intercourse, and majority of sexually active individuals are infected with STIs once in their lifetime (Kim *et al*., 2014). It has been reported that among women of reproductive age, the sub-Saharan Africa constituted about 40% of the burden of sexually transited infections with a prevalence of 20.2% of the curable STIs between 2009 and 2016 (Julian *et al*., 2020). The World Health Organization reported 170 million cases of sexually transmitted infections annually (Dessi *et al*., 2018). Early intervention helps prevent complications that can be caused by untreated sexually transmitted infections such as infertility as well as the transmission of the infections to healthy individuals. Jackson and colleagues in 2015 reported that women of reproductive age are at higher risk of acquiring STIs by being involved in risky sexual behaviors which includes: having multiple sexual partners, inconsistence use of condoms, engaging in sexual activities while under influence of intoxicants as well as having sex in exchange for money of drugs. Also, a report by the USPSTF in America stipulated that regardless of sexual behaviors nor age, black women are at an increased risk of acquiring *Chlamydia trachomatis* or *Neisseria gonorrhea* if they live in areas where the prevalence of sexually transmitted infections is high (Jackson *et al*., 2015). However, STIs can be controlled through 8 Sexual health and its linkages to reproductive health: an operational approach early identification and treatment, appropriate case management, improving health care-seeking behavior, partner notification, and preventing and managing complications (e.g. pelvic inflammatory disease).

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2.3 Chlamydia trachomatis

Chlamydia is a gram-negative, obligate intracellular bacterium that belongs to the family Chlamydiaceae (Zareba-Marchewka *et al*., 2020). It is known to cause chlamydia which is a sexually transmitted infection. Among the several chlamydia species, two species are major causes for chlamydial infections in humans. *Chlamydia trachomatis* and *Chlamydia pneumoniae*. *Chlamydia trachomatis* strains can be further divided into three biovars that are also further divided into three serovars. The first is trachoma biovar (which includes serovars A-C), and then the genital biovar (that includes serovars D-K) and last but not least the lymphogranuloma venereum biovar (which includes the L1-L3 serovars) (Shetty *et al*., 2021). Trachoma biovar is responsible for causing non-congenital blindness in developing countries, and the genital biovar is the most dominant sexually transmitted bacterium, whereas the lymphogranuloma venereum (LGV) biovar is the leading cause of invasive urogenital or anorectal infections, basically affects the lymphatic system (Hamill, 2022).

Chlamydia is one of the most common sexually transmitted infections globally, and its prevalence is a significant public health concern. Understanding the epidemiological trends of chlamydia worldwide is essential for developing effective prevention and control strategies. Research has shown that the prevalence of chlamydia varies significantly across different regions, with some areas experiencing higher rates of infection than others (Mafokwane *et al*., 2016). In Africa, *chlamydia* also poses a considerable health burden. The epidemiological trends of chlamydia in Africa reveal a complex interplay of socio-economic, cultural, and health system factors that contribute to the spread of the infection. Sub-Saharan Africa, in particular, faces unique challenges in addressing chlamydia infections due to various social and structural determinants of health.

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2.3.1 History of *Chlamydia*

Chlamydia was discovered in 1907 by the German dermatologist and radiologist Ludwig Halberstädter and an Austrian bacteriologist Stanislaus von Prowazek. They discovered chlamydial cytoplasmic inclusion bodies in the conjunctiva of an infected eye, which was later termed "Prowazek bodies" (Carolyn Black., 2013). The relationship between *chlamydia* and other sexually transmitted illnesses, however, was not discovered until the 1960s. Chlamydia's discovery was a critical turning point in our knowledge of the intricate nature of this bacterial infection.

Scientists had a difficult time figuring out the complex features of this enigmatic bacterium in the early years of Chlamydia research. They distinguished between the many Chlamydia trachomatis serovars, which allowed for the categorization of several strains linked to various clinical presentations. It became clear that chlamydia caused sexually transmitted illnesses and largely impacted the genitourinary system. Early research identified urethritis, cervicitis, and pelvic inflammatory disease as common signs of chlamydia. These results laid the groundwork for additional research into the causes, symptoms, diagnosis, and management of chlamydia.

2.3.2 Taxonomic classification of *Chlamydia trachomatis*

The taxonomic classification of *Chlamydia trachomatis* is as below:

Domain	Bacteria
Phylum	Chlamydiota
Class	Chlamydiia
Order	Chlamidiales

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Family	Chlamydiaceae
Genus	Chlamydia
Species	Chlamydia trachomatis

2.3.3 Morphological forms of chlamydia

Chlamydia exists in two morphological forms: (a) elementary body (EB), which is non-replicating and non-metabolically active yet infectious. The diameter ranges from 0.25 – 0.4. µm. The elementary body can survive in the extracellular harsh environment. It has rigid outer membrane which plays a role in its resistance, by binding to columnar epithelial cells and macrophages. (b) The reticulate body, it is not infectious and is larger than the elementary body with a size of between 0.8 µm to 1 µm. The reticulated body is found in less harsh environments intracellularly. It has fragile membrane, and is metabolically active and it replicates (Murray and McKay, 2021).

2.3.4 Epidemiology of Chlamydia

Chlamydia has a high global burden, affecting people of all ages and genders. According to the World Health Organization (WHO), an estimated 131 million new cases of chlamydia occurred globally in 2016 alone. The highest burden of chlamydia is observed among young people aged 15-24 years. In 2020, WHO reported a decrease in the number of cases reported globally, for which the estimate was 128.5 million cases amongst adults aged 15-49 years. However, it is important to note that chlamydia infections are often asymptomatic, leading to underreporting and a potential underestimation of the true burden. The global impact of chlamydia highlights the need for comprehensive prevention and control strategies to reduce transmission rates, improve diagnosis and treatment, and minimize the associated health consequences (Li *et al*., 2023).

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The genital biovar which causes sexually transmitted infection and LGV biovar which affects the lymphatic system are most prevalent in Africa, Asia and South America. Another type of Chlamydia, which is trachoma biovar is widely distributed. It is commonly found in developing countries where there is poverty and overcrowding populations. It's mostly endemic in Africa, Middle East, India and Southeast Asia and affects children. The most recognized transmission route is through contaminated birth canal. (Buder *et al*., 2019).

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2.3.5 Transmission of *Chlamydia trachomatis*

Chlamydia trachomatis is predominantly transmitted through unsafe sexual intercourse, but it can also be vertical via contaminated birth canal, contaminated clothing or droplets. It is highly transmissible, and the incubation period is 7-21 days. Most infections are asymptomatic, and re-infection is very ~~much~~ common. Perinatal transmission results in neonatal conjunctivitis in about 30- 50% of exposed newborns (Zikic *et al*., 2018). Risk-factors for *C. trachomatis* includes multiple partners, history of sexually transmitted disease infection, infection with another STD, oral contraceptive users and lack of barrier contraception (Jongen *et al*., 2021).

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2.3.6 Life cycle of *Chlamydia trachomatis*

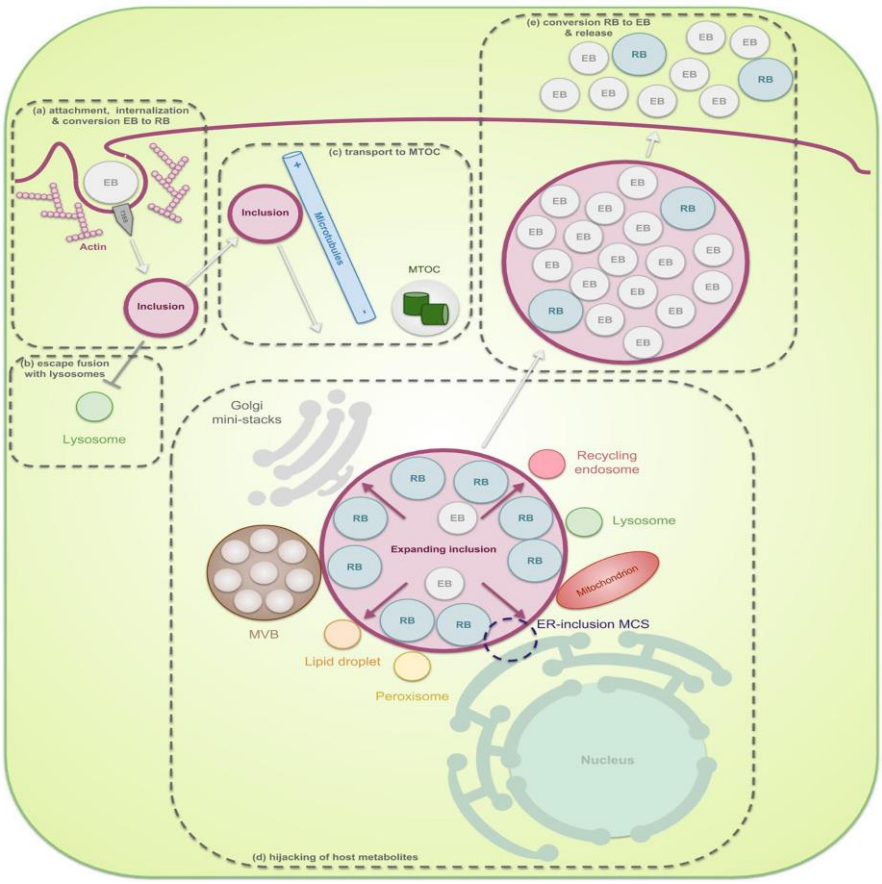


Figure 2.5: Life cycle of *Chlamydia* (Gitsels *et al*., 2019)

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The infection begins when the elementary body (EB) binds to host cell initiated by formation of trimolecular bridge between bacterial adhesins, host cell receptors and heparin sulfate proteoglycans and prevent the fusion of lysosomes with the chlamydia-containing phagosome, which in turn, allows for intracellular survival of the chlamydial elementary body. In order to transition to the non-infectious vegetative form (the reticulate body), the elementary body secretes glycogen which induces the transitioning process. For the next 18-72 hrs., the reticulate bodies begin to divide by binary fission every 2-3 hrs. and starts to fill the endosome (this is detectable via antibody specific stains in the host cells as inclusion bodies containing 100-1000 RBs). Post the division and incubation in the cytoplasmic inclusion, the reticulate body (RB) transforms into new infectious form (elementary body) which is released by exocytosis or by the rupture of the host cell.

2.3.7 Signs and symptoms of *Chlamydia* infection

In women, *C. trachomatis* causes asymptomatic infections in about 80% of cases. However, when symptoms appear, women experience cervicitis (infection of the cervix), urethritis and salpingitis (swelling of the fallopian tubes), postpartum fever and increased risk of premature birth and ectopic pregnancy. In males, about 75% of infected males are symptomatic. They experience urethritis, dysuria and pyuria and it is also the cause of non-gonococcal urethritis in 35 – 50% males, nevertheless it is a common cause of post-gonococcal urethritis (Jongen *et al*., 2021).

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During the first stage of infection by the LGV biovar, patients experience development of non-painful vesicular lesions at the site of infection, then this is followed by fever, myalgia and headaches. However, during the second stage of infection the draining lymph nodes start to swell

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up, accompanied by headache, fever and myalgia. The swollen draining lymph nodes can rapture and proctitis, ulcers or elephantiasis (elephantiasis of the draining tissue) develop (Samson, 2020).

2.3.8 Diagnosis, treatment and prevention *Chlamydia infections*

Chlamydia trachomatis can be diagnosed in the laboratory using different methods/techniques. Antigen detection, serology and nucleic acid amplification tests (NAATs). *Chlamydia trachomatis* can be treated with antibiotics including tetracycline, erythromycin and sulfonamides. Preventative measures that can be undertaken for *C. trachomatis* are to treat patients with their sexual partners, treatment should go hand-in-hand with proper sanitation and couples should be advised to practice safe sexual practices (Adachi *et al*., 2021). First line treatment of *C. trachomatis* is an oral dose of doxycycline (100mg) administered 2 times for 7 days. The second line treatment is an oral dose of 1g of Azithromycin (Peters *et al*., 2022).

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2.4 Trichomonas vaginalis

Trichomonas vaginalis is one of the leading causes of non-viral sexually transmitted infections. It's a parasitic protozoan pathogen belonging to the family Trichomonadidae under phylum Protista. It causes an infection called trichomoniasis and is transmitted through sexual intercourse with infected persons. Trichomoniasis occurs mainly in young women of reproductive age, but it infects both men and women and affects the urogenital system, urethra and the vagina. It's been associated with vaginitis, urethritis, tubal infertility, pelvic inflammatory disease and cervicitis (Adjei *et al*., 2019). It has been also demonstrated that *T. vaginalis* facilitates the acquisition of

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HIV and its transmission, cancer, pre-term birth and birth of underweight babies. An infection with *Trichomonas vaginalis* can be asymptomatic, but if not, clinical presentations resulting from trichomoniasis in women includes vulvar irritation, and yellowish-green frothy vaginal discharge and men experience burning sensation after urination and ejaculation, urethral irritation and mild discharge (Asmah *et al*., 2018). The severity of untreated trichomonas vaginalis infection is much more than previously acknowledged, in men it is associated with prostatitis, epididymitis and balanoposthitis and in women it is associated with cervical neoplasia and peri-natal morbidity (Patel et al., 2018). Pregnant women, and immunocompromised individuals are at high risk of disease complications, therefore require early interventions (Price *et al*., 2018).

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The World Health Organization reported that *T. vaginalis* accounted for 143 million new cases annually and in Africa it accounted for 25.64% of the burden of infections caused by *T. vaginalis* which is more than the incidence of infections caused by *Neisseria gonorrhea, Chlamydia trachomatis and syphilis*. In sub-Saharan Africa infections with *T. vaginalis* accounted for 11.5% in women of reproductive age (15 – 49 years) (Adjei *et al*., 2019, Masha *et al*., 2019). Early intervention for curable STIs like trichomoniasis such as immediate detection and treatment is appropriate to mitigate complications associated with the infections.

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2.4.1 Taxonomic classification of *Trichomonas vaginalis*

Below is a tabulation showing the classification of *Trichomonas vaginalis* from domain down to species level.

Domain	Eukarya
Kingdom	Protozoa

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Phylum	Metamonada
Class	Parabasilia
Order	Trichomonadida
Genus	Trichomonas
Species	*Trichomonas vaginalis*

2.4.2 History of *Trichomonas vaginalis*

Trichomonas vaginalis was first identified by Alfred Donne in 1836 as a motile microorganism in women who were presenting with vaginal discharge and genital irritation, their discharge was very purulent and had a frothy leukorrhea. In 1916, the term "trichomoniasis" was used to describe a clinical condition where *T. vaginalis* colonized the vaginal mucosa by O. Hohne. *Trichomonas vaginalis* was classified as a venereal disease amongst the group of soldiers of Italian origin who were serving in the Eastern army in Ethiopia by L. Proccaccini between 1934 and 1939. By 1940, it was discovered that *T. vaginalis* caused vaginitis in 9 out of 20 women through *T. vaginalis* culture by R.E Trussell and in 1959, two colleagues, D.H Clarke and E. Solomons established routine culture examination for *T. vaginalis*. Between 1960s – 1970s, more research was conducted with more emphasis on the biochemical test and microscopic examination of *T. vaginalis* in order to understand its characteristics and behavior and in the 60s, 5-Nitroimidazoles was used for the management of infection caused by *T. vaginalis*. The pathogenesis and immunology of *Trichomonas vaginalis* were studies through application of immunologic methods and molecular biology techniques from 1980s to 2000s. In 2007, *T. vaginalis* whole genome sequencing was done as a joint project, which identified *T. vaginalis* strain G, as approximately 160 megabase Mbp genome (Harp and Chowdhury., 2011).

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2.4.3 Structure of *Trichomonas vaginalis*

Trichomonas vaginalis is a flagellated eukaryotic protozoan parasite that exists in two forms, one as a trophozoite form and one as an amoeboid form. The trophozoite form is a free swimming flagellated pear-shaped cell, and the amoeboid form is characterised by increase in surface contact induced upon trophozoite contact in vivo with epithelial cells from the vagina, cervix, and urethra. *Trichomonas vaginalis* is a micro-aerototolerant amitochondrial flagellate residing in human urogenital tract. Each of the trophozoite form has 4 anterior flagella and a single recurrent flagellum embedded in an undulating membrane that is supported by a non-contractile costa. The cell body has a longitudinal piercing by a thin hyaline structure known as the axostyle, which projects posteriorly. The nucleus is contained within a porous shell. The structure is represented in figure 2.6.



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Figure 2.6: trophozoites of *T. vaginalis*, stained with Giemsa (Ifeanyi *et al*, 2018).

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2.4.4 Life cycle of *Trichomonas vaginalis*

Trichomonas vaginalis trophozoites inhabits the female lower genital tract and the male's urethra and prostate, where they replicate through longitudinal binary fission. The trophozoites are then transmitted to the next individual during sexual intercourse (CDC, 2024; Schwebke and Burgess, 2004).

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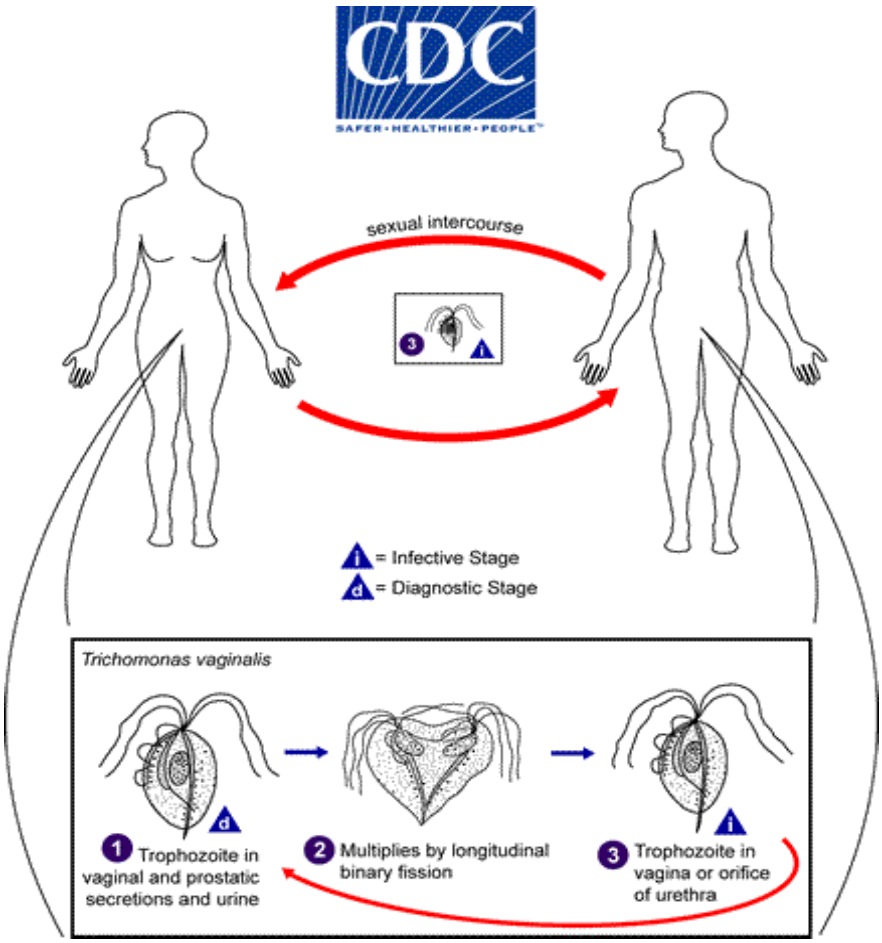


Figure 2.7: Life cycle of *Trichomonas vaginalis* (CDC, 2024).

2.4.5 Transmission of *Trichomonas vaginalis*

Trichomoniasis is a sexually transmitted infection, given that *T. vaginalis* infections are typically limited to the urogenital tract, nonsexual transmission is exceedingly rare, although other modes of transmission have been reported. For women, trichomoniasis frequently occurs in the lower genital tract i.e. vulva, vagina, cervix and urethra, and in men the penis (urethra) is the most

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common affected part. During unprotected sexual intercourse with an infected individual the parasite is transmitted from the penis to the vagina or from the vagina to the penis (Benjamin *et al.*, 2024).

According to a study conducted in Zambia amongst self-reported virgins, a prevalence of 24.7% of trichomoniasis was reported. There were significant associations with the use of pit latrines and bushes in place of toilets, this also included poor bathing conditions such as not using enough soap and sharing of bathing water (Crucitti *et al.*, 2011). Iatrogenic transmission (transmission due to medical examination) has also been described. Although very uncommon, this type of transmission usually occurs in low resource settings where proper hygiene is not practiced, an example is a case of a traditional healer who transmitted trichomonas during vaginal examination without using gloves. In addition to the aforementioned modes of transmission, non-sexual transmission through use of sex toys, wet wash cloths were reported from sexual activity between two females (Van Gerwen *et al.*, 2023).

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2.4.6 Pathogenesis of *Trichomonas vaginalis*

Two virulence factors: cytoadherence and cytotoxicity are known to be involved in the pathogenesis of *T. vaginalis*. Also, the presence of what is called *Trichomonas vaginalis* virus is also suspected of increasing the virulence of *T. vaginalis* (Graves *et al.*, 2019). The trophozoite form of *Trichomonas vaginalis* attaches to mucosal surfaces of the lower urogenital tract and replicates by longitudinal binary fission. The incubation period has been reported to be between 4 – 28 days. However, *Trichomonas vaginalis* can survive for longer depending on the varying acidic environment of the vagina. Rather than the columnar epithelia, *T. vaginalis* prefers to infect the squamous epithelia. The release of cysteine proteases, lactic acid, and acetic acid lowers the pH of

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the vaginal fluid and upsets the levels of glycogen. For *T. vaginalis* to survive, it needs to come into direct contact with the tissues of the vagina, urethra, or prostate. The parasite causes vaginal epithelium desquamation, metaplastic changes, and petechial hemorrhage. Intracellular edema, or chicken-like epithelium, is the most typical sign of trichomoniasis (Molgora *et al*., 2023).

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2.4.7 Trichomoniasis clinical manifestations

Most infections, especially in men, are asymptomatic, though some people experience urethritis, epididymitis, or prostatitis. Dysuria, dyspareunia, and an unpleasant yellowish-green foamy discharge are all possible side effects for women who may experience severe pruritic vaginitis (Khaskheli *et al*., 2021). There is a natural occurrence of cervical spine erosion. Endometritis and pyosalpingitis are uncommon complications. Symptomatic men develop a disease called persistent or chronic urethritis due to prolonged infection with *T. vaginalis*. Higher regions of the urogenital tract, the prostate, and seminal vesicles may be impacted in severe infection cases. Important indicators of a serious infection include ~~a~~ tender, swollen prostate, dysuria, nocturia, and epididymitis. In women, the diseases that they incur as a result of *T. vaginalis* infection is called persistent vaginitis. Infected women with persistent vaginitis have a foul-smelling, greenish-yellow liquid vaginal discharge after a 4–28-day incubation cycle (Ford *et al*., 2024). It is more likely that the vaginal acidity that occurs before and shortly after menstruation is the cause of the symptoms' worsening. There may even be chafing, burning, and scratching. Red punctate lesions can be seen when examining an infected woman's vaginal mucosa. Urethral involvement, dysuria, and increased frequency of urination are the most common symptoms. Less people get cystitis. Children with respiratory infections and conjunctivitis have both been reported to have *T. vaginalis*

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that is transmitted to them through the birth canal or during vaginal delivery, vaginalis trophozoites are transferred from an infected mother to the offspring (Ibáñez-Escribano *et al*., 2024).

2.4.8 Diagnosis and treatment of *Trichomoniasis*

The gold standard in diagnosing or testing samples for *T. vaginalis* has been culture, and women were also tested using microscopy which has 50-60% sensitivity. Recently nucleic acid amplification test has been available for testing *T. vaginalis* and is highly sensitive and specific and can be applied on many genitourinary specimens for men and women (Graves *et al*., 2019).

Trichomoniasis can be cured successfully by effective short course regimens that can be used between 1 and 7 days (Patel *et al*., 2019). The 5-nitroimidazole class of antimicrobials is the only class of drugs that has been approved for the treatment of infection caused by *T. vaginalis*. It is treated with metronidazole and tinidazole, but recently there has been evidence of resistance to the drugs (Dunne *et al*., 2003; Graves *et al*., 2019). Treating trichomoniasis with multidose of metronidazole (500mg 2 times a day for 7 days) has been shown to reduce the number of women who re-test positive by half after a month of their cure visit, in contrast with those who received a single dose (2g). Therefore, this is the recommended treatment for trichomoniasis by CDC. This was established on a randomized trial conducted on women who were HIV positive and had STDs (Kamb *et al*., 1998).

Another regimen that is used for the treatment of trichomoniasis and known to be more effective than metronidazole is tinidazole (a single dose of 2g orally). Tinidazole is also a drug of choice for both women and man. It has been shown to be more efficient than metronidazole, it has a longer half-life of 12.5 hours as compared to that of metronidazole which is 7.3 hours, and it can reach high levels in genitourinary tract and serum. The cure rate of metronidazole is 84%-98% and of

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tinidazole is 92%-100%. Therefore, other treatments like metronidazole gel are not recommended because it's less efficient and does not reach therapeutic levels in the urethra and perivaginal glands. Metronidazole is much more used than tinizadole mainly because tinizadole is more expensive (Marques-Silva *et al*., 2021; Schumann and Plasner 2023).

To avoid recurrent trichomoniasis infection, those who receive treatment for trichomoniasis are recommended to also have their sexual partners treated. And during the treatment period, they are also advised to abstain from sexual activities and they should re-test after a month of treatment.

2.4.9 *Trichomonas vaginalis* drug resistance

Treatment of trichomoniasis just like many other infections, is not always successful, it's also met with treatment failures. This is due to several factors including reinfection from untreated sexual partners and also patients not adhering to full course of treatment. Apart from that, the biggest treatment failure is due to resistance against the regimens used. Antimicrobial resistance to metronidazole has been reported to be around 4% - 10%, yet for tinidazole has been observed in 1% of the infections (Ghosh *et al*., 2018).

2.5 *Neisseria gonorrhea*

Neisseria gonorrhoeae (also known as gonococcus/gonococci) is an etiological agent that causes a disease known as gonorrhea. It is a gram-negative diplococcus bacterium and is passed on through sexual intercourse. It is only known to cause infections in humans. Base-line infection sites include columnar epithelium-lined mucous membranes of the urethra, rectum, endocervix, conjunctiva and the pharynx (Lin *et al*., 2021). In addition, *Neisseria gonorrhea* has been classified as a priority 2 microorganism on the Published WHO Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New antibiotics. This shows that it is

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vital to develop new antibiotics to treat Gonorrhea. It is an obligate human pathogen that causes mucosal surface infections in the reproductive tracts, rectum, pharynx as well on the conjunctiva. It has the ability to evade and modulate innate and adaptive immune systems in order to enhance replication and survival (Quillin and Seifert, 2018).



Figure 2.8: Illustration of *Neisseria gonorrhoea* (Horner, 2023)

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2.5.1 Taxonomic classification *Neisseria gonorrhea*

Below is a tabulation showing the classification of *Neisseria gonorrhea.*

Domain	Bacteria
Phylum	Pseudomonadota

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Class	Betaproteobacteria
Order	Neisseriales
Family	Neisseriacae
Genus	Neisseria
Species	*N. gonorrhoeae*

2.5.2 Discovery of *Neisseria gonorrhoea*

The term Gonorrhea goes back to the Greek clinician Galenus from Pergamon (131-200 AD) in the second century AD. He coined the diseases as seminal flow, although Lucio Celio Aureliano is the one who introduced the term gonorrhoea to describe an unwanted discharge from the urethra that occurred without an erection. The name was derived from the Greek "gonos" which referred to seed and "rhoia" which means river, this was reported in the chapter "De debilitate seminalium viarum". It was a chapter in his most famous book titled De morbis acutis et chronicisn (Vicentini *et al*., 2019; Jose *et al*., 2020)

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2.5.3 Virulence factors associated with *Neisseria gonorrhoea*

Neisseria gonorrhea has many virulence factors that it produces in order to facilitate the pathogenesis and cause the disease. These include~~s~~:

Capsules- these prevents phagocytosis of the *Neisseria gonorrhea*. In contrast to *Neisseria meningitis*, *N. gonorrhea* does not from true carbohydrate capsules. Instead, it forms a polyphosphate capsule that is loosely linked with its cell surface. The capsule is most apparent in newly isolated gonococci and has anti-phagocytic properties.

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Pilli- These are structures that are hair-like and they extend from the cytoplasmic membrane through the outer membrane. Pilli are made up of proteins known as pillins, which are repeating protein subunits. Protein pillins expression is controlled by the p gene complex. The pillins of all gonococci strains are antigenically different. As a result of chromosomal rearrangements, gonococcal pilli have a marked antigenic variation (Meyer *et al*., 2012). Gonococcal pilli are an important virulence factor because:

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- They play an important role in bacterial virulence
- They mediate attachment of *Neisseria gonorrhea* to non-ciliated epithelial cells
- They also contribute to virulence by preventing the uptake and killing of the gonococci by neutrophils

Por proteins- the por proteins are on the outer membrane proteins and they confer resistance to the serum killing of the *Neisseria gonorrhea* by preventing the fusion of phagolysosomes in neutrophils. **Opa proteins**- mediate the adhesion between bacterial cells and also between bacteria and the eukaryotic cells. **Rmp proteins**- produces antibodies that block the serum bactericidal activity against the gonococci cells (Shaughnessy et al., 2019). **IgA protease**- these proteases destroy IgA immunoglobulin. **Beta-lactamase**- these degrades the beta-lactam rings in the penicillins. **Plasmids**- plasmid-borne virulence determinants are linked with antimicrobial resistance. Other virulence factors such as **Lipo-oligosaccharide** has the endotoxin activity (Lenz and Dillard, 2018).

2.5.4 Transmission of *Neisseria gonorrhoea*

Since *N. gonorrhoeae* is an obligatory human colonizer, it is unable to thrive outside of its host. This is a requirement for a pathogen to be successful in spreading to new hosts. Sexual networks

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play a crucial role in the transmission of the disease between hosts. It starts in the core, high-risk population where most infections occur and moves to the fringe, medium-risk group where it is transmitted by members' partners and returned to the core group (Quillin and Seifert, 2018). Those with several sexual partners who engage in unprotected intercourse are considered high-risk populations. *N. gonorrhoeae* attaches itself to sperm and is easily transferred from men to their partners through them. Nevertheless, it is less clear how women maintain the effectiveness of transmission to their partners. It is therefore believed that bacterial sialidases, which are secreted by the cervicovaginal microbiota of women, must first desialylate *N. gonorrhoeae* lipooligosaccharide (LOS) in order to facilitate effective transmission from women to men. This is because the surface of *N. gonorrhoeae* must be free of sialic acid in order to successfully bind and enter urethral epithelial cells of men (Lenz and Dillard, 2018).

2.5.5 Pathogenesis of *Neisseria gonorrhoea*

Neisseria gonorrhoea primarily colonizes the genital mucosa, however, it also colonises the Nasopharyngeal, ocular and anal mucosa. Since *N. gonorrhea* does not express potent exotoxins, the activation of innate immune responses at the colonization sites results in damage. Gonococcus establishes infection by 1. Colonizing epithelial cells, 2. Invading the epithelial cells and 3. Trafficking into the sub-epithelial tissue (Quillin and Seifert, 2018).

2.5.6 *Neisseria gonorrhoea*: Sign and Symptoms

Signs and symptoms due to gonococcal infection differ depending on the site of infection. However, co-infection with other organisms such as *Chlamydia trachomatis, Mycoplasma genitalium*, bacterial vaginosis and *Trichomonas vaginalis* generally occurs. Urethral infection in men can occur in more than 90% of cases, with onset of discharge and dysuria within a week after

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being exposed to the infection (Malleswari *et al*., 2023). Patients rarely present with testicular and epididymal pain with swelling and tenderness during examinations. For women, urethral infection causes dysuria without frequent urination. If the infection site is the endocervix, individual's experiences increased vaginal discharge. This occurs in about 50% and lower abdominal pains ~~is~~ are usually reported in a quarter of cases. If there is co-infection with *Chlamydia trachomatis*, pelvic and lower abdominal pain may be experienced. When it comes to rectal infections many cases are asymptomatic, however, if symptoms occur, these include anal discharge and pains in the perianal area. The pharyngeal infection presents with the sore throat, however, most of these cases are asymptomatic. In addition, individuals could develop complicated gonococcal infection. Epididymo-orchitis, prostatitis and pelvic inflammatory diseases can occur as the result of transluminal spread of *Neisseria gonorrhea* from endocervix or urethra. Infected mucous membranes (Shephard *et al*., 2024).

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2.5.7 The epidemiology of *Neisseria gonorrhoea*

The World Health Organization (WHO) gave an estimation of 87 million new Gonorrhea cases globally in 2016. And then the burden increased to 106 new cases yearly on a global scale (Suay-Garcia and Perez-Garcia, 2018). However, in a 2020 report, it was estimated that 82.4 million cases were being reported globally. In high-burden countries, the prevalence of gonorrhea is highest in vulnerable groups, including men who have sex with males, sex workers, transgender women, teenagers, and young adults (Machado *et al*., 2022).

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2.5.8 Diagnosis of *Neisseria gonorrhoea*

One way to diagnose *N. gonorrheoa* is the syndromic approach, whereby a healthcare practitioner assesses the signs and symptoms and make a diagnosis. The syndromic technique may be sufficient

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for male urethral discharge, but it has low sensitivity and specificity for detecting infections in women and non-urethral infections in men. This could lead to inadequate treatment, which increases the likelihood of resistance developing (Mayer and Buder *et al*., 2020). The laboratory diagnosis of *N. gonorrheoa* makes uses of clinical samples such as first-catch urine or swab specimens from the pharynx, conjunctiva, anorectal, or urogenital regions for direct detection of the infection. There are currently a number of methods for detecting *N. gonorrheoa*, but the most effective ones are culture and nucleic acid amplification technologies (NAATs). In some circumstances, stained urogenital specimens can also be examined under a microscope. Nevertheless, because of their low sensitivity and specificity, DNA probe assays, antigen tests, and serology to identify antibodies against *N. gonorrheoa* are not advised for use in laboratory settings (Papp *et al*., 2014).

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2.5.9 *Neisseria gonorrhoea*: Antibiotic resistance

Because of its resistance to 3rd generation Cephalosporins and Fluoroquinolones, it has earned the position of organism of Priority 2, which highlights its importance on developing new drugs to treat it (Mlynarczyk-Bonikowska *et al*., 2020). Global concern was raised in 2007-2018 due to the emergence of resistant strains of *N. gonorrhoea* to ceftriaxone and Cefixime and in addition to that the strains are resistant to penicillins, tetraclyclines, fluoroquinolones and azithromycin (Peters *et al*., 2022).

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2.5.10 Treatment of infections caused by *Neisseria gonorrhoea*

The treatment of *Neisseria gonorrhea* includes the use of third generation Cephalosporins (Ceftriaxone,) and fluoroquinolones (Unemo *et al*., 2019). First line option is a 500mg single dose

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of ceftriaxone administered via intramuscular injection. The second line which is the effective substitute is an oral single dose of 800mg of Cefixime (Peters *et al*., 2022).

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Chapter 3: A Community-based Survey of the Knowledge, Attitude and Practice of Reproductive Age Women Concerning Contraceptives and their Usage in the Vhembe District of Northern South Africa

Submitted to Healthcare journal (written in the journal style).

Abstract

The world's population is constantly growing at a higher rate, especially in developing countries. The increase in population has an impact on the development of the economy. Apart from population growth there is a need to spread more knowledge and raise awareness on the use of contraceptives. Use of contraceptives saves lives by preventing unintended pregnancies, which in turn reduce infant and mother mortality as well as abortions. The objective of this study was to assess knowledge, attitude and practices of contraceptives amongst women of reproductive age in Northern South Africa. A cross-sectional study was conducted in Northern Limpopo, in the Vhembe district. Paper-based questionnaires were used to collect demographic data of participants as well as their knowledge, attitude and practice concerning contraceptives and their usage. Of the 364 women who participated in the study, 96.11% were aware of what contraceptives are. Participants acquired knowledge through more than one platform. With 41.67% through media, 41.38% via health workers and 31.61% through social networks. Participants had knowledge of at least one method of contraception. In practice, 75.1% were using at least one form of contraceptive and the most common methods were injectables (46.1%), male condoms (23.8%), and pills

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(17.4%). Most participants (96.9%) had a positive attitude towards contraceptives and 94.4% said they think contraceptives are beneficial. With 79.8% agreeing that the main benefit is to avoid unwanted pregnancy. The main reasons influencing the use of contraception from the study population is to avoid unwanted pregnancy and to maintain birth spacing. The rate of awareness of family planning methods is ~~high, yet~~high yet translating the knowledge to practice still needs improvement. More contraceptive awareness campaigns are needed especially to young females in the region.

Keywords: Attitude, Contraceptives, Family planning, Knowledge, Reproductive health, Practices

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3.1 Introduction

Each and every person has the right to decide on when to have children without feeling oppressed or discriminated against. Nowadays, people achieve that through employing family planning. According to the definition of family planning, it is a style of thinking and living that people voluntarily embrace based on their knowledge, attitude and responsible choices. It is a method employed by couples in order to limit or space the number of children they want to have [1, 2]. People turn to contraceptives for different reasons. For some it's about spacing birth or limiting number of birth or to avoid unwanted pregnancy or used as prescription treatment for some conditions. The use of contraceptives is perceived as necessary in improving people's health, it is preferable that a gap of 2-3 years between births be maintained and number of births be limited to 3 per woman [3]. In Sub-Saharan African countries, there is a high rate of unmet need in family planning. The increase in population, has an impact on the development of the economy [4]. Therefore, if the gap can be closed it could prevent about 76 million of unplanned pregnancies that occur in developing countries as it is reported that women in developing countries have low prevalence when it comes to practicing use of contraception [5]. As it stands, Africa has the highest fertility rate. The fertility rate per woman is 4.7 children [6]. The world's population is estimated to reach 9.7 billion by 2050. According to Statistics South Africa, in 2016 South African population was 55.6 million with a yearly increase rate of 1.23% per year. By 2022, it was estimated that the population would've reached 60.2 million [7].

Family planning is used to control population, and with a high rise in the world's population it is imperative that contraceptive use awareness is raised. This should be done to reduce the rate at

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which mothers give birth and at least to space birth between children in order to not stretch their health to extreme, as this leads to poor reproductive health and this problem can lead to untimely death of women [8]. Most African countries, including South Africa are facing teenage pregnancy as well, which is an important issue that needs to be addressed. This could be achieved by encouraging contraceptive use. This will also play a role in reducing poverty in many communities in South Africa.

There are several methods that are applied in family planning, such include injectables, pills, IUD and implants amongst others. Their period of use could be short term or long-term [9]. The use of contraceptives in many cases is regarded as women's responsibility, and how they perceive contraceptives can also be influenced by their partners. Unplanned and unexpected pregnancies have an impact on the economy and public health. This has been reported to increase mortality rate as well. Unintended pregnancies lead to people deciding to terminate as many would not be ready to have another child. About 40% of unwanted pregnancies happen to those who do not use contraceptives and yet do not want to become pregnant [10].

In South Africa, the prevalence of modern contraceptive use is estimated at 64.6%, indicating widespread reliance on hormonal and other contemporary methods of birth control.~~South Africa has a rate of 64.6% of contraceptive use of modern methods~~. However, as prevalent as the use of contraceptives is, South Africa still faces many women having unintended pregnancy and high rate of teenage pregnancy especially from low to middle class income families. At the end, some opt to terminate the pregnancies because they were not planned, and they will be financially strained. Thus, they end up going for illegal abortions [11, 12]. Encouraged use of contraceptives will reduce maternal and infant mortality addressing the Sustainable Development Goal of the United Nations

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No.3 which is good health and well-being in order to ensure healthy lives and promote well-being for all at all ages). This specifically addresses target 3.1 and 3.2 which are to reduce maternal mortality ratio to less than 70 in 100 000 live births globally and to end death of newborns and children under five years that could be prevented by reducing neonatal mortality to as low as 12 in 1000 live birth and to at least lower than 25 per 1000 live birth for children under 5 [13, 14].

Despite the availability of many contraceptive methods, individuals may lack adequate knowledge on the options, which may lead to unintended pregnancies, unsafe pregnancy terminations as well as maternal health risks. Understanding the attitude and practices of contraceptive users can aid policymakers and healthcare providers to design targeted interventions as well as improving the uptake, adherence and method choice for contraception. In addition, assessing knowledge and practices as well as attitudes helps to identify gaps in awareness, barriers and misconceptions as well as to identify side effects experienced by end-users. Therefore, this study aimed to assess the knowledge, attitude and practices of contraceptive use among women of reproductive age and to also understand their experiences with modern methods of contraception.

3.2 Materials and methods

3.2.1 Ethical consideration

Ethical clearance to conduct the study was obtained from the University of Venda Hhealth and Eethics Ceommittee (SMNS/20/MBY/05/1505). Permission was also obtained from study participants. The objectives of the study were clearly explained to the participants as well as their right to confidentiality and to withdraw from the study if they don't want to continue. Study participants were requested to sign a consent form before the study startedss.

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3.2.2 Study setting

A community based cross-sectional study was conducted to assess knowledge, attitude and practice of contraceptives among women of reproductive age, in the rural and semi-urban areas of Vhembe district. Participants were recruited in Thohoyandou and surrounding areas, namely: Maungani, Vuwani, Khubvi, Block-F, P-west, Golgotha and Nzhelele (Biaba, Tshirolwe and Mandala).

3.2.3 Study population

A total of 364 women, aged between 15 and 49 years, were eligible to participate in the study. Participants were selected using a convenience sampling method.~~A total of 364 women of ages between 15 and 49 years were eligible to participate in the study.~~

3.2.4 Study instrument

A paper-based questionnaire was developed by reviewing previous studies with similar objectives and adjusted to suit our study objectives and then by an expert panel. The questionnaire consisted of four sections (i) Demographic data (ii) Knowledge (iii) Attitude and (iv) Practice of contraceptives use among women of reproductive age. The questionnaire consisted of closed-ended questions with tick boxes to indicate responses and also had open-ended questions that required participants to write their responses. In section (i) participants were asked to provide demographics such as age, race, marriage status and~~,~~ number of children. Number of sexual partners, any idea of what contraceptives are, use of contraceptives and perception of contraceptives and many more. Section (ii) Consisted of questions that would allow the researcher

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to test if study participants have knowledge about contraceptives and how much knowledge do they have. Section (iii) Consisted of questions that would let the researcher know how study participants perceived contraceptives. And last but not least, section (iv) Consisted of questions that are based on the experience of the study participants with using contraceptives.

3.2.5 Pilot study and validation of the study tool.

The questionnaire was given to three independent scientists from the University of Venda from Ppublic Hhealth Department and from Ssocial Sscience Department for comment. All the requested corrections were made, and the validated questionnaire was used in the pilot study. A pilot study was completed from the 23rd of October – 06 November 2020 at Elim and Nzhelele Khalavha. A total of 34 participants were recruited and asked to sign the consent form and to answer the questionnaires supplied by the researcher. This was done in order to find out how long it will take to complete the questionnaire and to highlight methodological mistakes or ambiguity in questions. Study participants took approximately 30 mins to complete the questionnaires. No problems were raised concerning the questionnaire or the consent form. On that account, no changes were made on both outlets. Data from collected questionnaires was entered onto excel sheet and Cronbach's co-efficient was calculated in order to further validate the consistency of the questionnaire. A value of 0.82 was obtained, which indicated that the internal consistency was high.

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3.2.6 Data collection

A validated self-administered questionnaire was provided to study participants to fill -out. Convenience sampling was used to recruit a total of 381 participants to willingly take part in the study. Prior to distribution of the questionnaires, participants were made aware of what the research entails, its methods and that they had the right to withdraw should they wish to do so at any moment, ethical approval and how their data was going to be kept confidential and were asked to sign the consent form. It was taken into consideration to not include participants who took part in the pilot study in order to avoid biasedness as the participants would have seen the questionnaire already. Interviewers guided study participants in translating questions to the Tshivenda language for participants to answer and for those who could not write interviewers were writing answers for the participants.

3.2.7 Statistical analysis

Collected data was entered and cleaned on an excel spread sheet. SPSS software V27 and Stata 12 were used to analyze the data. Descriptive statistics was used to describe demographic, knowledge, attitudeattitude, and practice data. To determine the association between variablessS, chi square test (X^2) was used. And a *p* value of *0.05* between the associations was considered statistically significant.

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3.3 Results

3.3.1 Socio-demographic data analysis

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A total of 364 women of reproductive age participated in the present study. The age of the participants ranged from 15 to 50. The age group the most represented in the current study was the 20- 30 years, which was 60.16 %. Followed by 18.13 % of those between 15 and 19. The majority of the study participants were black Africans with 96.70% and 11 (3.02%) participants were of Indian origin and 1 participant (0.27%) was colored. A large number of women 142 (41.81%) ended their education at high school, ~~while~~while ~~44.07% of those who~~ reached tertiary levels (college and university) and 22 (6.21%) went further with their studies to postgraduate level. Only 4.80% of the study participants were illiterate and 3.11% had primary school education only. Most participants (94.23%) were Christians while 3.30 % were Muslims and 2.20 % followed the traditional religion and 1 participant (0.27 %) was an atheist. About 155 participants, who accounted for 42.58% had no children while 25% had one child, and 16.48% and 10.99% had two and three children respectively. Only 4.95% of the participants had four or more children. The results are shown in table 3.1.

Table 3.1: Socio-demographic characteristics of the study population.

Age group	**Frequency (n= 364)**	**Percent %**
15-19	66	18.13
20-30	219	60.16
31-40	52	14.29
41 and above	27	7.42
Race		

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Black	352	96.70
Colored	1	0.27
Indian	11	3.02
Education		
Illiterate	17	4.80
Primary school	11	3.11
High school	148	41.81
Tertiary	156	44.07
Postgraduate	22	6.21
Religion		
Islam	12	3.30
Christianity	343	94.23
Traditional	8	2.20
Atheist	1	0.27
Children		
No children	155	42.58
One child	91	25.0
Two children	60	16.48
Three children	40	10.99
4 children and above	18	4.95

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Marriage status		
Never married	267	73.35
Divorced	18	4.95
Widowed	8	2.20
Married only wife	54	14.84
Married first wife	10	2.75
Cohabitation	7	1.92

3.3.2 Knowledge of study participants on contraceptives

A large majority of the study participants (96.11%) had some knowledge as to what contraceptives are. They had knowledge of at least one method of contraception. Most of the participants (41.67%) reported that they acquired contraception knowledge through the media (mainstream and social media), followed by 41.38% who received their knowledge through the health workers in the health facilities. Others knew the different methods through Friends 83 (23.85%), colleagues 30 (8.62%), social network 110 (31.62%) and schools 79 (22.70%). Results are tabulated in table 3.2.

Table 3.2: Knowledge and source of knowledge of contraceptives among the study participants.

Know about contraceptives	**Yes**	**No**
	346 (96.11%)	14 (3.89%)

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Source of knowledge		
Media	145 (41.67%)	203 (58.33%)
Health worker	144 (41.38 %)	204 (58.62%)
Friends	83 (23.85 %)	265 (76.15%)
Colleagues	30 (8.62%)	318 (91.38%)
Schools	79 (22.70%)	348 (77.30%)
Social network	110 (31.61%)	238 (68.39%)

3.3.3 Knowledge of participants on contraceptive types

The study population were aware of several methods of family planning, with the knowledge acquired from different sources. The participants indicated that they knew more than one method. The most common method known to the participants was the injectables (88.41%), the male and female condoms were also commonly known with prevalent percentages of 79.13% and 63.19% respectively. Pills were also one of the dominant method known with the prevalence rate of. 85.51% (Table 3.3).

Table 3.3: Methods known by study participants

Method	Yes	No
Injectables	305 (88.41%)	40 (11.59-%)
Pills	295 (85.51%)	50 (14.49%)

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IUD	116 (33.62%)	229 (66.38 %)
Emergency pills	201 (58.26 %)	144 (41.74%)
Patch	108 (31.30%)	237 (68.70%)
Implants	99 (28.70%)	246 (71.30%)
Periodic abstinence	60 (17.39%)	285 (82.61%)
Vaginal ring	50 (14.53%)	294 (85.47%)
Spermicides	41 (11.92%)	303 (88.08%)
Sterilization	47 (13.66%)	297 (86.34%)
Withdrawal	140 (40.70%)	204 (59.30%)
Male condoms	273 (79.13%)	72 (20.87%)
Female condoms	218 (63.19%)	127 (36.81%)

3.3.4 Responsibility for contraceptives application

When participants were asked whom they think is responsible in making the decision of using contraception between man or woman or both partners, 70.70% said that both partners were responsible, and 27.70% percent believed that it was a woman's decision to use contraception, with only 1.50% saying they think it's a man who should make that decision. Results are shown in figure 3.1.

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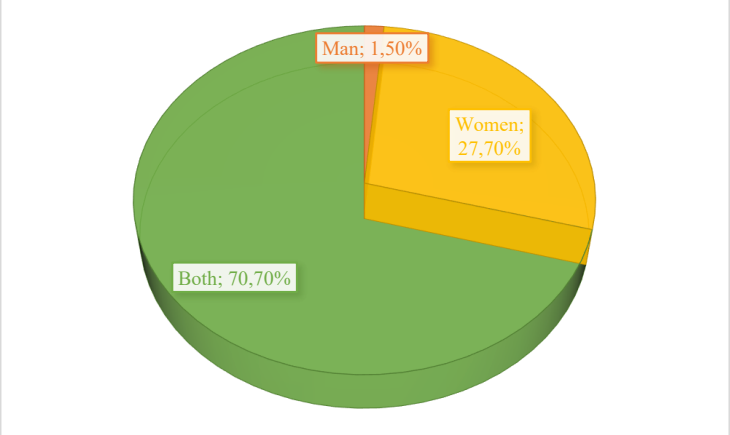


Figure 3.1: Responses of participants on decision making on the use of contraceptives~~Responses of participants on whom do they think is responsible or who should decide on the use of contraceptives.~~

3.3.4 Methods of contraception used by study participants

The most prevalent method of contraception used by study participants was the Injectables (46.1%), the second dominant method used by the participants was Male condoms with a percentage of 23.8%, the prevalence of those who were using pills was (17.4%). The least methods used included vaginal ring and spermicides with a percentage of 0.4%. And also, IUD, periodic abstinence had a low prevalence with 1.1% as well as sponge with 0.7%. Results are presented in figure 3.2.

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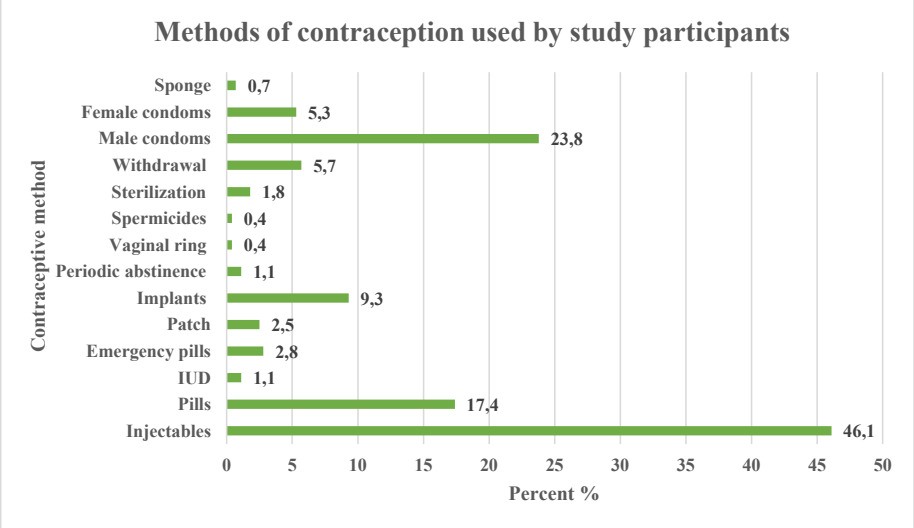


Figure 3.2: Common contraceptive methods used by study population

3.4.5 Factors influencing type of contraception used and Reasons for changing type of contraceptive use

When asked, "What is your priority when it comes to choosing the type of contraceptives to use", 58.2% of the participants priotise the effectiveness of the contraceptives, 32.2% said they choose type of method to use if it's comfortable to use. Others (19.5%) considered convenience of buying and using the contraceptive. Last but not least, 12.1% said the safety of the contraceptive method is what matters to them. Amongst the participants, some reported that they have stopped using contraceptives. When participants were asked if they have previously changed the type of

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contraception they use, 13.93% said “Yes” and 86.07% said “No”. We asked participants to provide reasons why they changed from one contraception to another. Some of the responses were *“it caused me to experience morning sickness all the time’, “I was gaining weight”, “I used injectables before but I fell pregnant while using them”, “I interchange between the injectables, because sometimes there is a shortage at public health facilities”, “the pill used to make me menstruate all the time”.* Other responses were that injectables causes them to menstruate on-stop or that some contraceptives caused them pain during sexual intercourse.

3.4.6 Impact of demographics on the use of contraceptives

Of the 364 women who participated in the study, 265 (75.07%) were using contraceptives. According to the age group, we found that women who were aged between 31-40 years were the ones who were using contraceptives more with a percentage of 77.78%. It seems from the results that, as the age went up, the use of contraception also increased, and the results were statistically significant with a *p value of 0.017*. Since the majority of the participants were African, about 264 of the participants were using some form of contraception and none of women of Indian origin were using any form of contraception. We also observed that educational status seemed to

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influence the use of contraception, where less participants with high school level of education used contraceptives, this occurrence was statistically significant with *P = 0.002*. Similarly, the more the participants had children, the higher the use of contraception as from the results, 66.67 % of women who have no children were using contraceptives and 83.33% of those who have 4 or more children were also using family planning methods. Results for this occurrence ~~was~~ were statistically significant with a *p value of 0.041*. Based on the religious status, many study participants fall under Christianity, followed by Islam, 259 (77.8%) whom are Christians are using any form of contraception and none of the Muslims are using the family planning methods. When it comes to marriage status, divorced women had high percentage of (100.0%) of contraceptive usage, followed by widowed women (87.50%), this was then followed by women who have never been married (75.68%) then those who are married and are the only wife with the least being ladies who are in cohabitation with their partners with a percentage of 42.86%. Table 3.4.

Table 3.4: Use of contraception among study participants in relation to demographics (n=)

Age group	**Do you use any form of contraception?**		**Statistics**
	Yes	**No**	
15-19	36 (59.02%)	25 (40.98%)	$X^2 = 10.2379$
20-30	168 (78.14%)	47 (21.86%)	
31-40	40 (80.0%)	10 (20.0%)	P = 0.017
41 and above	21 (77.78%)	6 (22.22%)	

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Race			
Black	264 (77.19%)	78 (22.81%)	$X^2 = 31.2687$
Colored	1 (100%)	0	P = 0.000
Indian	0	10 (100%)	
Education			
Illiterate	13 (76.47%)	4 (23.53%)	$X^2 = 17.031$
Primary school	10 (90.91%)	1 (9.09)	P = 0.002
High school	89 (63.57%)	51 (36.43%)	
Tertiary	128 (83.12%)	26 (16.88%)	
Postgraduate	17 (80.95%)	4 (19.05)	
Children			
None	100 (66.67%)	50 (33.33%)	$X^2 = 9.9569$
One	71 (81.61%)	16 (18.39%)	P = 0.041
Two	48 (81.36%)	11 (18.64)	
Three	31 (79.49%)	8 (20.51)	
Four and above	15 (83.33%)	3 (16.67)	
Religion			
Islam	0	11 (100.0%)	$X^2 = 37.4402$
Christianity	259 (77.78%))	74 (22.22%)	P = 0.000
Traditional	6 (75.0%)	2 (25.0%)	

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Atheist	0	1 (100.0%)	
Marriage status			
Never married	196 (75.68 %~~%~~)	63 (24.32%)	$X^2 = 13.5865$
Divorced	16 (100%)	0	P = 0.018
Widowed	7 (87.50%)	1 (12.50%)	
Married only wife	38 (71.70%)	15 (28.50%)	
Married first wife	5 (50.0%)	5 (50.0%)	
Cohabitation	3 (42.86%)	4 (57.14%)	

3.4.7 Contraceptive awareness amongst study participants in relation to demographics

In relation to contraception awareness, the 20-30 age group had a higher rate of awareness with 99.54%, and the 31-40 had an awareness of 96.08%, follow by 92.59% 0f the 41 and above and last but not least the 15-19 age group had an awareness of 85.94%. The results is statistically significant with a probability value of 0.000. Black participants had an awareness rate of 96.55% and Indian participants had a rate of 81.82%, these results were statistically significant with a p value of 0.044. Postgraduate participants had a high rate of contraceptive awareness with a

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percentage 100.0%, this was followed by participants who are at tertiary level with a percentage of 98.71%, followed by participants who were illiterate with a percentage of 94.12%. However, these results were not statistically significant. Participants who had one child and those who had four or more children were the most aware of contraception with a percentage of 100.0%, this was followed by study participants who had two children with 96.61%, followed by those with three and none with percentages 95.0% and 93.46% respectively. Nevertheless, the said results were not statistically significant. In terms of religion, Atheist and those who follow traditional ways were more aware with a percentage of 100.0%, subsequently Christians had a percentage of 96.76% and Muslims had a percentage of 75.0%. This was statistically significant with $p= 0.02$. ~~For~~ Pparticipants who are divorced and widowed had a percentage of 100.0%, following this ~~was~~were participants who have never been married and they were the only wives with 98.11% percentage. After this, ~~was~~ there were those who were never married with 96.21%, and subsequently ~~was~~ those who are cohabiting and those who are married and they are the first wives with 85.71% and 80.0% respectively. Nonetheless, the results reported were not statistically significant. Results are shown in table 3.5.

Table 3.5: Contraceptive awareness amongst study participants in relation to demographics.

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Age group	Do you know what contraceptives are?		Statistics
	Yes	No	
15-19	55 (85.94%)	9 (14.06%)	$X^2 = 25.4798$
20-30	217 (99.54%)	1 (0.46%)	
31-40	49 (96.08%)	2 (3.92%)	P = 0.000
41 and above	25 (92.59%)	2 (7.41%)	

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Race			
Black	336 (96.55%)	12 (3.45 %)	$X^2 = 6.2335$
Colored	1 (100%)	0	P = 0.044
Indian	9 (81.82%)	2 (18.18%)	
Education			
Illiterate	16 (94.12%)	1 (5.88)	$X^2 = 7.9478$
Primary school	10 (90.91 %)	1 (9.09%)	P = 0.094
High school	135 (93.10 %)	10 (6.90 %)	
Tertiary	153 (98.71%)	2 (1.29%)	
Postgraduate	22 (100 %%)	0	
Children			
None	143 (93.46%)	10 (6.54)	$X^2 = 9.957$
One	90 (100%)	0	P = 0.116
Two	57 (96.61%)	2(3.29%)	
Three	38 (95.0%)	2 (5.0%)	
Four and above	18 (100%)	0	
Religion			
Islam	9(75.00)	3 (25%)	$X^2 = 15.0211$
Christianity	359 (96.76%))	11 (3.24%)	P = 0.002
Traditional	7 (100%)	0	

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Atheist	1 (100%)	0	
Marriage status			
Never married	254 (96.21%)	10 (3.79%)	$X^2 = 10.5967$
Divorced	18 (100%)	0	P = 0.060
Widowed	8 (100.0%)	0	
Married only wife	52 (98.11%)	1 (1.89%)	
Married first wife	8 (80.0%)	2 (20.0%)	
Cohabitation	6 (85.71%)	1 (14.29)	

3.4.8 Attitude of study participants towards contraception

The majority of respondents demonstrated a high level of awareness and positive perception regarding contraceptive use. Specifically, 96.9% of participants agreed that it is important to know about contraceptives, while only 3.1% responded negatively. Similarly, 94.4% of respondents believed that contraceptives are beneficial, with only a small proportion (5.6%) expressing disagreement.

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When asked about specific benefits associated with contraceptive use, 79.8% of participants agreed that contraceptives help in avoiding unwanted pregnancies, whereas 20.2% disagreed. A lesser proportion (47.4%) agreed that contraceptives are useful for maintaining birth spacing, while 52.6% did not perceive this as a benefit. Notably, only 20.7% of respondents agreed that contraceptives play a role in limiting the number of births, with 79.3% expressing disagreement. Perceptions regarding maternal health benefits were particularly low, with only 4.0% agreeing that contraceptives improve the health of a mother, while a striking 96.0% disagreed with this statement.

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Table 3.6: Attitude of study participants towards contraception.

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Is it important to know about contraceptives?		**Percent %**
	Yes	96.9
	No	3.1
Do you think contraceptives are beneficial?		
	Yes	94.4
	No	5.6
Benefits of using contraceptives		
Avoid unwanted pregnancy	Agree	79.8
	Disagree	20.2
Maintain birth space	Agree	47.4
	Disagree	52.6
Limit number of birth	Agree	20.7

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	Disagree	79.3
Improve health of a mother	Agree	4.0
	Disagree	96.0

3.4 Discussion

In the present study we set out to assess the knowledge, ~~attitude~~attitude, and practice of family planning methods among community women of reproductive age. Most women who participated were from the age group 20 – 30 ~~who are young women and most of them already had one or two children~~ and also majority of study participants had no children. This study was assessing if women of reproductive age are aware of the different methods of family planning and how they feel towards them and if they are actively practicing any of those methods in order to determine the prevalence of use.

The current study demonstrated that 96.1% of the study participants were aware of what contraceptives are. Similarly, in a study done in India they also found a high rate (91%) of awareness as to what contraceptives are [15]. The results were also similar to a study conducted in Pakistan that also found high prevalence of contraceptive knowledge from study participants [9]. A study done by Bongongo [16] in South Africa also found high awareness rate of contraceptives which was 72.2% which was deemed satisfactory. In a study done in Nigeria among tertiary students, they found the awareness rate to be low with a percentage of 37.9% [17] Although study participants cited that they received their knowledge about contraceptives from more than one source, media was mentioned as the major distributor of contraceptive knowledge with 41.7%

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followed by 41.4% who received awareness through health workers at ~~Public~~public health facilities and social network with 31.6%. This shows that with improvement in technology, communication to raise awareness is being disseminated in various platforms.

Although awareness rate was high in the current study, in practice, only 75% of women were using contraceptives. This was not in accordance with the findings of a study done in Mpumalanga, South Africa who found that at least 81% of their study population was using contraception [18]. Others were not using due to side effects such as continuous menstrual spotting and pains, and others said they are not using them because they do not like them as well as restriction from partners. However, others revealed that even though their partners did not want them to use contraceptives they still do it. This shows that female counterparts need more awareness and counselling on contraceptives. In contrast to the current study, Mustafa and colleagues found contraceptive utilization of 53% among married women residing in rural area in Pakistan [19]. Also, a lower practice rate of contraception was observed in a study done by Aliya *et al* [3] in Pakistan as well.

The results revealed that most common methods of contraception known by study participants are injectables (46.1%), condoms (23.8%) and pills (17,4%); the results are in accordance with the findings in a study done in Ethiopia that found that common methods known by study participants were oral contraceptive pills, injections and condoms [10].

Education level did not seem to be a factor in the use of any family planning methods as the results varied in different levels of literacy, this is not in agreement with findings in other studies where they found that education literacy was influential when it comes to use of contraception [20, 21]. The major group of the participants went further than high school and the contraceptive use was high, 83% for tertiary and 81.0% for postgraduates and those who went as far as primary school

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also had a high rate of contraceptive use of 90.9%. This shows that although some study participants did not go further with their studies, they are still aware of the modern family planning methods, and they utilize them. This is in contrast with other studies, for example, a study done by Hameed *et al* in 2019 [9], found that low literacy was a limiting factor when it comes to utilizing contraception. It is not surprising that even those who are illiterate are aware of contraceptive methods and are using them, considering that in South Africa, there is an effort at public health facilities to raise awareness about these methods. However, with this effort being made, South Africa still faces a big problem when it comes to unwanted pregnancy or unplanned pregnancy as there is still a higher rate of abortion and the seemingly high prevalence of contraception use is believed to be masking other serious problems such as low quality of contraception service deliver, women's choice in using contraception of choice as well as the ability to use family planning method of choice consistently [22, 15]. This is supported by the views of the study participants as some had previously changed the type of contraception due to unavailability at the public health facilities as the majority of the study participants said they get their service from the clinics while others used contraceptive types interchangeable depending on what they find at the clinics.

In the current study, 70.7% of the participants said that both partners are responsible for family planning and 27.7% said they think it's only a women's responsibility to decide and use contraceptives. Among the priorities for choosing the type of method to use in family planning, most participants (58.2%) ~~P~~prioritize the effectiveness of the contraceptive method, and 32.2% consider the feeling of using and comfortability of the contraceptive tool. In addition, 19.5% of participants cited convenience of access as a key factor in their choice of contraceptive method, while 12.1% prioritized the safety of the contraceptive. These findings suggest that, among women in this study, effectiveness appears to take precedence over health-related considerations when

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selecting a contraceptive method.~~In addition, 19.5% considers convenience of getting the contraceptives, last but not least 12.1% consider the safety of the contraceptive tool. These findings indicate that women in this study do not seem to concern themselves more about health than the effectiveness of the contraceptive method.~~ This is contrast with what Aldabbah and Al-Qazz [23] reported, where women who participated in their study preferred tool safety more. In addition, study participants had several reasons for using contraceptives which included to avoid unwanted pregnancy, or unplanned pregnancy and to limit number of birth as well as to maintain birth spacing between children. These reasons are similar to those provided by study participants in a study done in Saudi Arabia by Sait *et al* [24].

This study revealed that the use of contraception decreased with age going up. However, this was not similar to findings in a study done by Semachew and colleagues in 2018 [1] who found that as the age increases, ~~also~~ the use of contraceptives also increased. Our study reports a 0% prevalence of contraception use among the Muslim participants, which is similar to what Berhanu *et al*~~., 2022~~ (10) found a low percentage of contraception use among Muslim women with a percentage of 7.7%. This could be ~~due to the fact that~~because in Islam, the practice of contraception is not encouraged, especially in unmarried women [1]. In conclusion, the rate of awareness of family planning methods is high, yet translating the knowledge to practice still needs improvement. More contraceptive awareness needs to be raised especially to female counterparts. There are few study limitations that should be considered when interpreting the results of this study. First one being that the study did not cover larger area and did not include male participants and some participants did not want to answer all the questions. Another drawback was using a non-probability method which could have resulted in selection bias. Lastly, the results cannot be generalized to other communities and other ethnic groups but be used as indication of what might be happening.

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3.5 Author's contribution

Conceptualization, M.M., A.S.; methodology M.M, A.S.; formal analysis M.M.; writing-original draft preparation, M.M.; writing- review and editing M.M, A.S, A.N.T.; supervision A.S. All authors have read and agreed to the published version of the manuscript.

3.6 Supplementary Materials

All the data that was generated or analysed in the study is included in this manuscript.

3.7 Institutional Review Board Statement

Ethical approval of the study was obtained from the University of Venda Health and Ethics Research Committee (project no: SMNS/20/MBY/05/1505)

3.8 Informed consent statement

Informed consent was obtained from all the participants by means of them signing an informed consent form prior to filling out the questionnaire provided.

3.9 Conflict of interest

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Authors declare no conflict of interest.

3.10 Acknowledgement

The authors would like to thank all the participants who took part in the study.

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CHAPTER 4: Molecular detection and distribution of *Chlamydia trachomatis, Neisseria gonorrhea and Trichomonas vaginalis* among women of reproductive age from semi urban and rural areas in Limpopo

Abstract

Sexually transmitted infections continue to be major causes of morbidity and mortality in many communities. Sexually transmitted infections have a substantial negative impact on public health, especially in low- and middle-income communities across the globe. However, their distribution varies significantly among communities. In the present study, urine samples (n=381) were collected from women of reproductive age between 18 and 45 years old; demographic data for study participants was collected using a questionnaire. Conventional PCR protocols were used for the detection of *Chlamydia trachomatis*, *Neisseria gonorrhea* and *Trichomonas vaginalis*. Sanger sequencing was also performed for confirmation of the organisms detected by PCR. In addition,

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phylogenetic analysis was performed to determine the relationship of the strains detected with others from South Africa and other countries. *C. trachomatis* was the most dominant with a prevalence of 17.39%, followed by *N. gonorrhea* with a prevalence of 5.43% while *T. vaginalis* was positive in 2.72% of the samples tested. Based on the age group *N. gonorrhea* had a high prevalence of 75.0 %, followed by *C. trachomatis* with 59.68 % and then *T. vaginalis* with a prevalence rate of 30.0% in those between 30-30 years old. Prevalence of the STI's in relation to education status varied with high prevalence (55.0%) of *N. gonorrhea* observed in those at tertiary level. About 30.0% of these participants were infected with *C. trachomatis* as well as *N. gonorrhea.* The prevalence of *C. trachomatis* was most noticeable in most of the methods compared to *N. gonorrhoea* and *T. vaginalis* which were not detected in participants who use other methods.

The findings of the study emphasize the need to normalize screening programs as individuals who tested positive on this study were asymptomatic and unaware of the infections. Though the study primarily focused on women of reproductive age, routine screening should be extended to all sexes and key populations such as sex workers and men who have sex with men (MSM) for early detection and treatment of STIs.

Keywords: Sexually transmitted infections; Infectious diseases, *Chlamydia trachomatis*, *Neisseria gonorrhea*; *Trichomonas vaginalis*; Family planning, Community-acquired infections

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4.1 Introduction

Sexually transmitted infections continue to be a major cause of morbidity and mortality among women of reproductive age. The high burden of STIs falls mostly in low-income countries (Kiweewa et al., 2019). Despite the preventative strategies and treatment of these curable STIs, the implications they have on the wellbeing and health of the young populations cannot be overlooked (Seidu et al., 2020). They affect quality of life, reproductive health as well as national and individual economies, in addition, it's been reported that STIs facilitate the transmission of human immunodeficiency virus (HIV) (Wi et al., 2019). The World Health Organization reported an estimate of 374 million new infections of four most common curable non-viral sexually transmitted infections caused by *Chlamydia trachomatis, Neisseria gonorrhea and Trichomonas vaginalis* and *Treponema pallidum* occurred in 2020 globally (Ekholuenetale et al., 2022).

Globally, the prevalence rates for *Chlamydia trachomatis, Trichomonas vaginalis*, and *Neisseria gonorrhoeae* among women are 3. 8%, 5. 3%, and 0. 9%, respectively (Rowley et al., 2019). The main route of transmission for the STIs is unprotected sexual intercourse with an infected individual. They can be asymptomatic for long periods of time, however, when symptoms present,

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they include genital ulcers, abdominal pains and genital discharges. If they are left untreated, they can cause more reproductive health complications such as infertility, mother-to-child transmissions, pelvic inflammatory diseases, genital tract inflammations, ectopic pregnancy and others. In addition, the risk of contracting HIV is greater (McGrath *et al*., 2022; Jarolimova *et al*., 2022).

In sub-Saharan countries STIs account for 40% of the worldwide STIs cases. South Africa has dominated in the prevalence of curable STIs and HIV for the past decade (Nyemba *et al*., 2022). The most common etiological causes associated with specific signs and symptoms are treated for STIs under syndromic management, which is the foundation of South African STI treatment guidelines. Syndromic management is time- and money-efficient, but it has significant downside as well such as missing lot of asymptomatic infections, particularly in women, and symptoms that are not STI-related are treated as such, which can result in overtreatment and possibly contribute to the emergence of antibiotic resistance. Inadequate epidemiological data adversely affects public health policies and guidelines and creates major gaps in population-level knowledge (Huyveneers *et al*., 2023). ~~Globally, the prevalence rates for *Chlamydia trachomatis, Trichomonas vaginalis,* and *Neisseria gonorrhoeae* among women are 3. 8%, 5. 3%, and 0. 9%, respectively (Rowley et al., 2019).~~

Ever since the wide spread of HIV and AIDS, managing and keeping STIs under control has proven to be challenging due to the fact that HIV causes immunosuppression. This has been observed mainly in those areas where HIV is highly prevalent (Balbeesi and Mohizea, 2018). Despite the fact that antibiotic resistance is a global public health emergency, front-line clinicians frequently underestimate the severity of antibiotic-resistant sexually transmitted infections (STIs). The US. Centers for Disease Control (CDC) have classified drug-resistant *Neisseria gonorrhoeae*,

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as one of the top five urgent threats to public health posed by antibiotic resistance alongside *Clostridium difficile*, and carbapenem-resistant Enterobacteriaceae.

The threat of drug-resistant infections becoming incurable was one of the reasons why the World Health Organization (WHO) prioritized control of gonococcal infections in their strategy report on STIs. Other common STIs have also been found to exhibit drug resistance; many of these can result in asymptomatic illness, which aids in the global spread of drug-resistant organisms (Tien et al., 2020). The spread of drug-resistant infections, including sexually transmitted infections, is facilitated by globalization and international travel. Traveling abroad has been linked to taking more risks when it comes to sex. Studies show that up to one-third of foreign visitors participate in casual sex abroad (CSA), and up to half report inconsistent condom usage. Sex tourism may be partially responsible for this activity (Paat et al., 2019). It is crucial to address the issue of antimicrobial resistance in STIs by promoting responsible antimicrobial use and stewardship (Shelke et al., 2023), as well as implementing strategies such as improved access to diagnostic laboratories, increasing surveillance for the emergence of resistance, regulating antibiotic use, training healthcare providers on appropriate prescribing practices and promoting public awareness about the importance of completing full courses of antibiotics, we can help slow down the spread of antimicrobial resistance in STIs. Additionally, the development of novel therapeutic antimicrobials and genetic AMR prediction tests can aid in preserving the efficacy of current and future antimicrobial treatments for STIs (Unemo et al., 2019). Furthermore, it is essential to improve the diagnosis, screening, and prevention of STIs, particularly in resource-limited settings. This can be achieved through increased investment in research and development, strengthening healthcare systems, promoting safe sexual practices and education, and ensuring access to affordable and high quality diagnostic tools and treatments. By adopting these comprehensive

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approaches, we can mitigate the impact of antimicrobial resistance on STIs and ensure that individuals receive timely and effective treatment (Toskin et al., 2017).

A "comprehensive STI control strategy," which should include effective clinical services, promotion and provision of prevention, targeted community-based interventions, and dependable data to inform response, was emphasized by the World Health Organization in 2009. Additionally, the World Health Organization highlights the importance of information and clinical service referrals because they eventually contribute to a decrease in the incidence and prevalence of sexually transmitted infections (Hidayani et al., 2021). However, the latest WHO global strategy on HIV and STIs places a strong emphasis on a data-driven strategy that shifts from syndromic management to active surveillance and causative treatment. Data is disaggregated by sex, age, and other important population characteristics. In this context, monitoring prevalence and incidence as well as diagnosing and treating etiology are all included in active STI surveillance (Newman et al., 2015). Many studies have been conducted to report on the prevalence of STIs in South Africa and other sub-Saharan countries, however, they mainly focus on cases that are reported in the healthcare facilities omitting asymptomatic cases. Therefore, this chapter reports on the incidence of three most common non-viral sexual transmitted infections in the communities that are otherwise undetected.

4.2 Materials and methods

4.2.1 Sample collection

Urine samples (n=381) were collected from study participants using urine collection bottles. Demographic data for study participants was collected using a questionnaire (refer to 3.2.6 in chapter 3). The samples were kept on ice during transportation to the University of Venda,

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parasitology lab for further analysis. Upon arrival at the laboratory, samples were cultured for the isolation of bacterial organisms, particularly *E. coli*, and *Staphylococcus*. Urine samples were divided into two parts, 2ml was aliquoted in a 2ml microcentrifuge tubes and centrifuged at 10 000 for 10 minutes and the pellet was stored until further analysis. Ten ml of the urine was aliquoted in a 15 ml tube~~s~~ for solid phase extraction of steroid hormones.

4.2.2 DNA extraction

Deoxyribonucleic acid (DNA) was extracted using bioflux, biospin omni genomic DNA extraction kit purchased from Separation Scientific, South Africa. Deoxyribonucleic acid (DNA) was extracted following the manufacturer's instruction. Briefly, the microorganisms were harvested by centrifuging urine samples at 5000 rpm for 5 mins, supernatant was discarded, and pellets were saved. Pellets were re-suspended in 180 µl of TES buffer and 20 µl of PK solution. The samples were mixed by vortexing intensively for 20s. Then incubated at 56 ^{0}C for 2 hrs. Then 200 µl lysis buffer were added and mixed thoroughly. After that, 200 µl of absolute ethanol was added and the tubes were mixed thoroughly. The mixture was transferred to a spin column and centrifuged at 12 000 rpm for 1 minute. Flow-though was discarded. After which 500 µl of PW buffer was added to the spin column. The mixture was centrifuged at 12 000 rpm for 1 minute, flow-through was discarded. Wash buffer (500 µl) was added to the spin columns and centrifuged at 12 000 rpm for I minute, the flow-through was discarded and the step was repeated. Spin columns were put in clean sterile microcentrifuge tubes and 200µl of elution buffer was added and incubated at room temperature for 2 mins. Then the spin columns were centrifuged at 10 000 rpm for 1 minute. And the flow-through DNA was stored at -20 ^{0}C for further analysis.

4.2.5 Polymerase chain reaction for the detection of sexually transmitted pathogens

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Conventional PCR was used for the detection of *Chlamydia trachomatis*, *Neisseria gonorrhea* and *Trichomonas vaginalis* using DNA extracted from urine samples as described by Krishnan et al., (2018). The Polymerase chain reaction was performed using a Bio-Rad T100 thermal cycler. Each reaction contained 12.5 µl Emerald Amp GT PCR master mix, 0.6 µl forward primer and 0.6 µl reverse primer, 0.25 µl BSA and 6.05 µl nuclease free water and 5 µl DNA. The amplified products were gel electrophoresed to identify sizes of the amplified DNA.

The amplicons were sent to Inqaba laboratories in Pretoria for sequencing. Once obtained the sequences were validated and used in different programs for analysis including Bioedit, ~~#~~Blast and Mega for drawing the phylogenetic trees.

Table 4.1: Primers used for screening of *Chlamydia trachomatis* and *Neisseria gonorrhea* as well as *Trichomonas vaginalis.*

Organism	**Target gene**	**Name**	**Primer sequence 5'-3'**	**Amplicon size**
Chlamydia trachomatis	Cryptic plasmid	CTF1 CTR1	TCCGGAGCGAGTTACGAAGA AATCAATGCCCGGGATTG	241bp
Neisseria gonorrhoeae	cppB gene	NGF1 NGRI	GCTACGCATACCCGCGTT CGAAGACCTTCGAGCAGACA	390bp
Trichomonas vaginalis		TV3 TV7	ATTGTCGAACATTGGTCTTACCCTC TCTGTGCCGTCTTCAAGTATGC	300bp

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Table 4.2: Cycling conditions used for screening of *Chlamydia trachomatis, Neisseria gonorrhea* and *Trichomonas vaginalis*

Primer name	**Cycling conditions**
CTF1 and CTR1 NGF1 and NGR1	Initial denaturation at 95 $^{o\theta}$C for 10 mins, denaturation at 94 $^{o\theta}$C for 1 min, annealing at 55 for 1 min, extension at 72 $^{o\theta}$C for 1 min and final extension at 72 $^{o\theta}$C for 5 mins.
TV3 and TV7	Initial denaturation at 95 $^{o\theta}$C for 5 mins, denaturation at 90 $^{o\theta}$C for 1 min, annealing at 60 $^{o\theta}$C for 30s, extension at 72 $^{o\theta}$C for 2 mins and final extension at 72 $^{o\theta}$C for 7 mins.

4.2.6 Statistics

All experiment data was recorded and organized on excel spreadsheet. Descriptive statistics was used to determine frequencies and chi-square test was used to assess associations. Statistical Package for the Social Sciences (SPSS) V27 was used.

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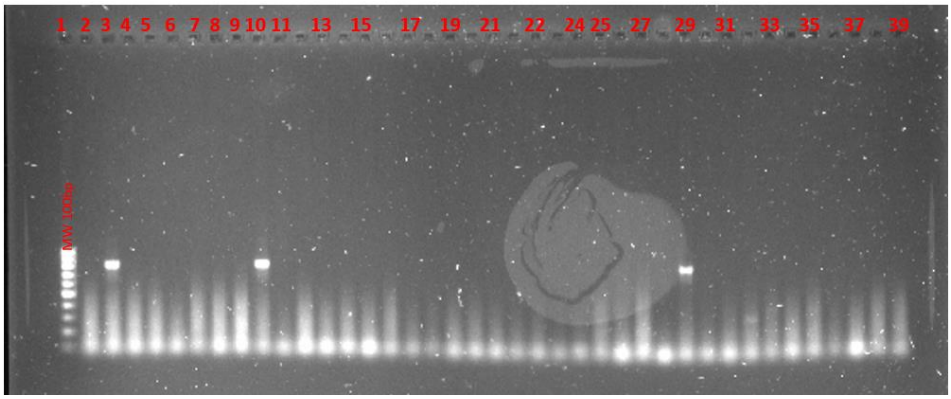
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4.3 Results

4.3.1 Detection of sexually transmitted pathogens

The amplified products were gel electrophoresed to identify sizes of the amplified DNA for the three pathogens: *Chlamydia trachomatis, Neisseria gonorrheoa and Trachomatis vaginalis*. The figures below (figure 4.1, 4.2 and 4.3) are gel representations showing the detection bands. Wells are represented in numbers from 1-39. Well no.1 is the molecular weight (100bp) and 2-39 is samples tested for *Chlamydia trachomatis*.



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Figure 4.1: PCR amplification products for *Chlamydia trachomatis* from urine samples, 2% of agarose gel stained with ethidium bromide.

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The figure 4.2 below is gel presentation showing the detection bands of *Neisseria gonorrhoea*, the amplification size 390bp. Wells are represented as L1-L20. Well no.1, L1 is the molecular weight (100bp) and L2-L20 are isolates tested for *Neisseria Gonorrhea*.

L1 L2 L3 L4 L5 L6 L7 L8 L9 L10 L11 L12 L13 L14 L15 L16 L17 L18 L19 L20

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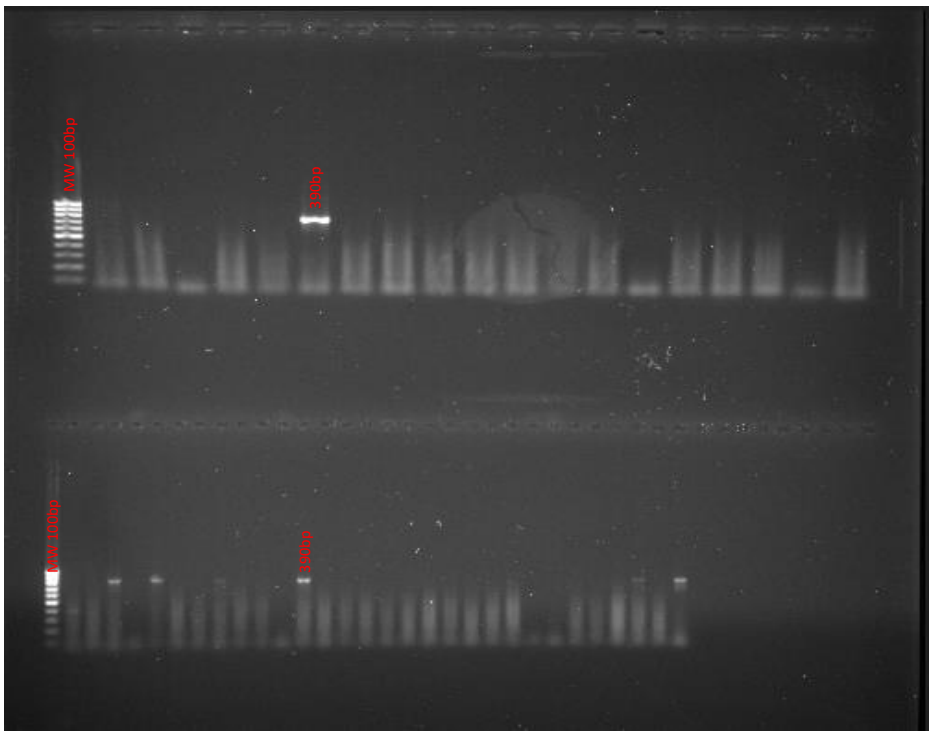


Figure 4.2: Detection of *Neisseria gonorrhea* in 2% of agarose gel stained with ethidium bromide.

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The figure 4.3 below is gel presentation showing the detection bands of *Trichomonas vaginalis*, the amplification size 300bp. Wells are represented as numbers from 1 to 38. Well no.1 is the molecular weight (100bp) and 2-38 is isolates tested for *Trichomonas vaginalis*.

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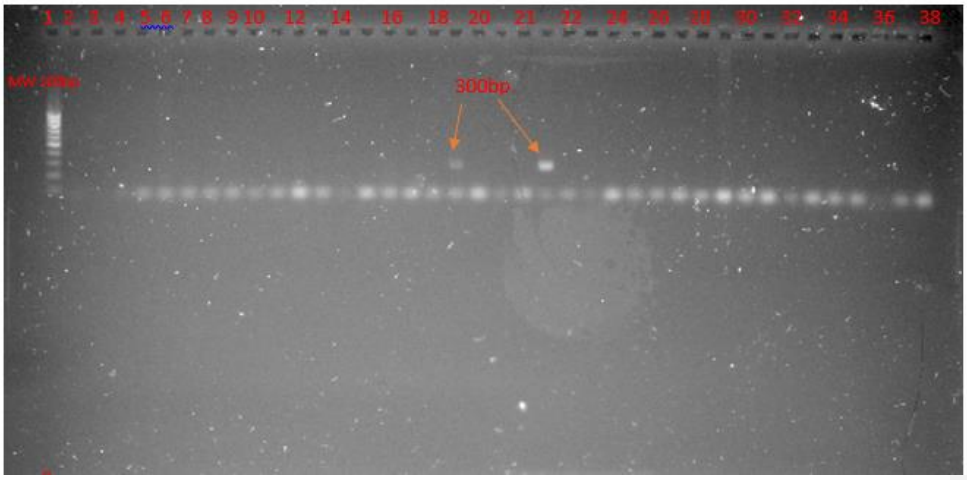


Figure 4.3: Detection of *Trichomonas vaginalis* in 2% of agarose gel stained with ethidium bromide.

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4.3.2 Overall prevalence of *C. trachomatis*, *N. gonorrhea* and *T. vaginalis*

Figure 4.4 below presents the overall prevalence of the three non-viral causative agents of STI's (*C. trachomatis, N. gonorrhea and T. vaginalis*) amongst the study participants. *Chlamydia trachomatis* was the most dominant with a prevalence of 17.39%, followed by *N. gonorrhea* with a prevalence of 5.43% and *T. vaginalis* had a prevalence of 2.72%.

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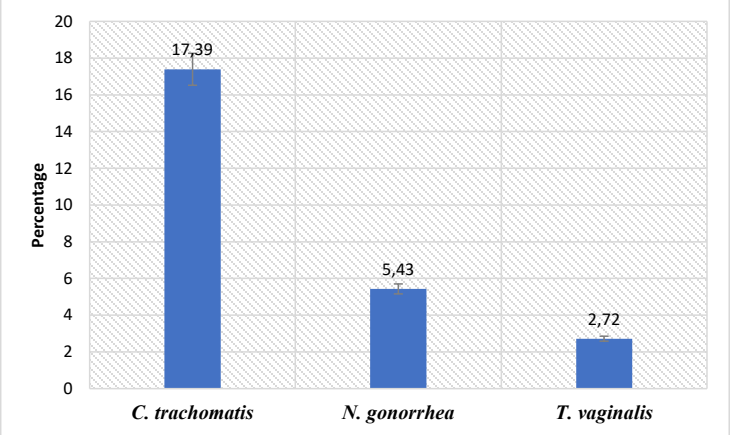


Figure 4.4: Prevalence of *C. trachomatis, N. gonorrhea and T. vaginalis*

4.3.3 Prevalence of *C. trachomatis, N. gonorrhea and T. vaginalis* in relation to demographic data

The prevalence of the three organisms varied across the study participants (Table 4.3). Based on the age group, the 20-30 years had a high prevalence of *N. gonorrhea*, followed by *C. trachomatis* with and then *T. vaginalis*. The 31-40 years age group also had a similar prevalence rate with the 20-30 age group. The prevalence rate of *N. gonorrhoea* varied by age group and the difference was statistically significant with $p= 0.022$. The prevalence of the STI's in relation to education status varied with high prevalence of *N. gonorrhea* observed among the study participants who studied up to high school, this was followed by high prevalence of *C. trachomatis* and *T. vaginalis* in those with high school education. Study participants who had no children had the highest prevalence of all three organisms. The occurrence was statistically significant with *C. trachomatis*

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and *T. vaginalis* with *p values* of *0.039* and *0.022* respectively. Participants who never married appear to be the most infected.

Table 4.3: Prevalence of *C. trachomatis, N. gonorrhea and T. vaginalis* in relation to socio-demographic data of study participants.

Variables	**Categories**	***C. trachomatis***	***N. gonorrhea***	***T. vaginalis***
Age group	15-19	7 (10%)	3 (4.5%)	2 (3%)
	20-30	37 (**16.9%**)	15 (**6.8%**)	3 (**1.4%**)
	31-40	10 (19.2%)	1 (1.9%)	3 (**5.8%**)
	41 and above	8 (29.6%)	1 (3.7%)	2 (7.4%)
Statistics		$X^2 = 5.141$ P = 0.162	$X^2 = 14.812$ P = **0.022**	$X^2 = 5.546$ P = 0.136
Race	Black	57 (16.2%)	20 (**5.7%**)	10 (**2.8%**)
	Colored	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Indian	5 (45.5%)	0 (0.0%)	0 (0.0%)
Statistics		$X^2 = 6.668$ P = 0.036	$X^2 = 0.7597$ P = 0.944	$X^2 = 0.350$ P = 0.839
Education	Illiterate	2 (11.8%)	0 (0.0%)	1 (5.9%)
	Primary school	3 (27.3%)	1 (9.1%)	0 (0.0%)
	High school	32 (**21.6%**)	7 (4.7%)	5 (**3.5%**)
	Tertiary	22 (14.1%)	11 (**7.1%**)	4 (2.6%)
	Postgraduate	1 (4.5%)	1 (4.5%)	0 (0.0%)
Statistics		$X^2 = 11.045$	$X^2 = 2.561$	$X^2 = 3.0814$

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		P = 0.050	P = 0.990	P = 0.687
Religion	Islam	5 (41.7%)	0 (0.0%)	0 (0.0%)
	Christianity	56 (16.3%)	1 (5.5%)	10 (2.9%)
	Traditional	1 (12.5%)	1 (12.5%)	0 (0.0%)
	Atheist	0 (0.0%)	0 (0.0%)	0 (0.0%)
Statistics		$X^2 = 6.755$ P = 0.149	$X^2 = 3.499$ P = 0.899	$X^2 = 1.742$ P = 0.0.783
Children	No children	21 (13.5%)	10 (6.5%)	3 (1.9%)
	One child	13 (14.3%)	5 (5.5%)	1 (1.1%)
	Two children	10 (16.7%)	3 (5.0%)	3 (5%)
	Three children	13 (32.5%)	2 (5.0%)	3 (7.5%)
	4 and above	5 (27.8%)	0 (0.0%)	0 (0.0%)
Statistics		$X^2 = 10.065$ P = **0.039**	$X^2 = 9.480$ P = 0.303	$X^2 = 6.337$ P = **0.022**
Marriage	Never married	43 (**16.1%**)	17 (**6.4%**)	6 (**2.2%**)
	Divorced	5 (27.8%)	1 (5.6%)	1 (5.6%)
	Widowed	4 (50.0%)	0 (0.0%)	0 (0.0%)
	Married only wife	7 (13%)	2 (3.7%)	3 (5.6%)
	Married first wife	3 (30%)	0 (0.0%)	0 (0.0%)
	Cohabitation	0 (0.0%)	0 (0.0%)	0 (0.0%)
Statistics		$X^2 = 11.045$	$X^2 = 2.561$	$X^2 = 3.081$

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		P = **0.050**	P = 0.990	P = 0.687

4.3.4 Co-infection of *C. trachomatis, N. gonorrhea and T. vaginalis* amongst study participants

The results below show the co-infection with organisms causing non-viral STIs amongst the study participants. About 30.0% of the participants were infected with *C. trachomatis* as well as *N. gonorrhea*, the results were statistically significant with *p = 0.027.* Furthermore, 20.0% had a co-infection with *C. trachomatis* and *T. vaginalis*. Also, co-infection was observed with *N. gonorrhoea* and *T. vaginalis*. However, the results were not statistically significant. Results are presented in table 4.4 below.

Table 4.4: Co-infection of *C. trachomatis, N. gonorrhea and T. vaginalis* amongst study participants.

Organisms	***C. trachomatis***	**Statistics**
N. gonorrhea	6 (30.0%)	$X^2 = 7.1880$ P = **0.027**
T. vaginalis	2 (20.0%)	$X^2 = 0.0487$ P = 0.0825
	T. vaginalis	**Statistics**
N. gonorrhea	1 (10.0%)	X2 = 0.4422 P = 0.0802

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4.3.5 Prevalence of *C. trachomatis, N. gonorrhea and T. vaginalis* in relation to type of contraceptive methods

The prevalence of the three organisms of interest in relation to the contraceptive methods used by the participants varied. The prevalence of *C. trachomatis* was most noticeable in many of the methods compared to *N. gonorrhoea* and *T. vaginalis* which were not detected in participants who uses other methods. Most prevalence was observed in participants using injectables with 18.60% predominance of *C. trachomatis*, followed by 7.75% prevalence rate for *N. gonorrhea* and 3.10% prevalence of *T. vaginalis*. *Chlamydia* and *Gonorrhea* was also observed in participants who are using pills with the former having a prevalence rate of 18.37% and the latter having a prevalence rate of 4.08%. Apart from the injectables *T. vaginalis* was detected in participants using two other methods which are Implants (3.85%) and male condoms (1.49%). None of the organisms were detected in participants using contraceptive methods such as vaginal ring, spermicides and withdrawal. Nonetheless, all the results observed were not statistically significant with p values that were over 0.05. Results are presented in table 4.5 below.

~~Following sequence analysis, the *C. trachomatis* sequences showed a diverse group of organisms (Figure 4.5). Although most were very close to each other in terms of evolution there was one strain that was completely out indicating a high divergence among the infecting *C. trachomatis* strains. The gonorrhea and *T. vaginalis* sequences did not give good results and therefore are missing in the analysis.~~

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Table 4.5: Prevalence of *C. trachomatis*, *N. gonorrhea and T. vaginalis* in relation to type of contraceptive methods

Methods	***C. trachomatis***	***N. gonorrhea***	***T. vaginalis***
Injectables	24 (18.60%)	10 (7.75%)	4 (3.10%)
Pills	9 (18.37%)	2 (4.08%)	0
IUD	1 (33.33%)	0	0
Emergency pills	2 (25.0%)	1 (12.50%)	0
Patch	1 (14.29%)	0	0
Implants	5 (19.23%)	1 (3.85%)	1 (3.85%)
Periodic abstinence	1 (33.33%)	0	0
Vaginal ring	0	0	0
Sponge	1 (50.0%)	0	0
Spermicides	0	0	0
Sterilization	1 (20.0%)	0	0
Withdrawal	0	0	0
Male condoms	10 (14.93%)	3 (4.48%)	1 (1.49%)
Female condoms	1 (6.67%)	1 (1.67%)	0

4.3.5 Phylogenetic analysis of *Chlamydia trachomatis* isolates

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Following sequence analysis, the *C. trachomatis* sequences showed a diverse group of organisms (Figure 4.5). Although most were very close to each other in terms of evolution there was one strain that was completely out indicating a high divergence among the infecting *C. trachomatis* strains. The gonorrhea and *T. vaginalis* sequences did not give good results and therefore are missing in the analysis. Strains such as **FN05 CT**, **FN05B CT**, and **MD06-CT** form a moderately supported clade with the globally referenced **CP054427** clade. This suggests that these strains may represent regionally circulating lineages within South Africa that still retain genetic similarities to internationally reported strains.

Strains **DR06 CT** and **TA36 CT** cluster closely with the reference strain **CP054427**, supported by a high bootstrap value (86), suggesting a strong genetic similarity. Reference strain CP054427 is a globally reported strain, and its proximity to local isolates implies possible global dissemination or shared ancestry, potentially due to travel, migration, or common transmission networks.

Multiple sequence alignment of the CT isolates, compared against the reference strain CP054427, revealed a high level of sequence conservation across the majority of nucleotide positions. However, several single nucleotide polymorphisms (SNPs) and point mutations were identified, indicating genetic variation among the isolates. TA36_CT and DR06_CT showed notable sequence variation, with several point mutations suggesting genetic divergence. TA49_CT and TA47_CT shared similar substitutions, indicating a close evolutionary relationship, while MA44_CT had a unique insertion (figure 4.6).

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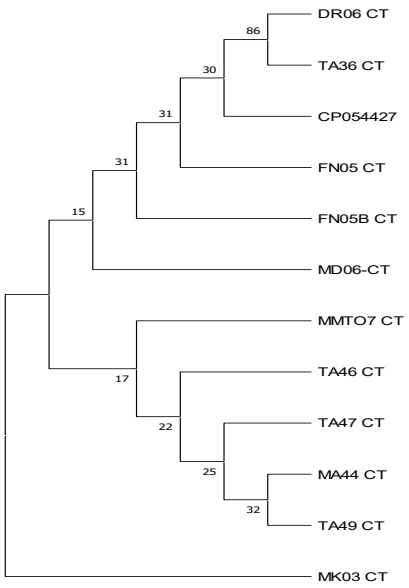


Figure 4.5. The evolutionary history of the *C. trachomatis* sequences inferred using the Neighbor-Joining method.

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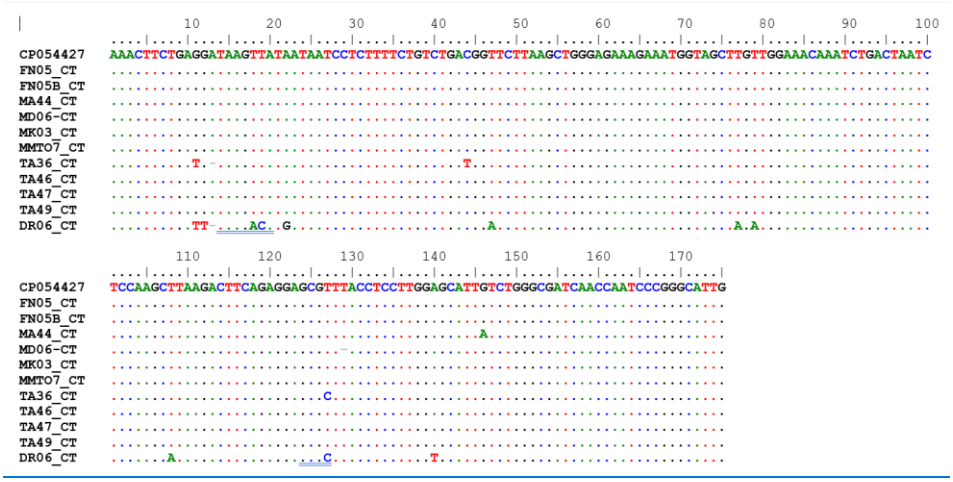


Figure 4.6: Sequence alignment of *C. trachomatis* isolates from female community members participating in the study.

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4.4 Discussion

The current study set out to measure the prevalence of three common non-viral sexually transmitted infections (*Chlamydia trachomatis, Neisseria gonorrhoea and Trichomonas vaginalis*) amongst women of reproductive age using non-invasive self-collected specimen (urine). The organisms were detected using conventional PCR. The most prevalent organism detected is *Chlamydia trachomatis (17.39%)*, followed by *Neisseria gonorrhea* (5.43%) with low prevalence of *Trichomonas vaginalis (2.72%)*.

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The most predominant organism detected in the study is *C. trachomatis*. This is concordant with the common knowledge that *C. trachomatis* is the commonest sexually transmitted bacterial infection globally (Mohseni *et al*., 2019). In addition, the results are in agreement with other scientific reports that found significantly higher prevalence rates for *C. trachomatis* (Kreisel *et al*., 2021; Svigals *et al*., 2020). This was followed by lower prevalence of *N. gonorrhea* and *T. vaginalis*. This had similarities with other literature that detected low rates of the two organisms (Silva *et al*., 2021).

The results from this study suggest that sexually transmitted infections are quite common in the study population, although differing with the type of ~~the~~ agent causing the infections. In terms of percentage, the rate of co-infections in the study populations were significantly higher between *C. trachomatis* and *N. gonorrhea* (30.0%) and non-significant for *N. gonorrhea* and *T. vaginalis* (10.0%) then *T. vaginalis with C. trachomatis* (20.0%). The comorbid infections with *C. trachomatis* and *N. gonorrhoea* ~~was~~ were consistent with other studies (Dionne-Odom *et al*., 2022; Guy *et al.,* 2014). It is not uncommon for multiple sexually transmitted infections to co-exist, one study did a survey at a hospital to assess burden of co-existing STIs found 37% of participants had concurrent STI infections (Choudhry *et al*., 2010). Also in 2022, Agustine *et al*, reported on a case of a male patient who presented with multiple STI infection of gonococcal urethritis and other infections.

In contrast, in a study conducted in India using urine specimen to detection of *C. trachomatis* and *N. gonorrhoea* they found low prevalence of both organisms in their study population (Krishnan et al., 2017). Additionally, a study conducted in Pemba, Tanzania also found low prevalence of *C. trachomatis* (4.6%) in pregnant women (Juliana *et al*., 2020). Furthermore, in a study done by Lowe *et al* in 2019 in a cohort of HIV-infected women in Zimbabwe, they found high prevalence

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of *T. vaginalis* of which suggested that it plays an important role in the transmission of HIV. However, low prevalence should not be considered as the true reflection of the infections amongst the study populations because as much as urine specimens are easily obtainable and accepted as a sample for diagnosis it also at the same time not the best specimen type. ~~And also~~And, it should be taken into consideration that urine used in this study was not the first void urine which would have been concentrated and having more organisms. Furthermore, comprehensive sexual health is still necessary even in populations where the prevalence is low.

Our data also revealed that risk factors associated with high infections of *C. trachomatis*, *N. gonorrhea* and *T. vaginalis* includes women in the age groups ~~20-30~~15-19 and ~~15-19~~20-30, single ~~marital status~~women ~~and having~~and those with ~~-~~no children. This is similar to other studies that have reported similar risk factors~~.~~, F~~f~~or example in a study conducted in Ghana, ~~where~~ they found that women in the ~~20-30~~ age group between 20-30 years are at high risk of contracting STIs (De la *et al*., 2019; Silveria *et al*., 2020). Although number of sexual partners was not included in the analysis as more than 90% of the participants reported one partner, this was to exclude analysis bias on the said risk factor. This could be the true reflection of the study population or not as other study participants may not have been truthful on the number of sexual partners when filling out the questionnaire.

The association observed in this study between the STIs and ~~method types~~types of contraceptive methods used by participants did not show any statistical significance. However, high prevalence rates of *C. trachomatis* were observed in participants using various methods of contraception. ~~Which~~ This indicates high risk of contracting *C. trachomatis* regardless of the method being used for contraception. The study observed varied prevalence of *Neisseria gonorrhea*, which renders our results inconclusive on whether the contraceptive methods ~~increases~~increase or reduce~~s~~ the

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risk of contracting *gonorrhoea*. The results concur with other studies, notably two studies that also did not find conclusive association on the acquisition of *Neisseria gonorrhea* with hormonal contraceptives (Morrison *et al*., 2009; McCarthy *et al*., 2019). The lower incidence of *N. gonorrhoea* might be ~~due to that~~because hormonal contraceptives reduce~~s~~ menstrual blood flow, thereby reducing iron sources for *N. gonorrhoea*. And because *N. gonorrhoea* is augmented in iron-rich menstrual blood, this may in turn prevent its growth (Jerse *et al*., 2002). None the least, the current study observed very low risk of acquisition of *T. vaginalis* associated with methods used by the participants. The low prevalence's were detected in participants who were using three contraceptive methods namely: injectables, implants and male condoms. The results reveal~~s~~ that the risk of~~to~~ acquiring~~e~~ *T. vaginalis* is relatively low regardless of the method used. Also, this could be ~~due to that~~because for *T. vaginalis* to establish infection it requires estrogen and androgen receptors, therefore, if there is competitive binding for receptors *T. vaginalis* may be outcompeted by progesterone receptors as they are for example highly expressed in DMPA contraceptives. Which in turn, inhibits *T. vaginalis* from binding to the androgen receptor (Brahmbhatt *et al*., 2014).

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4.5 Conclusion

In conclusion, the present study revealed that it is important to normalize STI screening, as participants who were positive for these three etiological agents of STIs were not aware of the infections. Although our study only focused on women of reproductive age, STI screening should be encouraged for both males and females. This is ~~. Because,~~because ~~the~~ majority of study participants reported that ~~to~~ they have one sexual partner~~s~~, yet they were infected with either of the three organisms~~.~~. T~~t~~his suggests that if the participants did not ~~misreport~~ under report on having one sexual partner, then their partners could be having more than one sexual partners leading to

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increased transmission of the infections. Also, the study did not include key population such as sex workers.

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CHAPTER 5: Potential association between Ethinylestradiol (EE2) and reproductive health

Abstract

Ethinylestradiol is present in almost all combination forms of birth control pills and is nearly the only estrogen used for this purpose. It shares structural similarities with estradiol, the estrogen found naturally in the body, but it is more effective when taken orally due to its higher bioavailability. It has strong estrogenic effects and is an essential part of combo oral contraceptives. To improve its stability and enable oral administration, ethinylestradiol is chemically modified by addition of an ethyl group at C17, meaning that the reactive beta-hydroxyl group on C17 position is now paired with an ethyl group in ethinylestradiol. While it offers various benefits, it is important to understand the potential risks associated with its use. Ethinylestradiol causes a wide variety of side effects, some of the side effects are more serious. Knowing how it affects long-term reproductive health, pregnancy, and fertility is crucial. ~~shares structural similarities with estradiol, the estrogen found naturally in the body, but it is more effective when taken orally due to its higher bioavailability. It has strong estrogenic effects and is an essential part of combo oral contraceptives. To improve its stability and enable oral administration, ethinylestradiol is chemically modified by addition of an ethyl group at C17, meaning that the~~

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~~reactive beta-hydroxyl group on C17 position is now paired with an ethyl group in ethinylestradiol.~~ Even at low quantities, ethinylestradiol still influences the estrogenic effects in women.

In the present study, ~~U~~urine samples (n=381) were collected from study participants using urine collecting bottles. The samples were inoculated on culture media for the isolation of bacterial organisms and determination of infection (UTI) caused by these bacterial organisms From the total volume, 20 ml of the urine was used for LC/MS and ELISA. A five-point calibration curve for EE2 standards was used for quantification of the ethinylestradiol using ELISA. Liquid chromatography mass spectrometry (LC/MS/MS) was used for the determination of the metabolomics characteristics of the samples. Urinary tract infections were found in 35.4% of the study population. The prevalence increased with age and was not influenced by education. In fact, our study showed that the higher the education the higher the prevalence of UTI. The use of contraceptives was significantly associated with higher prevalence of UTI (characterised by presence nitrites, leukocyte esterase, and bacteria in the urine) with 38.6% among individuals who have used contraceptives compared to 24.6% among those who never used contraceptives (p=0.034).

Ethinylestradiol (EE2) concentrations varied across the samples with the highest concentration of 804 pg/ml and the lowest concentration being 11 pg/mL~~l~~. Higher concentrations of EE2 were found in older age groups. Although there was no significant association between the use of contraceptives in general and the presence of EE2 in urine, individuals who used injectable contraceptives had significantly higher EE2 concentration and prevalence in their urine samples. The same situation was true for those who use implants and patches. The metabolomics analysis of 20 urine samples identified a number of compounds that might be of interest in the characterization of EE2 metabolism in h~~H~~umans. In conclusion, UTIs are a problem in these

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communities and is associated with the presence of EE2 in the urine of these community members. Further studies should expand with high study participant numbers and further investigations into the pharmacokinetics of EE2 in the community.

Keywords: 17α-ethinylestradiol; ELISA; Metabolomics; Contraception; Reproductive health; UTI

5.1 Introduction

Ethinylestradiol is a synthetic form of estrogen commonly used in hormonal contraceptives and hormone replacement therapies. Ethinylestradiol is present in almost all combination forms of birth control pills and is nearly the only estrogen used for this purpose (Kirigaya *et al*., 2006; Nasri *et al*., 2021). While it offers various benefits, it is important to understand the potential risks associated with its use. Knowing how it affects long-term reproductive health, pregnancy, and fertility is crucial. We can learn a great deal about the possible advantages and disadvantages of ethinylestradiol for people who are of reproductive age and use this knowledge to help create safer

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contraceptive methods and better reproductive healthcare. Ethinylestradiol shares structural similarities with estradiol, the estrogen found naturally in the body, but it is more effective when taken orally due to its higher bioavailability (Dhont, 2010; Chabi *et al*., 2022). It has strong estrogenic effects and is an essential part of combo oral contraceptives. To improve its stability and enable oral administration, ethinylestradiol is chemically modified by addition of an ethyl group at C17, meaning that the reactive beta-hydroxyl group on C17 position is now paired with an ethyl group in ethinylestradiol (in endogenous estradiol it's paired with an alpha-hydrogen atom). This synthetic hormone binds to estrogen receptors in a variety of tissues and functions similarly to endogenous estrogen, impacting human reproductive health in both positive and negative ways, causing a range of hormonal and physiological reactions (Katsiadaki *et al*., 2021; Wang *et al*., 2021).

This study's primary objective was to investigate how ethinylestradiol impacts the health of human reproduction. Ethinylestradiol causes a wide variety of side effects, some of the side effects are more serious. The primary pharmacodynamics impact of ethinylestradiol in females is the reduction of follicle-stimulating hormone and luteinizing hormone production from the pituitary gland, which results in the suppression of ovulation. Additionally, it changes the uterine lining to inhibit the implantation of a fertilized egg and thickens the cervical mucus, which makes it harder for sperm to enter the uterus (Shukla *et al*., 2017). Women who are using contraceptives containing ethinylestradiol, their health is significantly impacted. There was a possibility of developing hepatic cancer that was observed in rats that were continuously fed ethinylestradiol and this is one of the main cause for concern on adverse effects that the compound can elicits in human bodies~~The possibility of developing hepatic cancer that was observed in rats that were continuously fed ethinylestradiol and this is one of the main causes for concern on adverse effects that the compound~~

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~~can elicits in human bodies~~. Furthermore, even at low quantities, ethinylestradiol can influence the estrogenic effects in women (Mukanga *et al*., 2023). To completely comprehend the hazards and long-term impacts of ethinylestradiol usage in women, more research is required.

There are a number of risks and adverse effects linked to ethinylestradiol use in women. These may include an elevated risk of venous thromboembolism, breakthrough bleeding, nausea, and breast discomfort. Furthermore, the potential link between ethinylestradiol and idiopathic venous thromboembolic events has garnered increasing attention (Nelson *et al*., 2021). Research has also shown that long-term use of ethinylestradiol may pose risks to women's reproductive systems beyond its effects on fertility. These risks include potential alterations in menstrual cycle regularity, the development of benign ovarian cysts, and changes in hormone levels that can affect mood and overall well-being. These concerns provide the basis for considering the long-term implications of ethinylestradiol use (Lai *et al*., 2023). While the exact mechanisms underlying these associations are not yet fully understood, it is evident that ethinylestradiol effects extend beyond its primary role as a contraceptive agent. This study aimed to assess the potential associations of ethinylestradiol and reproductive health.

Urinary tract infections (UTIs) are among the most common bacterial infections affecting women of reproductive age, often resulting in significant discomfort, morbidity, and, if left untreated, long-term complications (Flores-Mireles *et al*., 2015; Sujith *et al*., 2024). Given the anatomical and hormonal factors that predispose women to UTIs, it is important to assess their prevalence in populations using hormonal contraceptives such as ethinylestradiol (EE2).

EE2, a synthetic estrogen commonly used in oral contraceptives, has been shown to influence the vaginal and urinary tract microbiota, potentially altering susceptibility to infections. Hormonal

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modulation may impact the host immune response, urogenital flora balance, or epithelial barrier integrity, all of which can affect the risk of UTI (Witikin *et al*., 2007).

Therefore, incorporating UTI assessment in this study allows for a more comprehensive evaluation of women's reproductive and urogenital health. By investigating the correlation between the presence of EE2 and UTI occurrence, this study aims to explore whether hormonal contraceptive use contributes to increased or decreased risk of urinary tract infections.

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In addition, urine specimens as complex matrix contains a variety of metabolites that could provide comprehensive insight into the metabolic activity that reflects the pathological and physiological state of an individual's body, thereby providing crucial information on health and disease states, and metabolic effects of medications, inclusive of contraceptives. Therefore, we also randomly selected a small proportion of the sample from three groups of contraceptive methods to perform untargeted metabolomics.

5.2 Materials and methodology

5.2.1 Data collection

Collection of participant's data and urine collection were described in the previous chapters. See section 3.2 for data collection in chapter 3 and for urine collection, see section 45.2 in chapter 45. In addition to urine collection, a urinalysis was performed immediately after sample collection to assess key indicators of urinary tract infection, including the presence of leukocytes, nitrites, protein, and blood. This rapid screening provided preliminary diagnostic information prior to microbiological culture and further analysis.

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5.2.2 Enzyme-linked immunosorbent assay (ELISA) to quantify EE2 concentration

ELISA kits (Ecologiena for environmental pollutants) were purchased from Inqaba biotechnical industries (South Africa), supplied by Tokiwa Chemical Industries. Due to the cost of running ELISA on all the samples, few samples (180) were randomly chosen. Competitive ELISA test was performedperformed, and the principle is based on competitive reaction chromogenic reaction and the quantitive analysisbinding reaction followed by a chromogenic reaction, allowing for quantitative analysis of the target analyte. Manufacturer's instructions were followed as per below:

Five wells were prepared for the 5 standards and one well for a control (blank standard diluent). Fifty microlitres (50 µl) of each dilution of standards and samples were added into the appropriate wells. And then, 50 µl detection reaction A was added to each well immediately. The plate was gently shaken and covered with a sealer. The plate was incubated for an hour at 37 $^{o\theta}$C. After an hour of incubation, the solution was aspirated and the plate was washed with 350 per well of the wash solution using a squirt bottle and was let to sit for 2 minutes. After which, the wash solution was removed and dried by snapping onto an absorbent paper. This was repeated 3 times. After the last wash the plate was dried by blotting it against the paper towel. Then 100 µl of detection reagent B was added to each well and incubated for 30 minutes at 37 $^{o\theta}$C covered with the plate sealer. After incubation the solution was decanted and the plate was washed with the wash solution and the washing was repeated 5 times. After the last wash 90 µl of substrate solution was added to each well, plate was covered and incubated for 20 minutes at 37 $^{o\theta}$C, the plate was protected from light. After this step the solution turns blue. After incubation the stop solution was added to each well, and the liquid immediately turned yellow. The solution was mixed by gently tapping the side of

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the plate. Then the absorbance was measured by reading the plate on the microplate reader at 450 nm wavelength.

5.2.3 Urinalysis and bacterial culture for UTI detection

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5.2.3.1 Isolation and Antibiotic Susceptibility Testing of *E. coli* from urine of study participants

Urinalysis was performed immediately upon sample collection to preserve key urinary indicators such as leukocytes, nitrites, and protein. In the laboratory, *Escherichia coli* was isolated using Eosin Methylene Blue (EMB) agar, where characteristic metallic green sheen colonies were used for presumptive identification. The confirmed *E. coli* isolates were subjected to antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method. Isolates were sub-cultured and incubated overnight, after which a 0.5 McFarland standard bacterial suspension was prepared. This suspension was inoculated onto Mueller-Hinton agar by uniformly streaking the surface in a four-way pattern to create a bacterial lawn. Antibiotic discs were then placed on the agar surface, and plates were incubated at 35–37°C for 18–24 hours. Following incubation, zones of inhibition were measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

5.2.3 Statistical analysis

All raw data were recorded in excel spreadsheets and the concentrations of all hormones were computed using Microsoft excel. Ethinylestradiol concentration in the samples was calculated based on the respective calibration curve using non-linear regression. The graphs were also computed using Microsoft excel as well.

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5.2.4 Liquid chromatography mass spectrometry (LC/MS/MS) to detect untargeted metabolites

5.2.4.1 Sample preparation

For metabolite extraction, uniform volume of the urine was placed on the solid phase extraction cartridge (C18-E silica-based cartridges, Stargate Scientific, South Africa) and were pre-conditioned with conditioning solvent (methanol). Once pre-condition is complete, samples were allowed to bind to the cartridge for 10 mins. Water in excess was removed by vacuum filtration and the cartridges were with water twice. To elute the metabolites, 1ml of an extraction solvent was used. The extracted metabolites were further purified by filtration using 0.22 µm nylon filters that are attached to 2 ml plastic syringe. The samples were analysed using the liquid chromatography hyphenated to a quadrupole time of flight detector.

5.2.4.2 Detection of compounds using UHPLC-QTOF-MS/MS

A triple quadrupole instrument: UHPLC-QTOF-MS/MS (Shimadzu Corporation in Kyoto, Japan) was used to analysed extracted urine samples. A Shimadzu Corporation Shim-pack Scepter C18 column (100 x 2.1 mm, 1.9 um) was used and the temperature was maintained at 55 ^{o0}C. The separation process consisted of a binary mobile phase gradient with a flow rate of 0.4 ml/min. Mobile phase A consisted of 0.1% formic acid solution in ultra-pure water, and mobile phase B consistent of methanol. The entire length of the gradient lasted for 53 minutes, beginning with 10% B for the initial 30 minutes, followed by an increase to 60% B by the 40-minute mark, remaining at that level until minute 43, then shifted to 90% B until minute 45 and finally returned to initial conditions for equilibration between minute 48 and 50.

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A high-resolution Q-TOF mass spectrometer with an electrospray interface (ESI) in positive ionization mode was used for mass spectral analysis. Amongst other things, the instrument parameters comprised of an interface voltage of 4.0 kv and an interface temperature of 300 ^{O}C. To achieve high mass precision, sodium iodide was used as the calibration standard, which facilitated the detection of ions within a m/z range of 100 to 1000 Da, with fragmentation tests being conducted using argon as the collision gas at an energy level of 30 eV. Sample acquisition was randomised, and to ensure invariable instrument performance, quality control samples were analysed every 10 minutes.

An Evosphere C18 column with a 1.7 µm particle size (100 × 2.1 mm) was used for separation of the anlaytes. The mobile phase consisted of two solvents: (A) water containing 0.1% formic acid and (B) methanol containing 0.1% formic acid, with a total flow rate of 0.45 mL/min. Chromatographic separation was achieved using a gradient method. Initially, the column was equilibrated with 10% B for 2 minutes. The percentage of B was then increased to 60% within 1 minute, followed by a further increase to 90% over 3 minutes, where it was maintained isocratically for an additional 3 minutes. Subsequently, the conditions were adjusted back to 60% B for 2 minutes and finally to 10% B for 1 minute. The column was then re-equilibrated at 10% B for another 2 minutes before the next injection.

5.2.4.3 Analysis of metabolites

MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/), an open-source software was used to analyse metabolomics data for various compounds of interest. In addition, Excel was also used to organise the metadata for samples analysed with MetaboAnalyst software. The analysis of the compounds was performed based on mass to charge ratio (m/z) using the software, mass spectra (MS/MS) and

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chromatographic retention time. This was achieved by comparing them to standard metabolites interpreted with reference databases such as the Human Metabolome Databases (HMDB), Massbank, Metlin, CHeBMI, Lidmaps, MeSH, FooDB. The process complies with the Metabolomic Standards Initiative (MSI) level 2.

5.3 Results

5.3.1 Standard curve calibration

A five-point calibration curve for EE2 standards was used for quantification. The curve obtained was non-linear with EE2 having correlation coefficient of (r^2) value of 0.99 as per the graph below. According to Asuero *et al*., 2006, the correlation coefficient value between the range of 0.90 to 0.99 is interpreted as strong correlation.

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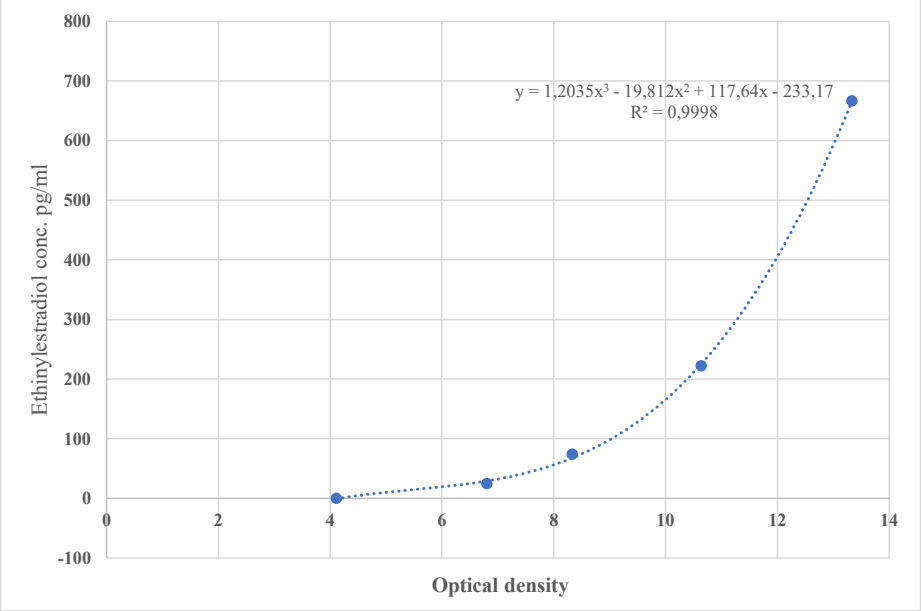


Figure 5.1: Ethinylestradiol (EE2) standard calibration curve

5.3.2 Ethinylestradiol concentration from study participants

The concentration of ethinylestradiol varied across the samples from low concentration of 11pg/mlL to the highest concentration of 804 pg/mL. Results are presented in a graph below (**Figure 5.2**).

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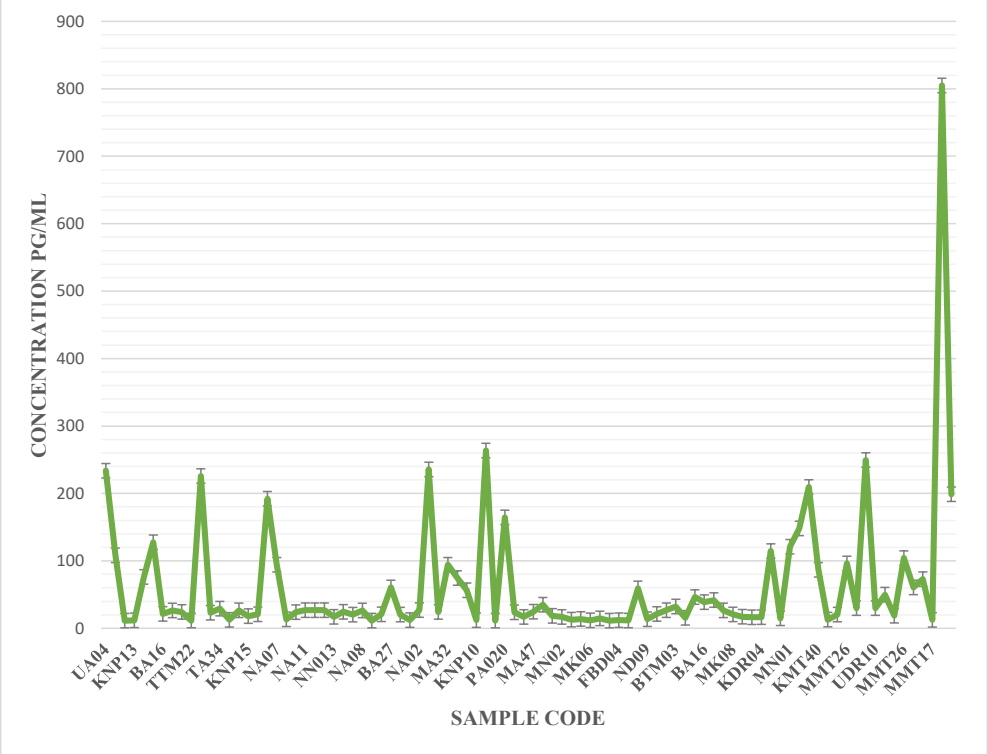


Figure 5.2: Varying concentrations of Ethinylestradiol (EE2)

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5.3.3 Ethinylestradiol concentration from study participants across different demographic groups

A total of 113 samples were tested for EE2. Of these 41 (36%) were negative while 72 (63.7%) were positive at different levels. Of those that were positive 60 (~~16.2~~53.1%) had a concentration below 100 while 12 (10.6~~3.2~~%) had higher concentrations above 100. The 20-30 group had high concentrations of EE2 with 48.6% on positive samples that had concentration of less than 100pg/ml and 12.5% had high concentration of EE2 on those above 100 pg/mL~~l~~. With 15-19 age group having 77.8% samples with concentrations that is ≤100pg/mL~~l~~. Furthermore, EE2 concentration varied across marriage groups with high concentrations observed among women who have never been married, women who are married as first wife had a 100% positivity rate with EE2 concentration of ≤ 100pg/mL~~l~~. Moreover, participants who had high school education and those who had tertiary education presented with high levels of EE2 on both ≤ 100pg/mL~~l~~ and ≥100pg/mL~~l~~, with 61.5% and 48.2% for high school and tertiary respectively. However, the was no significant association with the presented results. Results are present in table 5.1.

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Table 5.1: Distribution of EE2 according to age groups, marital status, education

Age groups	**EE2 positive**	**High EE2 concentration**	**Total**
15-19	7 (77.8%)	0	9
20-30	35 (48.6%)	9 (12.5%)	72
31-40	10 (58.8%)	1 (5.9%)	17
41 and above	18 (53.3%)	2 (13.3%)	15
Total	60 (53.1%)	12 (10.6%)	113
X2=1.045, p=0.790			
Marriage status			
Never married	46 (52.9%)	7 (8%)	87
Divorced	1 (33.3%)	0	3
Widowed	1 (50%)	1 (50%)	2
Married only wife	8 (53.3%)	3 (20%)	15
Married first wife	3 (100%)	0	3
Cohabitation	1 (33.3%)	1 (33.3%)	3
Total	60 (53.1%)	12 (10.6%)	113
Education			
Illiterate	4 (80%)	0	5
Primary school	1 (50%)	0	2
High school	24 (61.5%)	3 (7.7%)	39
Tertiary	27 (48.2%)	7 (12.5%)	56
Postgraduate	3 (60%)	0	5
Total	60 (53.1%)	12 (10.6%)	113

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5.3.4 Distribution of EE2 according to other clinical situations of the study participants

Ethinylestradiol levels were compared with other clinical categories in the study group to determine the positivity rate amongst the groups. The concentration of EE2 were compared with women who were using contraception, women who had abortions, women who had miscarriage and women who experiences pain during urination. across these four groups, it was observed that the positivity rate ranged from 55.08.3% to 61.9% amongst the positive samples and those with higher concentrations ranged from 8% to 20.8% across the groups. It was found that there was a strong association (*p-value 0.042*) in EE2 concentration and women who were using contraception with 55.0% being positive with EE2 concentration of ≤ 100pg/ml and 8.0% had EE2 concentration of greater than 100pg/ml. No significant association was observed in EE2 concentration and women who had abortion and miscarriage even those who experience pain during urination. Results are shown in table 5.2.

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Table 5.2: Distribution of EE2 according to other clinical situations of the study participants

		EE2 positive	**High EE2**	**Total**	
Currently using C's	Yes	55 (55.0%)	8 (8%)	100	$\chi 2=6.332$
	No	5 (38.5%)	4 (30.8%)	13	P=**0.042**
Had had abortion	Yes	13 (61.9%)	3 (14.3%)	21	$\chi 2=1.932$
	No	46 (51.7%)	8 (9%)	82	P=0.381
Had had miscarriage	Yes	14 (58.3%)	5 (20.8%)	24	$\chi 2=5.181$
	No	45 (51.1%)	7 (8%)	88	P=0.075
Pain when urinating	Yes	17 (58.6%)	4 (13.8%)	29	$\chi 2=2.37$
	No	23 (46.9%)	4 (8.2%)	49	P=0.129
Total	Total	60 (53.1%)	12 (10.6%)	113	

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5.3.5 Effect of EE2 on the occurrence of infection in the study population

Further analysis was conducted to determine the influence of EE2 concentration on the occurrence of infection. It was observed that 63.6% of study participants who were positive *C. trachomatis* also had a high rate of EE2 level, however the higher concentration (greater thant 100pg/mLł) was observed on participants who were negative for *C. trachomatis* with a rate of 11%. No strong correlation was observed between level of EE2 and *C. trachomatis* infection with a *p-value of 0.327*. No high EE2 concentration was found in any of the participants with gonococcal infection, while the positive individuals with presence of EE2 were 71.4%. Infection with T. vaginalis and occurrence of EE2 had a positivity rate of 66.7% with concentration ≤ 100pg/mLł. These occurrences also did not show any statistical significance with $p = 0.661$ for gonococcal infection and presence of EE2 and $p = 0.914$ for infection with *T. vaginalis* and presence of EE2. Although there is no statistical significance within these occurrences, samples positive co-occurrence of EE2 and these three infections tend to have high percentage of occurrence.

~~we~ We further analysed the urinalysis data to determine if there were individuals with indication of urinary tract infection (UTI), and the results revealed that of the 368 participants tested, 242 did not have UTI while 126 (34,2%) had UTI. In addition, of the 368 participants 241 (66.4%) had blood in the urine. Therefore, we also assess the effect of the presence of EE2 on those with UTI infection. It was found that 54.8% were positive for UTI and had EE2 levels of ≤ 100pg/mLł and 21.4% that were positive for UTI had EE2 concentration of ≥100pg/mLł. This occurrence showed strong correlation with p-*value of 0.006*. Infection with HIV did not show statistical significancet occurrence with $p = 0.900$. Results are presented in table 5.3.

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Table 5.3: The effect of EE2 on the occurrence of infection in the study population

Infection		**EE2 negative (41)**	**EE2 positive (60)**	**EE2 high (12)**	**Total**	**Statistics**
C. trachomatis	Negative	35 (38.5%)	46 (50.5%)	10 (11%)	91	χ^2=0.959
	Positive	6 (27.3%	14 (63.6%)	2 (9.1%)	22	p=0.327
N. gonorrhoea	Negative	39 (36.8%)	55 (51.9%)	12 (11.39)	106	χ^2=0.192
	Positive	2 (28.6%)	5 (71.4%)	0	7	P=0.661
T. vaginalis	Negative	40 (36.4%)	58 (52.7%)	12 (10.9%)	110	χ^2=0.012
	Positive	1 (33%)	2 (66.7%)	0	3	P=0.914
UTIs	Negative	31 943.7%)	37 (52.1%)	3 (4.2%)	71	**χ^2=10.248**
	Positive	10 (23.8%)	23 (54.8%)	9 (21.4%)	42	**p=0.006** **OR: 2.48** **95%CI: 1.059 – 5.80**
HIV	Negative	38 (36.9%)	54 (52.4%)	11 (10.7%)	103	χ^2=0.211
	Positive	2 (33.3%)	3 (50%)	1 (16.7%)	6	p=0.900

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5.3.3 Urinary tract infection (UTI) distribution in the study population

The distribution of UTI was studied amongst the study population across various demographics. It was found that UTI positivity rate varied across the different age groups, with a high prevalence observed in study participants who are 41 and above with a percentage of 20.7%, this was followed by a prevalence rate of 37% in individuals who are between the ages of 20-30. The association between age group and prevalence of UTI was not statistically significant. In addition, the prevalence rate of UTI compared with marriage status ranged from 30% for participants who are married as first wives to 57.1% in participants who are in cohabitation relationship. A prevalence rate of 41.1 % of UTI was observed in participants who have reached tertiary level and 36.4 in participants who reached postgraduate level. Finally, the prevalence rate of 25% was observed in individuals who were HIV positive. The association between urinary tract infections (UTI) and age group, marital status, education level and HIV status did not show significance statistically with p-values of 0.432, 0.752, 0.103 and 0.539 respectively. Results are shown in table 5.4.

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Table 5.4: UTI distribution in the study population

Characteristics	Category	UTI positive	Total	Statistics
Age groups	15-19	19 (28.8%)	66	χ2=2.747 P=0.432
	20-30	81 (37.0%)	219	
	31-40	15 (28.8%)	52	
	41 and above	11 (40.7%)	27	
Marital status	Never married	89 (33.3%)	267	χ2=2.663 P=0.752
	Divorced	8 (44.4%)	18	
	Widowed	3 (37.5%)	8	
	Married only wife	19 (35.2%)	54	
	Married first wife	3 (30%)	10	
	Cohabitation	4 (57.1%)	7	
Education	Illiterate	5 (29.4%)	17	χ2=7.704 P=0.103
	Primary school	3 (27.3%)	11	
	High school	40 (27.0%)	148	
	Tertiary	65 (41.1%)	156	
	Postgraduate	8 (36.4%)	22	
HIV status	Positive	3 (25%)	12	χ2=1.236 P=0.539
	Negative	113 (36.5%)	309	

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5.3.4 Occurrence of UTI in relation to demographic and clinical characteristic of study participants

Table 5.5 presents data analysed to investigate urinary tract infection occurrence with relations to ~~the~~ demographic characteristics and clinical characteristics amongst the study population. It was found that based on religion status, participants belonging to Christianity denomination and those who follows traditional practices had a UTI prevalence rate of 36.2% and 25% respectively. Participants from Muslim community and Atheist did not present with positive results for UTI The results did not show statistical significance with $p = 0.056$. Participants who are aware of contraceptive methods such as emergency contraception and intra uterine device (IUD) had high rates of UTI infections with prevalence rates of 39.8% and 37.0% respectively. This occurrence demonstrated strong relation between IUD awareness and occurrence of UTI with a *p-value of 0.027*. In addition, strong correlation was shown in individuals who ever used any form of contraception with a prevalence rate of 37.9% and a *p-value of 0.023*. No significant correlation was observed between UTI occurrence and study participants who are currently using contraceptives, had abortion, had miscarriage, had previous STIs and participants who experience pain during urination. However, 8.3% of pregnant participants revealed borderline statistically significance on the occurrence of UTI and pregnancy with $p = 0.046$.

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Table 5.5: The occurrence of UTI in relation to demographic and clinical characteristic of study participants

Characteristics		UTI positive	Total	Statistics
Religion	Islam Christianity Traditional Atheist	0 124 (36.2%) 2 (25%) 0	12 343 8 1	$\chi 2$=7.567 p=0.056
Emergency contraception	Yes No	80 (39.8%) 44 (30.6%)	201 144	P= 3.115 X2= 0.078
IUD	Yes No	51 (44%) 73 (31.9%)	116 229	**X2=4.886** **P=0.027**
Currently using C's	Yes No	98 (37%) 26 (29.5%)	265 88	X2=1.603 P=0.206
Ever used C's	Yes No	110 (37.9%) 15 (23.1%)	290 65	**X2=5.136** **P=0.023** **OR=0.491** **95%CI: 0.263 – 0.916**
Ever had miscarriage	Yes No	24 (47.1%) 99 (33.7%)	51 294	X2=3.394 P=0.065
Ever had abortion	Yes No	22 (40.7%) 100 (34.6%)	54 289	X2=0.748 P=0.387
Pain when you urinate	Yes No	26 (40.6%) 71 (35.1%)	64 202	X2=0.629 P=0.428
Have you had STIs	Yes No	9(23.7%%) 110 (35.7%)	38 308	P=0.141 X2=2.170
Currently pregnant	Yes No	1 (8.3%) 104 (36.4%)	12 298	**X2=3.965** **P=0.046**

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5.3.5 UTI in relation to urine characteristics among the study participant

Urinalysis data was further analysed to investigate the correlation of UTI and characteristics of urine. It was found that participants with presence of blood in urine had a positivity rate 41.1%. About 56.4% had a presence of nitrate in blood and 63.6% presented with ketones in their urine. Occurrence of UTI and presence of blood in urine, nitrate in blood and ketones in urine exhibited statistically significant relationship with *p=values* of *0.001* for blood and nitrate in blood and *0.038* for presence of ketones (see table 5.6).

Table 5.6: UTI in relation to urine characteristics among the study participants

Sample characteristics		**UTI positives**	**Total**	**Statistics**
Blood in urine	No blood in urine	25 (20.5%)	122	X2=15.263
	Blood found in urine	99 (41.1%)	241	**P<0.001** **OR=2.705** **95%CI: 1.626 – 4.500**
Nitrate in blood	No nitrate in the urine	71 (26.4%)	269	X2=27.854
	Nitrate found in the urine	53 (56.4%)	94	**P<0.001** **OR=3.605** **95%CI: 2.209 – 5.882**
Ketones in the urine	No ketones in the urine	117 (33.4%)	350	X2=4.316
	Ketones found in the urine	7 (63.6%)	11	**P=0.038** **OR=3.485** **95%CI: 1.00 – 12.14**

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5.3.6 Antibiotic resistance among *E. coli* isolates from the urine samples of the study participants

Antibiotic susceptibility test was performed for *Escherichia coli* isolates from study participants with urinary tract infection to determine resistance to antibiotics. The results show a high resistance rate of 77.2% to Ampicillin. This was followed by a resistance to 2nd and 3rd generation cephalosporins with resistance rates of 62.1% and 58.8% for ceftazidime and cefoxitin respectively. Resistance was also observed to carbapenems (last line of antibiotics used in treatment of infections caused by Gram-negative bacteria) antibiotics with resistance rates of ertapenem (25,8%) and imipenem (13.7%), results are presented in figure 5.3.

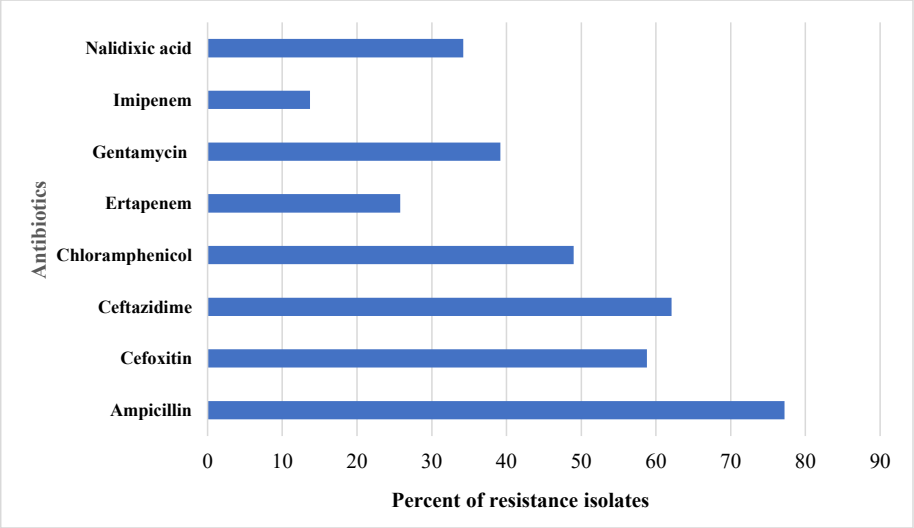


Figure 5.3: Antibiotic resistance among *E. coli* isolates from the urine samples of the study participants

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5.3.7 Heatmap of varying metabolites from three groups of contraceptives

The variation in concentration of various urine metabolites detected in individual who use different methods of contraceptives are shown below on figure 5.4. The Heatmap was generated through unsupervised hierarchical clustering analysis (HCA) suing MetaboAnalyst 6.0 and presents the top 52 metabolites identified from 20 urine samples of women using different contraceptive methods from three groups, namely: pills, injectables and implants.

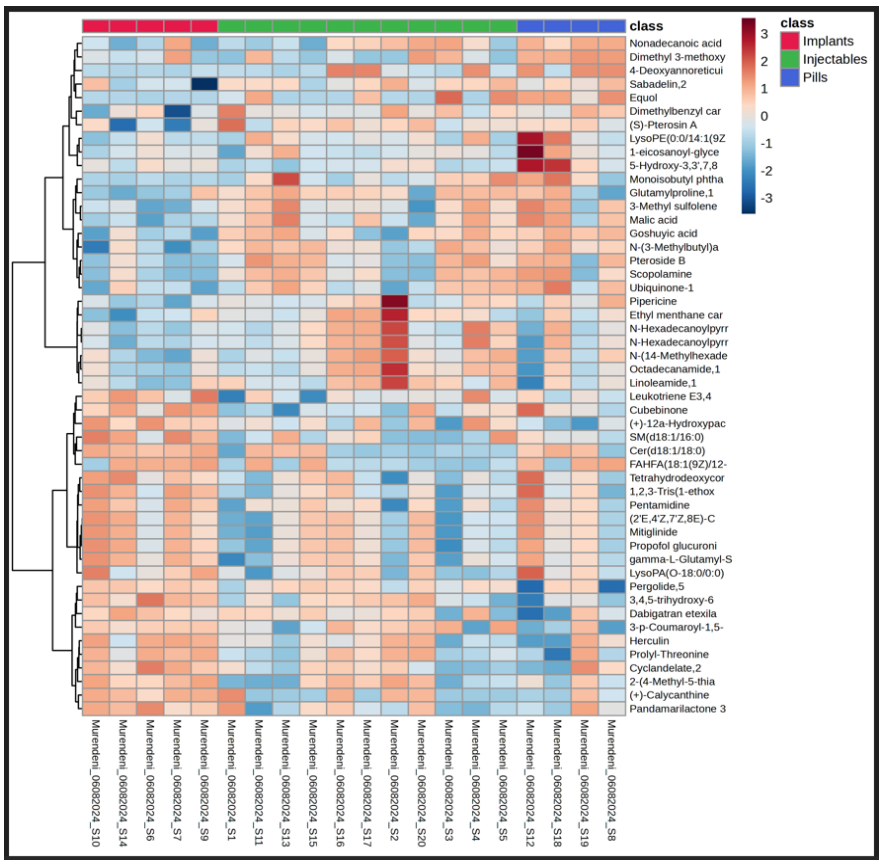


Figure 5.4: Heatmap of varying metabolites detect in urine sample

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5.3.8 Variable Importance in Projection score plot

The figure below (figure 5.5) depicts the variable importance projection score generated from OPLS-DA showing 15 metabolites that played a role in the distinction of the different groups of contraceptives i.e., Implants, Injectables and pills. From the data Equol, Goshuyic acid and Nonadecanoic had higher scores and are detected in urine of individuals using Pills.

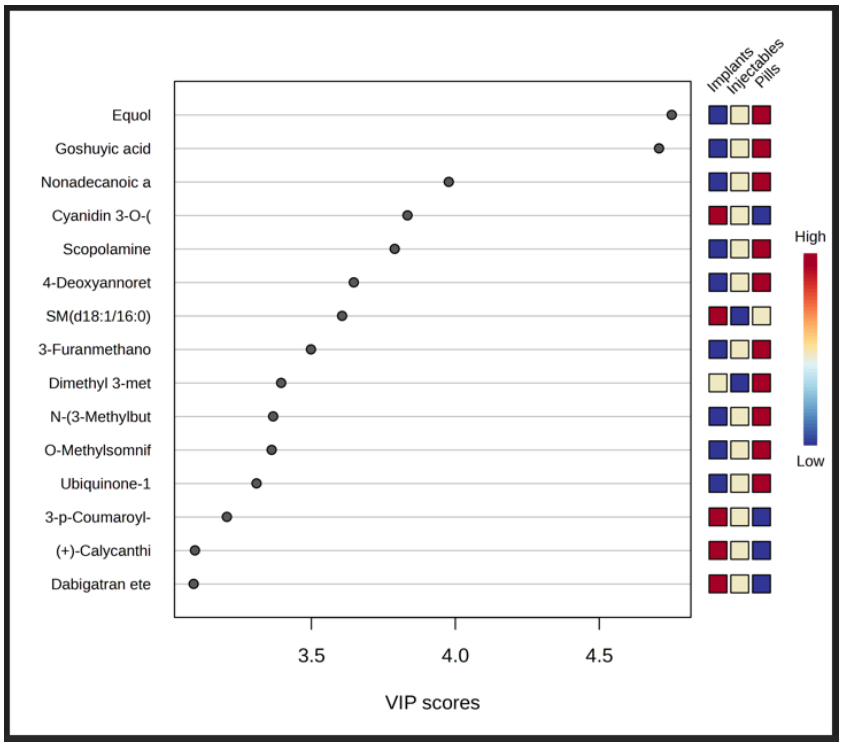


Figure 5.5: Variable Importance in Projection of detected metabolites in urine samples

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5.3.9 3D-PLS-A scores plot

The plot below (figure 5.6) shows the clear separation between the three groups of the contraceptive methods. The plot also shows very minimal overlap between these groups, which implies that there is a strong metabolic difference amongst the groups.

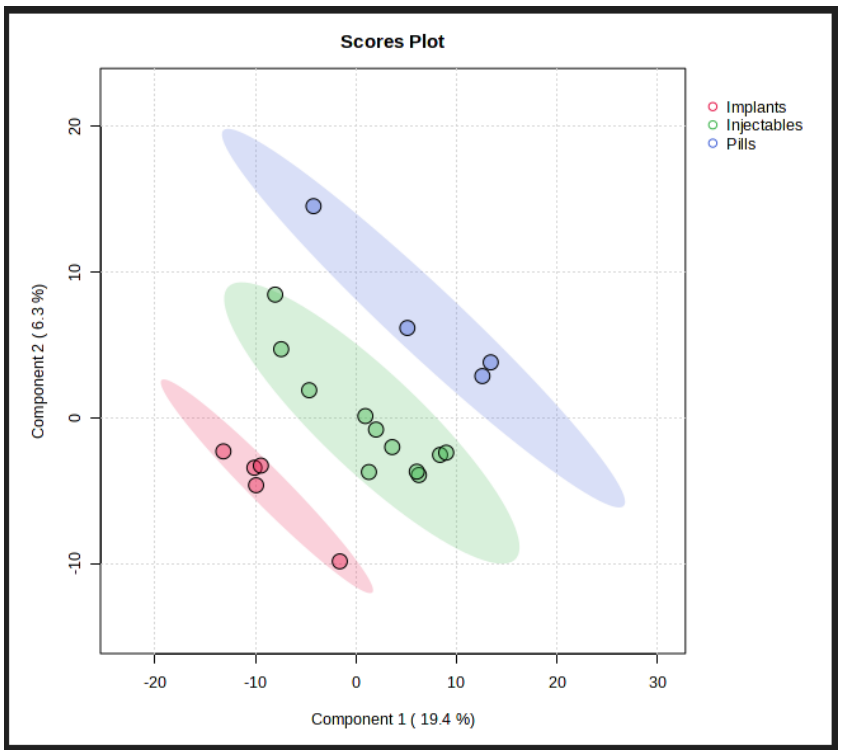


Figure 5.6: PLS-DA scores plot

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5.4 Discussion

Ethinylestradiol concentrations trends varied amongst study participants with the lowest concentration of 11pg/ml to the highest being 804 pg/ml. Based on various groups, for obvious reasons, high concentrations were observed in black women. This is mainly because more than 90% of the study population were black women. In addition, high concentrations varied between the age groups, with the 15-20 age group exhibiting high positivity rate of EE2 concentrations, however higher levels were not observed in this age group. With no statistical significance ($p = 0.790$) observed in the distribution of EE2 levels across the age group it suggests that exposure to EE2 is influenced by other factors. This was also the same case compared with marriage status and education level, where although some groups exhibited high positivity rate, no strong correlation was observed. The concentration of ethinylestradiol could also be influenced by other factors apart from contraception use for example environmental exposure, dietary intake, *etc*. This is also supported by some of the samples such as the outlier sample with the concentration of EE2 where the user self-reported that they do not use form of contraception that contains synthetic ethinylestradiol but uses condoms and the withdrawal method. Furthermore, some ~~of~~ ethinylestradiol concentration was also observed in study participants who are not using any form of contraception at all. However, significant association was found between EE2 presence and contraceptive use with a *p-value of 0.042*, which indicates that hormonal factors such as external estrogens contribute to increases levels of EE2.

The results also indicated statistically significant association between EE2 exposure and the occurrence of UTI ($P = 0.006$, OR = 2.48). The results suggest that individuals with exposure to EE2 are more than twice likely to develop urinary tract infection compared to those without exposure. This means that EE2 exposure may play a role in altering urinary microbiota or immune

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responses, which may lead to increasing susceptibility to organisms causing UTI. As it was observed within the study that participants using contraceptives tend to have high level of EE2 concentration, the results of EE2 occurrence and UTI could support the theory that contraception facilitates genital infections. This theory ~~was~~ also supported results in a study that analyses contraceptive use and UTI infection and found that general use of contraception (mostly injectables and pills) was negatively associated with UTI (Heidemann et al., 2022). Another study conducted in a cohort of reproductive women aged 18-52 revealed that there was increase in UTI infection with use of contraception (Čeprnja et al., 2023).

The current study analyzed distribution of urinary tract infections across demographic and clinical characteristics, as well as the antibiotic resistance of *E. coli* isolates. The findings revealed that urinary tract infection prevalence varied across age group, marital status, education level and HIV status, although no statistically significant association was observed in the groups. Clinical symptoms such as feeling pain when urinating and history of sexually transmitted infection did not show any significant correlation with the occurrence of UTI. However, pregnancy status demonstrated borderline significance with a p value of 0.046, indicating an increased risk of UTI among pregnant women. However, the results cannot be generalized as the study did not have many participants who were pregnant women. Additionally, characteristics of urine such as presence of blood ($P<0.001$), nitrates and ketones were significantly associated with prevalence of UTI. These findings align with established diagnostic biomarkers for UTIs, where hematuria, nitrituria are common indicators of UTI infection and kidney injury (Ogbole, 2021). Our findings were in contrast with a study conducted in Nigeria where urinalysis was performed in 200 women and no hematuria and nitrituria were observed from study participants (Okiki et al., 2021). This

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strengthens that these can only be detected in individuals with UTIs and for hematuria in individuals who also have kidney injury.

Antibiotic susceptibility revealed high resistance of *E. coli* isolates to commonly used antibiotics, with notable resistance to carbapenems and 2nd and 3rd generation cephalosporins as well as ampicillin. The results agree with a study done in Algeria where they observed high resistance to ampicillin with a rate of 75% (Merati et al., 2024). Resistance to carbapenems raises concerns on multi-drug resistance strains. Carbapenems are last-line drugs usually reserved for severe infections and resistance to these drugs poses a threat to public health (Sharma et al., 2024).

The presence of Equol, Pergolide, Leukotriene and Scopamine metabolites are indicative of potential roles in lipid metabolism changes, hormonal and estrogenic activity, inflammation and immune responses, neurological impact as well as oxidative stress regulation (Miyazaki et al., 2024; Ban et al., 2021; Melchoir et al., 2023). Equol, a metabolite produced by gut microbiota, is a very potent estrogen receptor modulator, it mimics the function of endogenous estrogen estradiol (Lv et al., 2024). Therefore, this behavior has an influence on hormone metabolism. It has been reported that equol may have an impact on hormone related conditions such as breast cancer and may enhance clearance of estrogen from the body which might reduce the estrogenic effects. In a study conducted in Japan amongst women aged between 20-70, they found equol and endogenous estrogen in high concentration in equol producers which indicates that equol promotes the excretion of estrogens (Fujitani et al., 2021).

Detection of equol in high concentration in urine suggests that the urine source is an equol producers as not everyone is an equol producer, and it can serve as a biomarker for endocrine disrupting exposure as well as an indicator for hormone imbalance. Clear separation of the groups on the PLS-DA score plot suggests that there is a metabolic difference between the three groups,

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and this clear clustering of the groups indicates that the different contraceptive methods induce distinct metabolic changes within the body.

5.5 Conclusion

The study revealed that exposure to ethinylestradiol (EE2) is a statistically significant risk for urinary tract infection (UTI). This preliminary finding calls for more research on the safety of the contraceptives contributing to these ~~in order~~ to save women from implication related to contraception. In addition, research need to be expanded in the food and water sectors to conduct investigation to determine levels of EE2 exposure from food and water consumption as our results also suggested that apart from contraception there are other factors that could be contributing to EE2 exposure. Variation of urine metabolomics is dependent on an individual physiology. Urine is from individuals ~~has~~ who have different composition and highly varied However, this variability in the concentrations of some of the detected metabolites is vital as it could be used as biomarkers for lifestyle factors, drug response and to monitor diseases. Ethinylestradiol can not only be found in individuals who are using ethinylestradiol-containing contraception and needs further studies to understand the origins of the synthetic compound as it seems there are more external factors contributing to levels of the synthetic compound in the body. Urinary tract infection distribution and antibiotic resistance highlights the importance of continuous surveillance of UTI and risk factors as well as antibiotic resistance patterns to guide appropriate prevention and treatment strategies.

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Chapter 6: Overall conclusion and recommendations

6.1 Conclusions

The current study assessed the potential effects of ethinylestradiol on reproductive health in relation to sSexually transmitted diseases. Because ethinylestradiol is a manmade estrogen that is highly potent and used in combinations as the main ingredient in hormonal-based contraception, we collected data that from study participants to assess their overall understanding and perceptions of contraception. We evaluated their knowledge, attitudeattitude, and practice of contraceptive methods amongst women of reproductive age. We observed that as much as awareness of the different methods of family planning is high, the actual practice of the methods is less than expected and needs improvement. Regardless of the high awareness rate, similar studies in India and Pakistan found discrepancy between the two variables *i.e.* knowledge and practice. This finding underscores the need to translate the knowledge into actual practice. One of the main

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findings was that amongst Muslim participants, it was observed that the prevalence of using contraception was low, which could be due to religious factors.

This project also investigated the prevalence of non-viral sexually transmitted diseases, caused by these three pathogens: ~~,~~ *Chlamydia trachomatis, Neisseria gonorrhea and Trichomonas vaginalis*. We observed high prevalence of *Chlamydia trachomatis*, followed by *Neisseria gonorrhea* and *Trichomonas vaginalis*. Because this this study is a community-based study, participants who tested positive for any of these infectious diseases are unaware of their statuses of the STIs. This highlights the importance of normalizing screening for sexually transmitted infections.

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Furthermore, the inclusion of UTI screening allowed for a broader understanding of genitourinary health. *Escherichia coli* was the predominant uropathogen isolated, and antimicrobial susceptibility testing revealed varying resistance patterns, underscoring the need for regular monitoring and prudent antibiotic use. The study also explored associations between contraceptive use—particularly those containing EE2—and the occurrence of UTIs and STIs. While correlations were observed, further research is needed to establish causal links.

Detection of the varied urine metabolites implies associations between neurological health, drug metabolism, inflammatory state and dietary intake. In women using hormonal contraceptives such as combined hormonal contraceptives (e.g., Pills) Equol can be used to evaluate the hormonal imbalance. It may indicate a risk of hormone related diseases, such as osteoporosis and breast cancer.

Collectively, these findings underscore the importance of integrated surveillance and diagnostic strategies that address hormonal exposure, STI prevalence, and UTI risk. The study provides a

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foundational framework for future research and public health interventions aimed at improving reproductive health outcomes, particularly in women using hormonal contraceptives.

6.2 Recommendations (These will be directed to Policy makers, researchers, pharmaceutical bodies, *etc.*)

i. **Family planning:** Targeted interventions could be employed to bridge the gap between knowledge and practice of family planning; this could be inclusive of counseling services, educational awareness and enhanced access to contraceptives. Future research could be conducted on a larger area and include male participants, sex workers to gain more understanding of family planning dynamics. Furthermore, sampling techniques could be improved to strengthen the future work. This study stresses the significance of not just raising awareness but to also encourage active practice of family planning. In addition, it is important to address cultural and religious factors that are influencing use of ~~contraception~~ contraceptives for the success of the interventions.

ii. **Sexually transmitted infection:** Given the asymptomatic nature of the infections, it is very important to encourage and normalize STI screening. Efforts to raise awareness should not be undermined, public health campaigns should be continued to inform about sexually transmitted infections, transmission dynamics as well as regular testing. Future research is needed in order to investigate the relationship between STI acquisition and methods of contraception. Inclusion of male participants would also in this case be of outmost importance in order to get a full picture of the transmission dynamics and prevalence of STIs.

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iii. **Urine metabolomics:** this is a powerful tool in providing important insight in metabolic responses related to various factors such as physiological conditions, drug metabolism, and environmental exposures.

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6.3 Limitations

i. **Reliance on self-reported data:** data collected from the study was self-reported by participants. Some of the variables are prone to bias as participants may not be honest when proving answers to questions such as number of sexual partners, HIV status.

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ii. **Sample size:** the sample size from the study was a small number which may not sufficiently represent the broader population.

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iii. **Population group:** Inclusion of some of the key populations such as sex workers, men who have sex with men would've helped in terms of generalizing the STI finding.

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iv. **Inclusion of other STIs:** the study only focused on non-viral sexually transmitted diseases and left out other critical infections such as Herpes simplex virus (HPV) and Human papilloma virus (HPV). Including a panel of a variety of critical STIs would provide a comprehensive understanding of the burden of STIs.

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v. **Specimen type:** the current study used urine as the specimen because it's non-invasive, however, urine may not the best specimen for detecting some urogenital

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infections, vaginal swabs would have been the best as urine could miss other infections.

vi. **Biased data:** As much as the study in metabolomics provides valuable insight into the metabolomic differences amongst contraceptive users, the differences in sample size per contraceptive group provides a limitation that does not allow for generalization or confirm the accuracy of the results, therefore future studies are needed with balanced sample sizes between the groups and a more advance statistical correction to minimize the effects of bias.

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Appendix A

Questionnaire for women in the Vhembe district

Knowledge, Attitude and Practice about Contraception and Reproductive Health

INSTRUCTIONS

This questionnaire seeks to investigate the Knowledge, Attitude and Practice of contraception and reproductive health in Vhembe district, Limpopo province of South Africa. The study is part of the researcher's Doctor of Philosophy in Microbiology Degree at the University of Venda (UNIVEN) and should help improve understanding of how contraceptives are affecting people and their reproductive health. No respondent will be identified or traced from this investigation whatsoever as confidentiality and anonymity are guaranteed. All data and information provided by you will be treated as strictly private and confidential. There are no 'right' and 'wrong' answers. The researcher is only interested in your response. You are also asked to provide your urine sample for laboratory testing for the presence of contraceptives and microorganisms in urine. You are therefore kindly requested to complete the questionnaire as honestly as you can. Thank you for participating in the current study.

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CODE ……

Section A: Demographic data Kindly indicate your response by placing a tick or a cross in the appropriate box at each statement or question		
	Name and surname	
	Year of birth	
	Gender	Female ☐ Male ☐
	Age	15-19 ☐ 20-30 ☐ 30-40 ☐ above 40 ☐
	Race	Black ☐ Colored ☐ Indian ☐ White ☐
	Marriage status	Married ☐ Single ☐ divorced ☐
	Occupation	
	Education	Illiterate ☐ Primary school ☐ High school ☐ Tertiary ☐ Postgraduate ☐
	Children	One ☐ Two ☐ Three ☐ four and above ☐
	HIV status	Positive ☐ Negative ☐
	Weight	
	Home setting	Rural ☐ Urban ☐
	Religion	Islam ☐ Christianity ☐ Atheist ☐ other (specify) ……

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Section B: Knowledge, Attitude and Practices of Contraception

1. How did you know about contraceptives? Media ☐ Social network ☐ Health worker Friends ☐ Colleagues ☐

2. What methods do you know? DMPA ☐ Pills ☐ Intra uterine device (IUD) ☐ Emergency contraception ☐ Male condoms ☐ Female condoms ☐ Other (specify) ……..

3. What are the benefits of contraceptives from your point of view? ……..……

……

……

4. What are some consequences of engaging in unprotected sex? ……...……

……

……

5. Do you find contraceptives to be beneficial? Yes ☐ No ☐

1b. Justify

……

……

6. Who do you think is responsible for contraception? Man ☐ Women ☐ Both ☐

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7. What are your feelings towards using contraceptives?

……

……

……

8. What are your feelings on the number of children you want to have?

……

……

……

9. What are your feelings on the sex of the first child and of the subsequent children?

……

……

……

10. Number of sexual partners?

……

11. Does family Planning make its users Promiscous?.....

12. Does family planning conflict with your moral/cultural/religious beliefs? ……

Explain

……

…….

13. Are you in use of contraceptives? Yes ☐ No ☐

1b. If not, do you think you will use in future, justify

……

……

……..

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14. How long have you been using contraceptives?

……..

15. Which type of contraceptives do you use? DMPA ☐ Pills ☐ intra uterine device (IUD) ☐ emergency contraception ☐ male condoms ☐ female condoms ☐

16. What is your reason for using contraceptives?

……

……

……

17. What are your feeling about involving your partner in the decision to use contraceptives?

……

……

……

18. What is your feeling in using male contraceptives?

……

……

19. Do you believe that contraceptives are effective in family planning?

……

……

20. What is your experience with contraceptives?

……

……..

21. Are contraceptives expensive?.....

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Appendix B

RESEARCH ETHICS COMMITTEE

UNIVEN Informed Consent

Appendix B

LETTER OF INFORMATION

Title of the Research Study : Ethinylestradiol (EE2) and Human Reproductive Health: Investigating the Potential Effects of Contraceptives on Human Reproductive Health and Sexually Transmitted Diseases in the Era of HIV and AIDS

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Principal Investigator/s/ researcher : (Manavhela Murendeni, MSc)

Co-Investigator/s/supervisor/s : (Prof Samie Amidou (Ph.D.) and Prof Traore AN, Ph.D.)

Brief Introduction and Purpose of the Study: I am doing a study about the effects of contraception on reproductive health and sexually transmitted diseases among women of reproductive age in Vhembe district of Limpopo province, South Africa. This study is a research project to obtain a degree of Doctor of Philosophy in Microbiology. Therefore, I want to collect information about your knowledge, attitude and practice of contraception.

Outline of the Procedures : You may participate in this project if you are a woman who is between the ages of 15- 50. If you are in use of any form or not of contraception. And you may not participate in the current project if you are under the age of 15 and if you are above the age of 50.

As a part of this study, I am going to hand you an informed consent that you will sign and questionnaire that you will have to fill, which will help me get the information mentioned above. You are among other volunteers who agreed to be part of the study. After you finish to fill the questionnaire, I ask that you give us a sample of your urine. The information you provide and the results of from the urine samples will help us in understanding the effects of hormones on the reproductive health and the association with sexual transmitted diseases (STDs).

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Risks or Discomforts to the Participant: (There will be no risks involved by taking part in the study.)

Benefits : (By participating in this study, you will not receive any direct benefit. However, you will help to increase our understanding about how hormones affect reproductive health and their association with sexual transmitted Diseases (STDs). I hope that the results of the study will improve the quality and utilization of the services currently available for you. Your participation in this study will not involve any risks to you.)

Reason/s why the Participant May Be Withdrawn from the Study: (Participants are completely free to take part in this study or to refuse to do so. Even after agreeing to participate in the study, they will be free to leave the discussion any time they wish. The decision to not to participate or to withdraw will not affect any future aspects of theirs.*)*

Remuneration : (The participant will not receive any renumeration for participating on the study*)*

Costs of the Study : (No Costs are expected to be covered by the participant*)*

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Confidentiality : (Confidentiality will be achieved by using codes on the samples that will be collected from study participants, and the questionnaires will also be allocated the same code as the samples)

Research-related Injury : (It is highly unlikely that there will be any injury as the investigators will not be handling the participants themselves, but should there be any reaction of some sort, then the participants will be taken to health care facilities.*)*

Persons to Contact in the Event of Any Problems or Queries:

(Prof Samie Amidou. samieamidou@yahoo.com). Please contact the researcher (tel no. 0671838394), my supervisor (tel no. 0159628186) or the University Research Ethics Committee Secretariat on 015 962 9058. Complaints can be reported to the Director: Research and Innovation, Prof GE Ekosse on 015 962 8313 or Georges Ivo.Ekosse@univen.ac.za

General:

Potential participants must be assured that participation is voluntary and the approximate number of participants to be included should be disclosed. A copy of the information letter should be issued to participants. The information letter and consent form must be translated and provided in the primary spoken language of the research population

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CONSENT

Statement of Agreement to Participate in the Research Study:

- I hereby confirm that I have been informed by the researcher, (*Murendeni Manavhela*), about the nature, conduct, benefits and risks of this study - Research Ethics Clearance Number: _,
- I have also received, read and understood the above written information (*Participant Letter of Information*) regarding the study.
- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.
- In view of the requirements of research, I agree that the data collected during this study can be processed in a computerized system by the researcher.
- I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.
- I understand that significant new findings developed during the course of this research which may relate to my participation will be made available to me.

Full Name of Participant Date Time Signature

…….. …… …… ……..

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I, (*Murendeni Manavhela*) herewith confirm that the above participant has been fully

Informed about the nature, conduct and risks of the above study.

Full Name of Researcher

…… Date…….. Signature……

Full Name of Witness (If applicable)

…….. Date …….. Signature……..

Full Name of Legal Guardian (If applicable)

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…… Date…… Signature……..

Please note the following:

Research details must be provided in a clear, simple and culturally appropriate manner and prospective participants should be helped to arrive at an informed decision by use of appropriate language (grade 10 level- use Flesch Reading Ease Scores on Microsoft Word), selecting of a non-threatening environment for interaction and the availability of peer counseling (Department of Health, 2004)

If the potential participant is unable to read/illiterate, then a right thumb print is required and an impartial witness, who is literate and knows the participant e.g. parent, sibling, friend, pastor, etc. should verify in writing, duly signed that informed verbal consent was obtained (Department of Health, 2004).

If anyone makes a mistake completing this document e.g. a wrong date or spelling mistake, a new document has to be completed. The incomplete original document has to be kept in the participant's file and not thrown away, and copies thereof must be issued to the participant.

References:

Department of Health: 2004. *Ethics in Health Research: Principles, Structures and Processes*

http://www.doh.gov.za/docs/factsheets/guidelines/ethnics/

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