DETECTION OF AZITHROMYCIN RESISTANCE IN *Neisseria gonorrhoeae, Chlamydia trachomatis* AND *Mycoplasma genitalium* AMONG SYMPTOMATIC WOMEN ATTENDING TWO HOSPITALS IN BUSIA COUNTY, KENYA

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DEPARTMENT OF PHARMACOLOGY, CLINICAL PHARMACY AND PHARMACY PRACTICE

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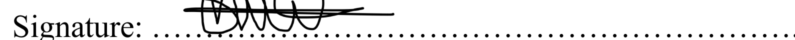

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DEDICATION

I dedicate this thesis to my wonderful mother Anne Ngina and my late grandparents Ladislaus and Cecilia Njogu for their unwavering love and support. They have been instrumental in encouraging and supporting me for the role of the future scientist I aspire to be. I hope this makes you proud.

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LIST OF ABBREVIATIONS AND ACRONYMS

AMR	Antimicrobial resistance
BLAST	Basic Local Alignment Search Tool
CDC	Centers for Disease Control and Prevention, the United States
CT	*Chlamydia trachomatis*
DGI	Disseminated gonococcal infection
DNA	Deoxyribonucleic acid
ERC	Ethics & Research Committee
ESCs	Extended Spectrum Cephalosporins
FSW	Female sex worker
GBV	Gender based violence
HIV	Human immunodeficiency virus
IgA	Immunoglobulin A
KNH	Kenyatta National Hospital
LGV	Lymphogranuloma venereum
LOS	Lipo-oligosaccharide
LPS	Lipopolysaccharide
MEGA	Molecular Evolutionary Genetics Analysis
MIC	Minimum inhibitory concentration
MG	*Mycoplasma genitalium*
MSA	Multiple sequence alignment
MSM	Men who have sex with men
MtrR	Multiple transferable resistance repressor
NAAT	Nucleic acid amplification test
NCBI	National Centre for Biotechnology Information
NF-Kb	Nuclear factor kappa B
NG	*Neisseria gonorrhoeae*
Opa	opacity- associated protein
PCR	Polymerase chain reaction

PID	Pelvic inflammatory disease
PorB	Porin protein B
rRNA	Ribosomal ribonucleic acid
STD	Sexually transmitted disease
STI	Sexually transmitted infection
UON	University of Nairobi
UV	Ultra violet
WHO	World Health Organization

DEFINITION OF OPERATIONAL TERMS

Antimicrobial resistance: Reduction or loss of effectiveness of a drug against target microorganisms.

Deoxyribonucleic acid (DNA) sequencing: The process of determining the nucleic acid sequence or the order of nucleotides in DNA.

Gene: Is the basic physical and functional unit of heredity passed from parent to offspring.

Multiplex PCR: A PCR technique that can be used to amplify several different DNA sequences simultaneously in a single reaction tube.

Mutation: Is a permanent change of the nucleotide sequence of the genome of an organism.

Polymerase chain reaction (PCR): A process of amplifying a small sample of DNA in a series of cycles of temperature changes, to make large enough amounts for further study.

Phenotype: The observable biochemical or physical characteristics of an organism as determined by both environmental influences and genetic makeup.

Primer: A short single stranded DNA sequence that defines the region of the DNA that will be amplified.

Resistant marker: A specific predetermined DNA or RNA sequence that is associated with the development of resistance to an antimicrobial agent.

ABSTRACT

Background

Neisseria gonorrhoeae and *Chlamydia trachomatis* are among the most prevalent organisms responsible for reproductive tract infections in both men and women, according to the World Health Organization (WHO). *Mycoplasma genitalium* is also being implicated as a cause more frequently. Several high-risk populations exist in Busia County and there have been increased reports of persistent sexually transmitted infections (STIs) in recent years. Women appear to be affected by these STIs more than men and are mostly asymptomatic which delays treatment. Health complications could arise if therapy is postponed. Treatment may become more challenging if the STIs become coinfected with one another. Sexually transmitted infections can raise the possibility of contracting and spreading the human immunodeficiency virus (HIV). The treatment of infections is increasingly threatened by the rising instances of antimicrobial resistance (AMR) to azithromycin.

Objectives

The study was carried out to determine the prevalence of three sexually transmitted infections: *N. gonorrhoeae*, *C. trachomatis* and *M. genitalium*, the prevalence of coinfections among the STIs, and to detect resistance markers involved in azithromycin resistance among symptomatic women attending Busia County referral hospital and Khunyangu sub county hospital in Busia County, Kenya.

Methods

Sexually active women of reproductive age attending STI, family planning and outpatient clinics in the two hospitals in Busia County were enrolled conveniently in this cross-sectional study. Endocervical swabs were collected from 424 women who presented with symptoms of sexually transmitted infections. Detection of the STIs was done using multiplex polymerase chain reaction. Amplification was then performed to detect resistant markers on the *mtrR* and 23S rRNA genes. The amplicons were then purified followed by Sanger sequencing and analyzed to detect point mutations.

Results

Among the symptomatic women, 23.6% had at least one sexually transmitted infection with prevalence as follows: *N. gonorrhoeae* (NG) at 17.7%, *C. trachomatis* (CT) at 6.8%, and

M. genitalium (MG) infection at 4%. The rates for coinfection were as follows: CT/NG at 2.1%, NG/MG at 1.9%, CT/MG at 1.7% and CT/NG/MG at 0.7%. None of the most commonly reported mutations were found for the 23S rRNA gene (A2058G and A2059G) or the *mtrR* gene (H105Y, A39T and G45D). Novel mutations that have not been previously reported or associated with resistance were found in this study. The most prevalent mutations were: P101A, H102Yand S110N for *N. gonorrhoeae*, G1987T (*Escherichia. coli* numbering) for *C. trachomatis* and G2010T (*E. coli numbering*) for *M. genitalium.*

Conclusion

There was a high prevalence of sexually transmitted infections among symptomatic women attending the two hospitals (23.6%). Markers for resistance that have not being previously reported were present and most of them were responsible for amino acid changes. There is a need for screening, surveillance, and treatment programs owing to the high prevalence of STIs in the study population. Antimicrobial susceptibility testing is needed to establish whether the reported mutations are associated with reduced azithromycin efficacy. Further surveillance and studies to investigate other mechanisms of azithromycin resistance should also be considered.

CHAPTER 1: INTRODUCTION

1.1 Background information

According to estimates from the World Health Organization (World Health Organization, 2022), there were 374 million instances of the four curable STIs chlamydia, gonorrhea, syphilis, and trichomoniasis in 2020. Global annual rates for *N. gonorrhoeae* among women stands at 2% with the Western Pacific region and Africa having the bulk of the infection (Rowley et al., 2019). Studies among the high risk 15-24 age group in East Africa have reported a prevalence of 8.2% (Torrone et al., 2018). For *C. trachomatis*, the prevalence in women stands at 3.8% with the highest occurrence in Africa and the Americas (Rowley et al., 2019). There is limited data on the global prevalence of *M. genitalium*. In a multi analysis study prevalence of *M. genitalium* in developing countries such as Kenya and Madagascar stands at 3.9% (Baumann et al., 2018).

In low- and middle-income countries, few large-scale studies have been done to determine STI prevalence. Youths aged 15-24 have the highest reported cases of STIs globally especially in low resource countries (Dehne & Riedner, 2001; Jespers et al., 2016). Several population-based studies in sub-Saharan Africa have found that STI prevalence is higher in women than in men and in younger women than in older women (Francis et al., 2018; Torrone et al., 2018). The higher prevalence especially among younger women could be attributed to several factors: increased biological susceptibility, higher risk of sexual coercion, older sexual partners, gender inequalities, and cultural norms that reduce access to reproductive health resources and reduced educational and economic opportunities (Yuh et al., 2020).

Busia County has an HIV prevalence of 7.7%, which is 1.6 times higher than the national prevalence, which stands at 4.9%, and is among the top five (5) counties with the highest HIV prevalence (Kenya, 2018). This county is a transit hub, and the high population mobility increases the risk of STI transmission. Prevalence data on other STIs are not available in Busia due to inadequate surveillance reports. Several studies conducted in Nairobi and Kisumu have found the prevalence of *N. gonorrhoeae* ranging from 0-6% (Maina et al., 2016; Otieno et al., 2015). Prevalence studies for *C. trachomatis* in Nairobi range from 6% (Marx et al., 2010) in one study to 12-13% in another study (Maina et al., 2016). In Western Kenya the prevalence found was 5.6% (Kinuthia et al., 2015) while in

Kisumu a lower prevalence of 2.8% has been reported among women being screened for HIV (Otieno et al., 2015). Most studies on *M. genitalium* in Africa have focused on cohorts of high risk women with prevalence of 12.9-16% in Kenya (C. R. Cohen et al., 2007; Gomih-Alakija et al., 2014), 14% in Uganda (Vandepitte et al., 2012) and 8.7% in South Africa (Hay et al., 2015).

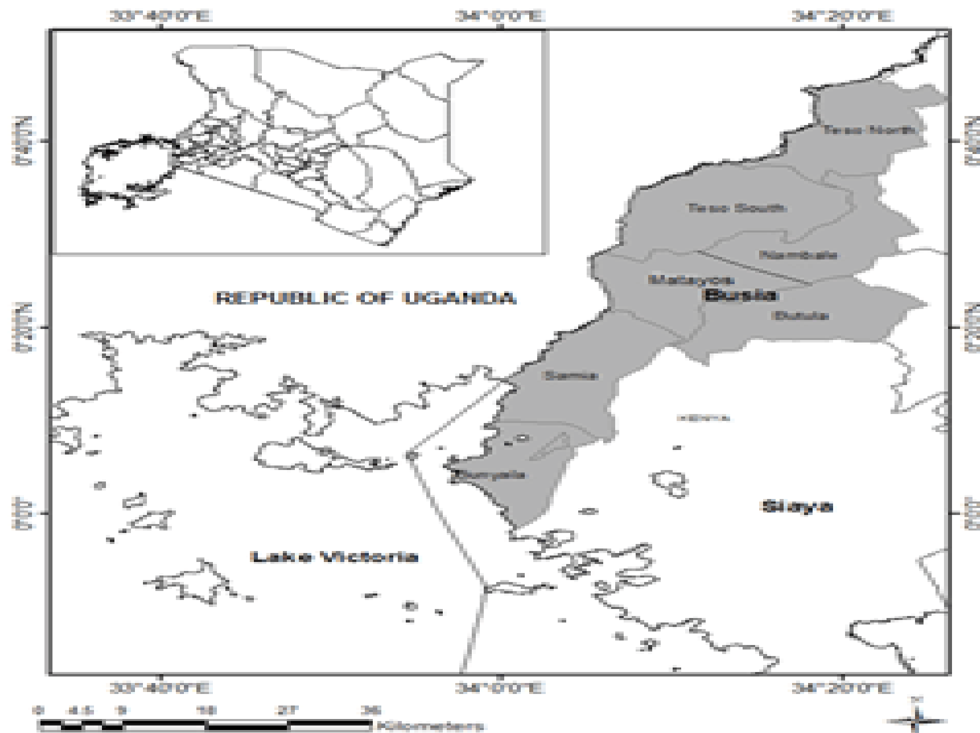

Figure 1.1 A map of Busia County, Kenya

Azithromycin, a macrolide, is currently in use for the treatment of *N. gonorrhoeae*, *C. trachomatis* and *M. genitalium* in both hospitals. There is a worrying trend with the increasing reports of the emergence of resistance. From several studies, *N. gonorrhoeae* isolates with high azithromycin MIC have emerged and are increasingly reported (Chisholm et al., 2010; Unemo & Shafer, 2014). The WHO (2012) put forth a universal alert on untreatable gonococcal infections. Mutations in the 23S rRNA gene have been linked to *C. trachomatis* resistance to macrolides from several studies (Misyurina et al., 2004; Sandoz & Rockey, 2010). Increased reports of *M. genitalium* macrolide resistance linked to mutations in the 23S rRNA gene have also been made in a number of different countries (Jensen et al., 2008; Nijhuis et al., 2015; Pond et al., 2014). In order to create a reliable

surveillance system, there is an urgent need for strong worldwide public health attempts (Tien et al., 2020).

This was a descriptive cross-sectional study conducted in two hospitals namely Busia County Referral hospital and Khunyangu sub county hospital. Samples were collected just once and analyzed using molecular techniques. A questionnaire was administered to each study participant and sociodemographic data, history of sexual behavior and symptoms were collected. The macrolide of interest, azithromycin, is among the first line drug recommended for the treatment of sexually transmitted infections so it was safe to conclude that most women who had been previously infected with a sexually transmitted infection had been treated with azithromycin. There was a high prevalence of sexually transmitted infections among symptomatic women attending the two hospitals (23.6%). Markers for resistance were also present and most of them were responsible for amino acid changes.

Conducting this study contributed to filling the knowledge gap on the prevalence of gonorrhea, chlamydia, and *M. genitalium* infections among symptomatic women attending hospitals in Busia County. This information will be valuable in informing evidence-based interventions to control the spread of these infections in this region. Furthermore, the identification of the molecular mechanisms of antimicrobial resistance in these STIs will provide valuable insights into the development of resistance and guide effective treatment strategies.

1.2 Statement of the problem

Infections of the urogenital tract caused by *N. gonorrhoeae* and *C. trachomatis* cause significant adverse reproductive and health outcomes. Women are disproportionally affected by the STIs and most of the infections remain asymptomatic thereby delaying treatment and resulting in problems including infertility and pelvic inflammatory disease (Gottlieb et al., 2014). *M. genitalium* is responsible for inflammatory reproductive tract syndromes and can lead to similar complications (Jensen & Bradshaw, 2015). Additionally, there is evidence that STIs raise the risk of HIV transmission and acquisition making STI control crucial (H. Ward & Rönn, 2010).

The impact of STIs, especially following antimicrobial resistance, on the economy, reproductive and psychosocial health remains underappreciated by frontline health care workers (Tien et al., 2020). Sexually transmitted infections can have a significant impact

on the economy through increased healthcare costs, decreased productivity, and lost wages. The STI and HIV-related medical expenses, particularly direct medical expenses (drugs, hospitalization), place a significant financial burden on society (Chesson et al., 2004; Owusu-Edusei, Chesson, et al., 2013). Both HIV and STIs, however, may also have an effect on other facets of the economy, including labor, homes, and education (Owusu-Edusei, Roby, et al., 2013). In order to ensure that the full economic impact of STIs and HIV on society is quantified and conveyed to policy/decision-makers, intersectoral costs associated with STIs and HIV need to be addressed (Schnitzler et al., 2021).

There hasn't been any research on the prevalence of STIs among women in Busia County as a whole. National STI prevalence data, except for HIV, also remains limited due to a lack of reporting systems and adequate surveillance. Knowledge on the prevalence of curable and other STIs can provide a basis for STI control measures for this population. The likelihood of coinfections among the STIs complicates treatment and there is a need to determine the prevalence and investigate regimens that provide efficacious treatment. This information is critical in developing effective treatment and control strategies to curb the spread of these infections in this region.

There is also limited information on the antimicrobial resistance patterns in Busia County which is a significant challenge to effective management and control of these infections. This situation is compounded by the overuse of antibiotics, including azithromycin, leading to the emergence of resistant strains. The increasing reports of azithromycin resistance to the selected STIs is an issue of general public health due to the lack of or limited availability of alternative therapy (Bissessor et al., 2015; Demczuk et al., 2016). Moreover, the lack of data on the molecular mechanisms of antimicrobial resistance in these infections hinders the development of effective treatment strategies.

1.3 Justification

Limited data exist on the prevalence of STIs, including gonorrhea, chlamydia, and *M. genitalium* infections, in symptomatic women attending Busia County referral hospital and Khunyangu sub county hospital in Busia County, located in the western region of Kenya. This information is critical in developing effective treatment and control strategies to curb the spread of these infections in this region. Moreover, the emergence of AMR to commonly used antibiotics, including azithromycin, is a significant concern. Therefore, there is an

urgent need to assess the prevalence of these infections and their antimicrobial resistance patterns in symptomatic women attending both hospitals in Busia County.

Understanding the molecular mechanisms of antimicrobial resistance is essential in guiding the choice of appropriate antibiotic therapy for effective management and control of these infections. Currently, there is little information on the presence of azithromycin resistance markers in *mtrR* gene in *Neisseria gonorrhoeae* and 23S rRNA gene in *Chlamydia trachomatis* and *Mycoplasma genitalium* in Busia County. Therefore, this study will provide valuable insights into the development of resistance and guide effective treatment strategies for the management and control of these infections. In addition to the public health implications, this study has significant scientific merit. It will contribute to the body of knowledge on the prevalence and antimicrobial resistance patterns of STIs, including gonorrhea, chlamydia, and *M. genitalium*, in Busia County. Furthermore, the study will provide valuable insights into the molecular mechanisms of antimicrobial resistance, which is critical in guiding the development of effective treatment strategies.

For the selected STIs, resistance markers have already been well characterized and therefore DNA based sequencing methods can be used to accurately and objectively predict antimicrobial resistance without the need for phenotypic AMR tests. Molecular prediction of AMR can then be used to identify settings that require further phenotypic AMR testing (Vernel-Pauillac et al., 2008). Molecular-based techniques are easier to carry out and provide rapid diagnostic results which improve patient care. With this technique, more samples can be easily collected from high-risk populations and screened for both *N. gonorrhoeae* and *C. trachomatis* which often appear concurrently. This is especially practical in low-resource settings such as the study location for this study. Molecular methods can help supplement the traditional culture-based AMR surveillance (Bachmann et al., 2010; Muralidhar, 2015; Unemo et al., 2016).

1.4 Research Questions

1. What is the prevalence of *N. gonorrhoeae*, *C. trachomatis*, and *M. genitalium* infections among symptomatic women attending Busia County referral hospital and Khunyangu sub county hospital in Busia County*?*
2. What is the prevalence of coinfections among the select STIs among

symptomatic women attending Busia County referral hospital and Khunyangu sub county hospital in Busia County?

3. Do resistance markers associated with azithromycin resistance exist in symptomatic women attending Busia County referral hospital and Khunyangu sub county hospital in Busia County?

1.5 Objectives

1.5.1 General objectives

The objective of the study is to determine the prevalence and detect resistance markers associated with azithromycin resistance for the three STIs: *N. gonorrhoeae, C. trachomatis*, and *M. genitalium* among symptomatic women attending two hospitals in Busia County, Kenya using molecular methods.

1.5.2 Specific objectives

1. To determine the prevalence of *N. gonorrhoeae, C. trachomatis*, and *M. genitalium* infections among women attending Busia County referral hospital and Khunyangu sub county hospital in Busia County.

2. To determine the prevalence of coinfections among the select STIs among symptomatic women attending Busia County referral hospital and Khunyangu sub county hospital in Busia County.

3. To detect the resistance markers associated with azithromycin resistance in *N. gonorrhoeae, C. trachomatis*, and *M. genitalium* among symptomatic women attending Busia County referral hospital and Khunyangu sub county hospital in Busia County.

1.6 Significance of the study

Information will be provided by the study's findings on the genetic mutations associated with azithromycin resistance across the select sexually transmitted infections. By presenting evidence- based knowledge on the resistance mechanisms, the study can help inform policy on STI control and also on diagnosis and treatment guidelines.

CHAPTER TWO: LITERATURE REVIEW

In developing nations, sexually transmitted illnesses place a significant economic and health burden. Sexually transmitted infections directly affect reproductive and child health and also indirectly increase the transmission of HIV thereby increasing the burden on mortality and morbidity. Women and children bear the greatest impact. Developing countries lack comprehensive and systematic surveillance and prevalence for most STIs is limited to specific subpopulations such as sex workers in most African countries including Kenya. The increasing reports of antimicrobial resistance in most STIs is a worrying global public health alarm that has necessitated the need for surveillance. Chlamydia and gonorrhea are among the four main curable STIs and are responsible for the high burden of infection in developing countries. *Mycoplasma genitalium* is also been increasingly reported to cause infections though there is little worldwide prevalence data. This study focused on three STIs and investigated the molecular mechanisms of azithromycin resistance in the selected STIs.

2.1 Epidemiology of the select STIs

Prevalence estimates in 2020 show that STIs are persistently endemic worldwide. The prevalence and incidence are highest in low-income countries, areas, and territories, particularly the western Pacific region dominated by China, which has a vast population. Fig. 2.1 below shows the prevalence rates for the four common STIs globally. Sexually transmitted infection acquisition is linked with a bigger risk of HIV spread through genital shedding of HIV. Prompt diagnosis and effective treatment reduce the risks of transmission and improve sexual and reproductive health (World Health Organization, 2022).

As the second most common sexually transmitted disease, gonorrhea is a significant source of morbidity, and is still a global public health problem. From WHO (2022) projections, there was an incidence of 82 million cases among 15 to 49-year-old adolescents and adults globally in 2020. The burden of infections contributes to morbidity and mortality, especially in developing countries. The greatest extent of infections was in the western Pacific and African regions. The global annual incidence rate in 2016 estimates was 2.6% for men and 2.0% for women. Men and women in Africa experienced incidence rates of 1.6% and 1.9%, respectively (Rowley et al., 2019).

Chlamydia is the world's most prevalent bacterial STI with 129 million new cases reported globally in 2020 according to WHO (2022) estimates. The prevalence rates from 2016 are

2.7% for males and 3.8% for females with the greatest occurrence in Africa and the Americas. The prevalence of infection is highest among 14–25-year-old men and women with increasing annual incidences due to the fact that the infection has no symptoms and inadequate partner treatment. Africa has the second- highest incidence of gonorrhea, with 11.4 million new cases annually (Rowley et al., 2019).

Concomitant gonorrhea and chlamydia infections complicate treatment. The regions of Asia, Africa, Latin America, and the Caribbean have the highest rates of co-infection. (De Schryver & Meheus, 1990). Teenagers, urban dwellers, and people with multiple sexual partners or those engaging in unprotected sex have the highest incidence of gonococcal infection (Weinstock et al., 2004). The development of resistance to antibiotics and some countries' inability to access antibiotics impedes the control of infections. Low levels of understanding about sexual health, the fact that infections have no symptoms, and the stigma attached to genital symptoms further negatively influence infection control (World Health Organization, 2022).

Nongonococcal urethritis in men and cervicitis are caused by *Mycoplasma genitalium.* Information on the worldwide etiology of *M. genitalium* is limited. Prevalence for the specific populations has been estimated as 3.2% in Men who have sex with men (MSM) and 15.9% in female sex workers (FSW) from a 2016 systemic review (Baumann et al., 2018). STIs remain underreported worldwide, mostly due to insufficient laboratory diagnosis services, undetected cases, underreporting and poor surveillance systems, and the reluctance of patients and clinicians to report the disease (Tapsall et al., 2009). Inadequate dissemination of STI preventive measures has contributed to the high gonorrhea and other STI prevalence (M. S. Cohen et al., 2000). The burden of infections, therefore, remains high in counties that are unlikely to control these factors.

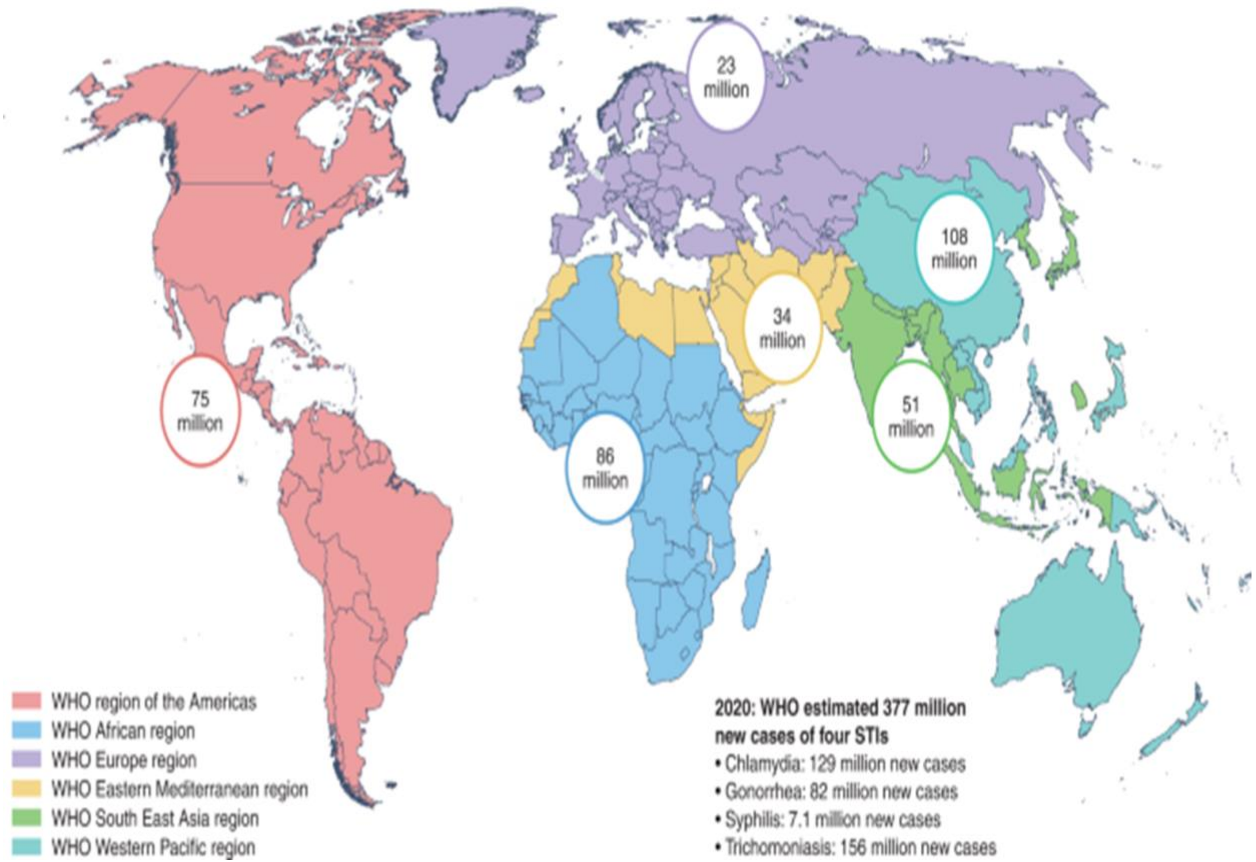

Figure 2.1 WHO global regions and the incident cases of four STIs, 2020 (chlamydia, gonorrhea, trichomoniasis and syphilis

2.2 Pathogenesis of the select STIs

2.2.1 Pathogenesis of *N. gonorrhoeae*

Several virulence factors allow for *N. gonorrhoeae* to adapt and invade the host microenvironment. Pathogenesis includes adhesion to epithelial cells, internalization, invasion, and spread. Surface proteins like pili and opacity associated (Opa) act as adherence ligands independently or cooperatively to facilitate binding on host epithelial cells. Porin protein B (PorB), lipooligosaccharide (LOS), and opacity-associated (Opa) proteins are required for *N. gonorrhoeae* invasion (Edwards & Apicella, 2005).

Infection with gonorrhea happens when the bacterium adheres to the mucosal surface of the epithelial cell which it permeates leading to massive neutrophil infiltration. The neutrophils attack polymorphonuclear cells causing the development of tiny abscesses and pus exudation. By releasing neutralizing agents like the IgA protease enzyme or undergoing

antigenic alteration on attachment sites like the Opa outer membrane proteins and pili, some bacteria may be able to evade the host cell immune system (Edwards & Butler, 2011).

2.2.2 Pathogenesis of *C. trachomatis*

Chlamydia infection happens when the organism attacks reproductive tract epithelial cells which are the principal targets of infection. By secreting chemokines, which attract inflammatory leukocytes to the infection site, the epithelial cells then start and spread immunological responses. To initiate and intensify the cellular inflammatory response, cytokines are also produced. The mediators cause tissue damage that is immediate. With re-infection, the inflammatory immune system is amplified rapidly. The infected cells and infiltrating inflammatory cells release proteases, growth factors, and clotting factors leading to tissue damage and scarring. Common chronic infections will, therefore, cause repeated tissue damage and scarring (Darville & Hiltke, 2010; Rasmussen et al., 1997).

2.2.3 Pathogenesis of *M. genitalium*

The pathogenesis of *M. genitalium* is unique and is not very well understood. *M. genitalium* attaches to the host reproductive tract epithelial cell resulting in acute inflammatory responses mediated by innate immune mediators such as the toll-like receptors. The receptors interact with the organism resulting in NF-κB activation which leads to gene activation and subsequent activation of proinflammatory signals such as chemokines which result in leukocytes being brought to the location of infection and subsequent inflammation. Reactive oxygen species and nitric oxide can be released as metabolic byproducts of the host cell immune system and cause tissue damage. *M. genitalium* causes chronic urogenital infections due to its outstanding capability to escape the host immune defense system (McGowin & Totten, 2017).

2.3 History and characteristics of the select STIs

A summary of the history and characteristics of the select STIs is given below;

2.3.1 History and characteristics of *N. gonorrhoeae*

Neisseria gonorrhoeae was the initial species of genus *Neisseria* to be described in 1879 by Albert Ludwig Sigismund Neisser. Leistikow and Loffler successfully cultivated *N. gonorrhoeae* in 1882 (Mandell et al., 1979). The organism is sensitive to variations in temperature, UV rays, dryness, and other environmental factors. *Neisseria* are cocci with a

size of 0.6-1.0 μm and include at least 21 members. Most of the members are harmless commensal species, though some can cause opportunistic infections like *N. lactamica*. The disease-causing organisms in humans are *N. gonorrhoeae* and *N. Meningitidis*. *Neisseria gonorrhoeae* is a fastidious gram-negative bean- shaped diplococcus with opposing surfaces appearing flattened intracellularly. The best growth conditions occur at 37˚C in 5% CO_2 on chocolate agar. It uses glucose rather than maltose or sucrose and is rapid oxidase positive (Mandell et al., 1979).

The normal gram-negative cell envelope of *N. gonorrhoeae* consists of an outer membrane made up of lipopolysaccharide (LPS) and other components. The outer membrane of *N. gonorrhoeae* releases fragments called blebs as the bacterium grows. These fragments have a unique oligosaccharide structure that lacks the repeating O- antigen subunits and are referred to as lipooligosaccharide (LOS). Anti-LOS antibodies are resisted by LOS, which has endotoxin action and is responsible for invading epithelial cells. Obscure colonies seen on mucosal surfaces are caused by opa proteins, which are expressed for by the *opa* gene. They influence cell adhesion and invasion in addition to controlling the immune response. The surface proteins play a vital role in the host's invasion, virulence activity, and resistance development. Porin protein allows for the movement of substances across the external membrane and exists in two antigenic forms PorB1A and PorB1B encoded by *porB* gene (Bachmann et al., 2010).

2.3.2 History and characteristics of *C. trachomatis*

There is little information regarding the evolutionary origins of *C. trachomatis* but they appear to share a common ancestor with environmental chlamydiae and likely evolved with humans around 700 million years ago (Clarke, 2011). Chlamydiae are obligate intracellular pathogens that infect a variety of hosts and cause disease (Sandoz & Rockey, 2010). T'ang and colleagues in China initially isolated *C. trachoma* in embryonated hens' eggs in 1957 (T'ang & Chang, 1957). Chlamydiae consist of an infectious metabolically inactive elementary body (0.3μm) responsible for the infection. Initially, the elementary body was referred to as chlamydozoa, which means "cloak and animal". The elementary body separates from the non-infectious metabolically active reticulate body (~ 1 μm). The development of the organism takes place within an inclusion which is a type of intracellular structure, a modified cellular organelle of *chlamydia* (M. E. Ward, 1983).

2.3.3 History and characteristics of *M. genitalium*

Mycoplasma genitalium a species of *mycoplasma* was first discovered in 1981 and was first cultured using special media from urethral exudates of men with urethritis, not of gonococcal origin (Tully et al., 1981). It acquired its name from the host cell tissue location from which it was isolated (Tully et al., 1983). *M. genitalium* has similar characteristics with *M. pneumoniae* like the terminal tip like structure and its flask shape (Lind et al., 1984). The organism has a small size of approximately 0.18 µm and lacks a cell wall (McGowin & Totten, 2017).

2.4 Transmission and risk factors for STIs

Transmission of STIs is primarily through the sexual route and depends on sexual partners within sexual networks. Gonorrhea, and other STIs, are network diseases since the establishment of the link is through sexual contact, with every contact being important in outlining the sexual linkage (Day et al., 1998). A small group of high-risk people called a core group is primarily responsible for transmission and is linked either directly or indirectly. They facilitate transmission through their sexual partners to the general population (Jolly & Wylie, 2002; Yorke et al., 1978). The low- risk groups that act as links between the core infected and general populations are called bridging populations. For effective and proper control of infections, core groups should be well-identified. Instead of targeting the low-risk general population, intervention programs should concentrate on these core groups and bridging groups (World Health Organization, 2007).

The social or sexual behavior of an individual significantly affects STI acquisition and the rate of transmission (LaMontagne et al., 2004). Lack of condom use or other barrier use and multiple sexual partners also increases the risk of transmission (Manavi, 2006). Sexual barriers should be readily offered to sexually active and high-risk groups since they greatly lower the chance of transmission. Women have a 50% risk of acquiring gonorrhea for each instance of an unprotected sex with a male who is infected, while males have a 20% risk with each encounter with an infected female. Women with many sexual partners or whose partners have several partners have a higher risk of exposure (Embree, 2011).

There are a number of factors that raise the chance of at-risk behavior and the risk of gonococcal infection in pregnant women, i.e., drug abuse, low-income professions, unmarried status, and homelessness (Weinstock et al., 2004). Factors that cause the risk of

acquiring and transmitting STIs include multiple sexual partners, unprotected anal sex, male homosexuality, history of coexisting or past STDs, engaging in sexual activity from an early age, low socioeconomic status, transactional sex, and use of illicit drugs (Loza et al., 2010; Manavi, 2006).

2.5 Clinical manifestations and complications

Most sexually transmitted infections present with common symptoms making it difficult to clinically differentiate between different STIs. It is therefore important to test for several STIs when a patient presents with genito-urinary signs and symptoms. Currently, commercial NAATs are available to enable the diagnosis of more than one STI especially those that occur concurrently. Differential diagnosis can then enable effective treatment that covers the coinfections.

2.5.1 Clinical manifestations and complications in *N. gonorrhoeae*

The endocervical canal in women is the primary location of gonococcal infection, which results in cervicitis. Burning while urinating, unusual vaginal or penile discharge, pelvic or abdominal pain, and untreated bleeding between periods are frequent symptoms. Long-term side effects include infertility in both men and women as well as pelvic inflammatory disease (PID), ectopic pregnancy, and blindness in newborns (Goire et al., 2014; Tapsall et al., 2009). High viral loads, gonococcal infection, and the acquisition of HIV are all strongly correlated, particularly in high- risk populations. In areas where both illnesses are present, this circumstance increases the prevalence of HIV infections (van der Elst et al., 2013). Newborns may acquire gonococcal ophthalmia neonaratum from an infected mother during passage through the birth canal. Conjunctivitis presents with pus-filled discharge and periorbital edema. if untreated it progresses to ulceration of the cornea, rupture, and blindness (Hammerschlag, 2011).

Pelvic inflammatory is a complication that occurs in 10-20% of cervicitis and may initially manifest as dyspareunia, unusual vaginal bleeding, and pelvic pain. Perihepatitis, also known as Fitz-Hugh-Curtis syndrome, can occur in conjunction with gonorrheal infection and PID and manifests as fever, pleuritic right upper quadrant pain, nausea, and vomiting. Uterine tubal scarring in PID may cause involuntary childlessness, ectopic pregnancy, and chronic pelvic pain. Severe symptoms include fever, abdominal pain, tubo-ovarian abscess, and systemic infection. Since PID also presents asymptomatically or with mild symptoms,

it can lead to undetected damage of uterine tubes. Among 85 percent of women who experience treatment delay develop PID which progresses to cause further complications (Hillis et al., 1993; Weström et al., 1992).

In men, the main site of infection is the urethra leading to urethritis presenting with copious purulent discharge and dysuria. While there is an incubation period of 1 to 14 days, most men begin to exhibit symptoms in 2 to 5 days. Complications such as lymphangitis, periurethral abscess formation, and edema are rare. Another complication could be epididymitis which presents with pain and swelling of the epididymis (Bolan et al., 1999).

Disseminated gonococcal infections (DGI) occur when an untreated infection spreads through the bloodstream. It manifests as acute purulent arthritis, arthralgia, tenosynovitis, dermatitis, or a combination of all the symptoms. Although rare, meningitis, endocarditis, and osteoarthritis may occur (Rice, 2005).

2.5.2 Clinical manifestations and complications in *C. trachomatis*

C. trachomatis has several serotypes; Serotype A-C which cause ocular infections, Serotype D-K responsible for classical genitourinary infection and Serotype L1-L3 which cause lymphogranuloma venereum (LGV), an emerging disease in MSM often leading to proctitis (Morre et al., 2000). Chlamydia is often asymptomatic with 50% of men and 70% of women not aware of the infection. The incubation period lasts between 7-21 days before symptoms present. Besides the genitourinary infections, chlamydia can also infect the eye to cause chlamydial conjunctivitis, the rectum leading to discomfort and discharge, and the pharynx often without any symptoms.

Women may have abnormal discharge, painful urine, intermenstrual hemorrhage or bleeding after sex, lower back pain, and intensely painful sex. Ascending infections in women can lead to endometriosis which can result in PID. PID can subsequently cause perihepatitis, ectopic pregnancy and ultimately cause infertility. In men, symptoms include urethritis, dysuria, and urethral discharge. The infection can spread and lead to testicular pain and epididymo-orchitis or epididymitis. Infertility can ultimately result if no treatment is given to the infection. Chlamydia can also cause sexually acquired reactive arthritis which involves inflammation of eyes, joints, and urethra, a condition that is more common in men (Miller, 2006).

2.5.3 Clinical manifestations and complications in *M. genitalium*

One of the main causes of non-gonococcal urethritis is *M. genitalium* and is often asymptomatic in 40-75% of women and 70% of men. Women who have *M. genitalium* infection often have painful urination, changed or increased vaginal discharge, intermenstrual or bleeding after sex, cervicitis, and lower abdominal pain. Left untreated, complications can arise such as PID, sexually activated reactive arthritis, poor pregnancy outcomes and infertility due to the tubal factor. In men, signs and symptoms include; urethritis, urethral discharge, dysuria, and proctitis. Complications such as epididymitis, sexually activated reactive arthritis, and in rare cases, conjunctivitis may arise if no treatment is given for the infection (Sethi et al., 2017).

2.6 Sexually transmitted infection control

There are no STI vaccines, and control depends on several strategies, including rapid and early diagnosis, appropriate antibiotic treatment, and contact tracing/partner notification. Primary diagnosis based on clinical symptoms and laboratory diagnosis is definitive in identifying STIs. It is necessary to incorporate STI services into primary and reproductive healthcare settings. Sexual health education and condom promotion should be strengthened. It is also crucial to optimize STI diagnosis and screening, especially for high-risk populations. Novel point-of-care diagnostic tests and new drug development are required for the treatment of STIs, especially for gonorrhea. For global STI prevention and control, developing STI vaccines remains the only sustainable option (Gottlieb et al., 2014).

2.6.1 Diagnosis of STIs

2.6.1.1 Diagnosis of N. *gonorrhoeae*

Laboratory diagnostic methods include examining culture, stained smears, genetic or immunochemical tests to identify clinical isolates recovered from the site of infection. Based on the gram-negative intracellular diplococci found in polymorphonuclear leucocytes by microscopy in initial samples, *N. gonorrhoeae* can be presumptively identified as the source of the infection. Despite being widely accepted due to its affordability, its low sensitivity prevents it from being recommended as the only approach for diagnosis (Unemo & Nicholas, 2012). It has been proven that using culture as a sensitive and repeatable diagnostic approach yields correct results. Additionally, it enables testing of the organism for AMR. The main downside is that testing takes 2-4 days to complete and

rigorous optimization of the testing environment is necessary since gonococci are impacted by harsh environmental conditions (Taxt et al., 2020). Although culture remains the gold standard for diagnosis, the practice is no longer carried out in most countries.

The collection of specimens is easier and less invasive with nucleic acid amplification tests (NAATs), especially when prevalence is high and genital examination is not possible. Screening for STIs can therefore be performed on urine samples to give similar performance to that of endocervical swabs. Another advantage of NAATs is that a single specimen can be confirmed for both *N. gonorrhoeae* and *C. trachomatis*. For effective surveillance of antimicrobial resistance trends, cultures are the recommended method. The use of both culture and NAATs is highly recommended for rapid diagnosis and AMR and should be implemented in countries with adequate resources (Unemo & Dillon, 2011).

2.6.1.2 Diagnosis of *C. trachomatis*

For detection of chlamydia, the gold standard is a culture-based method with 100% specificity due to its ability to detect infectious elemental bodies making it the preferred choice in medico-legal issues. Cultures also allow for antimicrobial susceptibility testing. However, the method has low sensitivity which could be caused by inadequate specimen collection, storage, and transportation, contamination by commensal microbes, and toxins. Other disadvantages include: long turnaround time, labor intensities, need for experienced technicians, and lack of standardizations. Therefore, culture methods are rarely used for diagnosis (Manavi, 2006; Meyer, 2016).

The discovery of NAATs transformed the diagnosis of chlamydia. By amplifying and detecting nucleic acids present in chlamydia, the assays have high sensitivity and specificity. Due to the lack of requirement for viable microorganisms and the use of non-invasive self-collecting specimens like urine and swabs, they are appealing to the majority of patients. They are currently the preferred diagnostic methods for chlamydia (Manavi, 2006).

2.6.1.3 Diagnosis of *M. genitalium*

The meticulousness and slow development of *M. genitalium* makes its isolation and use of in-vitro tests for diagnosis and AMR testing very difficult (Samra et al., 1988). Although culture methods have been developed, the method remains cumbersome making NAAT-based methods more preferable (Jensen et al., 1991).

2.6.2 Contact tracing and partner notification

Partner notification is a critical aspect of STI control following diagnosis and treatment of the primary case. For all positive gonorrhea cases, recommendations state that partners be notified and the matter be investigated. In some countries, including the USA, Canada, and China, gonorrhea is a notifiable disease (Canada, 2008; St Cyr et al., 2020; Wang et al., 2012). It is imperative to test the sexual partners of an infected individual and treat those who are infected. Both partners should be tested for additional STIs and given advice on the dangers and implications of STIs. The treatment given to the primary case should also be administered to a partner who does not get tested (Golden et al., 2005; Manavi, 2006; Sethi et al., 2017).

2.6.3 High-risk groups

High-risk groups appear to bear the highest burden of gonorrhea and other STIs. Therefore, there is a need to develop targeted interventions for these groups to prevent further transmission to the rest of the population. Strategies can include expanded testing and screening and ensuring screening recommendations are adhered to. For low-income countries with limited resources, promoting condom use as the primary prevention could be an effective control measure (Kirkcaldy et al., 2019).

2.7 Treatment of select STIs

2.7.1 Treatment of Gonorrhea

Ceftriaxone 250 mg intramuscularly is recommended by the WHO (2016) as a single dose. Additionally, either 400 mg of cefixime or 1 g of azithromycin can be taken orally in a single dose. A single oral dose of 1g of azithromycin is further administered for the treatment of simple vaginal and anorectal infections.

Treatment failures to the remaining recommended treatment options have been observed with cefixime and ceftriaxone. Failure has been reported, particularly in treating pharyngeal gonorrhea using extended spectrum cephalosporins (ESCs) (Unemo et al., 2012). Recent reports in the United Kingdom (UK) in 2016 indicate suspected treatment failure with ceftriaxone and azithromycin dual therapy (Fifer et al., 2016). These gonococcal resistance strains are likely to circulate the globe given the ease of international travel and commercial sex tourists' ability to travel to different countries.

2.7.2 Treatment of Chlamydia

As first-line therapy for chlamydial urethritis or cervicitis, current treatment guidelines for chlamydia recommend either azithromycin 1g as a single dose or doxycycline 100 mg twice daily for 7 days. Doxycycline is prescribed for Lymphogranuloma venereum during a 21-day period. The 7-day doxycycline regimen is favored for anorectal infections due to its increased efficacy (World Health Organization, 2016).

2.7.3 Treatment of *M. genitalium* infections

Based on the existence or lack of macrolide resistance, and also based on whether the infection is uncomplicated or complicated, the European guidelines have advised a course of treatment. Macrolides are still recommended as first-line treatment. For macrolide-resistant infections or as a second-line treatment, moxifloxacin is recommended. A single 1-gram dose of azithromycin for empiric treatment of urethritis is recommended by the CDC with moxifloxacin as an alternative therapy. However, based on evidence that a 1-g single dose treatment has a higher chance of development of macrolide resistance, several studies recommend alternative dosing. A preferable option would be to use the 1.5g extended course (500mg once, followed by 250mg daily for 4 days) (Anagrius et al., 2013; Horner et al., 2014; Manhart et al., 2015).

2.8 Drug resistance mechanisms in the select STIs

This study's focus was on azithromycin resistance because it is the primary recommended treatment of the select STIs. A brief discussion below summarizes a brief history and resistance mechanisms for the recommended treatment for each STI.

2.8.1 Resistance mechanisms in *N. gonorrhoeae*

The gonococcus appears to have a long history of developing resistance against drugs that were once effective. The ability of *N. gonorrhoeae* to develop resistance to all medications used to treat it has been demonstrated. *N. gonorrhoeae* can acquire resistance genes through transformation or conjugation processes (Morse et al., 1986). Several resistance mechanisms can co-exist in *N. gonorrhoeae* to cause resistance against a specific antibiotic. Alternatively, some of the resistance mechanisms can cause cross-resistance to several unrelated classes of antibiotics.

N. gonorrhoeae uses a number of different resistance mechanisms: a) chromosome or plasmid- mediated deletions or alterations to the target site b) reduced drug uptake through

reduced permeability, thus reducing access of the drug to the target site c) enzyme production that causes the drug to become inactive or less effective before it can reach the target site and d) active efflux or pumping of the drug outside the cell ensuring effective drug concentration that would harm the bacteria are not reached (Alekshun & Levy, 2007; Shafer et al., 2010).

2.8.1.1 Resistance to Azithromycin

The antibiotic azithromycin, which is a member of the macrolide class, prevents the production of proteins by attaching to the bacterial ribosome's 50s subunit (Parnham et al., 2014). Azithromycin is one of the recommended dual treatment therapies (Unemo et al., 2019). Macrolide resistance and reduced susceptibility emerged in the 1990s, and variant strains are still being reported (Chisholm et al., 2010). The overproduction of the mtr (CDE)-encoded efflux pump caused by mtrR mutations is the primary cause of *N. gonorrhoeae*'s decreased sensitivity to azithromycin. Adenine deletions (A-) or dinucleotide inserts (TT+) in the mtrR promoter region or mutations in the *mtrR* structural gene (H105Y, G45D, and A39T) cause low-level macrolide resistance. (Cousin et al., 2003; Warner et al., 2008). A study conducted in Kenya, in four different geographical regions, found a low-level resistance to azithromycin (Kivata et al., 2019). Further surveillance is required to identify the emergence and spread of resistance.

Post-translational methylation of 23S rRNA by methylase enzymes which are encoded for by ermA/ermB/ermC/ermF can lead to mutations. These mutations prevent azithromycin from binding to the target site, 23S rRNA, and therefore inhibit protein synthesis (Chisholm et al., 2010; Gomes et al., 2017). Mutations in 23S rRNA or the presence of rRNA methylases have been associated with the development of low to high resistance. In high-level azithromycin resistance, mutation of the specific base A2509G is present (Demczuk et al., 2017). The presence of other enzymes such as phosphotransferases and esterases, which are encoded by the genes *ere* and *mph*, may cause changes to macrolide structure and lead to the development of resistance (Gomes et al., 2017). Overexpression of efflux pumps such as MtrCDE encoded by *mef* genes can lead to macrolide resistance.

2.8.2 Resistance mechanisms of *C. trachomatis*

Although there are few recognized cases of antimicrobial resistance in chlamydia that is pathogenic to humans, there is evidence that the organism can express resistant phenotypes.

Chlamydiae can develop antibiotic resistance in-vitro through mechanisms such as genetic transformation, mutagenesis, and recombination (Sandoz & Rockey, 2010).

Culture methods have several challenges in detecting antibiotic-resistant strains and therefore cannot be used for accurate antimicrobial resistance surveillance. The lack of a universal testing methodology for cultures, the challenging and time-consuming techniques make it challenging to detect and monitor antibiotic resistance. Additionally, clinical isolates are extremely fastidious with slow growth rates, and high likelihood of persistence or cytotoxicity, and low recovery rates using culture (Sandoz & Rockey, 2010).

Despite the lack of significance antibiotic resistance in chlamydia species that are pathogenic to humans, the ability of the species to evolve and develop resistance through transformation warrants the need for vigilance. A phenotype of tetracycline resistance has been discovered in Chlamydia suis found in pigs indicating the likelihood of such a case developing in humans (Donati et al., 2016). Clinical isolates showing azithromycin resistance have been found to lead to recurrent infections (Somani et al., 2000). Azithromycin-resistant *C. trachomatis* may be caused by changes in the peptidyl transferase region of the 23S rRNA gene. Two copies of the gene exist but there is a low possibility of the bacteria having mutations in both alleles. Mutations at A2058 have been linked to extensive resistance to all macrolides (Misyurina et al., 2004).

2.8.3 Resistance mechanisms of *M. genitalium*

Resistance to macrolides which are the first-line drugs and alternative drugs such as fluoroquinolones and tetracyclines is rapidly increasing globally. The emergence of resistance raises concerns due to the limited options for alternative therapies (Hughes & Saunders, 2018). Isolation of strains from clinical isolates using culture-based methods is still very difficult and labor-intensive and molecular approaches have therefore been developed to investigate the prevalence of *M. genitalium* infections and detect gene mutations that are implicated in resistance. Understanding molecular mechanisms of resistance and surveillance of the resistant rates are important in informing treatment guidelines that give the most effective treatment for the infections (Dumke et al., 2016).

The first cases of macrolide resistance emerged in Australia in 2008 and the resistance was followed by Europe and Japan and has spread worldwide and is estimated at 10-30%. The resistance has been linked to mutations in region V of the 23S rRNA gene (Lau et al., 2015;

Nijhuis et al., 2015; Taylor-Robinson & Jensen, 2011). A study conducted in Sub Saharan Africa also confirmed the presence of macrolide-resistant *M. genitalium* isolates among women in rural South Africa (Hay et al., 2015). The most commonly observed mutation is the A2058G mutation but other mutations have been observed such as A2073G in Germany (Dumke et al., 2016). It is recommended that routine identification of macrolide resistance-related mutations be carried out to prevent treatment failure and curb the transmission of macrolide-resistant *M. genitalium*. Increasing treatment failure has been reported for moxifloxacin which is the second-line treatment that further complicates effective management of *M. genitalium* infections (Couldwell et al., 2013; Dumke et al., 2016).

CHAPTER 3: METHODOLOGY

3.1 Study design

This was a descriptive cross-sectional study conducted at a hospital. Samples were collected just once and analyzed using molecular techniques.

3.2 Study site

The study was carried out in STI, family planning and out-patient clinics of two hospitals in Busia County: Busia County referral hospital and Khunyangu sub-county hospital. Busia County is a transit region located near the Kenya-Uganda border. Busia county referral hospital is located approximately 1km from the Kenya- Uganda border and therefore has a wide catchment population from both countries. Being a referral hospital, it serves a major population from Busia County as well as the population from the border town in the neighboring country Uganda. Khunyangu sub county hospital is in the rural setting and serves the population from Busia County and the neighboring County of Siaya. The hospitals were also selected on the basis of having a -80°C refrigerator which was needed for storage of the samples.

3.3 Study population

The study participants were sexually active females of reproductive age attending STI, family planning and out-patient clinics in the two hospitals in Busia County. The recruitment process begun after approval was given by the Kenyatta National Hospital-University of Nairobi Ethics & Research Committee (KNH-UoN ERC) attached as Appendix G.

3.3.1 Inclusion criteria

In order to participate in the study, participants had to meet the following requirements:

i. Sexually active females of reproductive age above 15 years old
ii. Should present with signs of vaginal discharge, vaginal itching, or burning sensation
iii. Willing to participate in the study by giving informed consent

3.3.2 Exclusion criteria

Participants who met the following criteria were not allowed to participate in the study:

i. Females who fail or otherwise incapable/ unable to provide consent for study participation
ii. Females experiencing vaginal bleeding

3.4 Sampling procedure

Up until the necessary number was reached, participants were recruited conveniently. The inclusion criteria for potential study participants were identified. Clinic nurses or clinical officers provided explanations of the trial's goals, risks, and benefits in English (Appendix A) or Swahili (Appendix B), and those who signed a consent form indicating their willingness to participate were enrolled in the study.

3.5 Sample size determination

Cochran's formula by Fisher *et al*., 1998, was used for sample size determination.

$n=Z^2pq/d^2$

Where;

n= desired sample size

Z= standard normal deviation at 95% confidence level (1.96)

p= estimated prevalence of the STIs; since the prevalence of STIs in the target population is not known, it was estimated at 50% (0.5%), which yields the largest target sample size.

q= 1-p

d^2= degree of accuracy desired (0.05)

A level of significance of 95% and an error margin of $\pm 5\%$ were considered acceptable for this study. Substituting;

$n= (1.96)^2(0.5)\ (0.5)/\ (0.05)^2 =384$

To adjust for error; $384 \times 0.05 = 19$.

There were 334 participants from Busia County referral hospital and 90 participants from Khunyangu sub county hospital.

3.6 Sampling and recruitment procedures

Eligible participants who met the inclusion criteria were recruited conveniently as identified until the required number was attained. Potential study participants were identified as they sought medical services at different clinics in the hospitals (STI, family planning and outpatient). The research assistants attending to patients provided study information to potential participants and got written informed consent from willing patients.

3.7 Data Collection Procedures

The research assistants for the study included clinic nurses and clinical officers who were

responsible for the recruitment and collection of study participants' bio data and clinical samples. Before recruitment and data collection, research assistants underwent training on the study objectives and their roles. They then interviewed the participants to collect socio-demographic data, history of sexual behavior and symptoms using a questionnaire attached as Appendix C. Data collected from the questionnaire was then uploaded to a KoboCollect application using their mobile devices. Data was uploaded daily and aggregated to the Kobo Toolbox interface from the different devices and then downloaded at the end of the study using Excel for analysis.

3.7.1 Genital Sampling

An endocervical swab was collected by a trained clinician or nurse during speculum examination using sterile swabs. The swab collection was done for participants who satisfied the inclusion criteria in a side room to maintain participant privacy. The genital sampling was done by inserting the speculum one to three centimeters deep into the endocervix, inserting the swab to the top of the vagina, and rotating for 20 to 30 seconds, to obtain the discharge. The swabs were then promptly stored at -80˚C refrigerators which were available for use in both study sites. The samples were later delivered to Pan African Hub for infectious diseases, Kenya Medical Research Institute, in Nairobi using dry ice for molecular processing.

3.7.2 Molecular testing

3.7.2.1 Lysate Preparation for Direct PCR

Following standard protocol, genomic DNA was extracted from the 424 samples, using MightyPrep reagent with slight modifications (Takara Bio Inc, Lot: AJG2464A). The swab was cut and placed in a 1.5ml Eppendorf tube, 200 µL of MightyPrep reagent for DNA was added, spun at 15,000 rpm for 1 minute and heated at 95°C while shaking at 800rpm and later cooled down on a heat block. Hard vortexing was then done for 1 minute, spun at 15,000rpm for 2 minutes then stored at 30°C awaiting downstream processing.

3.7.2.2 Multiplex Polymerase Chain Reaction

Multiplex PCR was used to detect and identify each of the selected STIs. The PCR tubes were labeled with the sample identifications. Specific primers for each organism were used (Table 3.1). The primers were then prepared (Table 3.2). The positive controls used were PCR product extracts from previous positive samples stored in the laboratory. PCR water

was used as a negative control. The master mix was mixed well by finger tapping and spinning and 18μL of the master mix and 2μL of the DNA template were transferred to each of the labeled PCR tubes.

Table 3.1 Primers for the detection of the select STIs

Target	Forward primer (5'-3')	Reverse primer (5'-3')	Base pair
MG	TACATGCAAGTCGATCGGAAGTAGC	AAACTCCAGCCATTGCCTGCTAG	433
CT	TCTTTTTAAACCTCCGGAACCCACTT	GGATGGCATCGCATAGCATTCTTTG	361
NG	CGGCAGCATTCAATTTGTT	AAAAAGCCGCCGTTTTTGTA	162

MG, *Mycoplasma genitalium*; CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*.

Polymerase chain reaction (PCR) was performed using Promega GoTaq® following the manufacturer's protocol with slight modifications: 10μl Promega GoTaq® G2 Hot Start Green Master Mix (LOT: 0000382417), 1.2μl primer mix, 6.8μl of water, and 2μl of the sample (Table 3.3). The tubes were set in the SimpliAmp Thermal Cycler (Applied Biosystems) and the reaction of 35 cycles started. The following conditions were used for the PCR process: denaturation at 95°C for 60 seconds, annealing at 59°C for 60 seconds, elongation at 74°C for 26 seconds, and prolonged elongation at 74°C for 5 minutes. After the reaction, the samples were electrophoresed on a gel, stained, and examined under an ultraviolet light (UV).

Agarose powder was measured (2 mg) and 100 ml of Tris Acetate EDTA (TAE) buffer added and transferred in a glass bottle. The gel was then melted in a microwave oven with a stirrer, allowed to cool, and poured onto the gel-making dock. The gel was allowed to stay for at least 15 minutes, after which it was transferred to the mucid agarose tank containing the running buffer. An amount of 5μL of 100bp ladder marker (LOT: 74000L, Nippon Gene) was loaded in the first and the last well. An amount of 5μL of the samples and the positive and negative controls were loaded onto the rest of the wells. The gel was then allowed to run at 100V for 35-40 mins. GelRed stain was prepared by dissolving 10μL GelRed in 50ml water. After running, the gel was then stained in gel red for 40 mins with shaking, after which it was viewed under UV and photographed. The samples that tested

positive for each of the STI were therefore identified.

Table 3.2 Preparation of Primer Mix for detection of select STIs

	Target	Product size	primer	Tm (CLC)	f/c(uM)	Vol/reaction	Vol for primer mix
1	*C. trachomatis*	361 bp	CT_F	63	1.0	0.2	130.0uL
			CT_R	64	1.0	0.2	130.0uL
2	*M. genitalium*	433 bp	MG_F	60	1.0	0.2	130.0uL
			MG_R	57	1.0	0.2	130.0uL
3	*N. gonorrhoeae*	162 bp	NG_F	57	1.0	0.2	130.0uL
			NG_R	56	1.0	0.2	130.0uL
Total						1.2	780

Table 3.3 Preparation of Master Mix for detection of select STIs

Component	f/c	X1 (µL)	X105
Water		6.8	714.0
GoTaq G2 Hot Start Green Master Mix 2X	1x	10.0	1050
STD primer mix	1.0µM	1.2	126.0
DNA Template		2.0	Unique
Total		20.0	

3.7.2.3 PCR amplification for *N. gonorrhoeae mtrR* gene

Isolates that tested positive for *N. gonorrhoeae* were analyzed to determine the existence of macrolide-resistant markers. The primers for the amplification of the *mtrR* gene were: mtrR-F 5' GCCAATCAACAGGCATTCTTA 3' and mtrR -R 5' GTTGGAACAACGCGTCAAAC 3'-380 base pairs (Lucas et al., 1997).

The PCR mixture reaction contained 10µl Promega GoTaq® G2 Hot Start Green Master Mix (LOT: 0000382417), 0.4 µl primer mix, 7.6 µl of water, and 2µl of the sample (Table 3.4). Amplification was performed on SimpliAmp thermocycler as follows: an initial

denaturing step at 95° for 2mins followed by 40 sequential cycles of 95°C, 60 secs for denaturation, 55°C, 60 secs for annealing, 74°C, 23 secs for elongation, and extended elongation at 74°C for 5 mins. The DNA products were then run on an agarose gel electrophoresis then underwent staining and visualization under UV light.

Table 3.4 Preparation of Master Mix for NG_ *mtrR* gene amplification

Component	**f/c**	**X1 (µL)**	**X80**
Water		7.6	608
Go Taq G2 Hot Stat Green PCR Master Mix (2X)	X1	10.0	800
Primer mix_ NG_ *mtrR*	1.0µM	0.4	32
DNA Template		2.0	Unique
Total		20.0	

3.7.2.4 PCR amplification for *C. trachomatis* 23S rRNA gene

Isolates that tested positive for *Chlamydia trachomatis* were analyzed to determine the presence of macrolide-resistant markers. Primers for the 23S rRNA gene used for amplification were CT_23SrRNA-F 5'AAGTTCCGACCTGCACGAATGG 3' and CT_23SrRNA-R 5'TCCATTCCGGTCTCTCGTAC 3'-725 base pairs (Misyurina et al., 2004).

The PCR mixture reaction contained 10µl Promega GoTaq® G2 Hot Start Green Master Mix (LOT: 0000471776, Promega Corporation), 0.4 µl primer mix, 7.6 µl of water, and 2µl of the sample (Table 3.5). The reaction conditions were as follows: initial denaturation at 90°C for 2 mins followed by 40 cycles of denaturation at 95°C for 60 secs annealing at 62°C for 60 secs, elongation at 74°C for 44 secs and an extended elongation at 74°C for 5 mins. The DNA products were stained and examined under UV light after being passed through an agarose gel electrophoresis.

Table 3.5 Preparation of Master Mix for CT_23SrRNA gene amplification

Component	f/c	X1 (µL)	X35
Water		7.6	266
Go Taq G2 Hot Stat Green PCR Master Mix (2X)	X1	10.0	350
Primer mix_CT_23S rRNA	1.0µM	0.4	14
DNA Template		2.0	Unique
Total		20.0	

3.7.2.5 PCR amplification of *M. genitalium* 23S rRNA gene

Isolates that tested positive for *M. genitalium* were analyzed to determine the presence of macrolide-resistant markers. Primers for the 23S rRNA gene used for amplification were MG_23SrRNA-F 5’ TGAAATCCAGGTACGGGTGAGGAC 3’ and MG_23SrRNA-R 5’CGGTCCTCTCGTACTAGAAGCAAAG 3’- 580 base pairs (Jensen et al., 2008).

The PCR mixture reaction contained 10µl Promega GoTaq® G2 Hot Start Green Master Mix (LOT: 0000382417), 0.08 µl primer mix, 7.92 µl of water, and 2µl of the sample (Table 3.6). The reaction conditions were as follows: Reaction conditions were an initial denaturation at 95°C for 15 mins followed by 35 cycles of denaturation at 94°C for 60 secs, annealing at 64°C for 60 secs, elongation at 72°C for 60 secs, and extended elongation at 72°C for 10 min. After running the DNA products via an agarose gel electrophoresis, they were stained and visualized under UV light.

Table 3.6 Preparation of Master Mix for MG_23SrRNA gene amplification

Component	f/c	X1 (µL)	X35
Water		7.92	158.4
Go Taq G2 Hot Stat Green PCR Master Mix (2X)	2.5U	10.0	200
Primer mix_MG_23S rRNA	0.4µM	0.08	1.6
DNA Template		2.0	Unique
Total		20.0	

3.7.2.6 Gel extraction and purified DNA analysis

Using a scalpel, the DNA bands were excised from the gel on an Ultra Slim Blue Light Transilluminator (Maestrogen). The excised gels were put into labelled tubes for DNA extraction and purification using a QIAquick® gel extraction kit (cat Number 28704 and 28706) under the manufacturer's protocol. One volume (100 mg gel in 100 µl) received three volumes of buffer QG before being incubated at 50 °C for ten minutes. Additionally, a quantity of isopropanol equal to one volume of gel was added, mixed, and spun for one minute at 13,000 rpm. Additionally, 500 µl of QG buffer and 750 µl of PE buffer were added to the sample, which was then spun at 13,000 rpm for 1 minute. The final column was then transferred to a new Eppendorf tube and eluted by adding 50 µl of buffer EB and spinning at 13,000 rpm for 1 minute. A nanodrop DNA concentration analysis was conducted using a Thermoscientific Nanodrop 2000 spectrophotometer. For sequencing analysis, the purified DNA samples were sent to Macrogen Europe B.V. Amsterdam in the Netherlands (Sanger sequencing).

3.7.2.7 Sequence analysis and detection of mutation sites

The chromatograms generated from sequencing were trimmed using the Bioedit Sequence Alignment Software. The Basic Local Alignment Tool (https://blast.ncbi.nlm.nih.gov) was then used to compare the trimmed nucleotide sequences to sequences from the database to find regions of similarity using the Nucleotide Blast default search parameters. This was done to confirm the identity of the organisms. After blasting, the forward and reverse sequences were used to generate consensus sequences using the Bioedit Sequence Alignment Software. These consensus sequences were the used compared against a part of

the reference genome sequences retrieved from NCBI to detect any mutations.

This was achieved by first generating multiple sequence alignment (MSA) using the reference sequence and the generated consensus sequence of each sample using the Molecular Evolutionary Genetics Analysis (MEGA) software. Analysis was then done to determine if they led to any amino acid changes. The wild type strain FA19 (GenBank Accession number CP012026) was used to detect resistance related mutation sites in *Neisseria gonorrhoeae* positive samples. The sequence of the reference D/UW-3/CX 23SrRNA (GenBank accession number NR_076160) was used to compare and detect resistance related mutation sites in the consensus sequences generated for *C. trachomatis* after sequencing. The consensus sequences were compared to reference strain G-37 23s ribosomal RNA gene (GenBank Accession number NR_077054).

3.8 Ethical considerations

This investigation was evaluated and approved before the study began by the Kenyatta National Hospital-University of Nairobi Ethics & Research Committee (KNH-ERC/A/229). Participants under the age of 18 gave written informed assent and written informed consent was provided by the parent/ guardian. Participants under the age of 18 who were attending the hospital on their own were considered emancipated and were treated as adults. All patients were treated equally with no discrimination and privacy was maintained by collecting samples in a separate private room. Instead of using patient names, distinct patient identifiers were utilized to protect confidentiality.

Microbiological processing for diagnosis and treatment for the STIs was provided separately to the patients as per the treatment guidelines in the hospitals. Once the trial was over, the results were shared with the clinicians to help direct the patients' future treatment.

3.9 Data management

Only the primary investigator had access to any documented that connected the patient to the data that was gathered, and it was only given to the ERC and the quality control team upon request for auditing purposes. Weekly backups of the data were made at a different secure location. A spreadsheet in Excel was filled out with data. To restrict access, the database was password- protected.

3.10 Quality assurance

The lead investigator double-checked the information collected from patient samples before

entering it. Additionally, this was done for data derived through sequencing. According to the protocols, the final report received review and a quality audit. Any deviation from the standards and protocols was noted, reviewed, and reported in the final report if it had an impact on the study's validity.

3.11 Statistical analysis

The prevalence of infections and coinfections among the study participants and sociodemographic characteristics were presented as frequency tables and percentages. Descriptive data analysis was conducted on all the variables. To determine variables that were normally distributed, the Shapiro-wilk test was used. Variables that were not normally distributed were expressed as median and interquartile range (IQR). The statistical analysis was considered significant at $P< 0.05$ and it was carried out using STATA version 13 (College Station, Texas, TX, USA).

3.12 Study results dissemination plan

Findings from this study will be published in a peer-reviewed journal. A soft copy of the thesis will be deposited in the electronic repository of the University of Nairobi. The results of the study will be disseminated to the relevant hospital staff once the study is completed.

CHAPTER 4: RESULTS

4.1 Prevalence of the selected sexually transmitted infections

The prevalence of sexually transmitted infections among the sampled symptomatic women attending hospitals within Busia County stood at 23.6% (95% CI, 19.4, 27.7). Most of the women were infected with a single organism, and among the STIs, *N. gonorrhoeae* had the highest prevalence at 17.7% (95% CI 16.7., 18.7), followed by *C. trachomatis* at 6.8% (95% CI 5.82, 7.78) and *M. genitalium* at 4% (95% CI 3.02, 4.98) (Table 4.1).

4.2 Prevalence of coinfections

Overall, the prevalence for those with all the three STIs was 0.7% (95% CI 0, 1.68), for MG/NG was 1.9% (95% CI 0.92, 2.88), for those with CT/MG was 1.7% (95% CI 0.72, 2.68) while those with CT/NG was 2.1% (95% CI 1.12, 3.08) (Table 4.1).

Table 4.1 The prevalence of selected sexually transmitted infections and the rate of coinfections

	Prevalence n (%)	95%CI
Presence of STI	100 (23.6%)	22.6% -24.6%
CT	29 (6.8%)	5.82% - 7.78%
MG	17 (4%)	3.02% - 4.98%
NG	75 (17.7%)	16.7% -18.7%
Coinfections		
CT/MG	7 (1.7%)	0.72% - 2.68 %
CT/NG	9 (2.1%)	1.12% - 3.08%
MG/NG	8 (1.9%)	0.92% - 2.88%
CT/MG/NG	3 (0.7%)	0 - 1.68%

CT *(Neisseria gonorrhoeae),* MG *(Chlamydia trachomatis),* NG *(Mycoplasma genitalium*

4.3 Sociodemographic characteristics of the study participants

The findings revealed that 48.8% (n =207) of women had secondary level education, 44.1% (n =187) were self-employed. The median age at first sexual encounter was 17 (IQR: 15 – 18) years, 28.8% (n =122) had new sexual partner in last six months with a median of 1(IQR: 0 – 1). The maximum number of new partners in the last six months was 3. In assessing the frequency of condom use, 11.1% (n =47) of women stated that they always use condom during sexual intercourse while 39.6% (n =168) of women used condom during last sexual intercourse. The findings also revealed that 96.9% (n =411) of the respondents

had history of HIV testing where 53.3% (n =219) were HIV positive. The findings established that 71.2% (n =302) of the respondents had vaginal discharge, 67.9% (n =288) of them had lower abdominal pain while 32.8% (n =139) had abnormal vaginal bleeding (Table 4.2 and Table 4.3)

Table 4.2 Sociodemographic characteristics of participants presenting with symptoms of sexually transmitted infection

Factors	Median (IQR)	Frequency	Percent
Demographic factors			
Age	33(25 - 40)		
17 - 24 years		41	9.7
25 - 34 years		188	44.3
35 years and above		195	46
Highest level of education			
No formal education		17	4
Primary level		141	33.3
Secondary level		207	48.8
Tertiary level		59	13.9
Occupation			
Unemployed		130	30.7
Student		9	2.1
Casual labourer		61	14.4
Self-employment		187	44.1
Salaried employment		37	8.7

Table 4.3 History of sexual behaviour and symptoms of study participants

Sexual behaviour history	Median (IQR)	Frequency	Percent
Age at first sexual experience (Years)	17(15 - 18)		
≤17 years		253	59.7
18 - 19 years		109	25.7
≥20 years		62	14.6
Currently Expectant			
Yes		10	2.4
No		414	97.6
New sexual partner in last six months			
Yes		122	28.8
No		302	71.2
Number of new partners in last six months	1(0 - 1)		
Frequency of condom use during intercourse			
Always		47	11.1
Sometimes		204	48.1
Never		173	40.8
Use condom during last intercourse			
Yes		168	39.6
No		256	60.4
Family planning uptake			
Yes		259	61.1
No		165	38.9
History of STD treatment			
Yes		275	64.9
No		149	35.1
History of HIV testing			
Yes		411	96.9
No		13	3.1
HIV status (n =411)			
Positive		219	53.3
Negative		192	46.7
History of symptoms			
Genital Pain		198	46.7
Vaginal discharge		302	71.2
Burning urination		184	43.4
Genital itching		219	51.7
Abnormal vaginal bleeding		139	32.8
Lower abdominal pain		288	67.9

4.4 Multiplex PCR results for detection of the organisms

Following the multiplex PCR, gel electrophoresis results were as follows: 75 samples (17.7%) showed bands at the target size for *N. gonorrhoeae* (162bp), 29 samples (6.8%) showed bands at the target size for *C. trachomatis* (361bp) and 17 samples (4%) showed bands at the target size for *M. genitalium* (433bp).

Figure 4.1 shows the Ultraviolet gel image showing 21 samples run on a 26-well gel. The ladder is at position 1 and 26 (Gel Pilot®. The clear bands represent the respective organisms at the respective target size. The positive control (PC) at position 23, 24 and negative control (NC) at position 25.

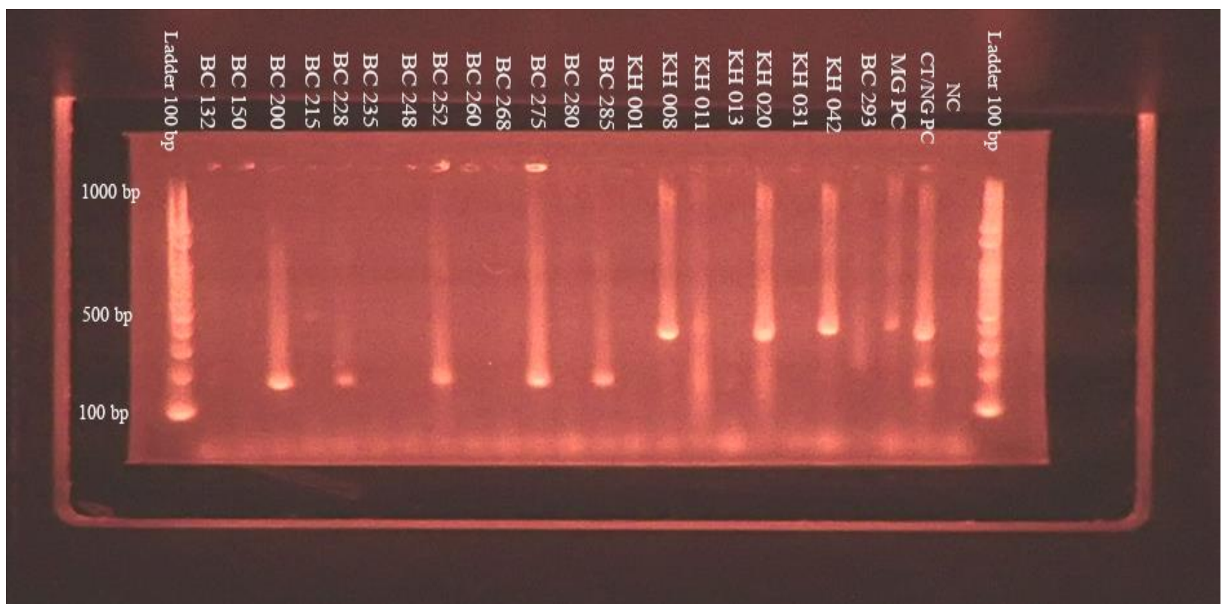

Figure 4.1 First representative gel showing detection of *N. gonorrhoeae*, *C. trachomatis* and *M. genitalium* using multiplex PCR.

4.5 Markers associated with azithromycin resistance in the organisms

4.5.1 Mutations in the *mtrR* gene of *Neisseria gonorrhoeae*

Following gene amplification, gel electrophoresis was performed (Figure 4.2). The PCR products of 6 isolates (1.4%) of *N. gonorrhoeae* isolates showed bands of resistance after they were purified, quantified and sequenced. The sequenced data was analyzed using Bioedit and MEGA software. Most of the samples had similar mutations: C→G transversion at position 301 leading to amino acid P101A and C→T transitional substitution at position 304 leading to amino acid change H102Y. There was a transitional substitution

C→T at position 355 in two of the samples that did not lead to an amino acid change (Table 4.4).

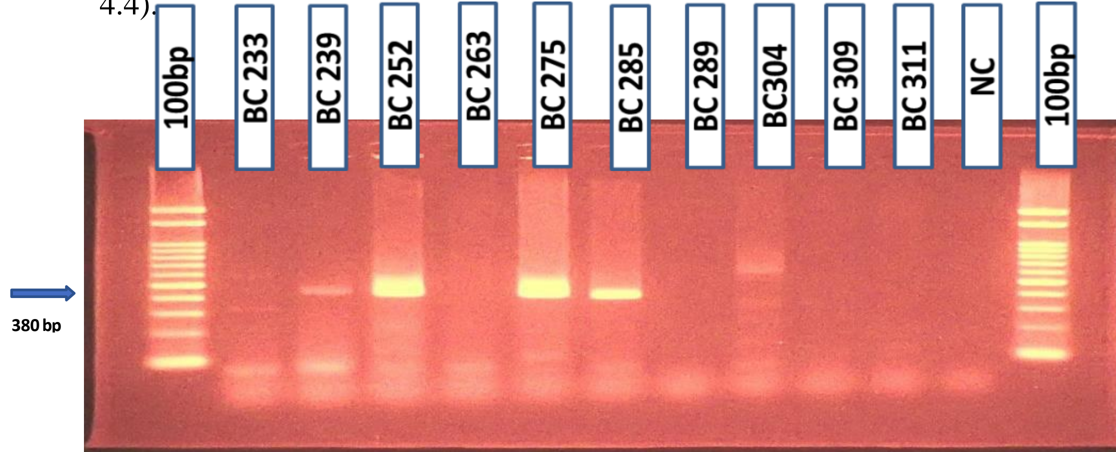

Figure 4.2 Ultraviolet gel image showing PCR products run on a 13-well gel. The ladder is at position 1 and 13 (Gel Pilot®). The clear bands represent the samples that showed resistance genes at the target size of 380bp

Table 4.4 Mutation in *mtrR* gene of selected resistant *N. gonorrhoeae* isolates

Patient Code	Mutation position in the *mtrR* region	Mutation	Amino acid change	
BC 060	301	C→G	P101A	Pro→Ala
	304	C→T	H102Y	His→Tyr
	329	G→A	S110N	Ser→Asn
BC 076	301	C→G	P101A	Pro→Ala
	304	C→T	H102Y	His→Tyr
	329	G→A	S110N	Ser→Asn
BC 155	332	G→C	A111P	Arg→Pro
	355	C→T	L119L	No change
BC 252	301	C→G	P101A	Pro→Ala
	304	C→T	H102Y	His→Tyr
	329	G→A	S110N	Ser→Asn
BC 275	355	C→T	L119L	No change
BC 285	301	C→G	P101A	Pro→Ala
	304	C→T	H102Y	His→Tyr
	329	G→A	S110N	Ser→Asn

4.5.2 Mutations in the 23S rRNA gene in *Chlamydia trachomatis*

The PCR products for the positive chlamydial samples underwent Sanger sequencing following amplification, purification and quantification (Figure 4.3). Of these, 3 samples (0.7%) had mutations. Following analysis, a majority of the point mutations were G→A transitional substitutions found in several positions and resulting in several amino acid changes. Other samples had a transversion substitution G→T at position 1987 (*E. coli* numbering) with amino acid changes G667V. A silent mutation was also found following a transitional substitution C→T at position 2027 (*E. coli* numbering) (Table 4.5). Insertion-deletion mutations (Indels) were also identified. In one sample there was a guanine insertion between positions 2720-2721 of the 23S rRNA gene. On another sample, there was an adenine insertion (position 2014-2015) and a guanine insertion (position 2077-2078). A common adenine guanine insertion (AG) was found in three of the samples.

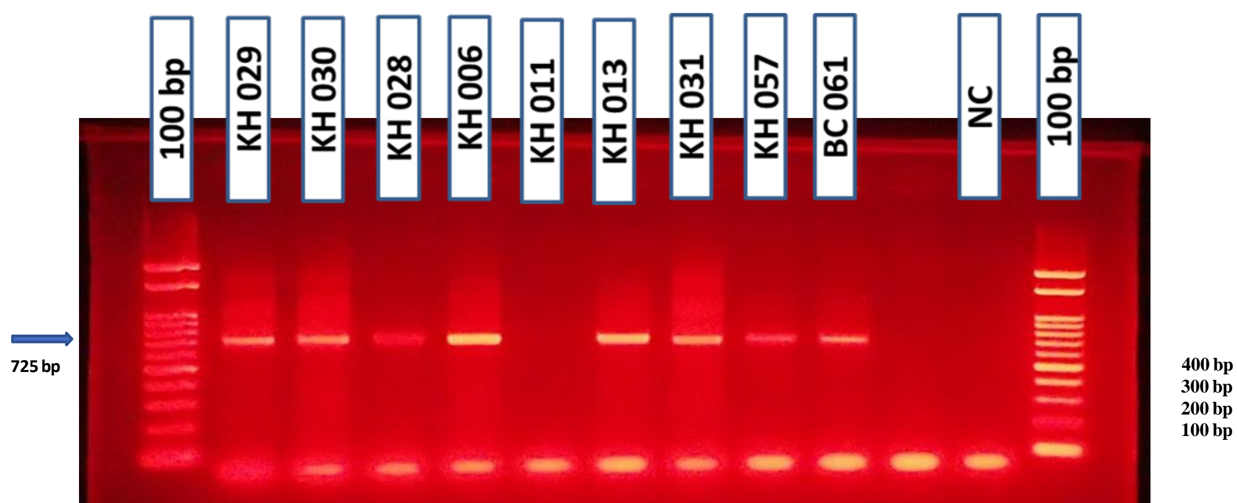

Figure 4.3 Ultraviolet gel image showing PCR products run on a 13-well gel. The ladder is at position 1 and 13 (Gel Pilot®). The clear bands represent the samples that showed resistance genes at the target size of 725bp.

Table 4.5 Mutations in the 23S rRNA gene of selected resistant *C. trachomatis* isolation

Patient Code	Mutation position on 23S rRNA gene	*E. coli* numbering	Mutation	Amino acid change	
BC 323	2000	1987	G→T	G667V	Gly→Val
KH 006	2000	1987	G→T	G667V	Gly→Val
KH 031	2040	2027	C→T	H680H	No change
	2041	2028	T→G	C681G	Cys→Gly
	2074	2061	G→A	A692T	Ala→Thr
	2131	2118	T→G	Y711D	Tyr→Asp
	2177	2164	G→A	R726K	Arg→Lys
	2392	2379	G→A	G798S	Gly→Ser
	2404	2391	G→A	D802N	Asp→Asn
	2426	2413	G→A	R809Q	Arg→Gln
	2627	2614	G→A	R876Q	Arg→Gln

4.5.3 Mutations in the 23S rRNA gene in *Mycoplasma genitalium*

Out of the samples that were positive for *Mycoplasma genitalium*, 2 samples (0.5%) had mutations after being sequenced following amplification, purification and quantification of the 23S rRNA gene (Figure 4.4). Both samples had substitution by transversion point mutations at positions 2009 and 2010 (*E. coli* numbering). Mutation G2009T resulted in a silent mutation S674S while G2010T resulted in amino acid change V675L (Table 4.6).

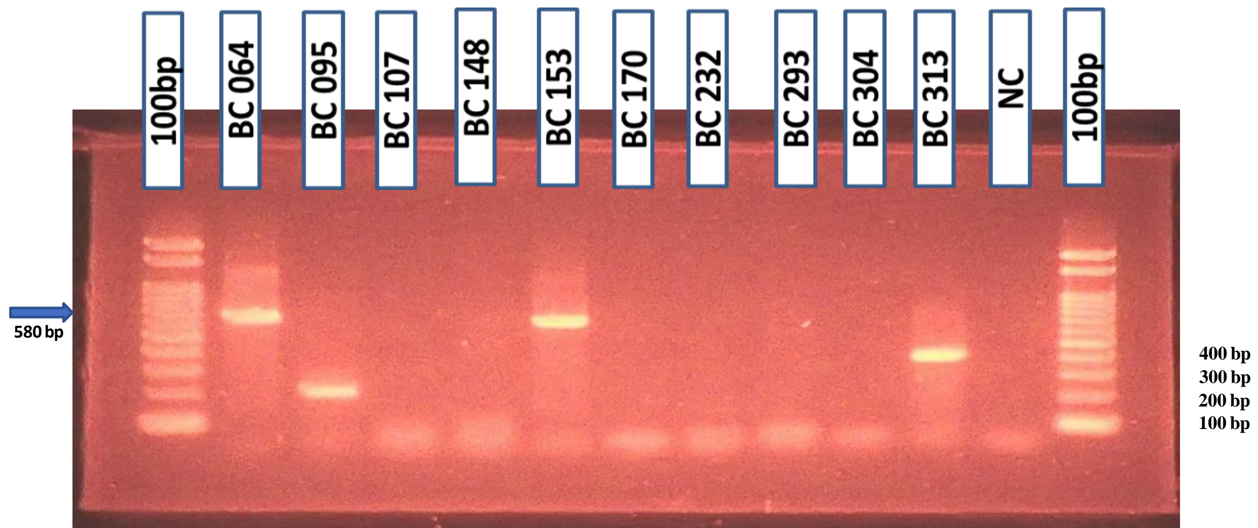

Figure 4.4 Ultraviolet gel image showing PCR products run on a 13-well gel. The ladder is at position 1 and 13 (Gel Pilot®). The clear bands represent the samples that showed resistance genes at the target size of 680bp.

Table 4.6 Mutations in the 23S rRNA gene of selected resistant *M. genitalium* isolates

Patient Code	**Mutation position in 23S rRNA**	***E. coli* numbering**	**Mutation**	**Amino acid change**	
BC 064	2022	2009	G→T	S674S	No change
	2023	2010	G→T	V675L	Val→Leu
BC 153	2023	2010	G→T	V675L	Val→Leu

CHAPTER 5: DISCUSSION

5.1 Discussion

The prevalence of sexually transmitted infections among symptomatic women attending clinics in the two hospitals sampled stood at 23.3% (95% CI, 19.4, 27.7). Globally, the number of STI cases increased by 58.15 percent between 1990 and 2019, rising to 769.85 million from 486.77 million (Fu et al., 2022). An incidence cohort study in Kisumu also found a similar prevalence of 24.4% among women undergoing HIV screening (Otieno et al., 2015). The high prevalence found from women in this study is also consistent with findings from other regions in Sub Saharan Africa. There was a prevalence of 19.4% in Swaziland among women attending several hospitals for routine health care (Ginindza et al., 2017). In South Africa, a study screening sexually active women reported a prevalence of 13% (Naidoo et al., 2014). A prevalence of 36% was reported during screening of women of reproductive age in Uganda (Patra, 2016). These findings signify the high burden of sexually transmitted infections among women of child-bearing age which is worrying given the adverse effects of these infections on women (Gottlieb et al., 2014).

Global estimates show a prevalence of 1.9% for *N. gonorrhoeae* among women from the general populations in the African region (Rowley et al., 2019). In comparison, the prevalence of gonorrhea was predominantly high among symptomatic women in Busia County at 17.7%. A meta-analysis study carried out in South Africa in 2017 found a prevalence of 6.6% among women from populations representing the general population (Kularatne et al., 2018). Lower prevalence rates have been reported in Kenya; 0% among women attending family planning clinic in Nairobi and 6% among women undergoing HIV screening in Kisumu (Maina et al., 2016; Otieno et al., 2015). The high prevalence in Busia could have been because the study only sampled symptomatic woman which may have exaggerated the prevalence. The high prevalence of gonorrhea could also be as a result of the increasing reports of resistance towards antibiotics (Kivata et al., 2019; Unemo & Nicholas, 2012; Wi et al., 2017).

The prevalence level of *Chlamydia trachomatis* (6.8%) found in Busia County is higher than that found in Sub Saharan Africa. Community based studies have found low prevalence (1.6-3.2%) of chlamydia in the general population in sub–Saharan Africa (Orroth et al., 2003; Pépin et al., 2004). Prevalence estimates of 14.7% were found among women from

the general populations in a meta- analysis study in South Africa (Kularatne et al., 2018). A study reported a prevalence of 6% in women visiting an outpatient clinic in Nairobi (Marx et al., 2010) which is comparable to the prevalence among symptomatic women in Busia County. In Nairobi, a study among women attending a family planning clinic found the prevalence of chlamydia to be higher (12-13%) (Maina et al., 2016). In western Kenya, a study reported a prevalence of 5.6% among HIV negative pregnant women (Kinuthia et al., 2015), while another study in Kisumu found a lower prevalence of 2.8% among women being screened for HIV (Otieno et al., 2015).

The prevalence of *Mycoplasma genitalium* among symptomatic women in Busia County stood at 4%. In a multi analysis study, the prevalence of *M. genitalium* among the populace as a whole in developing countries stands at 3.9% and 1.3% in highly developed countries (Baumann et al., 2018). A study in Europe found a prevalence of 9.3%, 6% and 11% in Denmark, Norway and Sweden respectively among women attending STI clinics (Unemo et al., 2018). Most studies on *M. genitalium* in Africa have concentrated on cohorts of high-risk women with prevalence of 12.9- 16% in Kenya (C. R. Cohen et al., 2007; Gomih-Alakija et al., 2014), 14% in Uganda (Vandepitte et al., 2012) and 8.7% in South Africa (Hay et al., 2015). The prevalence of *M. genitalium* appears to vary from region to region, is low among the general population and higher among high-risk populations. There is need to continue with surveillance due to the adverse reproductive health outcome of *M. genitalium* in women (Lis et al., 2015).

Little data exists on the prevalence of coinfections in sexually transmitted infections. Coinfection rate of CT/NG in this study was 2.1% which was lower compared to a study among women undergoing HIV screening in Kisumu that had a prevalence of 7.1% (Otieno et al., 2015). A prevalence of 5% was found in a pilot study that set to determine CT/NG coinfection in New Delhi, India (Aravinda et al., 2022). Chlamydia tends to coexist concurrently with gonorrhoea infections. Studies from the 1980's led to recommendations that all patients receiving treatment for gonorrhoea should also be presumptively treated for chlamydia (Centers for Disease Control (CDC), 1985), and were reiterated again in the early 2000's (Lyss et al., 2003). Despite the low prevalence of NG/CT coinfection in this study, co-treatment for both organisms should still be continued.

Mycoplasma genitalium has been found to occur concurrently with other STI's including *Chlamydia trachomatis* at prevalence of 4.8 %- 42.9% (Chernesky et al., 2017; Getman et al., 2016; Ljubin-Sternak et al., 2017; Xiao et al., 2019). This study reported the prevalence of MG/CT coinfection of 1.7% which was lower than the prevalence reported from other studies. Two studies from STD clinics in United States (US) cities found higher prevalence of 36.1% in one study (Manhart et al., 2015) and 25% in another study (Gaydos et al., 2009). Another study conducted in Birmingham found MG/CT prevalence level of 7.3% (Harrison et al., 2019). Although there are limited studies focusing on MG/CT coinfection, the prevalence of *M. genitalium* and *C. trachomatis* coinfections has been found to be population dependent (Harrison et al., 2019). Despite the fact that both infections can be treated with azithromycin, the dose used to treat chlamydia does not efficiently cure *M. genitalium* infections. This can lead to persistent *M. genitalium* infections with adverse effects on the reproductive health in women (Manhart et al., 2015).

The prevalence of *Mycoplasma genitalium* and *N. gonorrhoeae* coinfection stood at 1.9%. Studies from several countries have also identified the existence of MG/NG coinfections. In the USA, the prevalence was 1.9% which is similar to this study (Getman et al., 2016). Prevalence was higher in other countries; 4.8% in Spain (Fernández-Huerta & Espasa, 2019), 2.4% in Singapore (Hart et al., 2020) and between 5-7.9% in Australia (Richardson et al., 2021; Stewart et al., 2020). The clinical significance of *M. genitalium* infections remains controversial and misunderstood. Most infections may therefore remain unknown and go untreated since routine screening is not recommended. A study in Australia has recently confirmed that the 1g administered for treatment of gonorrhoea could potentially treat *M. genitalium* infections (Richardson et al., 2021).

Prevalence of infections and coinfections could have been higher because this study sampled only symptomatic women. Having a sexually transmitted infection increases the likelihood of attaining other STIs including HIV by amplifying inflammatory processes and interfering with genital mucosal environment. The presence of co-infections consequently complicates treatment and can increase HIV infectiousness (H. Ward & Rönn, 2010; Wolday et al., 2004). Co-treatment has been recommended and shown to be effective for chlamydia and gonorrhoea infections (Lyss et al., 2003) but remains ineffective in other coinfections such as MG/CT. The increasing reports of macrolide resistance further

complicate treatment of infections and coinfections (Sweeney et al., 2019; Xiao et al., 2019; Yang & Yan, 2020) and could be responsible for high prevalence in some of the STIs. There is therefore need for other interventions such as screening, treating and promotion of condom use (Menezes et al., 2018).

In this study, three mutations (P101A, H102Y, and S110N) were identified in the mtrR region of *N. gonorrhoeae* isolates that were resistant to azithromycin. These mutations were not previously reported in other studies, indicating the emergence of new mutations associated with azithromycin resistance in *N. gonorrhoeae*. A previous study has shown that mutations in the DNA-binding domain of MtrR can interfere with its ability to bind to the promoter region of the mtrCDE operon, resulting in increased expression of the efflux pump and decreased susceptibility to antibiotics, including azithromycin (Zarantonelli et al., 1999). The P101A, H102Y, and S110N mutations identified in this study may have a similar effect by altering the structure of the DNA-binding domain of MtrR and reducing its affinity for DNA, thereby leading to increased expression of the mtrCDE efflux pump and azithromycin resistance.

Mutations in the efflux systems in charge of the active efflux of antibacterial agents have been linked to the development of resistance to antibiotics (Grkovic et al., 2001). The transcriptional repressor (MtrR) is produced by the *MtrR* gene, which also regulates the expression of the cell envelope protein-encoding mtrCDE complex (MtrC-MtrD-MtrE) (Rouquette-Loughlin et al., 2002). Gonococcal strains have been found to harbor a variety of mutations, either in the mtrR gene or in the promoter region (Cousin et al., 2003), which result in low-level macrolide resistance due to upregulation of the MtrCDE efflux pump and downregulation of *MtrR* expression (Chisholm et al., 2010).

A metadata study analyzing *Neisseria gonorrhoeae* samples from 68 countries found nonsynonymous SNPs A39T, G45D, G120S, and A121S (Manoharan-Basil et al., 2021). Mutations A39T and G45D have been implicated in low-level macrolide resistance and found to be more common in patients previously exposed to azithromycin compared to non-exposed patients (Demczuk et al., 2016; Wind et al., 2017). Frequent use of azithromycin in high risk populations may cause resistance by increasing the MICs (Kirkcaldy et al., 2015; Shigemura et al., 2015; Wind et al., 2017). Azithromycin resistance (MIC 2–8 mg/L) has been reported to be caused by substitutions in the MtrR protein for G45S, A86T, and

Y105H, as well as for D79N, A39T, L99G, or H, which increased the MIC value but most frequently did not result in resistance (Ohneck et al., 2011). A study by Kivata et al reported low-level azithromycin resistance in *N. gonorrhoeae* from isolates collected from several regions in Kenya (Kivata et al., 2020). These mutations highlight the complexity of mtrR mediated regulation of azithromycin resistance. This study's resistance findings emphasize the value of ongoing surveillance and the necessity for more investigation.

Although reports on azithromycin resistance are rare, point mutations causing amino acid changes were detected in this study. We can speculate that the resistance in *C. trachomatis* could have been caused by reduced affinity of drug binding. Because the mutations occurred in regions that were in close proximity to the binding site for macrolide, tertiary structure could have been affected by the amino acid changes. A highly conserved region makes up the 23S rRNA's peptidyl transferase loop. High levels of macrolide resistance in *C. trachomatis* have been highly correlated with mutations in the 23S rRNA gene region V at locations A2058G and A2059G as they are the binding sites of macrolides. Mutations T2611C, A2057G and C2452T have been implicated in low level resistance (Vester & Douthwaite, 2001). A study in Russia found A2058C, and T2611C mutations in the 23S rRNA gene of several isolates (Misyurina et al., 2004). In another study wild type mutations A2057G, A2059G were observed *in vitro* for the first time and T2611C was also identified. While clinical strains have been previously reported in the laboratory, the reverse has not been reported. This is because rRNA mutations are only detected clinically once treatment with the drug fails (Jiang et al., 2015). None of these mutations were found in this population which is consistent with a study in Japan that did not find mutations in the 23Sr RNA gene in clinical chlamydial isolates (Deguchi et al., 2018).

Furthermore, the fidelity of transcription in *C. trachomatis* may have been affected by increasing the distance between the distinct parts of the 23S rRNA gene by a single nucleotide (A/G) or dinucleotide (AG), resulting in a decrease of binding affinity. These indel mutations have not been previously reported or linked to resistance (Vester & Douthwaite, 2001). The novel mutations found in this study might be a cause for alarm and may indicate that isolates in this population are highly mutative and that mutation may not be restricted to the previously reported regions. Further investigation is necessary to confirm whether the mutations cause clinical resistance. This will be important to help

determine whether azithromycin is still effective against chlamydial infections in this population.

The *M. genitalium* positive samples had a silent mutation G2009T which corresponded to position 2022 in the 23S rRNA gene. Both samples had mutation G2010T (*E. coli* numbering) which led to an amino acid change. These mutations have not been reported elsewhere in the literature and have not been linked to macrolide resistance in other bacteria (Vester & Douthwaite, 2001). We can hypothesize that they could contribute to resistance owing to the change in amino acids which could have affected the tertiary structure and reduced affinity of the binding site for the drug. A meta-analysis study using data from 21 countries reported a rise in the prevalence of macrolide resistance to *M. genitalium* from less than 10% before 2010 to 51.4% in 2016-2017 (Machalek et al., 2020). From several studies, A2058G and A2059G are the most commonly reported mutations for *M. genitalium* with the less commonly reported being A2058T and A2058C (Nijhuis et al., 2015; Salado-Rasmussen & Jensen, 2014). Such mutations have been reported globally in Australia, Europe, and Japan (Taylor-Robinson & Jensen, 2011). A study in South Africa found A2058G mutation in 9.8% of the *M. genitalium* positive samples tested for macrolide resistance (Hay et al., 2015). Further investigation is needed to confirm whether the mutations cause resistance against azithromycin so as to determine treatment options.

The use of nucleic acid amplification-based testing for sexually transmitted infections was a key strength of this investigation which provided exceptional specificity and sensitivity for the detection of *N. gonorrhoeae*, *C. trachomatis,* and *M. genitalium*. Polymerase chain reaction (PCR) - based amplification of known resistance markers was also used to determine whether azithromycin resistance is present and is spreading in this population. The results of this study address the huge knowledge gap on the prevalence of resistant strains among women in this region and the molecular techniques involved in azithromycin resistance in the selected sexually transmitted infections. Furthermore, the prevalence data from this study will fill the gaps where tangible data on the burden of STIs is scarce or unavailable.

5.2 Study Limitations

The study had a number of drawbacks. Firstly, drug susceptibility testing in vitro was not done for this study and we could therefore not compare minimum inhibitory concentration

(MIC) levels to determine efficacy of the macrolide. However, detecting resistance genes has been shown to be a preferable assay since they are more convenient and cost effective in predicting the efficacy of treatment compared to drug susceptibility testing (Shao et al., 2020). Due to time, logistical and cost issues, other genes that are responsible for azithromycin resistance in the selected organisms were not analyzed in this study.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Sexually transmitted infections are highly prevalent among symptomatic women in Busia County and more data is needed to establish the extent of the spread to the rest of the general population. Specifically, there was a higher prevalence of gonorrhea in the county compared to studies carried out in other regions in the country. The most commonly reported mutations responsible for azithromycin resistance were not detected in isolates for this study. Novel mutations in the resistance markers of interest among the selected microorganisms causing STIs were detected. The results of this study help fill the knowledge gap on the prevalence of STIs among symptomatic women in Busia County. This study emphasizes the value of ongoing surveillance to detect the presence of antimicrobial resistance among the sexually transmitted infections.

6.2 Recommendations

There is need for interventions to reduce the burden of sexually transmitted infections among women in this population. Enhancing advocacy can help spread knowledge about STI risk factors, STI prevention strategies, and the value of early diagnosis, contact tracing, and treatment. Other intervention measures include education, socioeconomic development, and women's empowerment.

There is need for further surveillance and studies to establish whether the novel mutations are responsible for reducing efficacy of treatment with azithromycin. Additionally, cultures can be done to allow antibiotic susceptibility testing to determine whether the mutations reduced azithromycin efficacy. Studies to investigate mechanisms of resistance in other genes can also be undertaken. There is need to promote evidence- based treatment based on current recommendations. Antimicrobial stewardship should be encouraged and measures put in place to minimize antibiotic misuse and overuse.

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APPENDICES

Appendix A: Consent Form (English Version)

Title of the Research Study: Molecular characterization of macrolide resistance in select sexually transmitted infections among symptomatic women attending hospitals in Busia County, Kenya

Investigators: Dr. Primrose Muthoni Ndungu

Study location: Busia County Referral Hospital and Khunyangu Sub- County hospital.

You are being asked to volunteer for a research study. It is up to you whether you choose to participate or not. There are no consequences, and you will not lose anything if you decide not to join or if you decide to quit after joining. This research aims to investigate resistance to the treatment of sexually transmitted infections, which will help inform treatment guidelines. You should understand the following general principles which apply to all participants in medical research.

i. Your agreement to participate in this study is voluntary.

ii. You may withdraw from the study at any time without necessarily giving a reason for your withdrawal.

iii. Refusal to participate in the research will not affect the services that you are entitled to receive in this Clinic.

Procedure to be followed

Should you agree to participate in the study, you will be required to answer a few questions and have a swab collected to test for several sexually transmitted infections. Please remember that participation in the study is voluntary, and you are free to ask for clarification at any point.

Duration of the procedure

Should you agree to participate in the study, your involvement will last approximately 30 minutes to answer some questions and have a vaginal swab collected from you.

Discomforts and Risks

If you take part in the study, you will experience very minimal risk. A vaginal swab will be

administered, and you might experience slight discomfort, bruising, or bleeding. Some questions might be uncomfortable but please answer all if possible.

Potential Benefits

You will receive free counseling on STI prevention and control strategies. Your participation in the study will help us learn more about mutations that could lead to treatment failure. The results of this study could help inform policymakers on the need to review treatment guidelines and help in providing more effective treatment for sexually transmitted infections.

Confidentiality

All data collected from you will be coded to protect your identity. Only the researcher and study staff will have access to the information. At the end of the study, there will be no way to link your name with your data. Any additional information about the study will be provided to you.

You are free to withdraw or refuse to answer any questions at any time without any consequences.

Contact Information

For any further information about this study, you may contact me, my co-investigator or the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee using the contacts provided below:

Dr. Primrose Muthoni Ndungu,

Department of pharmacology and pharmacognosy,

School of Pharmacy,

University of Nairobi

P.O Box, 19676-00202, Nairobi. Tel: 0723 598 587

Prof Nzou Muuo,

Institute of Tropical Medicine and Global Health, Nagasaki University,

Kenya Medical Research Institute

P.O BOX 54840-00200, Nairobi. Tel : 0729 020 763

The Secretariat

KNH-UON Ethics and Research Committee

2726300 Ext 44102. Email: uonknh_erc@uonbi.ac.ke

Participant Statement

I have read and understood the nature of the study, I understand my responsibilities as a study participant, risks/benefits have been explained to me, all my questions and concerns have been addressed satisfactorily. I understand that my participation is voluntary and that I may withdraw at any time. I freely agree to participate in the study.

I agree to participate in the study	YES	NO
I agree to have vaginal swab preserved for later study	YES	NO

Name of study participant……………………………………………………….

Signature or thumbprint of study participant………………………. Date…………………….

(Use the following signature blocks for representative, parents, and guardians, only if applicable) Your signature below indicates you are legally authorized to act on behalf of the participant and have read this document. You will receive a copy of this document.

Name of Legally Authorized Representative ……………………………………….

Relationship to the Participant**………………………………………………………**

Signature of Legally Authorized Representative……………………… Date…………………

Administering individual's statement

I, the undersigned have fully explained the relevant details of this research study to the participant named above and I believe she has understood and has willingly and freely given her consent.

Name of Administering individual……………………………………………

Signature of person obtaining consent……………………………… Date……………………….

Name of Witness…………………………………………………………………

Signature of witness…………………………………………. Date……………………...

Appendix B : Fomu Ya Kibali (Kiswahili Version)

Mada ya Utafiti: Kuelewa mabadiliko ya mwelekeo wa upinzani wa madawa yanayotumiwa kutibu magonjwa ya ngono kwa wanawake wanaohudhuria hospitali za Kaunti ya Busia, Kenya

Mchunguzi: Dr. Primrose Muthoni Ndungu

Eneo la Utafiti: Busia County Referral Hospital na Khunyangu sub county hospital

Unaulizwa ushiriki katika utafiti huu kwa hiari yako. Una haki ya kushiriki au kutoshiriki kwa utafiti huu kwa hairi yako. Taarifa hii itatumika na wizara ya Afya kuelewa mabadiliko haya yanayosababisha upinzani wa madawa ya kutibu magonjwa ya ngono na hivyo kubadilisha miongozo ya matibabu haya nchini. Unafaa kuelewa mambo yafuatayo ya kimsingi yanayofaa kuzingatiwa na washiriki wote.

i. Mwitikio wako wa kushiriki katika utafiti huu ni wa hiari.
ii. Unaweza kujitoa kutoka kwa utafiti huu wakati wowote pasipo kuhitajika kutoa sababu za kujitoa.
iii. Kutoshiriki katika utafiti huu hakutaadhiri upokezi wa matibabu katika kliniki hii.

Utaratibu wa kufutualiwa

Kushiriki kwa utafiti huu kutahitaji kwamba ujibu maswali kadhaa na kipimo kimoja kitachukuliwa kwako kwa ajili ya vipimo vingine vitakavyofanywa baadaye. Tafadhali kumbuka kwamba kushiriki katika utafiti huu ni kwa hiari yako. Unaweza kuuliza maswali kuhusiana na utafiti wakati wowote.

Muda wa kufanya utafiti

Utafiti huu utachukua takriban dakika thelathini kuchukua ujumbe kwa njia ya maswali na pia kutoa sampuli kutoka kwako.

Usumbufu na hatari

Ikiwa unashiriki katika utafiti huu, hatari kwako ni kidogo sana. usufi wa uke utatolewa na unaweza shuhudia uchungu, usumbufu au kuvuja damu kwa muda kidogo tu. Maswali mengine ni nyeti lakini wahimizwa uyajibu yote.

Faida

Wakati wa majadiliano, utapokea ushairi kuhusu jinsi za kujizuia na kupunguza uwezekano wa kupata magonjwa ya ngono. Ikiwa utashiriki katika utafiti huu utasaidia kutupa ujumbe ya jinsi ya kutoa tiba bora kwa maambukizi ya magonjwa ya ngono

Usiri

Ujumbe wote utakaopeana katika utafiti huu utalindwa kwa kutumia msimbo ili kulinda kitambulisho chako. Mchunguzi na wanaohusika na utafiti huu pekee ndio watakaoweza kupata ujumbe ambao utatupa katika utafiti huu. Kufikia kikomo cha utafiti huu, hakutakuwa na njia ya kulinganisha kitambulisho chako na ujumbe wowote utakaotoa. Ujumbe wowote wa zaidi kuhusu utafiti huu utawasilishwa kwako iwezekanavyo. Una hiari ya kuhusika au kutohusika kutoa ujumbe wowote kwa wakati wowote bila adhari yoyote.

Maelezo ya mawasiliano

Ikiwa una maswali yoyote kuhusu utafiti huu, unaweza wasiliana na mchunguzi, msimamizi wa utafiti au sekretari wa kamati ya ukaguzi wa maadili ya chuo kikuu cha Nairobi kupitia mawasiliano haya :

Dr. Primrose Muthoni Ndungu,
Department of pharmacology and pharmacognosy,
School of Pharmacy,
University of Nairobi
P.O Box, 19676- Nairobi. Tel: 0723 598 587

Prof Nzou Muuo,
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P.O BOX 54840-00200. Tel: 0729 020 763

The Secretariat
KNH-UON Ethics and Research Committee
2726300 Ext 44102. Email: uonknh_erc@uonbi.ac.ke

Taarifa ya washiriki

Nimesoma na kuelewa asili ya utafiti huu, naelewa jukumu langu kama mshiriki wa utafiti, nimeelezwa kuhusu usumbufu, hatari na faida za kushiriki katika utafiti huu, naelewa kuwa kushiriki kwa utafiti huu ni kwa hiari yangu na nina uhuru wa kutoshiriki, na kwamba maswali yangu yameshughulikiwa vilivyo.

Nakubali kushiriki katika utafiti huu	NDIO	APANA
Nakubali sampuli ya usufi kutumika katika siku zijazo	NDIO	APANA

Jina la mshiriki…………………………………………….

Sahihi au Kidole gumba…………………………. Tarehe……………………

Tumia nafasi hii kwa mwakilishi, mzazi ama mlezi kama inahitajika. Sahihi yako inaonyesha kwamba umeruhusiwa kisheria kumsimamia mshiriki na umesoma fomu hii. Utapokea nakala hii.

Jina la aliyeidhinishwa kisheria………………………….

Uhusiano na mshiriki**…………………………….**

Sahihi ya aliyeidhinishwa kisheria**………………………** Tarehe……………………...

Taarifa ya Mchunguzi

Nimempa mshiriki maelezo yote kuhusu utafiti hu una naamini kuwa mshiriki huyu ameelewa na amekubali kushiriki katika utafiti huu kwa hiari yake na amepeana idhini yake kwa uhuru.

Jina la mchunguzi………………………………………………….

Sahihi ya Mchunguzi………………………………… Tarehe…………………….

Jina la shahidi…………………………………………………

Sahihi ya Shahidi…………………………………… Tarehe……………………

Appendix C: Questionnaire

STUDY TITLE: MOLECULAR CHARACTERIZATION OF MACROLIDE RESISTANCE IN SELECT SEXUALLY TRANSMITTED INFECTIONS AMONG SYMPTOMATIC WOMEN ATTENDING HOSPITALS IN BUSIA COUNTY, KENYA

Name of facility: ………………………………………………………….

Date of interview: ………………………………………………………….

Clinic: ………………………………………………………………….

Code of the Participant: …………………………………………………….

SECTION A: SOCIODEMOGRAPHIC CHARACTERISTICS

1. Age of participant (Umri)……………………………………………

2. Where do you live? (Unaishi wapi)

Subcounty (Kijimbo)……………………………….

Village (Kijiji)…………………………………….

3. Highest level of education (kiwango cha juu zaidi cha masomo)

a)	Primary	Shule ya msingi
b)	Secondary	Shule ya sekondari
c)	College/University	Chuo kikuu
d)	No education	Sijaenda shule

4. Occupation (Kazi unaoifanya)

a)	Student	Mwanafunzi
b)	Casual labourer	Kibarua
c)	Self-employed	Kujiaijiri kibinafsi
d)	Salaried employment	Kazi ya kuajiriwa
e)	Unemployed	Sijaajiriwa

SECTION B: HISTORY OF SEXUAL BEHAVIOUR

5. At what age did you have your first sexual experience? (Ulikuwa umri wa miaka ngapi uliposhiriki ngono kwa mara ya kwanza?) ……………………………………..

6. Are you pregnant? (Je, una mimba?)

YES	NDIO
NO	APANA

7. Have you had a new sexual partner in the last six (6) months? (Je, umekuwa na mpenzi mpya ambaye umeshiriki naye ngono katika miezi sita (6) iliyopita?)

YES	NDIO
NO	APANA

8. How many sexual partners have you had in the last six (6 months? (Je, umeshiriki ngono na watu wangapi katika miezi sita (6) iliyopita?)

0	3
1	4
2	$\geq$4

9. How often do you use condoms during sex? (Je, mara ngapi unatumia mpira wakati wa kushiriki ngono?)

Always	Kila wakati
Sometimes	Wakati mwingine
Never	Situmii kamwe

10. Did you use a condom during your last sexual intercourse (Je, ulitumia mpira wakati wako wa mwisho kushiriki ngono?)

YES	NDIO
NO	APANA

11. Are you on any family planning method? If yes, which one? (Je, unatumia njia gani kuzuia mimba?

……………………………………………………………………………………………………

12. Have you been treated for a sexually transmitted infection before? (Je, umepimwa ugonjwa wa ngono hapo awali?)

YES	NDIO
NO	APANA

13. Have you been tested for HIV before? (Je, umepimwa ukimwi hapo awali)

YES	NDIO

NO APANA

14. If yes, what is your HIV status? (Kama ndio, hali yako ya HIV ni gani?)

Positive

Negative

15. Were you on any drugs e.g., alcohol during your last sexual intercourse? (Je, ulitumia dawa za kulevya kama pombe, mara yako ya mwisho kushiriki ngono?)

Always Kila wakati

Sometimes Wakati mwingine

Never Situmii kamwe

SECTION C: HISTORY OF SYMPTOMS

16. Have you experienced any of these symptoms in the last six (6) months? (Je, umeshuhudia dalili zozote kwa miezi sita (6) iliyopita?)

a) Genital pain/swelling Maumivu/kufura sehemu ya siri

b) Vaginal discharge Kutokwa kwa uke

c) Burning urination Kuchoma mkojo

d) Genital itching Kuwashwa sehemu ya siri

e) Abnormal vaginal bleeding Damu isiyo ya kawaida ukeni

f) Lower abdominal pain Maumivu ya chini ya tumbo

17. Have you been treated for any of the symptoms above in the past six (6) months? (Je, umepata matibabu ya dalili zozote kwa miezi sita (6) iliyopita?)

YES NDIO

NO APANA

Name of interviewer ……………………………………………………………………….

Interviewer signature……………………………………………………………….

Date … ………………………………………………………………………………………….

Appendix D: Nano drop Concentration

Organism	S/No	Code	Conc(ng/µl)	A260	A280	260/280	260/230
N. gonorrhoeae	1	BC040a	0.4	0.007	-0.023	-0.31	0
	2	BC040b	0.5	0.05	-0.011	-4.56	0
	3	BC042	0.7	0.014	-0.011	-1.29	0
	4	BC043	0.2	0.005	0.011	-0.44	0
	5	BC049	-0.7	-0.014	-0.023	0.61	0
	6	BC060	2.9	0.059	0.025	2.38	0.02
	7	BC076	2.3	0.045	0.01	4.46	0.01
	8	BC083	0.5	0.03	0.003	10.49	0.01
	9	BC113	-0.6	0.012	-0.018	-0.68	0
	10	BC143	-0.3	-0.006	-0.19	0.3	0
	11	BC155	5.8	0.019	0	113.14	0
	12	BC171	0.4	0.014	-0.002	-8.81	0
	13	BC197	0.6	0.018	0.008	2.23	0.01
	14	BC200	0.1	0.001	-0.004	-0.29	0
	15	BC228	0.1	0.002	0.003	0.68	0
	16	BC239	4.8	0.021	-0.006	-3.64	0
	17	BC252	6.6	0.131	0.033	4.02	0.01
	18	BC275	5.9	0.119	0.042	2.82	0.01
	19	BC285	3.5	0.035	0.01	3.39	0.01
	20	BC342	0.5	0.03	0	249.24	0.01
	21	BC343	-0.3	-0.006	-0.007	0.76	0
	22	KH026	0.5	0.095	0.034	2.77	0.01
	23	KH033	-0.2	-0.005	-0.006	0.82	0
	24	KH035	-0.3	-0.005	-0.003	1.86	-0.001
M. genitalium	1	BC064	6.5	0.129	0.061	2.13	0.05
	2	BC095	1	0.047	0.017	2.78	0.01
	3	BC153	3.3	0.066	0.026	2.59	0.02
	4	BC313	0.2	0.064	0.03	2.14	0.02
	5	BC343	1.2	0.024	0.003	7.94	0.01
	6	KH005	0.8	0.048	0.018	2.65	0.03

Organism	S/No	Code	Conc(ng/µl)	A260	A280	260/280	260/230
C. trachomatis	1	BC049	0.4	0.228	0.038	5.99	0.02
	2	BC061	0.2	0.083	0.038	2.17	0.02
	3	BC064	0.3	0.066	0.004	14.72	0
	4	BC084	0.7	0.12	0.043	2.77	0.01
	5	BC143	0.4	0.069	0.023	3.01	0.02
	6	BC178	0.4	0.108	0.014	8	0.01
	7	BC197	0.1	0.042	-0.009	-4.8	0
	8	BC202	0.4	0.088	-0.014	6.12	0.01
	9	BC239	1	0.02	-0.008	-2.59	0
	10	BC293	0.9	0.059	0.027	2.15	0.01
	11	BC304	0.2	0.043	0.011	3.98	-0.25
	12	BC323	5.7	0.114	0.022	5.13	0.01
	13	BC327	0.6	0.093	0.036	2.57	0.01
	14	BC344	0.9	0.018	0	291.4	0.01
	15	KH002	0.3	0.045	0.007	6.09	0
	16	KH006	6.8	0.055	0.019	2.87	0.01
	17	KH011	0.9	0.137	0.054	2.53	0.01
	18	KH013	1.3	0.084	0.018	4.62	0.01
	19	KH028	1	0.061	0.014	4.43	0.01
	20	KH029	1.5	0.101	0.017	6.01	0.1
	21	KH030	1.7	0.066	0.031	2.09	0.02
	22	KH031	4.2	0.084	0.039	2.14	0.02
	23	KH057	0.4	0.047	0.02	2.35	-0.91
	24	KH061	0.5	0.151	0.028	5.45	0.01

Appendix E: Generated Consensus Sequences

B060 *Neisseria gonorrhoeae*

>Consensus

TTGCCAATCAACAGGCATTCTTATTTCAGGATATAAAAACCGCCTGCTTTGATACCCGAATG
TTCGAACGGGTTGCAAAGCAGGTTATACCTGTTTCAAAGTTGAGATGCAGTCTCAATTTTAT
GGGTTTCATTATACATACACGATTGCACGGATAAAAAGTCTTTTTATAATCCGCCCTCGTCA
AACCGACCCGAAACGAAAACGCCATTATGAGAAAAACCAAAACCGAAGCCTTGAAAACCA
AAGAACACCTGATGCTTGCCGCCTTGGAAACCTTTACCGCAAAGGGATTGCGCGTACCTCG
CTCAACGAAATCGCCCAAACCGCCGGCGTAACGCGCGGCGCGCTCTATTGGCATTTCAAAAA
TAAGGAAGACTTGTTTGACGCGTTGTTCCAACAA

BC 076

>Consensus

AGACCTRAWRMACCGKSCSWKCTTTGMTAMCMSAATAARTCTMGWACSRRTTGCAAGAGMATG
RCTYRTACCTRKTWTCAAACTCTRTCCTSMSATKCAGWCTCAATWTWAWRRGTTTCATYAYACA
TCAACACTGATTTCGCAAAATTCTACCAGSATAAGTTWAAGTATCTWKGTTYATARTCTYGCYCT
ACGTCARACCKACSYGAWAYRWWAACGYMRTYWTGCCAGAAMAMMCAARMYYAKAMTGCCW
YGRWARCCWWWKRWCACMTSATGMTTKCCGCCATAGGGTSAGTAMACATTCTTAYTACGGTCT
ATATCAGATCAACTCAGKGATTGCAKCSTACMTYGCATCTAWTCKAAAYCARMCMAAAGTMCG
ACYGSTATTATCGCTWACGCATKCKGCGCKYTACTRTTCGGTTTTACCTCATTAYCAAWCTWWTC
WAGKAASATCWYSTTTKATCRMRTWRTTMMARCAAACAAMAAGKSTTGYYSTKWTTYKCATGT
TYMYTACAAAKMAGCGAKMCTCAAATAKTCASASACRTWCKAYCTATKATWKASGKMATGGAG
AMRKGCCCAATARTMARTATWSWTRMTCRARTAGTATSMTMSAAGSRSTSKWRACGGACTGTRA
TTGTKTTAAAAAACCCACAAAATCWRKATTRTKWTCTATARTACATSACYSWKSTRTACTATTTC
AAYSATCAKATKYAAKMYMTCSTAASGSSKTCCAGCAAKAKAGKTKSMATKTCCCCMGTKKAAM
RWGASAAWCAKTTCAYGMSTTTCCTASKGYTSSTSAKSTSWAGTACCGTACGGSASCTTGYATART
RACWWTCGCTCTTSTSAKAWGYMGTTTRCMSYSSSARWATKSSSATMTASGASRKGTAWTCAKRT
STASSATCAACTCATARGTCGMTGTACAWRAWRRAGTGACAGWWAWACTAATCCTGYGCRMAT
RATTGGCTSAASSTYYKAYYGYATSCAGWAATATTASGARWSSWSAKWAATCWAAGGCAGRGAT
AMAWCAATTACSSWSWTMWARKSCTARWASWSCTAMSTTTWYACCTGCTATGGTATMAGTKWA
SAWWKSAAAARKATTACACRATCACCACATAAWCWAAWASMRRARAWACATSTTTCCATCCRT
MKWSTTGAKATCCRAAKAYAATTCTCCCMAWMTACRCACATACARTCRTWAARATATTTTWTTC
GAGGATWWGRGATWWGMMAATCAACAGGCATTCTTATTTCAGGATATARRARCYGCMWGYTT
WGATACCCGAATGTTMGWAMGGGTTGSAGTAWGCAGTRTYAGGATGASMKGTWTTSAWAGTK
GRRAWGMRGWCTSARKTTTATGASGTWKMRTWAATAGYATWYAYGAYTRCAGTCTGGRTAAA
WRGTSKTTKTTATAATCCGMSCTSGTCARACCGRYMYGWAAYGAAWACGCCATTRTGAKMRAA
ACCWAWACCGAMGCMTTGCWWAWCCRAWGTAWGYASCWGWWGCGWWGSYRYSTTGGAAAC
CTWKTACCGMACWRGGGAYWGCGATACGKASMTCGMTCAASGARATMGMSCAAAYMGYMGRC
GWAAMGCGYGGSKSGSTCTATGGCATCRAWAWTWAGGATTK

BC155 *Neisseria gonorrhoeae*

>Consensus

TGCCAATCAACAGGCATTCTTATTTCAGGATATAAAAACCGCCTGCTTTGATACCCGAATGT
TCGAACGGGTTGCAAAGCAGGTTATACCTGTTTCAAAGTTGAGATGCAGTCTCAATTTTAT

GGGTTTCATTATACATACACGATTGCACGGATAAAAAGTCTTTTTTATAATCCGCCCTCGTCA
AACCGACCCGAAACGAAAACGCCATTATGAGAAAAACCAAAACCGAAGCCTTGAAAACCA
AAGAACACCTGATGCTTGCCGCCTTGGAAACCTTTTACCGCAAAGGGATTGCCCGCACCTCG
CTCAACGAAATCGCCCAAGCCCCCGGCGTAACGCGCGGCGCGCTTTATTGGCATTTCAAAAA
TAAGGAAGACTTGTTTGACGCGTTGTTCCAACA

BC252 *Neisseria gonorrhoeae*

>Consensus

TTGCCAATCAACAGGCATTCTTATTTCAGGATATAAAAACCGCCTGCTTTGATACCCGAATG
TTCGAACGGGTTGCAAAGCAGGTTATACCTGTTTTCAAAGTTGAGATGCAGTCTCAATTTTAT
GGGTTTCATTATACATACACGATTGCACGGATAAAAAGTCTTTTTTATAATCCGCCCTCGTCA
AACCGACCCGAAACGAAAACGCCATTATGAGAAAAACCAAAACCGAAGCCTTGAAAACCA
AAGAACACCTGATGCTTGCCGCCTTGGAAACCTTTTACCGCAAAGGGATTGCGCGTACCTCG
CTCAACGAAATCGCCCAAACCGCCGGCGTAACGCGCGGCGCGCTCTATTGGCATTTCAAAAA
TAAGGAAGACTTGTTTGAMGCGTTGTTCCAACA

BC275 *Neisseria gonorrhoeae*

>Consensus

TYGCCAATCAACAGGCATTCTTATTTCAGGATATAAAAACCGCCTGCTTTGATACCCGAATG
TTCGAACGGGTTGCAAAGCAGGTTATACCTGTTTTCAAAGTTGAGATGCAGTCTCAATTTTAT
GGGTTTCATTATACATACACGATTGCACGGATAAAAAGTCTTTTTTATAATCCGCCCTCGTCA
AACCGACCCGAAACGAAAACGCCATTATGAGAAAAACCAAAACCGAAGCCTTGAAAACCA
AAGAACACCTGATGCTTGCCGCCTTGGAAACCTTTTACCGCAAAGGGATTGCCCGCACCTCG
CTCAACGAAATCGCCCAAGCCGCCGGCGTAACGCGCGGCGCGCTTTATTGGCATTTCAAAAA
TAAGGAAGACTTGTTTGACGCGTTGTTCCAACAAAA

BC285 *Neisseria gonorrhoeae*

>Consensus

TKTGCCAATCAACAGGCATTCTTATTTCAGGATATAAAAACCGCCTGCTTTGATACCCGAAT
GTTCGAACGGGTTGCAAAGCAGGTTATACCTGTTTTCAAAGTTGAGATGCAGTCTCAATTTT
ATGGGTTTCATTATACATACACGATTGCACGGATAAAAAGTCTTTTTTATAATCCGCCCTCGT
CAAACCGACCCGAAACGAAAACGCCATTATGAGAAAAACCAAAACCGAAGCCTTGAAAACC
AAAGAACACCTGATGCTTGCCGCCTTGGAAACCTTTTACCGCAAAGGGATTGCGCGTACCTC
GCTCAACGAAATCGCCCAAACCGCCGGCGTAACGCGCGGCGCGCTCTATTGGCATTTCAAAA
ATAAGGAAGACTTGTTTGACGCGTTGTTCCAACA

BC064 *Mycoplasma genitalium*

>Consensus

TKTTGAAATCCCAGGTACGGGTGAAGACACCCGTTAGGCGCAACGGGACGGAAAGACCCCG
TGAAGCTTTACTGTAGCTTAATATTGATCAAAACACCACCATGTAGAGAATAGGTAGGAGCA
ATTGATGCAAGTTCGCAAGGATTTGTTGATGTGAAATGTGGAATACTACCCTTGGTTATGTTT
TGTTCTAATTGGTAACTGTGATCCAGTTTCAAGACAGTGTTAGGTGGGCAGTTTGACTGGGG
CGGTCGCCTCCTAAAAGGTAACGGAGGCGCACAAAGGTACCTTCAGTACGGTTGGAAATCG
TATTTAGAGTGTAATGGTATAAGGGTGCTTGACTGTGAGACTTACAGGTCGAACAGGTGAGA
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CGATGTCGACTCATCTCATCCTCGAGCTGAAGCAGGTTCGAAGGGTTCGGCTGTTCGCCGAT
TAAAGAGATACGTGAGTTGGGTGCAACCGTCGTGATACAGGTGGGTGCCATATCTATTACGC
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CGCATTATCGTAGCACTCTCTTGCATATGCATGCAGCTACTAAGTGAAAGCTTAAATGTAGT
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TTAGTTGATATGAACTAACGAATTATAAGTCTGTCTATGTATGATTGAATAATTATGCTTCAC
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CGGTTGCCGACAGTATATTAACGTTTCTACATGATGGAAGGACATGCATAGACTAAGGCATC
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GGCATGATCACTTTAGTAAATGATATATTTAGTCAGGATCCTTTCACCAGTATAACGTGGGG
GTGTGCAATCATAGTATGCTTAGAGGGAGAATTCTATATGTGGTTCGTTAGCAACGTATGCT
GATTGTTAAATGCCCAGGGATGAAAATGATTAATGATGCTTGAAAGAAAGCAAAATAATAC
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GTAAGTAACGACTATTAGCTCCATGAAATGGTTAGTTGAGTAGATAGTGCATGTGTTGGCCT
TAATACGCTTATTATTTTACGACCTTAAAGTATTATTGTCTTTTTTCAGAGTGTTGTTACTCTT
GATCAGTTCTAACCGAAAAATTTAAGCAAATGACACGCGATACGTAGATGAGTATTGAGRTT
CGCATAGAAATATCATGCTGTGATGACGGATTTCAATAGCTCGCCAGTGTGGTGAGTTAAAT
TTTGTGAACCAAC

BC153 *Mycoplasma genitalium*

>Consensus

TTGAAATCCAGGTACGGGTGAAGACACCCGTTAGGCGCAACGGGACGGAAAGACCCCGTGA
AGCTTTACTGTAGCTTAATATTGATCAAAACACCACCATGTAGAGAATAGGTAGGAGCAATT
GATGCAAGTTCGCAAGGATTTGTTGATGTGAAATGTGGAATACTACCCTTGGTTATGTTTTGT
TCTAATTGGTAACTGTGATCCAGTTTCAAGACAGTGTTAGGTGGGCAGTTTGACTGGGGCGG
TCGCCTCCTAAAAGGTAACGGAGGCGCACAAAGGTACCTTCAGTACGGTTGGAAATCGTATT
TAGAGTGTAATGGTATAAGGGTGCTTGACTGTGAGACTTACAGGTCGAACAGGTGAGAAAT
CAGGTCATAGTGATCCGGTGGTTCAGTATGGAATGGCCATCGCTCAACGGATAAAAGCTACT
CCGGGGATAACAGGCTGATACTGCCCAAGAGTTCATATCGACGGCAGTGTTTGGCACCTCGA
TGTCGACTCATCTCATCCTCGAGCTGAAGCAGGTTCGAAGGGTTCGGCTGTTCGCCGATTAA
AGAGATACGTGAGTTGGGTTCAAACCGTCGTGAGACAGGTTGGTCCCTATCTATTGTGCCCA
CAGGAAGATTGAAGAGCTTTGCTTCTAGTACGAGAGGACCGA

BC323 *Chlamydia trachomatis*

>Consensus

TTAAGTTCCGACCTGCACGAATGGTGTAACGATCTGGGCACTGTCTCAACGAAAGACTCGGT
GAAATTGTAGTAGCAGTGAAGATGCTGTTTACCCGCGAAAGGACGAAAAGACCCCGTGAAC
CTTTACTGTACTTTGGTATTGATTTTTGGTTTGTTATGTGTAGGATAGCCAGGAGACTAAGAA
CACTCTTCTTCAGGAGAGTGGGAGTCAACGTTGAAATACTGGTCTTAACAAGCTGGGAATCT
AACATTATTCCATGAATCTGGAAGATGGACATTGCCAGACGGGCAGTTTTACTGGGGCGGTA
TCCTCCTAAAAAGTAACGGAGGAGCCCAAAGCTTATTTCATCGTGGTTGGCAATCACGAGTA
GAGCGTAAAGGTATAAGATAGGTTGACTGCAAGACCAACAAGTCGAGCAGAGACGAAAGTC
GGGCTTAGTGATCCGGCGGTGGAAAGTGGAATCGCCGTCGCTTAACGGATAAAAGGTACTC
CGGGGATAACAGGCTGATCGCCACCAAGAGTTCATATCGACGTGGCGGTTTGGCACCTCGAT
GTCGGCTCATCGCATCCTGGGGCTGGAGAAGGTCCCAAGGGTTTGGCTGTTCGCCAATTAAA
GCGGTACGCGAGCTGGGTTCAAAACGTCGTGAGACAGTTTGGTCTCTATCCWTCGTGGGCGC
AGGATACTTGAGAGGAGCTGTTCCTAGTACGAGAGAGGACCGGGAATGGAA

KH006 *Chlamydia trachomatis*

>Consensus

TTAAGTTCCGACCTGACACGAATGGTGTAACGATCTGGGCACTGTCTCAACGAAAGACTCGG
TGAAATTGTAGTAGCAGGTGAAGATGCTGTTTACCCGCGAAAGGACGAAAAGACCCCGTGA
ACCTTTACTGTACTTTGGTATTGATTTTTGGTTTGTTATGTGTAGGATAGCCAGGAGACTAAG
AACACTCTTCTTCAGGAGAGTGGGAGTCAACGTTGAAATACTGGTCTTAACAAGCTGGGAAT
CTAACATTATTCCATGAATCTGGAAGATGGACATTGCCAGACGGGCAGTTTTACTGGGGCGG
TATCCTCCTAAAAAGTAACGGAGGAGCCCAAAGCTTATTTCATCGTGGTTGGCAATCACGAG
TAGAGCGTAAAGGTATAAGATAGGTTGACTGCAAGACCAACAAGTCGAGCAGAGACGAAA
GTCGGGCTTAGTGATCCGGCGGTGGAAAGTGGAATCGCCGTCGCTTAACGGATAAAAGGTA
CTCCGGGGATAACAGGCTGATCGCCACCAAGAGTTCATATCGACGTGGCGGTTTGGCACCTC
GATGTCGGCTCATCGCATCCTGGGGCTGGAGAAGGTCCCAAGGGTTTGGCTGTTCGCCAATT
AAAGCGGTACGCGAGCTGGGTTCAAAACGTCGTGAGACAGTTTGGTCTCTATCCTTCGTGGG
CGCAGGATACTTGAGAGGAGCTGTTCCTAGTACGAGAGAGGACCGGAATGGAAA

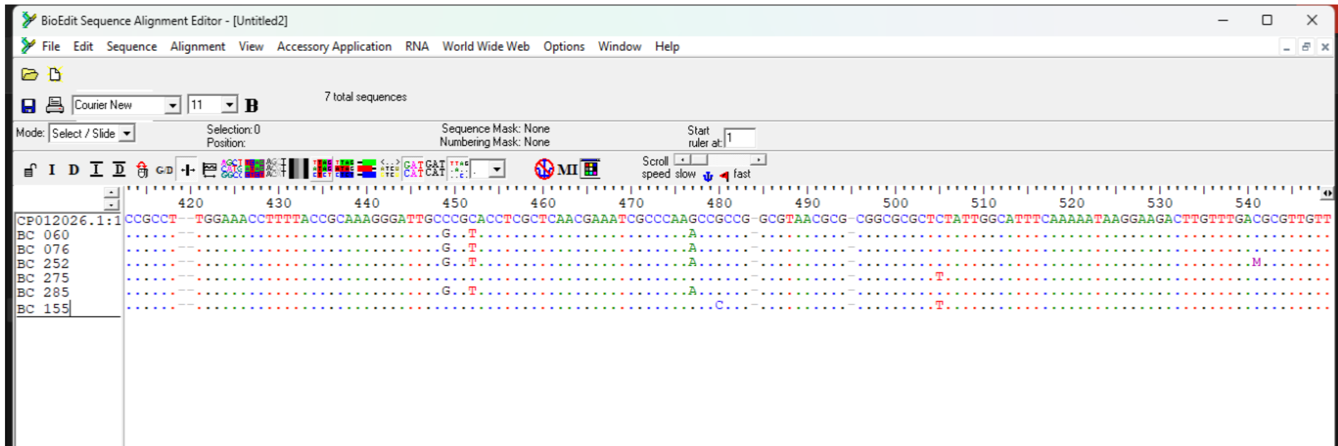
KH031 *Chlamydia trachomatis*

>Consensus

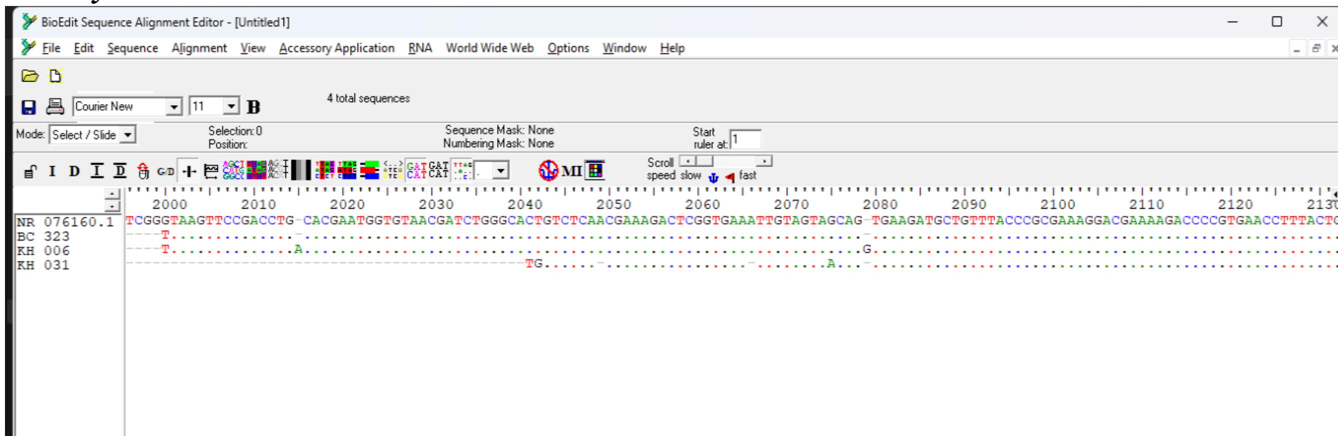
TGGTCTCACGAAAGACTCGGTGAATTGTAGTAACAGTGAAGATGCTGTTTACCCGCGAAAGG
ACGAAAAGACCCCGTGAACCTTTACTGGACTTTGGTATTGATTTTTGGTTTGTTATRTGTAGG
AYAGCMAGGAAACTAAGAACACTCTTCTTCAGGAGAGTGGGAGTCAACGTTGAAATACTGG
TCTTAACAWGCTGGGAATCTAACATTATTCCATGAATCTGGAAGATGGACATTGCCASAYGG
GCAGTTTTACTGGKGCGGTATCCTCCTAAAAAGTAWCGGAGGAGCCCAAAGCTTATTTCATC
GTGGTTGGCAATCACGAGTAGAGCGTAAAGGTATAAGATAAGTTGACTGCAAAACCAACAA
GTCGAGCAGAGACAAAAGTCGGGCTTAGTGATCCGGCGGTGGAAAGTGGAATCGCCGTCGC
TTAACGGATAAAAGGKACTCCGGGGATAACAGGCTGATCGCCACCAAGAGTTMATATCGAM
GTGGCGGTTTGGCACCTCGATGTCGGCTCATCGCATCCTGGGGCTGGAGAASGTCCCAAGGG
TTTGGCTGTTCGCCAATTAAAGCGGTACGCAAGCTGGGTTCAAAACGTCGTGARACASTTTG
GTCTCTATCCTTCGTGGGCGCAGGATACTTGAGAGGAGCTGTTCCTAGTACGAGAGAGGAM
CGKWATGKAA

Appendix F: Multiple Sequence Alignment

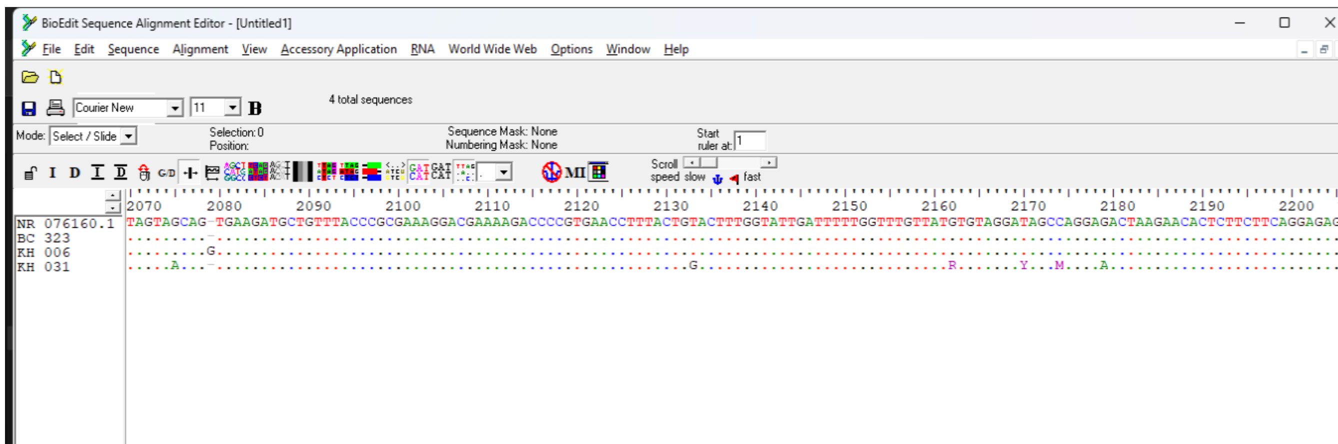
Neisseria gonorrhoeae MSA



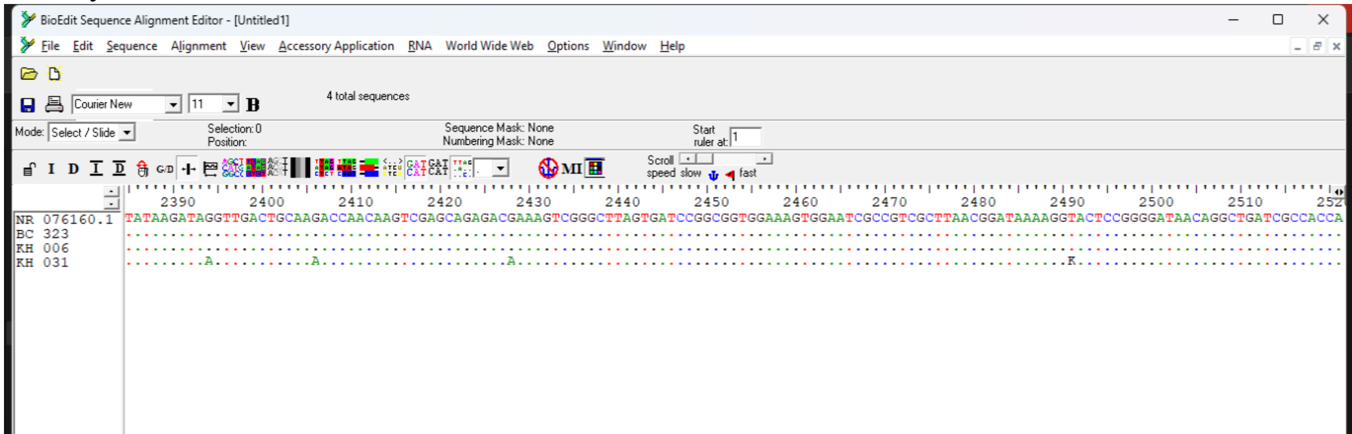
Chlamydia trachomatis MSA 1



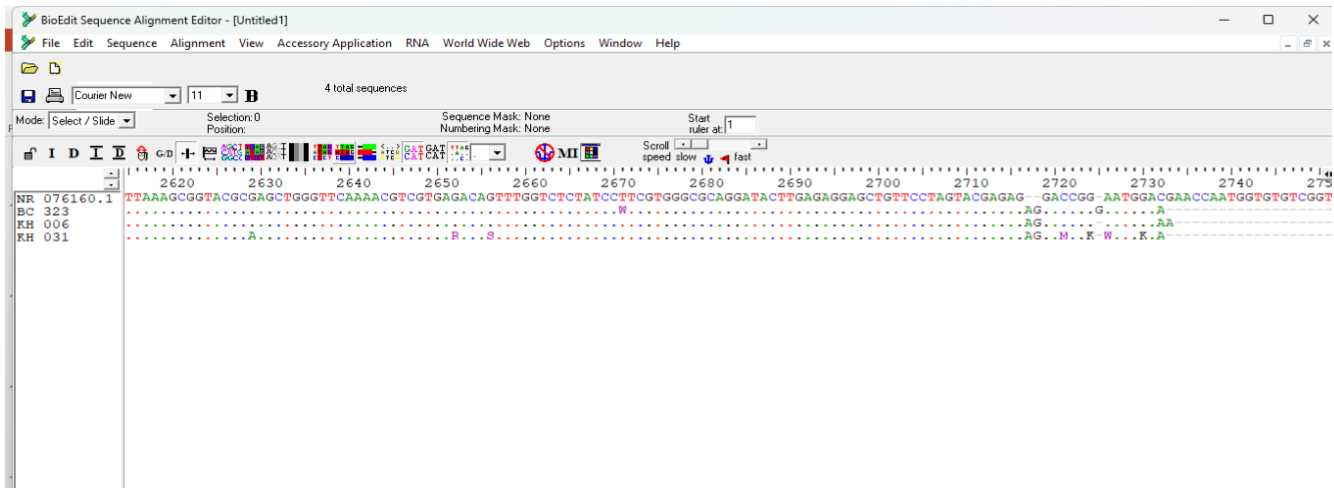
Chlamydia trachomatis MSA 2



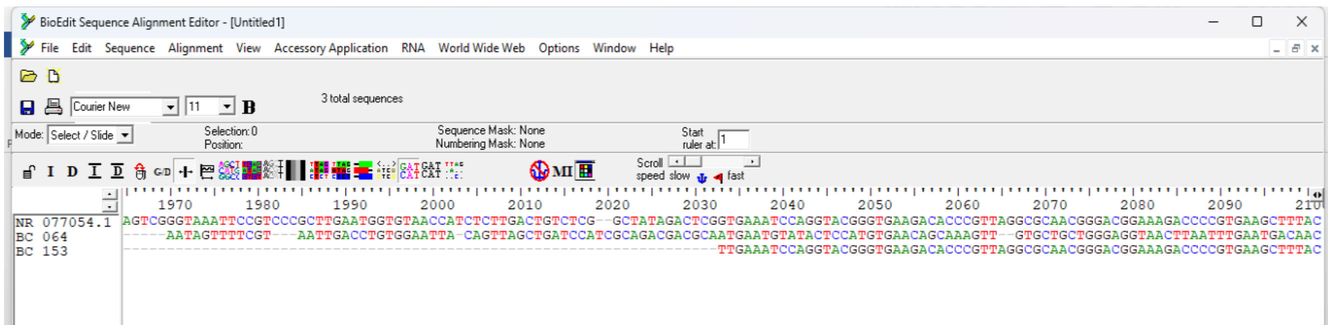
Chlamydia trachomatis MSA 3



Chlamydia trachomatis MSA 4



Mycoplasma genitalium MSA



Appendix G: KNH-UON Ethical Review Committee Approval

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Ref. No.KNH/ERC/R/114

5th July, 2022

Ndungu Primrose Muthoni
Reg. No. U52/35140/2019

Pharmacology & Pharmacognosy Unit
Dept. of Pharmacy
Faculty of Health Sciences
University of Nairobi

Dear Primrose,

Re: Approval of Annual Renewal- Molecular characterization of macrolide resistance in select sexually transmitted infections among symptomatic women attending hospitals in Busia County, Kenya (P135/03/2021)

Your communication dated 24th June 2022 refers.

This is to acknowledge receipt of the study progress report and hereby grant annual extension of approval for ethics research protocol P135/03/2021 for data analysis only.

The approval dates are 30th June 2022 - 29th June 2023.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc.) will be used.
b) All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH- UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH- UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. *(Attach a comprehensive progress report to support the renewal).*

n) Clearance for export of biological specimens must be obtained from KNH- UoN-Ethics & Research Committee for each batch of shipment.

g) Submission of an *executive summary* report within 90 days upon completion of the study.
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

Yours sincerely,

k1cE AMUGUNE SECRETARY, KNH-UoN ERC

cc. The Dean, Faculty of Health Sciences, UoN The Senior Director, Clinical Services, KNH The Chair, KNH-UoN ERC