ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *NEISSERIA GONORRHOEAE* ISOLATED FROM URETHRAL DISCHARGES AND MEN WHO HAVE SEX WITH MEN IN NAIROBI

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DECEMBER, 2018
DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Sign ......................................................... Date ..............................................

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We confirm that the candidate, under our supervision, carried out the work reported in this thesis

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DEDICATION

To my entire family who stood by me throughout my professional career. To wife Matilda Kai, daughters Chelsea, Cynthia and Son Christian Ziro for their support, understanding and constant encouragement. Finally, I would like to glorify the name of the ALMIGHTY GOD, for giving me health, power and the ability to accomplish this work.
I take this opportunity to express my sincere gratitude to the Almighty God for the strength has always given me during and after the period of collecting data, analysis and writing this thesis and my entire family for their encouragement during this study period. I extend my heartfelt gratitude to my employer the Kenya Aids Vaccine Initiative – Institute of Clinical Research (KAVI-ICR) especially Mr. Bashir Farah who allowed me some time to attend course work and provided materials and reagents for carrying out laboratory tests. I also appreciate the support I received from my supervisors Dr. Anthony Kebira and Prof. Omu Anzala from the Department of Medical Microbiology in Kenyatta University and University of Nairobi respectively for their helpful comments and suggestions throughout my academic time, I thank you for taking time out of your busy schedules to meet with me, email with me, and read my manuscripts.

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<tbody>
<tr>
<td>AMR</td>
<td>Antimicrobial Resistance</td>
</tr>
<tr>
<td>AST</td>
<td>Antibiotic susceptibility test</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Culture Type Collection</td>
</tr>
<tr>
<td>CBD</td>
<td>Central Business District</td>
</tr>
<tr>
<td>Ceph-R NG</td>
<td>Cephalosporin resistant Neisseria gonorrhoea</td>
</tr>
<tr>
<td>CSW</td>
<td>Commercial Sex Worker</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for Disease Control</td>
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<tr>
<td>Ct</td>
<td>Cycle threshold</td>
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<tr>
<td>CLSI</td>
<td>Clinical Laboratory Standards Institute</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DGI</td>
<td>Disseminated Gonococcal Infection</td>
</tr>
<tr>
<td>ESC</td>
<td>Extended spectrum cephalosporins</td>
</tr>
<tr>
<td>FTD</td>
<td>Fast track diagnostics</td>
</tr>
<tr>
<td>GASP</td>
<td>Gonococcal Antimicrobial Surveillance Programme</td>
</tr>
<tr>
<td>GISP</td>
<td>Gonococcal Isolate Surveillance Project</td>
</tr>
<tr>
<td>GC</td>
<td>Gonococci</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>IC</td>
<td>Internal Control</td>
</tr>
<tr>
<td>MDR</td>
<td>Multidrug Resistance</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum Inhibitory Concentration</td>
</tr>
<tr>
<td>MSM</td>
<td>Men who have Sex with Men</td>
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<tr>
<td>NAATs</td>
<td>Nucleic acid amplification tests</td>
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<td>NG-MAST</td>
<td>Neisseria gonorrhoea-Multi-Antigen Sequence Typing</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
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<tr>
<td>NICD</td>
<td>National Institute of Communicable Diseases</td>
</tr>
<tr>
<td>NHLS</td>
<td>National Health laboratory Services</td>
</tr>
<tr>
<td>OPA</td>
<td>Opacity related proteins</td>
</tr>
<tr>
<td>RMP</td>
<td>Reduction modified protein</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Real-Time Polymerase Chain Reaction</td>
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<tr>
<td>LOS</td>
<td>Lipooligosaccharide</td>
</tr>
<tr>
<td>QRNG</td>
<td>Quinolone Resistance <em>Neisseria Gonorrhoea</em></td>
</tr>
<tr>
<td>PBP</td>
<td>Penicillinase Resistance <em>Neisseria Gonorrhoea</em></td>
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<tr>
<td>PID</td>
<td>Pelvic inflammatory disease</td>
</tr>
<tr>
<td>PPNG</td>
<td>Penicillin Producing <em>Neisseria Gonorrhoea</em></td>
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<tr>
<td>PorB</td>
<td>Porin Proteins</td>
</tr>
<tr>
<td>ST</td>
<td>Sequence Typing</td>
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<tr>
<td>STC</td>
<td>Special Treatment Centre</td>
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<tr>
<td>STD</td>
<td>Sexually Transmitted Disease</td>
</tr>
<tr>
<td>STI</td>
<td>Sexually Transmitted Infection</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>TRNG</td>
<td>Tetracycline Resistant <em>Neisseria Gonorrhoea</em></td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WSW</td>
<td>Women who have sex with women</td>
</tr>
<tr>
<td>WPR</td>
<td>Western Pacific Region</td>
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**ABSTRACT**

*Neisseria gonorrhoea* has progressively developed resistance to previously used drugs and recently to fluoroquinolones. Currently, there have been reports of emergency of *N. gonorrhoea* strains with reduced susceptibility and some exhibiting extensive multidrug resistant to the cephalosporin class of antibiotics that remains to be the remaining option for management of gonorrhoea. In Kenya, published reports regarding the drug susceptibility of *N. gonorrhoea* is scanty because of lack of surveillance programs and reliance of syndromic management established 20 years ago. Currently, management of gonococcal infections still utilizes a fluoroquinolone in combination with a macrolide; a treatment option that was revised more than three years ago. This study was undertaken in order to determine the antimicrobial susceptibility profile of *Neisseria gonorrhoea* cultured from symptomatic men and asymptomatic men who have sex with men (MSM) attending Casino Special Treatment Centre (STC) clinic in Nairobi. A total of 264 participants were consecutively sampled of which 73 were symptomatic men patients that presented with urethral discharges and 191 were asymptomatic MSM during the period between September 2015 and August 2016. Swabs were directly inoculated on modified thayer martin agar plates and transported to the University of Nairobi department of medical microbiology laboratory in candle jars for analysis. Colonies that were suggestive of gonococci were identified and *N. gonorrhoeae* was confirmed using standard procedures. The Minimum Inhibitory Concentrations (MIC) using Etest for penicillin, tetracycline, ciprofloxacin, spectinomycin, erythromycin, azithromycin, gentamicin, cefixime and ceftriaxone were determined and WHO reference strains used as controls. Data analysis was done using SPSS and descriptive statistics used to analyse both demographic and risk sexual behaviour of MSM. The Chi-square test was used to determine level of significance age category and risk sexual behaviour. There was a significant association between age category and the variables of number of partners and insertive anal sex one MSM was engaged for the last 3 months. The overall isolation rate was 23.8% (63/264) where 20.1% (53/264) were recovered from symptomatic men and 3.7% (10/264) from symptomatic MSM. All *N. gonorrhoea* strains recovered urethral discharges were highly susceptible to cefixime, ceftriaxone and spectinomycin; gentamicin demonstrated moderate susceptibility (94.3%) levels. High resistance levels were observed in penicillin (49.1%), tetracycline (96.2%), ciprofloxacin (49.1%) and azithromycin 16.9%). Of the total 3.7% *N. gonorrhoea* strains isolates from asymptomatic MSM, 4/9 (1.5%) were recovered from the pharynx, 5/9 (1.9%) urethral and 0/9 from the rectal. All the gonococci isolated from the pharynx were highly susceptible to cefixime, ceftriaxone, spectinomycin and gentamicin (100%). Urethral strains were highly sensitive (100%) to spectinomycin and gentamicin only. Susceptibility to both cefixime and ceftriaxone was at 80% and 60% respectively. One strain showed high level MIC to both ceftriazone and cefixme; while one showed cefixime MIC increased level (≥2.0µg/ml) and (≥0.160µg/ml) respectively both recovered from the urethra. Cefixime and ceftriaxone currently used in the management of gonorrhoea are still effective in our local gonorrhoea conditions. The traditional antibiotics previously used to manage gonorrhoea are not effective and there is an emerging resistance of *N. gonorrhoea* strains towards azithromycin. There is therefore a need to conduct continuous surveillance of gonococcal strains and screen high risk group as they harbour gonococcal strains that are resistant to antibiotics. The study recommends molecular characterization of these two gonococcal strains isolated from the urethra of MSM to further understand if they are the same strains previously associated with extensive multidrug resistance in other parts of the world.
CHAPTER ONE

INTRODUCTION

1.1 Background Information

*Neisseria gonorrhoea* (also referred to as “gonococcus”), is the causative agent of gonorrhoea, is the second most common cause of sexually transmitted disease (STD) that accounts for most sexually related illnesses and is therefore of great public health concern worldwide. In 2012, more than one million gonococcal cases were reported globally, which showed an increase of 21% since 2005 (WHO, 2012). Gonorrhoea is mainly transmitted through sexual intercourse and also through the vaginal canal of an infected mother to foetus during birth. After infection, *N. gonorrhoea* colonises and replicates in sites such as the cervix, uterus and fallopian tubes in females, and in men in the urethra. However, the bacteria can also multiply in other secondary body sites like the mouth, throat, eyes and anus depending on acquisition. Complications may arise as a result of persistent colonization at site of infection by the bacterium which leads to disseminated diseases. Examples of these disseminated diseases are like: infertility, blindness in neonates and pelvic inflammatory disease. The control of gonococcal disease depends on strategies that target effective treatment to prevent acquisition and transmission to unaffected people (Tapsall *et al*., 2009). There is also a strong association between human immunodeficiency virus (HIV) acquisition, high viral loads and gonococcal infection among high risk populations hence increasing the burden of HIV infection in areas where both infections exist (Sanders *et al*., 2013).

Since the discovery of antimicrobial agents that dates back the early 1930s to date, *N. gonorrhoea* has progressively developed resistance to almost all drugs known to treat infections caused by this bacterium. The gonococcus has the ability to utilise almost all possible known mechanisms of drug resistance which may be both intrinsic and externally
acquired for example; plasmid mediation, transformations, conjugation and antigenic variations (Achchehe Lal Patel et al., 2011).

Most African national departments of health have not undertaken gonococcal susceptibility surveys since the introduction of syndromic management of sexually transmitted infections (STI) initially introduced and adopted in the late 1990s. This is despite the efforts by the World Health Organization (WHO) recommendations that such routine surveillance or screening for STIs especially on high risk population groups must form an integral part of the syndromic management approach (WHO, 2003). In countries where routine surveillance programs are being undertaken several challenges are still being experienced, for instance; there exist differences in testing methodologies especially in the determination of antimicrobial resistance and the type of media used for testing. Several other programs face the challenge of inappropriate or in availability of control panels for N. gonorrhoea control strains that are used to interpret or report gonococci of susceptible, intermediate and resistant phenotypes for the specific antibiotic that is being evaluated. Additionally, there is also lack of national reference laboratories that are to be used for confirming the findings of susceptibility work from the tertiary laboratories (Lewis et al., 2011).

In Kenya gonococcal surveillance programs were conducted sporadically mainly on case by case basis and with small target population groups for instance; men who have sex with men (MSM), female commercial sex workers (FCSW), Intravenous drug users (IDU) but there has never been community based surveillance studies to report on disease incidences and antimicrobial trends over time. For instance; the initial study on gonococcal prevalence and drug susceptibility profile in Kenya was conducted in the early 1970s by Verhagen and workers (Verhagen et al., 1971). It took a decade for a similar study to be conducted by Perin and colleagues in 1980 who reported on gonococcal prevalence and drug susceptibility profile although STI centres had already been established by this time country wide (Perine et al., 1980). Subsequent work done in 1991 by Daly and colleagues reported a gonococcal
prevalence rate of 3.2% among women attending a family planning clinic in a national referral hospital in Nairobi (Daly et al., 1991) and later on in the early 2000 some gonococcal prevalence and drug susceptibility profile was reported specifically on high risk population groups like CFSW, MSM and IDU which formed a significant section of the community that is now responsible in the acquisition and spread of STIs and human immunodeficiency virus (HIV) (MARPS, 2005, Sanders et al., 2013).

Gonorrhoea was one among the first infections to be treated successfully using penicillin from the early1940’s without any reported cases of verified resistance and hypersensitivity. After several years of misuse, high level penicillin resistance was reported in many parts of the world making it less useful in the management of gonococcal infections worldwide (Unemo et al., 2014). Tetracycline was later introduced in the market as an alternative choice to manage gonococcal infections that were resistant to penicillin and its use was not long-lived as gonococcal strains emerged much sooner and fluoroquinolones like ciprofloxacin replaced them until in 2003 where the extended spectrum cephalosporin (ESC) were recommended for use to date. The progressive replacement of treatment regime to management infections caused by *N. gonorrhoea* is due to this bacterium’s ability to develop a myriad of novel drug resistant mechanisms to the various antimicrobial agents discovered and subsequently put into use.

A study conducted in rural Western Kenya investigating STIs in men for *N. gonorrhoea* from 2002 to 2009 showed that 65% of 168 gonococcal isolates obtained from patients had penicillinase producing *Neisseria gonorrhoea* (PPNG) strains and 97% were plasmid-mediated tetracycline resistance *Neisseria gonorrhoea* strains (TRNG) (Mehta et al., 2009). In South Africa, among 209 gonococcal isolates, 54(22.8%) were resistant to penicillinase-producing *N. gonorrhoea* (PPNG) and 154 (73.3%) TRNG producing strains (Fayemiwo et al., 2011). On fluoroquinolone resistance, It is now more than eight years since these classes of antibiotics such as ciprofloxacin were no longer recommended for use in the treatment of
gonococcal infections because of rapidly rising rates of resistance (WHO, 2003). The study conducted in rural Western Kenya by Mehta et al., (2003); reported 11% of the gonococcal strains resistant were ciprofloxacin resistance while Philippe et al., (2012) reported ciprofloxacin resistance of 53.2% in young uncircumcised men in Kisumu. In addition, some drugs like azithromycin which is currently used as a dual therapy with cephalosporin, some levels of resistance rates are emerging and have been reported some parts of the world which makes the treatment for gonorrhoea quite uncertain (Lewis et al., 2013; Unemo et al., 2012).

The World Health Organization (WHO) STI treatment regime for managing gonococcal infections recommends that local gonococcal resistant profile data be used to determine the choice of therapy (both for dual and single therapy). The current treatment recommendation for gonorrhoea utilises a ceftriaxone 250g intramuscularly as a single dose PLUS azithromycin 1g taken orally as a single dose or cefixime 400g as a single dose taken orally PLUS azithromycin 1g orally as a single dose (WHO, 2003). However; in Kenya current treatment policy protocol on the management of gonorrhoea has not been revised to meet the WHO recommendation probably due to lack of long time derived surveillance data on local antimicrobial susceptibility trends on gonococcal cases. The ESC group of antibiotics have not been used extensively in managing gonococcal infections in the African continent due to availability and coast implication, but already reports of reduced susceptibility has been reported in many parts of the world like Japan, France, Switzerland and Norway (Deguchi et al., 2003, Tapsall et al., 2009, Unemo et al., 2010). It is not surprising that these multidrug gonococcal strains could easily spread in Kenya undetected probably through commercial sex tourists.

In Kenya Supriya et al., (2002) reported an increased oral cephalosporin minimum inhibitory concentration (MIC) creep of gonococcal strains from uncircumcised men in Kisumu (Supriya et al., 2002). These increasing MICs levels for this class of drugs that was observed increases the growing concern regarding multi-drug resistance (MDR) N. gonorrhoea
particularly in individuals involved in high sex behaviour. The inability to manage gonococcal infections is of great concern globally. The gonococcal antimicrobial susceptibility program (GASP) was introduced in African countries that did not have routine STI programs that monitor the antimicrobial susceptibility profile of *N. gonorrhoea* routinely. This is a global effort to ensure monitoring of antibiotic profile is performed by conducting standardised laboratory procedures and routinely reporting disease incidences and drug trends to authoritative government healthcare providers.

High risk populations are at risk of acquiring gonococcal infection and the level of risk rises with the number of sexual partners and also the presence of other sexually transmitted diseases (STDs). Homosexuals (men who have sex with men) are much more likely to acquire and harbour *N. gonorrhoea* without showing symptoms of gonococcal infection. They also harbour *N. gonorrhoea* strains that have far higher rates of antibiotic resistance or multidrug resistant strains at their secondary sites of infection. The presence of untreated STIs (both those which cause ulcers and those which do not) also increases the risk of both acquisition and transmission of HIV by a factor of up to 10. The prompt treatment of gonococcal infections is not only important for curbing further spread of the infection to the healthy population but also as a strategy reducing the risk of HIV acquisition for those practising unsafe sex. *Neisseria gonorrhoea* strains can rapidly spread as history has previously thought us; it is likely that an era of untreatable gonorrhoea may be approaching posing a major public health problem especially when HIV and gonorrhoea shall be co-existing. It will be important to seek concerted efforts in the implementation of plans globally and at nationally level to establish effective control strategies including enhancing surveillance of gonococcal resistance, monitor treatment failures and antimicrobial use or misuse. Efforts aimed at improving prevention especially of HIV transmission, early diagnosis and treatment of gonorrhoea will be critically important. Novel treatment strategies,
new antimicrobials (other compounds) and novel vaccine strategies must be developed in order to combat this STI.

1.2 Statement of the problem

The success of any STD control programme depends on effective treatment regimens that ensure the pathogenic organism has totally been eradicated from the site of infection. However, to achieve this and maintain a high level of drug efficiency, the knowledge of the current local bacterial susceptibility pattern is of utmost importance so as to select the most appropriate antibiotic to treat that infection, which has not been the case in most African healthcare setups. This is because the susceptibility to antibiotics especially *N. gonorrhoea* strains changes overtime, resistance patterns varies with anatomical site of infection even within the same individual and has a wide geographical variation (Lewis *et al*., 2011).

According to WHO incidence reports of 2012, *N. gonorrhoea* is the second commonest STI after *Chlamydia trachomatis* in the world. Several studies have already reported reduced drug susceptibility levels not only to ceftriaxone but also the oral cefixime which remains the only alternative in the management of gonococcal infections in the world. The two previous reports from Japan on gonococcal strain exhibiting extended drug resistance (XDR) are especially disturbing. A high level ceftriaxone and cefixime resistant gonococci was isolated from a commercial sex worker in Japan rendering both drugs unusable. (Deguchi *et al*., 2003, Tapsall *et al*., 2009, Unemo *et al*., 2010). Later in France and Norway, Unemo and colleagues reported that other strains of gonococci with high levels of resistance that seem to be due to novel altered penicillin binding proteins were isolated (Unemo *et al*., 2010).

*Neisseria gonorrhoea* often presents without obvious symptoms when it colonises other anatomical sites like the anorectum and pharynx allowing it to be passed from different sexual partners and subsequently to the general community through heterosexuality. There is a strong association between gonococcal infection and HIV acquisition especially in high risk sexual groups like MSM which happens due to presence of broken skin barriers. The WHO
recommends regular screening of asymptomatic risk taking populations at least once a year to be able to detect these silent infections (carriers of gonococcal strains) and timely treat them to avoid HIV acquisition and spread. Unfortunately, such strategies of screening MSM and other high risk sexual populations have not fully been implemented in Kenya. There is need to revitalise gonococcal laboratory STI surveillance programs especially in high risk groups like MSM so as to monitor emerging strains of *N. gonorrhoea* and give appropriate advice to health policy makers concerning treatment strategies for gonococcal infections.

1.3 Justification

Historically; the gonococcus has progressively demonstrated its ability to resist a wide range of antibiotics previously used to manage gonococcal infections. In many of the Western countries with robust STI surveillance programs like the Gonococcal Isolate Surveillance Project (GISP), patterns of drug susceptibilities are closely being monitored and information gathered is being used to update or inform drug resistance trends overtime and subsequently influence health policy decisions to take care of infections caused by *N. gonorrhoea* in their respective regions or countries. In 2003, the CDC made a recommendation for the new treatment policy that included a combination of both injectable ceftriaxone (250 mg single dose intramuscularly) with either azithromycin (1g) or doxycycline (100 mg) orally for 7 days as effective treatment for uncomplicated gonorrhoea (CDC, 2011). It is unclear whether this recommendation has been effected into the health policy document in Kenya probably due to inadequate information originating from STI programs that could leverage the implementation of such policy changes.

A study by Philippe and colleagues in 2012, reported gonococcal strains with high levels resistance to fluoroquinolones in four different regions of Kenya (53.2%); while in a study conducted in Kisumu between 2002 to 2009 on young men to determine if circumcision could prevent HIV acquisition, the rate of quinolone resistance of gonococci isolates was at 11% (Supriya *et al.*, 2011). Generally, a 5% antibiotic resistance is generally accepted to
change the treatment regimen for gonococcal treatment as recommended by CDC for the effective management of gonorrhoea. Despite the high level of resistance level of local gonococcal isolates to ciprofloxacin, health policy makers in Kenya have not published revisions to the treatment policy on gonorrhoea. This could probably be due to inadequate progressive data on surveillance of *N. gonorrhoea* from various high risk populations like MSM in Kenya. The only studies conducted in this region focused on high risk populations and acquisition or transmission of HIV/Aids. Information on the prevalence of *N. gonorrhoea* on various anatomical sites on MSM is scanty and the antimicrobial susceptibility.

Pharyngeal gonococcal infection is usually very difficult to treat and as recommended by WHO require ceftriaxone 250g intramuscularly single dose plus 100g azithromycin single dose (WHO, 2003). These treatment regimens require to be monitored for its effectiveness and revised accordingly based on available local susceptibility data. Already there are reports from several parts of the world of verified treatment failures on gonococcal strains to the remaining treatment and it’s strongly believed that these strains may be spreading in many parts of the world (Deguchi *et al.*, 2003, Tapsall *et al.*, 2009, Unemo *et al.*, 2010). These sites (urethral, anal and pharynx) therefore require regular screening through culture or other detection methods to establish disease burden and drug susceptibility profile. Gonococcal strains from pharynx and rectum tend to easily develop resistance to antimicrobial agents and therefore need to be monitored for resistant strains. In Kenya there have been no strategies or studies to monitor the burden of carried gonococcal strains among MSM and their drug susceptibility profile despite the increasing numbers of MSM in the community. This information is important to guide on the formulation of treatment protocols based on site of infection of gonococci to be determined by available accumulated data on antimicrobial susceptibility of gonococcal isolates.
1.4 Hypotheses

i. *Neisseria gonorrhoea* is not prevalent in both men presenting with urethral discharges and asymptomatic men who have sex in men in STD Casino, Nairobi.

ii. There are no levels of drug resistance in *N. gonorrhoea* isolated from both men presenting with urethral discharges and asymptomatic men who have sex with men recovered from different anatomical sites: urethral, anorectal and pharyngeal.

iii. There are no significant levels of resistance between the various antibiotics tested at the various anatomical sites (urethra, rectal and throat) of asymptomatic MSM.

1.5 Study Objectives

1.5.1 General Objectives

To determine the antimicrobial susceptibility profile of *N. gonorrhoea* that are recovered from male patients presenting with urethral discharge and asymptomatic men who have sex with men attending an STD clinic in Nairobi.

1.5.2 Specific objectives include:

i. To determine prevalence of *N. gonorrhoea* in both men presenting with urethral discharges and asymptomatic men who have sex in men in STD Casino, Nairobi.

ii. To determine antimicrobial susceptibility profile of *N. gonorrhoea* isolated from both men presenting with urethral discharges and asymptomatic men who have sex with men recovered from different anatomical sites: urethral, anorectal and pharyngeal.

iii. To determine if there is a significant level of resistance between the various antibiotics tested at the various anatomical sites (urethra, rectal and throat) of asymptomatic MSM.

1.6 Significance

The control of gonococcal infection is an important strategy which requires prompt detection and effective treatment so as to break the transmission chain. To achieve this, knowledge of local susceptibility patterns of *N. gonorrhoea* based on time points, site of infection, sexual
risk taking behaviour and early detection of disease needs to be understood. This requires routine and continuous high quality surveillance programs that will monitor susceptibility trends of both past and current antimicrobial agents; however; there is scanty information on antimicrobial resistance profile especially on high risk populations in Kenya. The current study sort to provide an insight into the antibiotic resistance profile of *N. gonorrhoea* isolated from asymptomatic high risk population in Nairobi; Kenya and men that voluntarily seek medical assistance due to presumed gonococcal infection as they presented with sign of urethral discharge. Surveillance data would significantly assist in reviewing the effectiveness of the current treatment regime and where possible the formulation of new treatment guidelines. This will also go a long way in putting up measures of how to prevent the spread of any emerging gonococcal strains especially for the ESC group of antibiotics from further spreading to the general community.
CHAPTER TWO

LITERATURE REVIEW

2.1 Characteristics of *Neisseria gonorrhoea*

*Neisseria gonorrhoea* is a species of Gram-negative bean shaped diplococci bacteria responsible for the sexually transmitted infection (STI) gonorrhoea. Transmission is through sexual contact and the gonococcus dies quickly when outside the human host. *Neisseria gonorrhoeae* is able to evade host immune system and can survive without necessarily destroying the cells it has colonised and this allows to be transmitted to other hosts. This ability to adapt in the host without being destroyed makes it to stay alive and spread over long period of time. In women, gonococcal infections manifests as a mild diseases without obvious symptoms (Achchhe Lal Patel *et al.*, 2011).

*Neisseria gonorrhoea* has a very important characteristic of inherently demonstrating antibiotic resistance through phenotypic and genotypic variability. Phenotypic variability is achieved through producing differential existing regions or parts of the genetic makeup on the surface of the bacterium, while genotypic variability is by inserting new genetic material by either conjugation or transformation processes. The ability to acquire drug resistant plasmids by *N. gonorrhoeae* has been associated to development of resistance to penicillin and other drugs. The other characteristic that makes it unique is antigenic variability where it’s able to acquire and incorporate genetic material in its genome from other related organisms (Achchhe Lal Patel *et al.*, 2011).

2.2 Physiology and structural characteristics of *Neisseria gonorrhoea*

The gonococcus is a fastidious aerobic bacterium that requires nutritious media for growth and prefers humid atmosphere that contain approximately 5% carbon dioxide. The bacteria have an optimal growth temperature of 35-37°C, and have minimal chances to survive at low temperatures. It produces oxidase, catalase and oxidizes glucose if available. *N. gonorrhoeae* do not expose flagella and produces no endospores. Examined in the microscope, the
bacterial cells have a characteristic coffee bean shape with adjacent side flattened in the microscope. In older colonies of *N. gonorrhoeae*, the cells may appear swollen and round due to autolysis. The cell wall structure of *N. gonorrhoeae* is similar to other Gram negative bacteria with an inner cytoplasmic membrane and an outer membrane composed of proteins, phospholipids, and lipopolysaccharide (LPS), and a thin peptidoglycan layer between the inner cytoplasmic membrane and the outer membrane. The LPS in the *N. gonorrhoeae* are referred to as lipooligosaccharides (LOS), due to that they consist of a shorter saccharide chain compared to the LPS. Several proteins are exposed on the cell surface of the *N. gonorrhoeae*, pili, porin proteins (PorB), Rmp proteins (Reduction modifiable protein) and Opa (opacity protein) proteins. They are all thought to have different roles in invasion of the host and virulence activity. *N. gonorrhoeae* is a fastidious aerobic bacterium. It requires nutritious media for growth and prefers humid atmosphere that contain approximately 5% carbon dioxide. The bacteria have an optimal growth temperature of 35-37°C, and have minimal chances to survive at low temperatures. It produces oxidase, catalase and oxidizes glucose if available. *N. gonorrhoeae* do not expose flagella and produces no endospores (Bachmann *et al.*, 2009)

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2.3 Pathogenesis and clinical manifestations of Neisseria gonorrhoea

The mechanism of entry into the mucosa of the human host and subsequent colonization of *N. gonorrhoea* is summarised in Figure 2.2. Gonorrhoea is transmitted by contact with exudates from mucus membranes of infected persons primarily through sexual activity. This can occur during vaginal, oral or anal sexual activity. Gonococcal ophthalmia neonatorum can occur in neonates who have had contact with the mother’s infected birth canal during birth. Bacterial infection happens when the organism attaches itself to the mucosal surface of the epithelium cells where it then penetrates the epithelial cells reaching the submucosal tissues within 24 – 48 hours. These infected epithelial tissues are then infiltrated by massive numbers of neutrophils which attracts polymophornuclear cells at the site of invasion resulting in the formation of submucosal micro abscesses and exudative pus. Some of the bacteria may escape the host immune reactions at this stage and continue to replicate inside cells as part of its strategy of surviving the host cell immune system. The bacterium has mechanisms of escaping the host immune system by releasing some neutralizing factors like the enzyme IgA protease, it can also undergo antigenic variation at its attachments sites (pilli) and other membrane sites like the colony opacity-associated (Opa) outer membrane proteins (Bachmann et al., 2009).

Women are protected naturally from suffering specific gonococcal infections like vaginitis by producing high levels of oestrogen hormone and cornification of the vaginal epithelium. The incubation period in women is 10 days while in men is 2-3 days, and the common signs of disease include painful inflammation of the cervix, vaginal discharges and heavy menstrual flow in women (Bachmann et al., 2009). In most of the cases (10-20%), if the infection is not well managed using effective antibiotics, the bacteria descends to the endometrium and colonise the fallopian tubes. Complications may arise due to persistent bacterial colonization.
of the fallopian tubes. Such complications may include conditions like pelvic inflammatory disease (PID), inflammation of the endometrium, infertility, abortion, abscess and inflammation of the surrounding of uterus and fallopian tubes. If these complications are not treated promptly they may cause permanent damage to the affected organs especially in women where total loss of fertility and other consequences of great public health concern will occur. In men, gonorrhoea is manifested by a sudden inflammation of the urethra accompanied by discharge of pus through the penile pore, painful urination and sudden inflammation of the epididymis. Contrary to women where gonococcal infection exhibits serious long term consequences, in men complications are as a result of persistent infection leading to conditions like painful infection of joints, bone disease, reddening of the skin, inflammation of meninges of the brain and rarely does it lead to disseminated gonococcal infection (Holmes et al., 1980).

Figure 2.1: The pathogenesis of *Neisseria gonorrhoea* into the human host (Todar’s online textbook of bacteriology)

Key: PMN – Polymorphomononuclear cell; LPS – Lipopolysacharides; TNF – Tumor necrosis factor
2.4 Epidemiology and risk factors of *N. gonorrhoea* acquisition

The global incidences of disease burden caused by *N. gonorrhoea* for the year 2005 is summarised in Figure 2.5. Sexually transmitted infections present an enormous burden of morbidity and mortality, especially in developing countries; greater than 80% of all cases worldwide occur in these countries and mostly affect reproductive health and the health of young children (Moodley *et al.*, 2006). It is believed that STIs, excluding HIV, are the second most common cause of loss of healthy life years in young women in developing countries; these infections constitute an enormous health burden, accounting for 17% of the total economic losses from disease, and they are among the top ten reasons for visits to health centres (WHO, 2006). Treatment of gonorrhoea has increased in price as a result of resistance to the antibiotics that were previously used, and some developing countries cannot purchase these antibiotics at such high prices, which affects infection control. Other factors that influence infection control include the social stigma associated with genital symptoms, the asymptomatic nature of gonorrhoea, and the low levels of awareness regarding sexual health (WHO, 2006).

In most of the developing countries, data are obtained from incidence studies, surveys, and sentinel surveillance systems, which usually distort rates of infection. According to the WHO, in 2006, 27 million new cases of gonorrhoea were estimated to have occurred in Southeast Asia where the highest rates of gonorrhoea incidence worldwide that were registered, with 18.5 million cases occurring in Africa and 7.5 million cases occur in Latin America and the Caribbean (WHO, 2006).

Africa is the continent with the second highest incidence of gonorrhoea, but sub-Saharan Africa has one of the world's highest incidences of gonorrhoea, with 17 million new cases per year in a population of 269 million adults (Johnson *et al.*, 2007). These high rates are the result of a combination of behavioural risk factors, poor health services, high rates of migration, and the spread of the HIV/AIDS epidemic. It should be noted that in Africa, risky
sexual practices have also increased. In 2005, the prevalence of gonorrhoea in Senegal was 5.4% among MSM (Caceres et al., 2007).

In industrialised countries, there are large differences in the prevalence of gonorrhoea depending on race, socioeconomic status and sexual orientation. In the United State (US) for example, gonorrhoea is the second most reported bacterial STI with an incidence of 99.1 cases per 100,000 inhabitants in 2009, which has declined slightly since 2006 (CDC, 2010). In Canada, the incidence of gonorrhoea has continued to increase since 1997. In 1999, there were 17.6 cases per 100,000 inhabitants and in 2008 this rate doubled to 38.2 cases per 100,000 inhabitants (CDC, 2010). As in most industrialised countries, in Australia, the incidence of gonorrhoea has increased in the last decade among both heterosexuals and homosexuals (CDC, 2010).

Risk factors for acquisition and transmission of *N. gonorrhoea* include unprotected sex especially unprotected anal intercourse, multiple sexual partners, male homosexuality, low socioeconomic status, transactional sex, history of concurrent or past STDs, early age of onset of sexual activity and illegal drug use (Loza, et al., 2010).

The gonococcal infections frequently found in concomitant with other STIs especially *Chlamydia trachomatis* which further complicates the treatment (DeSchryver et al., 1990). In 1999 DeSchryver, reported the greatest incidence of both infections that occurred in parts of Asia followed by Africa, Latin America and the Caribbean (DeSchryver et al., 1999). High rates of gonococcal infection are reported amongst the teenage (<24), those that reside in highly populated urban areas or slum dwellers, people who have multiple sex partners, and those that engage in unprotected sexual behaviour (Weinstock et al., 2000). The individual social or sexual behaviour significantly affects gonorrhoea acquisition and rate of transmission since the number of sexual partners one has and engaging in unsafe sex are directly associated with high acquisition and transmission of infection. Women are always at risk of contracting gonorrhoea up to 50% for every episode of unprotected sexual encounter.
with an infected male, while men can get infected up to 20% with infected females). The use of barrier methods during sexual activity significantly reduces the risk of spreading the infection and should be encouraged. Such sexual barriers should be advocated or freely provided to high risk groups to enable them practise safe sex and therefore break the transmission cycle.

The prevalence of *N. gonorrhoeae* in the population or network in which a woman socializes and chooses her sexual partners determines the likelihood of exposure to this pathogen. Women who have multiple sexual partners or whose partners have multiple sexual contacts increase their risk of exposure to *N. gonorrhoeae*. Women who do not use condoms or other barrier protection increase their risk of acquisition of *N. gonorrhoeae* infection on exposure to the organism. It is unknown whether women who are HIV-positive have an increased risk of infection by *N. gonorrhoeae* on exposure to it. Factors associated with an increased likelihood of at-risk behaviour that results in an increased risk of gonococcal infection among pregnant women include younger age, unmarried status, homelessness, problems with drug or alcohol abuse, prostitution, low-income professions (Weinstock *et al.*, 2000).

**2.5 Screening and detection of antimicrobial resistant *Neisseria gonorrhoea* strains**

Gonorrhoea has the ability to infect several host sites that include: urethra, cervicovagina, oropharyngeal, rectal and conjunctiva of the eye. Gonococci of the urethra are always symptomatic in men; contrary to women where 80% are asymptomatic (Gerald *et al.*, 2009). As recommended by the WHO, surveillance of *N. gonorrhoea* should comprise routine screening of high risk populations by using standardised laboratory tests. This strategy will form the basis of an effective treatment, monitoring and control program as it help cut the transmission cycle of any STI. In MSM, it is estimated that a significant proportion of urethral gonorrhoea occur simultaneously with pharyngeal gonorrhoea (Kinghorn *et al*, 2010) due to bisexual practise and other unsafe sexual practise; and most of such cases are localised to sites other than the urethra (Marcus *et al.*, 2011). Extra-genital infections are
overwhelmingly asymptomatic (Marcus et al., 2011) and under-diagnosed. It is worrying therefore that the continuous presence of asymptomatic gonococcal infections of the throat and rectum in one region is a potential reservoir for new acquisition and subsequent transmission to other susceptible hosts.

All STI surveillance programs must aim at reducing acquisition and transmission of gonococci and more importantly limit the possibility of acquiring both a gonorrhoea and HIV/AIDS as these pathogens can easily be acquired and carried simultaneously by the same especially those involved in unsafe sex. The CDC in 2003 published some screening guidelines for the regular detection of HIV/AIDS and other STDs in order to improve on sexual health of MSM and other risk groups (CDC, 2003). These guidelines are specific for *N. gonorrhoea* and *C. trachomatis* are as follows:

- Yearly urethral or urine screening for both infections among sexually active MSM, performing cultures of the pharynx for MSM with oral-genital exposure and rectal chlamydia and gonorrhoea cultures for MSM who have had receptive anal sex.
- Performing regular screening tests (3-6 months) for individuals involved in high risk behaviour.
- Performing screening tests for all individuals engaging in both anal insertive and receptive sex despite reported condom use.

Surveillance programs should therefore follow these screening recommendations as an important strategy in controlling increasing incidences of gonorrhoea in high risk population groups. However, there are no designated STD screening programs and gay men health centres in Kenya and Africa at large with the exception of South Africa that conduct both rectal and pharyngeal gonococcal screening in asymptomatic risk population groups. There is a need to continually evaluate these screening strategies to determine their suitability in detecting gonorrhoea so as to generate accurate and precise data especially among high risk
population groups whose practise is gradually being accepted in the African society (MARPS, 2012).

*Neisseria gonorrhoea* in MSM causes infections without obvious signs of disease and in cases where these clinical signs appear, they are commonly non-specific in nature. These non-specific presentations require appropriate diagnosis for confirming accurate diagnosis and case by case finding if the targeted causative organism has to be eliminated completely. To diagnose *N. gonorrhoea*, there are various laboratory techniques currently in use to either detect the presence of bacteria and/or its genetic material in genital or extra genital specimens. They include microscopic examination stained smears, growth of the organism using artificially prepared media, or nucleic acid amplification tests (NAATs). Routine testing of gonococcal isolates on frequently used drugs is the cornerstone of every STI antibiotic surveillance program. Unfortunately, STI centres in Kenya are not enough and treatment strategies do not follow detection of the bacteria in clinical samples but based on evidence of symptoms suggestive of gonococcal infection established twenty years ago (Philippe *et al.*, 2012).

The presumptive identification of gonococci is based on detection or finding intracellular diplococci in polymorphonuclear leucocytes by microscopy as Gram negative in primary samples. The method is universally accepted because it has the merit of being affordable, highly sensitive and specific in diagnosing men with symptoms of urethral discharges. Despite having these merits, microscopy is not recommended as the only method for diagnosis of cervical, pharyngeal or rectal gonorrhoea, or for asymptomatic patients due to its low sensitivity, it depends on the experience of the microscopist and cannot be used for AMR testing (Unemo *et al.*, 2014).

Culture of genital samples has been used for a long time as an accurate basic diagnostic technique with sensitivity and reproducibility of test results (if appropriate species-verification assays are applied) and also allows for complete AMR testing of the organism.
However, its major short coming is that it requires some relative length of time between 2 – 4 days to carry out the test and also requires strict optimisation of the test conditions from pre-analytical, analytical and post analytical stages of testing since gonococci are affected by extreme environmental factors (GASP-LAC lab Manual, 2011).

Since the introduction of NAAT based techniques over 20 years ago, many laboratory settings rapidly changed from culture which is laborious to the rapid and convenient molecular methods of diagnosis. The use NAATs may have many advantages, including detection of nonviable gonococci; they have a sensitivity detecting pharyngeal and rectal specimens; and they utilise non-invasive samples like urine for males and vaginal swab for females. NAATs have the advantage that they can be used for rapid testing, can fully be automated, and several pathogens can be identified simultaneously if contained in the same specimen. However, NAATs also have disadvantages such as not allowing AMR testing data, test of cure has not yet been defined, and are only commercially available (Unemo et al., 2014). The presence of Neisseria species existing as normal flora normally at the extra genital sites, but uncommon at the urinary tract, makes it share some genetic similarities with pathogenic N. gonorrhoea and this may affect the result of NAAT through cross reactions (Unemo et al., 2014).

The Etest which is a gradient two fold drug sensitivity technique is used to establish the minimum inhibitory concentration (MIC) in μg/ml of the tested antibiotic in an agar plate. This technique has the following shortcomings: the test is costly, is labour intensive and cannot be used for routine drug susceptibility assays (Unemo et al., 2014). The disc diffusion susceptibility technique is routinely encouraged for routine testing only when MIC testing cannot be established for N. gonorrhoea in such circumstances the disc susceptibility test should be confirmed with MIC test (Unemo et al., 2014).

Gonococcal surveillance and epidemiological studies require routine AMR testing, culture and phenotypic testing to confirm the identity of the organism. The use of both culture and
NAATs where resources are available for both rapid diagnosis and generate data on AMR is highly recommended and implemented in countries with enough resources. To enhance collection of data prevalence on gonococcal infection it is all regions that have limited STI treatment centres must also build their local capacity in carrying out standardised laboratory tests that can yield accurate and reliable laboratory reports. The use of both culture and molecular techniques in the same facility would increase both sensitivity and specificity of isolating *N. gonorrhoea* in clinical samples only when resources are available (Unemo *et al.*, 2014).

Currently, there is only one globally recommended effective treatment option for treatment of gonococcal infections which is already under threat. Limited screening strategies may not only be hindering the detection of asymptomatic cases spreading in the community but also a source for harbouring some resistant strains. For example; the throat is not only a reservoir of gonococcal infection, it is a reservoir of antimicrobial resistant gonococcal strains where commensal *Neisseria species* pass resistant genes through processes such as transformation and conjugation. This has long term implications for the spread and acquisition of resistant strains from the MSM and other risk groups to the general population. To be able to routinely monitor new and emerging gonococcal strains that may spread in the general community, healthcare providers should enforce policies that would seek to follow up on unsafe sex practise in MSM; and individuals reporting such practises should be tested regardless of symptoms (CDC, 2006). This will enable early identification to curb spreading of such gonococcal strains with possible drug resistances more importantly the first line drugs (cephalosporin). These resistant gonococcal strains are known to spread easily and extensively as reported probably through commercial sex tourists and other international travellers.
2.6 Working case definitions for cephalosporin resistant Neisseria gonorrhoea

The working case definition for cephalosporin resistance are worked relying on data collected from monitoring cephalosporin treatment failure of *N. gonorrhoea* (Ceph-R NG) and/or laboratory-based AST (antibiotic susceptibility test) results. Evidence based management of individual cases of gonococci in most parts of Africa is hampered by lack of AST data to treat local gonorrhoea infections, and as such it has been difficult to appropriately define an agreed laboratory criteria for Ceph-R NG levels due to lack of routine AST data. To date the Clinical Laboratory Standards Institute (CLSI) has not defined MIC breakpoints for resistance (CLSI, 2011) of gonococci since there have been few reported cases of cephalosporin resistance globally. It also poses a challenge when correlating the bacterial breakpoint which is yet to be determined and cephalosporin treatment failure.

The MIC breakpoints for cefixime used in the case definitions differ from the breakpoints for ceftriaxone because cefixime MICs are generally one-dilution higher than ceftriaxone MICs. These working case definitions will be made available once data on Ceph-R NG is available but the current used case definition for *N. gonorrhoea* treatment as below:

- MIC ≥ 0.25µg/ml for cefixime
- MIC ≥ 0.125 µg/ml for ceftriaxone

These MIC break points were used as critical alert values to detect an early warning sign for an emerging resistance for cefixime and ceftriaxone *N. gonorrhoea* strains.

2.7 Pharyngeal and anorectal Neisseria gonorrhoea as a reservoir of drug resistant strains

*Neisseria gonorrhoeae* has the propensity of infecting various genital organs mainly by sexual contact and infection of the other extra genital sites like the throat and rectum allows it to acquire resistance mutants to various classes of antibiotics. The presence of lipids contained in faecal materials and other bacteria within the rectum gives an opportunity for *N. gonorrhoea* to development drug resistance due to acquisition of resistant plasmids and also
making the bacteria to mutate at certain region within the bacterial cell such as the mtr locus due to selective pressure exerted by hydrophobic molecules (Unemo et al., 2014). On the contrary, pharyngeal infections provides room for genetic rearrangement where there is co-existence between N. gonorrhoeae and other Neisseria species (Unemo et al., 2014) for example; the mosaic penA allele, is responsible for the decreasing cephalosporin susceptibility. This gene is believed to have evolved between the recombination of penA gene from commensal Neisseria species and pharyngeal infections (Unemo et al., 2014).

Furthermore, gonorrhoea of the throat is usually asymptomatic, not easy to treat using antibiotics as compared to urethral and rectal gonorrhoea. This therefore provides a further opportunity for genetic selection of resistance due to mutation at the pharynx where bacterial commensalism is common. It is therefore evident that the first high level ceftriaxone resistant gonococcus was a mutant of the pharynx as a result of selective pressure (Unemo et al., 2014). Most cases of drug resistance and/or MIC level increase as observed amongst gay men, bisexual men and MSM among whom both rectal and pharyngeal infections are common are attributable to cross infections (Unemo et al., 2014).

It is common practice in routine and STI programs to collect urethral swabs while carrying out AMR in gonococci as they are easy to collect. As a consequence there is limited published data that compares susceptibility patterns of N. gonorrhoea between the various anatomical sites especially the rectal and throat. The only available published report shows that both rectal and pharyngeal gonococcal strains are less susceptible to common drugs compared to urethral strains, though the study was short of evaluating and controlling for sex of sex partners, as was a potential confounder of association between the anatomical sites and drug susceptibility.

The CDC and WHO have always relied on both national and other international STI programs to gather data in determining gonorrhoea case management policies and treatment reviews for extra genital infections. It is therefore imperative for any country to understand if
the antibiotic susceptibilities profiles of urethral isolates are similar to those isolated at both the pharynx or rectal anatomical as they are difficult to obtain due to stigma and cultural beliefs.

2.8 *Neisseria gonorrhoea* treatment history and development of antimicrobial resistance

Before the advent of antibiotics, gonorrhoea was treated using living a healthy life that involved eating well and abstaining from sexual activity once infected with *N. gonorrhoea*. Treatment improved after the second half of the 19th century where gonococcal infections were managed using traditional herbs from trees of cubebs and copaiba. Later on these herbs were prepared using mixtures of licorin magnesium hydroxide or ammonium carbonate to improve on taste and then made into capsules to reduce toxicity. Other treatment measures included the use of certain detergents and water enemas to reduce the retention of urine and the use of warm baths to avoid irritation and if needed catherization were also performed. Urethral irrigation was used to treat acute urethritis by using dilutions of warm potassium permanganate for several weeks (Unemo *et al*., 2014).

The need for specific antibacterial agents began towards the end of 1800 years when the search for various metallic compounds was intensified and tested so as to have better and effective treatment for the management gonorrhoea. This led to significant discoveries in the search for more advanced treatment options. For example; during the first world war soldiers suffering from gonorrhoea were treated using concoctions of condoms and metallic ointment (silver compounds), while postcoital treatment canters used urethral irrigation facilities to treat people suffering from gonorrhoea. The use of mercury compounds later diminished due to adverse effects experienced especially when used as an antiseptic agent to cure urinary tract infections cause by *N. gonorrhoea*. To reduce these adverse effects associated with use of mercury compounds and also boost effectiveness, mixtures of mercury compound and glucose solution as an intravenous injection were used which had fewer adverse effects and
more efficacious. Other than the introduction of intravenous mercuric mixtures, further treatment involved the use of a silver-protein complex instilled into the urethra, or the seminal vesicles irrigated with potassium permanganate (Unemo et al., 2014).

The history of antimicrobial compounds discovery, introduction of various antimicrobial agents, evolution of drug resistance and recommendations in first-line antimicrobial(s) use for *N. gonorrhoea* are summarized on Figure 2.2. Antimicrobial agents were first introduced into use in 1935 when a sulphonamide for the first time discovered as a natural antibacterial compound. The use of sulphonamides in managing gonococcal infections was short-lived as by 1945 reports of gonococcal strains resistant to this class of antibiotics had already emerged in most parts of the world (Lewis et al., 2010). Penicillin was later discovered and introduced into use as an alternative option to treat gonococcal strains resistant to the sulphonamides. Penicillin become very effective as the drug of choice and was used in small doses with cure rates exceeding 95%. This drug then became very popular and was used extensively to treat gonorrhoea and other mild infection like upper respiratory tract infections. Drug resistance later emerged due to constant drug exposure leading to selective pressure on the bacteria hence development due to mutational changes and release of enzymes that target the functionality of the drug. The minimum inhibitory concentrations (MICs) then gradually started increasing with time due to mutational changes targeting key genes within the bacterial genome (*penA, penB, ponA, mtr* and *pem*) (Lewis et al., 2010). Later on, chromosomally mediated penicillin resistance gonococci emerged, and this further rendered penicillin less a less effective drug that was cheap and affordable for use in the management of gonorrhoea (Achchhe La Patel et al., 2011).

Tetracycline, as with penicillin was discovered in the late 1940s, and was also effective in treating patients with gonorrhoea allergic to penicillin. Tetracycline resistance just like with penicillin resistance bacterial resistance was as a result of alterations of genomic composition
at specific sites within the bacterial cell (*rpsJ, penB, mtr, tem*). Later, the isolation of *Neisseria gonorrhoea* strains with high-level plasmid mediated tetracycline-resistant (TRNG) strains in in the United States and Netherlands was reported that further lowered its chances for use in managing gonorrhoea as resistance levels were increasingly evident exceeding the acceptable cure rate (Roberts *et al.*, 1985). TRNG strains are now common and circulating across the globe as demonstrated by the 73% prevalence rate recently reported in South Africa (Fayemiwo *et al.*, 2011).

Spectinomycin was discovered in the early 1960s to specifically treat gonorrhoea that was resistant to penicillin and tetracycline. However; this drug did not find much use in the developing countries because of its limited availability and cost. *Neisseria gonorrhoea* can also easily develop resistance to spectinomycin when used in a large scale as reported in South Korea in the 1980s, where there was high level resistance when it was used in a clinical study (Boslego *et al.*, 1987). However, kanamycin can be the better alternative as is affordable and can be used effectively in some resource-limited countries. For example, it has been used in Zimbabwe and Mozambique without reported levels of resistance (Boslego *et al.*, 1987).

Gentamicin is cheap and available can be used in many resource limited countries being in an aminoglycoside. This drug was used as a monotherapy for treating gonorrhoea in Malawi for more than a decade and had no gonococcal resistant strains were reported against gentamicin and also no pharmacological effects were reported. Gentamicin has been in use for many years and its pharmacological efficacy in the management of gonococcal infections has not been verified although some countries have used on a larger scale on the management of gonococcal infection. It is therefore an antimicrobial agent that has the potential for use as the best option in cases of detected MDR gonococcal infections (Brown *et al.*, 2010).

Fluoroquinolones, for example ciprofloxacin, was first used in the 1980s in the management of gonococcal infections until 2003 where it was eventually replaced with the extended
spectrum cephalosporins (ESC). The use of fluoroquinolones lasted for a few decades but later, treatment failures were reported in parts of Asia and later spread all over the world (Lewis et al., 2010). Quinolone-resistant *N. gonorrhoeae* (QRNG) developed as a result of the ability of the bacteria to form specific mutations within the chromosomal genome targeting specific genes responsible for bacterial replication like *gyrA* and *parC* genes. The emergence of QRNG was and is still of great concern as since *N. gonorrhoea* was declared a “superbug” due to its ability to resist multiple treatment options and now the only treatment option available in the market which are the ESC (Lewis et al., 2011).

Cephalosporins: This compound was first discovered from the growth of the fungus *Cephalosporium acremonium*, in 1948 and since then it underwent several modifications like the second and third generation cephalosporins. The extended spectrum cephalosporin replaced the fluoroquinolones in the management of gonococcal infection since 2003. They are administered either orally (cefixime) or injectable (ceftriaxone) in the treatment of gonorrhea either of the genital or extra genital sites and have remained to be the first-line treatment and the only remaining option (Bignell et al., 2012).

In the recent years, gonococcal strains exhibiting MDR and extensive multidrug resistance (EMDR) were isolated and characterised in Japan and now spreading to different parts of the world (Denguchi et al., 2003; Ito et al., 2004; Ison et al., 2004). These strains have now demonstrated clinical treatment failures to the only treatment option (cefixime and ceftriaxone) especially increasing MIC levels for these classes of antibiotics have been reported for gonorrhea affecting the pharynx (Denguchi et al., 2003; Ito et al., 2004; Ison et al., 2004). These gonococcal strains are believed to be circulating around the world and can easily be passed from one individual to another or one population to another through unprotected sex and commercial sex tourists travelling across different countries or continents.
2.9 Mechanisms of drug resistance *Neisseria gonorrhoea*

The ‘gonococcus’ is an old discovered bacterium with a long history of antibiotic resistance but it was once very susceptible to drugs (*Johnson et al.*, 1988). This organism once exposed to an environment that is hostile for example; persistent exposure to antibiotics is able to rapidly select for multiple mutations targeting several drugs. The various mechanisms of drug resistance developed by *N. gonorrhoea* against the various classes of antibiotics are amongst the following:

- Reduced access of the drug target sites that is achieved by reducing the permeability of its cell wall thereby preventing the drug from penetrating into the cell.
- Actively exporting or pumping the drug outside the cell using the efflux pumps ensures that the higher drug concentrations are not achieved that would cause harm to the bacterium.
- Rendering the drug inactive or less effective before reaching the target site
- Making deletions or alterations to the target site through chromosomally mediated or the use of plasmids (*Johnson et al.*, 1988).

*Neisseria gonorrhoea* is known to interact with other commensal *Neisseria spps* and acquire resistance genes through either the process of transformation or bacterial conjugation.
Acquisition of these drug resistance genes can either be chromosomally-mediated or plasmid mediated, but plasmid mediated resistance spreads much faster than chromosomally mediated drug resistance (Johnson et al., 1988), which is confined to specific classes of drugs like penicillin and tetracycline. For conjugation to happen between one bacterial and another presence of a conjugate plasmid is required to carry or disseminate the resistant gene to the recipient bacteria. Therefore either the pathogenic and commensal Neisseria bacteria present within the niche should be able donate a plasmid containing a resistant gene to the pathogenic one (recipient).

2.9.1 Resistance to penicillins

Since the beginning of the antibiotic era, N. gonorrhoea has exhibited high level susceptibility to all available antibiotics discovered and tested for use. Penicillin was discovered in the early 1900s and was extensively used to treat gonococcal infections successful using 150,000 units. Two decades later decreased susceptibility and penicillin treatment failures were reported worldwide. Penicillin contains the target compound called β-lactam ring also referred to as penicillin binding proteins (PBPs), which are enzymes contained within bacterial cell surface and are responsible for cell wall metabolism. Penicillin resistance happens when there are alterations in PBP-2 and PBP-1 that brings about reduced affinity to penicillin, and organism uses other alternative mechanisms to manufacture its cell wall avoiding the use of the drug (Sparling et al., 1975) while changes to the mtr and penB locus produces an additive effect to the bacterial resistance. The mtr locus mediates resistance to a wide range of antibiotics, detergents and dyes through an active efflux system (Guymon et al., 1975, Hagman et al., 1995). Mutations that develop within the penB locus affect the structural alignment of the porins that subsequently reduce the permeability of the bacterial cell envelope which inhibits certain antibiotics especially those compounds with hydrophilic properties from penetrating (Johnson et al., 1975, Gill et al., 1998). The presence of porA
‘pseudogene’ in *N. gonorrhoeae* which has not been fully exploited is associated drug resistance (Feavers *et al.*, 1998).

### 2.9.2 Resistance to quinolones

Quinolones were extensively used in the treatment of gonorrhoea for several decades until 2003 where they were replaced with the third generation cephalosporin. Quinolone resistance involves several changes on the chromosomal gene that may include alterations that affect absorption of the drug and mechanisms that pump out the drug by efflux action to prevent the drug to accumulate to attaining toxic levels. The main target site quinolones within the bacterial cell mainly are bacterial enzymes like topoisomerases and the chromosomal DNA gyrase (Achchle Lal Patel *et al.*, 2011) which are responsible for gene replication. High-level quinolone resistance is mainly brought about by alteration of the DNA gyrase gene and other mutations within the chromosome (Enders *et al.*, 2006). Multiple amino acid substitutions have also been reported like the *parC* gene that encode for the production of the topoisomerase gene.

### 2.9.3 Resistance to cephalosporin antibiotics

The third generation cephalosporin was recommended as first line therapy for the management of both genital and extragenital gonococcal infections both for oral treatment (cefixime) and injectable (ceftriaxone) after failure of the floroquinolones. Since their recommendation and adoption in the management of gonococcal infection in 2003, they were effective until most recently where treatment failures started being reported in several parts of the world (Denguchi *et al.*, 2003, Ison *et al.*, 2004, Ito *et al.*, 2004). Resistance to cephalosporin occur when the cell wall through which the drug enters the bacterial cell is altered through mutational changes and hence altering the cell wall permeability for the drug to enter and exert its effects (Johnson *et al.*, 1975, Bygdeman *et al.*, 1984).
Other resistance mechanisms involve cross-resistance between penicillins and early generation cephalosporins such as cefuroxime due to similarities in chemical structures and functionalities between the two families of drugs (Bygdeman et al., 1984, Rice et al., 1986) unlike the third generations of cephalosporin like cefixime they have undergone advanced structural modifications. Gonococcal mutations that develop at the TEM-1 gene and a mosaic cephalosporin producing \textit{N. gonorrhoea} are responsible for the drug resistance in cephalosporins.

In the past five years, gonococci with decreased susceptibility to ceftriaxone and cefixime have been reported in many parts of the world though the resistance mechanism are not yet clear but has linked to the presence of many key genes that are responsible to various classes of antibiotics (Akasaka et al., 2001, Denguchi et al., 2003, Ison et al., 2004, Ito et al., 2004).

\textbf{2.10 Emergence of cephalosporin resistance \textit{Neisseria gonorrhoea} strains}

Non-susceptible gonococci with elevated Extended Spectrum Cephalosporin (ESC) MICs have been reported with increasing incidences in several parts of the world (Lewis et al., 2011, Cohen et al., 1997, Tanaka et al., 2011). These strains that have been isolated and spreading are typically MDR strains demonstrating more resistance to the ESC, quinolones and the previously used drugs (Lewis et al., 2011, Deguchi et al., 2003). What worries most is that these strains have emerged to be demonstrating Extensively Drug Resistant \textit{N. Gonorrhoea} (XDR \textit{N. gonorrhoea}) and have originated from commercial sex workers (CSW) and MSM groups. They have already shown increasing MIC levels to ceftriaxone (Lewis et al., 2010, Ison et al., 2010). The first gonococcal strain (H041) had a ceftriaxone MIC of 2–4 mg/l and was recovered from the pharynx of a Japanese female CSW in Kyoto in 2009 (Ison et al., 2010). After the isolation of this XDR strain, the search for the spread of this strain was undertaken in other parts of Japan (2010–2012) to establish the extent of spreading within the normal population (Ohnishi et al., 2011). Similar MDR gonococcal strains were later isolated in France, Norway, Sweden, and Canada and were thought to be having
similarities with the Japanese strain (Lewis et al., 2011, Deguchi et al., 2003, Unemo et al., 2010).

The surveillance *N. gonorrhoea* isolates that were tested in Japan from the various risk population groups report some encouraging data about the spread of this resistant strain. All the gonococcal strains tested were categorised as susceptible to both cefixime and ceftriaxone (MIC ≤0.25mg/l). This then pointed out that the H041 gonococcal strain isolated in Japan may not have been transmitted further into the community. Later in France, a gonococcal strain (F89) with elevated MIC to ceftriaxone (MIC 2mg/l) was recovered from the urethral discharge of an MSM; while in Spain a resistant strain was recovered in the rectum and urethra with increased MIC level to ceftriaxone (ceftriaxone MIC 1.5 mg/l) (Unemo et al; 2010, Lewis et al., 2010). All these three gonococcal isolates are similar despite geographical areas of origin as they share the same *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST) which is sequence type ST 1407 but are different from the Japanese H041 strain which has sequence type NG-MAST ST 4220 (Lewis et al., 2010, Ison et al., 2010).

There is now a growing concern globally that the future of treatment for gonococcal infections is unknown. The only treatment option now available is already under threat and increasing levels of ESC are growing in number day by day. Most European countries, Asia, USA and other regions of the world have surveillance programmes that monitor gonococcal susceptibility patterns overtime. In Africa only South Africa has for a long time been involved in surveillance of gonococcal resistance susceptibility monitoring. In Kenya and other regions of Sub-Saharan Africa surveillance programmes should be initiated to monitor the spread of these resistance strains. More important is to institute screening strategies to gather local information on high risk populations that harbour resistant gonococcal strains in various anatomical sites that can easily spread through commercial sex tourists.
CHAPTER THREE

MATERIALS AND METHODS

3.1 Study location
This study was conducted at the Casino Special Treatment Centre (STC) clinic which is a referral centre for sexually transmitted diseases, it is an accessible clinic managed by the County Government of Nairobi. The clinic is a public health centre that has a long history of managing STI care from the entire East African region. The clinic has an enrolment of approximately 45,000 out-patient cases and 3,000 STI cases annually. It is located on the central business district of the Nairobi metropolitan city where it serves majority of the commercial sex workers within the city with diverse economic and social activities.

3.2 Study population
This study was approved by the Kenyatta University ethical committee before the recruitment process of participants commenced. Study participants were men aged between 18 to above 60 years.

3.3 Sampling procedure
Participants were recruited consecutively until the required number was attained. All the men who met the inclusion criteria were identified from the STI clinic attendance register. After identification, the potential study participants were taken through the informed consent process whereby the study objectives, risks, benefits and study procedures were explained in English (Appendix A) or Kiswahili (Appendix B) or translated into a language of the participants preference by the clinic nurses. Only those who agreed to participate by signing the consent form were included in the study.

3.4 Inclusion criteria
The study clinician or nurse examined the participants to be enrolled into study who were meeting the following requirements:
i. Adult male patients irrespective of sexual orientation

ii. Adult male of age ≥18 years of age

iii. Should present with signs of urethral discharge.

iv. Should have been involved in active risk sexual work for at least the past 1 month

v. Willing to participate into the study by giving informed consent

### 3.5 Exclusion criteria

Participants that were not meeting the following requirements were excluded from the study:

i. Male patients aged less than 18 years

ii. Participants who could not give consent to participate

iii. Dysuria without genital discharge.

iv. Not having been involved in active risk sexual activity in the past 1 month

v. Showing signs of gonorrhoea of the throat and rectal

vi. A participant with any significant acute/chronic condition or unconscious that in the judgement of the investigator or designee, would preclude provision of informed consent, or otherwise interfere with achieving the study objective.

### 3.6 Sample size determination

Sample size was determined by the method of Fisher et al., 1998

\[ n = \frac{Z^2pq}{d^2} \]

Where \( n \) = desired sample size

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

\( P \) = Estimated prevalence of \( N. \) gonorrhoea (2.2%) in the general population (WHO, 2012)

\( q = 1 - p \)

\( d^2 \) = degree of accuracy desired (0.05)
According to the WHO estimates of 2012, the prevalence of *Neisseria gonorrhoea* from the general population in Africa in men was 2.2% (WHO, 2012).

For this study, a specified level of significance of 95% and an error margin of ±5% were considered acceptable based on similar studies elsewhere.

Substituting:

\[ n = (1.96)^2 (0.22) (0.78) / (0.05)^2 \]

\[ = 263 \]

Therefore, 263 was the minimum number of participants that was required for this cross sectional study.

### 3.7 Questionnaire

The study instrument constituted a structured questionnaire which was both categorical and open ended. It was administered to all eligible men. The questionnaire is attached in Appendix A (English version) or Appendix B (Kiswahili version).

### 3.8 Specimen collection

Each study participant meeting the inclusion criteria above three different types of swabs were collected from the urethra, throat and rectal for those that were gay or men who had sex with men and this was considered as one complete sample. Participants who could not agree to remove all the specimens were disqualified from the study. Participants that presented with urethral discharge a urethral swab were collected only. Male participants both asymptomatic MSM and those that presented with urethral discharges were sampled consecutively until the required number was obtained. Swabs were collected using dacron or rayon swabs because calcium alginate may be toxic to gonococci. To minimise the inhibitory effects of unknown substances in the specimen, the swabs were inoculated directly onto growth medium and placed in a CO₂ jar immediately after sampling and incubated on site at
37°C until transport was available to be transferred to the University of Nairobi department of medical microbiology laboratory for further incubation.

3.9 Collection of urethral samples
Consenting participants were advised on the pain expected during the sample collection procedure of the different genital samples that were collected. Urethral specimens were collected by inserting the dacron wire cotton swab into the penile pore (3 – 4 cm) and allowed to absorb the material for about ten seconds. The first swab was used to inoculate the Modified Thayer Martin agar (Polyvitex/VCAT3-Biomeriex®), (MTM) by rolling the swab across the agar surface in a Z- pattern. The inoculated plate was then placed in a candle jar and incubated immediately at 37°C awaiting transport, while the second swab was used to prepare a smear for Gram staining. Colonies suspected to be Neisseria gonorrhoea were confirmed using biochemical tests (GASP-LAC lab Manual, 2011).

3.10 Collection of rectal samples
Anorectal samples were collected by inserting a clean proctoscopy into the anal canal of the participant and then inserting a cotton swab 2-3 centimetres and rotated to collect the specimen. Specimens with faecal materials were discarded and the procedure repeated until no faecal matter was visible. Only one swab was collected and inoculated onto MTM agar plate for culture as described above (GASP-LAC lab Manual, 2011).

3.11 Collection of throat samples
Throat samples were collected by depressing the tongue with a sterile spatula so as to visualise the pharynx and a sterile cotton swab inserted to swab the pharynx. The swab was then inoculated onto the MTM agar plate as previously described. Inoculated plates were then transported to the gonococcal antimicrobial surveillance program (GASP) laboratory in the University of Nairobi, Department of Medical Microbiology. On arrival, the inoculated plates were incubated at 35°C in a moist environment containing 5% CO₂ for 24 to 48 hours. Colonies that had a morphological appearance suggestive of N. gonorrhoea were further
followed by performing Gram staining, and further confirmed using their positive reaction to catalase, oxidase and sugar fermentation tests (API NH) (Biomeriex®, Inc, France). Positive colonies were sub-cultured to be confirmed using multiplex polymerase chain reaction (PCR), (FTD-ABI®7500). All positive colonies were subcultured on GC agar plate (Polyvitex/CAT3-Biomeriex®) for susceptibility testing (GASP-LAC lab Manual, 2011).

3.12 Cultural characteristics of *Neisseria gonorrhoea* on MTM agar

Colonies of *N. gonorrhoea* on MTM agar base after 18 – 24 hours of incubation in a 5% CO₂ concentration appeared as medium to large, round, smooth, convex, colourless-to-grey and opaque with no decoration of the medium even after prolonged incubation as shown in plate 3.12. Characteristic colonies were selected and Gram stained to further ascertain their staining characteristics and cellular arrangements.

Plate 3.12: Colonial appearance of *N. gonorrhoea* on MTM agar plate (Magnification 300x300)
3.13 Gram stain technique

A smear of suspect colony was prepared on glass slide and after heat fixing stained using crystal violet and then counterstained with Gram’s iodine for 1 minute respectively. After washing to remove excess stain the smear was decolourized with absolute acetone and washed immediately, then counterstained with neutral red for one minute. Slides were then dried and examined with the oil immersion objective lens for the presence of Gram negative both intracellular and extracellular (direct smear) kidney shaped diplococci organism as demonstrated in Plate 3.13 (GASP-LAC lab Manual, 2011).

Plate 3.13: Gram staining microscopic appearance of *Neisseria gonorrhoea* (Magnification 200x150)

3.13 Catalase test

The test was performed by picking an overnight colony of suspect colony and suspending it on a drop of hydrogen peroxide placed on a microscope slide. After mixing the organism with the reagent some effervescence effect was immediately observed for organisms that produced the enzyme catalase which breaks down the hydrogen peroxide into oxygen and water. *N. gonorrhoea* was able to produce catalase enzyme that breaks down hydrogen peroxide into oxygen and water (GASP-LAC lab Manual, 2011).
3.14 Cytochrome oxidase test

A filter paper was moistened with 1-2 drops of the oxidase reagent (*phenylene diamine tetra cetic acid*) and an applicator stick used to pick a suspect colony and rubbed on the moistened filter paper containing the oxidase reagent. Formation of purple colour at the organism inoculation site was a positive test and demonstrated the production of the cytochrome oxidase enzyme produced by *N. gonorrhoea*; while no colour change at the site of inoculation signified in ability to release of the cytochrome enzyme and a negative result (GASP-LAC lab Manual, 2011).

3.15 Rapid API NH test

API NH (BioMeriéux, La Balme-les-Grottes, France) is used to identify *Neisseria species*, *Haemophilus species* and *Moraxella catarrhalis*. It is based on biochemical properties in the metabolism of the bacteria. The test strip has 10 wells which contain different dehydrated substrates, and are able to perform 12 identification tests (enzymatic reactions or sugar fermentation), and in addition detect penicillinase production. According to manufacture; after incubation with bacteria suspension, a metabolic reaction is visualized as change of color in the well. Evaluation of the test result can be performed manually or by using the API LAB software (ATB). The reagents are API NH test-kit, 0.9 % saline, JAMES reagent, ZYM B and mineral oil (BioMeriéux, La Balme-les-Grottes, France).

The test was performed by preparing a heavy suspension of overnight culture of suspected *N. gonorrhoea* colonies corresponding to McFarland standard no. 4 in sterile normal saline. The test strip was tilted and 50 µl added into the wells from penicillin to urea; additionally, mineral oil was added into these wells. The wells containing lipase to β-galactosidase were added with 150µl of the bacterial suspension. The strip was then sealed with the enclosed lid and placed in a humid chamber and incubated at 37°C for 2 hours. The colour changes were compared following manufactures instructions. The result was then transferred to the API lab software for predication and identification of *Neisseria gonorrhoea*. 
Plate 3.15: Rapid API NH for the identification of *N. gonorrhoea* based on enzymatic and sugar fermentation tests (Magnification 250x200).

### 3.16 Multiplex Polymerase chain reaction for confirming *N. gonorrhoea* isolates

All the nine *Neisseria gonorrhoea* isolates recovered from the urethra, throat and rectal anatomical sites of asymptomatic MSM subjects were stored frozen at -80°C in brain heart infusion medium (Difco BD Bioscience) with 20% glycerol until use. Frozen isolates were thawed and aliquots from frozen inoculum were cultured on Gonococci (GC) medium (Difco BD Bioscience) supplemented with 1% vitox supplement medium (Difco BD Bioscience). Culture plates were incubated at 35°C with 5% CO₂ in a humid environment overnight as described earlier. A WHO F strain was also cultured and included a positive known control strain. Colonies of gonococci from the overnight culture growth were selected and emulsified in a 200µl of Buffer ALT for use in bacterial DNA extraction.

*Neisseria gonorrhoea* isolates from the throat and urethra of MSM was confirmed by the multiplex polymerase chain reaction (PCR) by amplifying the specific region of the bacterial genome using specific primers. In real-time polymerase chain reaction (RT-PCR, the amplified product was detected using fluoresce dye (SYBER- Green). The dye is linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluoresce intensifies during the real-time PCR allows the detection of accumulating product without opening the reaction tubes after PCR run. The presence of specific pathogen sequences in the reaction was detected by the increase in fluorescence observed from the relevant dual-labelled probe and was reported as a cycle threshold (Ct) by the Real-time (RT) thermocycler.
DNA extraction

The DNA was isolated using a QIAamp mini kit (QIAGEN, USA) according to manufacturer’s instructions. Briefly, fresh overnight cultures were suspended in 200 µl of Buffer ALT 200µl and centrifuged at 8000 rpm for 10 minutes. The bacterial pellet was then resuspended in 200µl Buffer ALT and 20µl of proteinase K added into each specimen, mixed thoroughly by vortexing and incubated for 10 minutes at 56°C. After incubation, 200µl of Buffer AL (QIAamp, USA) was added into each tube, mixed briefly, followed by 200µl of ethanol 96% and mixed by vortexing. The mixture was pipetted into a QIAamp Mini column spin (QIAGEN) and placed in a 2ml collection tube then centrifuged at 8000 rpm for 1 minute. The flow-through and collection tube were then discarded and the QIAamp Mini column spin placed in a new collection tube, after which 500µl Buffer AW1 (QIAamp, USA) was added to the column and centrifuged at 8000 rpm for 1 minute. The flow-through and collection tubes were discarded and the QIAamp Mini column spin placed in a new collection tube, 500µl Buffer AW2 (QIAamp, USA) was added into the column and centrifuged at 14000 rpm for 1 minutes. The QIAamp Mini column spin was then placed in a clean 1.5 ml microcentrifuge tube and 100µl Buffer AE (QIAamp, USA) was pipetted directly into the spin column membrane and incubated at room temperature for 5 minutes and later centrifuged 8000 rpm for 1 minute to elute the DNA. The assay used murine CMV (mCMV) as a positive extraction control and WHO F strain as a positive known control. DNA was quantified using a Nanodrop 2000 spectrophotometer (Thermo Fisher Sciences Inc.) and 50ng/µl of DNA from each isolate was used for Real Time (RT)-PCR reaction. An internal control (IC) was introduced into each sample in order to control the extraction process of each individual sample and identify possible reaction inhibition.

Multiplex polymerase chain reaction technique

The multiplex RT-PCR technique, was performed using a reaction containing 15µl of 2X SYBER-Green Mastermix, 0.25µl of each of the forward primers (10µM), 0.25µl of the
reverse primer (10µM) and 10µl of DNA template (50ng/µl) using an RT-PCR (AB) Stepone PLUS™ system (Applied Biosystems®, Thermo Fisher Scientific) on a 96-well platform. The final reaction volume was adjusted to 25µl with PCR water. The PCR technique was conducted according to the manufacture’s guidance as follows: initial holding and activation at 95°C for 15 minutes, followed by a secondary holding at 95°C for 3 minutes. PCR was performed for 40 cycles at 94°C for 8 seconds and 60°C for 34 seconds. Both positive and negative controls (not extracted) were included in every run to verify the correctness of the whole PCR process.

Results were interpreted using the rotor-gene (Applied Biosystems®, Thermo Fisher Scientific) software through the presence of fluorescence curve on the green channel. Results were accepted only when the negative control was below the threshold level; the positive controls demonstrated a positive amplification trace or a threshold level of below 33 cycle threshold (Ct). A sample was considered positive for *N. gonorrhoea* if its Ct value as defined in the result grid in the Green channel the fluorescence curve crossed the threshold line (Ct > 33).

### 3.17 Antimicrobial Susceptibility Test (E-test)

Susceptibility of the *N. gonorrhoea* isolates to cefixime (256-0.016µg/ml), ceftriaxone (256-0.002µg/ml), azithromycin (256-0.016µg/ml), ciprofloxacin (256-0.002µg/ml), tetracycline (256-0.016µg/ml), erythromycin (256-0.016µg/ml), spectinomycin (1024-0.064µg/ml), penicillin (32-0.002µg/ml) and gentamicin (256-0.016µg/ml) was tested using E-test (BioMerieux®, Marseille, France) and minimum inhibitory concentrations determined. The E-test was carried out on GC agar plates with added growth factors (GC agar with 1% vitox).

Overnight grown bacterial colonies were directly inoculated in sterilised inoculating fluid to make turbidity comparable to 0.5 McFarland standard. Within 15 minutes, the bacterial inoculum was picked using a cotton swab and then seeded evenly by spreading it on top of
the GC agar plate and allowed to air dry. After the absorption of the inoculum, E-test strips that had been allowed to attain room temperature were applied aseptically. These inoculated plates with E-test strips were placed at 35°C incubator containing 5% CO₂ for 18 – 24 hours and a WHO strain F was used as control organism (GASP-LAC lab Manual, 2011).

Plate 3.17: E-test MIC for *N. gonorrhoea* on GC agar plate (Magnification 300x300)

Minimum inhibitory interpretative criteria to determine whether the organism was sensitive, intermediate and resistant followed recommendations of Clinical and Laboratory Standards Institute (CLSI) document M100-S24: MICs of ≥2 µg/ml (penicillin), ≥2 µg/ml (tetracycline), ≥1 µg/ml (ciprofloxacin), and ≥128 µg/ml (spectinomycin), ≤0.25 µg/ml for cefixime and ceftriaxone was considered susceptible (CLSI, 2014). *Neisseria gonorrhoea* isolates that demonstrated increased susceptibility to cefixime and ceftriaxone were confirmed using polymerase chain reaction (PCR) for the detection of nucleic acid.
3.18 Analysis of data

Data analysis was done using Statistical Package for Social Sciences (SPSS) version 17.0. Demographic and risk sexual behaviour was a descriptive statistics that was done using frequency tables and cross tabulation to check for members of subjects between age categories and risky behaviours. Inferential statistics was done by chi-square test to compare the level of resistance between the various antibiotics at the various anatomical sites and the level of significance was extrapolated from the p-value of chi-square test.
CHAPTER FOUR

RESULTS

4.1 Demographic and risk sexual behaviour of MSM

This study involved both men that presented with urethral discharges and asymptomatic MSM without symptoms of gonorrhoea during the period between September 2015 and August 2016. Demographic data on the recruitment process was obtained from MSM subjects that were both seeking treatment for a possible STI infection and routine screening at the Casino STC clinic within the Nairobi County. A total of 264 men were consecutively recruited (73 were men patients who presented with urethral discharge) and 191 MSM without any signs of gonorrhoea. All participants were between the age group of 18 – 60 years with a mean age of 32 years. The age category of 40 - 49 years reported having been involved with too many sexual partners that they could not remember 4(15.4%) followed by age category 30 - 39 years 1(1.8%) in the period of last 3 months (Table 4.1). There was a significant association between age category and the number of sexual partners that an individual MSM was involved in sexual activity for the last three months (p = 0.009). On frequency of condom use, the age category 30 – 39 reported using condoms always 33(60.0%) followed by the age category of 40 – 49 years 14(53.8%) each time they were involved in sexual activity. The age categories of 18 – 29 and 30 -39 years reported 5(4.8%) and 1(1.8%) respectively to have never used condoms while performing sex. There was no significant association between age category and frequency of condom use (p = 0.098).

For receptive anal sex 43.3% (n = 45) of the participants reported having had received anal sex were within age category 18 – 29 years which was followed by 30 – 39 years 20(36.4%) for the last 3 months. On giving anal sex (insertive anal sex); 64(61.5%) of the subjects reported having given anal sex were within age category 18 – 29 years followed by age categories 30 – 39; 29(52.7%) and 40 – 49 years 10(38.5%) respectively (Table 4.2). There
was a significant association between age category and the number of sexual partners an individual MSM was engaged in insertive anal sex for the last three months.
Table 4.1: Summary of relationship between number of partners and frequency of condom use by MSM in last three months

<table>
<thead>
<tr>
<th>Age Category</th>
<th>No. of sexual partners engaged in sexual intercourse</th>
<th>Frequency of condom use</th>
<th>Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (n) (%)</td>
<td>2 (n) (%)</td>
<td>3 (n) (%)</td>
<td>4 (n) (%)</td>
</tr>
<tr>
<td>18 - 29</td>
<td>95 (92.2%)</td>
<td>8 (7.8%)</td>
<td>1 (1.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>30 - 39</td>
<td>50 (90.9%)</td>
<td>4 (7.3%)</td>
<td>0 (0.0%)</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>40 - 49</td>
<td>19 (73.1%)</td>
<td>3 (11.5%)</td>
<td>0 (0.0%)</td>
<td>4 (15.4%)</td>
</tr>
<tr>
<td>≥50</td>
<td>6 (100.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

Key
1: 10 and below; 2: 11 – 20; 3: 21 – 30; 4: too many to remember
Table 4.2: Summary of relationship between age category and number of sexual partners that MSM were involved in either receptive or insertive anal sex in last three months

<table>
<thead>
<tr>
<th>Age Category</th>
<th>No. of sexual partners that received anal sex in the last 3 months</th>
<th>Chi-square</th>
<th>P-value</th>
<th>No. of sexual partners that had insertive anal sex in the last 3 months</th>
<th>Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO (n) (%)</td>
<td>YES (n) (%)</td>
<td>Don’t Know (n) (%)</td>
<td>Not applicable (n) (%)</td>
<td></td>
<td>Missing data</td>
</tr>
<tr>
<td>18 - 29</td>
<td>53 (51.0%)</td>
<td>45 (43.3%)</td>
<td>1 (1.0%)</td>
<td>2 (1.9%)</td>
<td>13.064</td>
<td>0.364</td>
</tr>
<tr>
<td>30 - 39</td>
<td>33 (60.0%)</td>
<td>20 (36.4%)</td>
<td>0 (0.0%)</td>
<td>1 (1.8%)</td>
<td></td>
<td>22 (40.0%)</td>
</tr>
<tr>
<td>40 - 49</td>
<td>18 (69.2%)</td>
<td>6 (23.1%)</td>
<td>0 (0.0%)</td>
<td>2 (7.7%)</td>
<td></td>
<td>14 (53.8%)</td>
</tr>
<tr>
<td>≥50</td>
<td>3 (50.0%)</td>
<td>2 (33.3%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td></td>
<td>2 (33.3%)</td>
</tr>
</tbody>
</table>
4.2 Prevalence of *Neisseria gonorrhoea* from both men presenting urethral discharge and Asymptomatic MSM

In total 264 swabs were collected from both men that presented with urethral discharges and asymptomatic MSM during the period September 2015 to August 2016 as illustrated in Table 4.2. A total of 191 swabs were collected from high risk asymptomatic MSM; each participant was collected three swabs from the rectum, urethra and throat which was assigned the same study number. Out of the 264 participants, 73 swabs were collected from men that presented with urethral discharges. MSM subjects that we could not collect all the 3 swabs from the different sites, these were disregarded for in the final analysis. For each participant involved in this study, all the 3 different swabs were collected and cultured from all the 191 asymptomatic study participants. The overall prevalence of *N. gonorrhoea* during this period was 23.4% where 3.4% (9/264) were recovered from asymptomatic MSM and 53/264 (20.1%) from men that presented with urethral discharge. Out of the total 9 *N. gonorrhoea* strains that were isolated from asymptomatic MSM participants 5/9 strains were from the urethra, 4/9 from the throat and there was none isolated was the rectal anatomical site. All the 9 gonococci strains that was isolated from the throat and urethra of asymptomatic MSM their identity was confirmed by amplifying their *PorA* gene and detected using real-time PCR, where all were positive.

Table 4.3: Prevalence of *N. gonorrhoea* from both men presenting with urethral discharges and asymptomatic MSM

<table>
<thead>
<tr>
<th>Study participants</th>
<th>Anatomical site</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic MSM</td>
<td>Urethra</td>
<td>Throat</td>
<td>Rectal</td>
<td>Total</td>
</tr>
<tr>
<td>n = 5</td>
<td>n = 5</td>
<td>n = 4</td>
<td>n = 0</td>
<td>n = 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n = 191</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(3.4%)</td>
</tr>
<tr>
<td>Men presenting with urethral discharges</td>
<td>Urethra</td>
<td>Throat</td>
<td>Rectal</td>
<td>Total</td>
</tr>
<tr>
<td>n = 73</td>
<td>n = 73</td>
<td>-</td>
<td>-</td>
<td>n = 53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(20.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n = 264</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(23.5%)</td>
</tr>
</tbody>
</table>
4.3 Antibiotic susceptibility profile of *N. gonorrhoea* isolates recovered from symptomatic male patients and those with urethral discharge

The antimicrobial susceptibility profile of 53 strains of *Neisseria gonorrhoea* to penicillin, tetracycline, cefixime, azithromycin, ceftriaxone, ciprofloxacin, gentamicin, erythromycin and spectinomycin was assessed and demonstrated in Table 4.4 - 4.5.

All the gonococcal strains tested were susceptible to ceftriaxone, cefixime (MIC ≤ 0.25µg/ml) and spectinomycin (MIC ≤32 µg/ml). Intermediate susceptibility was detected in gentamicin 50/53 (94.3%) (MIC ≤ 0.38 µg/ml) and azithromycin 41/53 (77.3%) (MIC ≤ 0.023 µg/ml) and erythromycin 27/53 (50.9%) (MIC ≤0.047µg/ml). Resistance to penicillin was observed in 26/53 (49.1%) (MIC ≤0.032 µg/ml), tetracycline 51/53 (96.2%) (MIC ≤0.032 µg/ml) and ciprofloxacin 26/53 (49.1%) (MIC ≤0.003µg/ml). The WHO strain F was used as an internal quality control to interpret the zone of inhibition as either susceptible, intermediate or resistant.

Table 4.4: Antibiotic ranges and MIC of antibiotic used to manage *N. gonorrhoea* strains

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (µg/ml)</th>
<th>WHO F Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&lt;0.003 – 2</td>
<td>0.938568</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&lt;0.016 – 0.023</td>
<td>0.016</td>
</tr>
<tr>
<td>Cefixime</td>
<td>&lt;0.016 – 0.016</td>
<td>0.015622</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>0.038 – 16</td>
<td>5.952973</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>0.023 – 0.32</td>
<td>0.244703</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.032 - &gt;32</td>
<td>9.553378</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.047 – 0.75</td>
<td>0.449757</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.032 - &gt;32</td>
<td>9.040541</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.38 – 8</td>
<td>1.858649</td>
</tr>
</tbody>
</table>
Table 4.5: Antibiotic susceptibility profile of *N. gonorrhoea* isolates recovered from symptomatic men patients with urethral discharges from Casino STC clinic

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive n</th>
<th>%</th>
<th>Intermediate n</th>
<th>%</th>
<th>Resistant n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>8</td>
<td>15.9</td>
<td>20</td>
<td>37.7</td>
<td>26</td>
<td>49.05</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>53</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cefixime</td>
<td>53</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>53</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>41</td>
<td>77.3</td>
<td>9</td>
<td>16.9</td>
<td>3</td>
<td>5.6</td>
</tr>
<tr>
<td>Penicillin</td>
<td>7</td>
<td>13.2</td>
<td>21</td>
<td>39.6</td>
<td>26</td>
<td>49.1</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>23</td>
<td>43.4</td>
<td>27</td>
<td>50.9</td>
<td>3</td>
<td>5.6</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>5.6</td>
<td>51</td>
<td>96.2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>50</td>
<td>94.3</td>
<td>3</td>
<td>5.6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

4.4 Antibiotic susceptibility profile of *N. gonorrhoea* strains isolated from throat and urethra of asymptomatic MSM

The antimicrobial susceptibility profile of *N. gonorrhoea* strains from throat and urethral anatomical sites is illustrated in Figures 4.2 – 4.3. All strains recovered from the pharynx (n=4) were highly susceptible (100%) to cefixime, ceftriaxone, gentamicin and spectinomycin (Figure 4.2). Intermediate susceptibility was observed in tetracycline (100%), ciprofloxacin (n=1); (25%), azithromycin (n=2); (50%) and erythromycin (n=3); 75%), while resistance levels were demonstrated in azithromycin (n=2); (50%), ciprofloxacin (n=1); (25%) and erythromycin (n=1); (25%).

The antimicrobial susceptibility profile of urethral *N. gonorrhoea* strains is as illustrated in Figure 4.3. Out of the 5 isolates, maximum sensitivity was observed in gentamicin and spectinomycin (100%); while ceftriaxone and cefixime sensivity levels were at 80% and 60% respectively. Two strains of *N. gonorrhoeae* demonstrated increased MIC level to ceftriaxone only (MIC ≥0.038µg/ml) and the other to both ceftriaxone and cefixime (MIC ≥0.016µg/ml);
beyond the recommended working definition for cefixime (MIC ≥0.25µg/ml) and ceftriaxone (MIC ≥0.125µg/ml) respectively. Intermediate susceptibility was observed in erythromycin (n = 4); (80%), tetracycline (n=3); (60%), penicillin (n=2); (40%) and ciprofloxacin (n=1); (20%). Resistance was high in ciprofloxacin (MIC ≥ 3.0µg/ml); (n=3); (60%), penicillin (MIC ≥ 6.0µg/ml); (n=1);(20%) and azithromycin (MIC ≥ 4.0µg/ml); (n=2); (40%).

![Antibiotic Susceptibility Profile](image)

Figure 4.1: Antibiotic susceptibility profile of *N. gonorrhoea* isolated from throat anatomical site of asymptomatic MSM

S – Sensitive; I – Intermediate; R – Resistant

Figure 4.2: Antimicrobial susceptibility profile of *N. gonorrhoea* of urethral isolates from asymptomatic MSM

S – Sensitive; I – Intermediate; R – Resistant


4.5 Comparison of resistance levels drugs used to treat *N. gonorrhoea* in various anatomical sites

Table 4.4 – 4.5 Illustrate the comparison in levels of drug susceptibility levels used to treat *N. gonorrhoea* infecting from the various anatomical sites. There were no significant variations in the resistance levels between the various categories of antibiotics used to treat of *N. gonorrhoeae* from the various anatomical sites (p=0.099).
Table 4.6: Comparison between antibiotic resistance levels for \textit{N. gonorrhoea} isolated at the throat anatomical site

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>THROAT</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive (n) (%)</td>
<td>Resistant/Intermediate (n) (%)</td>
<td>Chi-square</td>
</tr>
<tr>
<td>CIP</td>
<td>3(75.0%)</td>
<td>1(25.0%)</td>
<td>2.723</td>
</tr>
<tr>
<td>CEFT</td>
<td>4(100.0%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>CEFX</td>
<td>4(100.0%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>SPEC</td>
<td>4(100.0%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>AZITH</td>
<td>1(25.0%)</td>
<td>3(75.0%)</td>
<td></td>
</tr>
<tr>
<td>PEN</td>
<td>2(50.0%)</td>
<td>2(50.0%)</td>
<td></td>
</tr>
<tr>
<td>ERY</td>
<td>0(0.0%)</td>
<td>4(100.0%)</td>
<td></td>
</tr>
<tr>
<td>TET</td>
<td>0(0.0%)</td>
<td>4(100.0%)</td>
<td></td>
</tr>
<tr>
<td>GENT</td>
<td>4(100.0%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
</tbody>
</table>

Key:
CIP: Ciprofloxacin; CEFT: Ceftriaxone; CEFX: Cefixime; SPEC: Spectinomycin; AZITH: Azithromycin; PEN: Penicillin; ERY: Erythromycin; TET: Tetracycline; GENT: Gentamycin

4.5 Comparison of drug resistance level for \textit{N. gonorrhoea} strains isolated at the urethra

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>URETHRA</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
<td>Resistant/Intermediate</td>
<td>Chi-square</td>
</tr>
<tr>
<td>CIP</td>
<td>1(20.0%)</td>
<td>4(80.0%)</td>
<td>2.723</td>
</tr>
<tr>
<td>CEFT</td>
<td>4(80.0%)</td>
<td>1(20.0%)</td>
<td></td>
</tr>
<tr>
<td>CEFX</td>
<td>3(60.0%)</td>
<td>2(40.0%)</td>
<td></td>
</tr>
<tr>
<td>SPEC</td>
<td>5(100%)</td>
<td>0(0.0%)</td>
<td></td>
</tr>
<tr>
<td>AZITH</td>
<td>2(40.0%)</td>
<td>3(60.0%)</td>
<td></td>
</tr>
<tr>
<td>PEN</td>
<td>2(40.0%)</td>
<td>3(60.0%)</td>
<td></td>
</tr>
<tr>
<td>ERY</td>
<td>1(20.0%)</td>
<td>4(80.0%)</td>
<td></td>
</tr>
<tr>
<td>TET</td>
<td>1(20.0%)</td>
<td>4(80.0%)</td>
<td></td>
</tr>
<tr>
<td>GENT</td>
<td>4(80.0%)</td>
<td>1(20.0%)</td>
<td></td>
</tr>
</tbody>
</table>

Key:
CIP: Ciprofloxacin; CEFT: Ceftriaxone; CEFX: Cefixime; SPEC: Spectinomycin; AZITH: Azithromycin; PEN: Penicillin; ERY: Erythromycin; TET: Tetracycline; GENT: Gentamycin
 CHAPTER FIVE

DISCUSSION, CONCLUSIONS, RECOMMENDATIONS

5.1 Discussion

Demographic data on high risk sexual practice on MSM in Kenya and Africa in general is still scanty because of the stigma and MSM has not yet been accepted within the African culture. In this study, demographic data showed that young teenage age were enrolled in high risk sexually behaviour as early as 19 years while the highest age limit was 59 years (Table 4.1). The average age category was 34 years and the age group that had the highest number of sexual partners (too many to remember) was 40 – 49 years (15.4%); while the age category of >59 years had the least number of sexual partners that they engaged in sex in the last 3 months. This can be explained by the fact that at teenage age majority of people still have depend on parents and guardians and hence have the fear of being identified as MSM and but as age advances they become independent and the fear of identity slowly disappears with time. Most participants reported consistent use of condoms when engaging in sex irrespective of the age and the nature of sex being undertaken. This can be attributed to the massive awareness of acquisition and transmission of STI and HIV.

On anal sexual behaviour (receptive or insertive) the age category of 18 – 29 years was both mostly involved (43.3% and 61.5% respectively) in either giving and receiving anal sex and there was a significant association between age category and giving anal sex (p = 0.009). Majority of the MSM therefore preferred giving anal sex than receiving because mostly they would exchange sex for monetary gain. The young MSM significantly sell anal sex in exchange for money. This observation is in agreement with a recent study in the Netherlands that reported young MSM visiting STI clinics were at higher risk for both a single STI infection and STI co-infections than older MSM (Dukers et al., 2007). This study has also reported that the age category 18 - 29 years was commonly involved in giving (insertive) anal
sex (61.5%) and also had the highest number of subjects that had anal sex without use of condom (63.5%). Both receptive and insertive anal (unprotected anal sex) is often considered to be the primary direct risk factor for STI and HIV acquisition among MSM due to its higher per-act transmission (Dukers et al., 2007). These findings agree with other studies in many parts of the world (Sunita et al., 2010, Unemo et al., 2013).

Factors that contribute to high risk sexual behaviour by MSM are mainly due to peer group influence in school or college level as early as middle school level, social medium interactions, drug use and the desire to live a higher standard of life with no stable source of income or poverty. In a CDC young men’s survey involving 3,500 young MSM reported that approximately 90% of alcohol users in the past 6 months, 66% were reported to have used illicit drugs and 28% were polydrug users (Mutchler et al., 2011). Sunita et al., 2010; reported the use of internet to be the most common method used by MSM for meeting sex partners and which can consequently can be used for future health promotion interventions in MSM especially in rehabilitation centres. In order to reduce the transmission cycle in MSM, measures should be undertaken to ensure that the transmission cycle of STIs is broken through practising safe sex, reducing drug use, reducing the number of sexual partners and other preventive measures that are relevant to correcting sexual behaviours of both male and females engaging risk behaviours in the society (Sanders et al., 2013, Salve et al., 2015).

The overall gonococcal prevalence from both men presenting with urethral discharges and asymptomatic MSM in this region was 23.5% (Table 2). In MSM, 3 sites are commonly infected: pharynx, rectum and urethra. The prevalence of N. gonorrhoea from asymptomatic MSM was 3.4% with 5/9 and 4/9 strains isolated from urethra and throat respectively. The prevalence of N. gonorrhoea from men presenting with urethral discharges was 20.1% which is in agreement with many other studies conducted in many other parts of the world (Sanders et al., 2013, CDC, 2012, Salve et al., 2015). Most STI clinics conducting surveillance studies have previously reported the overall prevalence (for all anatomic sites – pharynx, rectal and
urethral) of \textit{N. gonorrhoea} among MSM, regardless of symptoms, range from 2-12\% (Cook \textit{et al.}, 2002 and Kent \textit{et al.}, 2003). These reports are consistent with our current findings where the overall prevalence of \textit{N. gonorrhoea} in MSM was 3.4\% as recovered in all extra-genital sites where culture swabs were performed. In the contrary; a study conducted by Krishneel \textit{et al.}, 2015 reported a higher prevalence of rectal gonorrhoea (26.4\%) and pharyngeal (9.4\%) by culture from asymptomatic MSM and also in a Seattle clinic study reported the proportion of MSM with pharyngeal, urethral and rectal gonorrhoea was 6.5\%, 9.5\% and 5.5\% respectively (Barbee \textit{et al.}, 2014). A study done in Senegal in 2005, the prevalence of gonorrhoea was 5.4\% among MSM (Caceres \textit{et al.}, 2005) which is in agreement with our study findings. In a study conducted in five Central Americus countries, 2.4\% of MSM had gonorrhoea (Soto \textit{et al.}, 2007), while in Brazil a gonococcal prevalence rate of 18.4\% in MSM was reported (Cook \textit{et al.}, 2004) which was higher than what is reported in this study. This study lacked generalization due to study duration (one year) and also had a small sample size. Culture of pharyngeal and rectal samples has low sensitivity but high specificity and hence the low rate of prevalence is illustrated in this study. The success of this study lies on the fact that culture of extra-genital swabs was used to detect \textit{N. gonorrhoea} and 9 strains were recovered from the asymptomatic MSM which were further confirmed using multiplex PCR technique.

The existence of \textit{Neisseria gonorrhoea} at the pharynx and rectal anatomical sites in MSM without exhibiting obvious signs of gonorrhoea can be attributed to frequent biological activities like cough and defecation that constantly reduce numbers at these ecological niches. Rogstad and co-workers reported a lower partner rate and partner notification of a potential gonococcal infection as compared with heterosexuals significantly contributes to gonococci being carried for longer period of time silently by high risk population groups as a risk factor for more acquisition and transmission (Rogstad \textit{et al.}, 1999). This therefore requires that MSM and other risk population groups be regularly be screened for harbouring STI on the
extra-genital sites and be treated promptly. Other factors that may lead to longevity of the infection is lack of reporting of the individual MSM to a healthcare clinic to be treated due to stigma.

The gonococci colonizing the extra-genital sites especially the pharynx has a higher propensity of developing drug resistance through acquiring resistant genes through plasmids and by transformation (Barbee et al., 2014). Neisseria gonorrhoea colonizing the rectum may develop resistance through exposer to faecal lipids that subject the bacterium to selective pressure leading to the development of drug resistance due to mutations at specific target regions like the mtr locus that later confer further resistance to some hydrophobic compounds (Shafer et al, 1995). In Kenya, MSM as a sexual behaviour has not fully been accepted and for the sake of this study there was a lot of difficulty identifying and consenting participants to be enrolled and eventually collect extra-genital specimens for culture. Our findings should form the basis of conducting more structured and laboratory standardized studies on MSM and gonococcal susceptibility profile isolated from extra-genital sites since no similar studies have been conducted previously.

The antibiotic susceptibility profile of N. gonorrhoea isolates recovered from symptomatic men that presented with urethral discharge demonstrated increased levels of resistance to the drugs previously recommended for the management of gonococcal infections like penicillin (49.1%), tetracycline (96.2%) and ciprofloxacin (49.1%). The occurrence of resistance to these drugs exceeded the recommended allowable limit of 5% beyond which the specific drug should be withdrawn for use (Workowski et al., 2008). These high resistance rates observed in this study have been reported in several studies conducted in many parts of the world (Bates et al., 2009, Filipius et al., 2009).

Ciprofloxacin was previously used as the first line therapy for the management of gonorrhoea, high resistance rates globally resulted to revision in treatment regime to now the third generation cephalosporins (ceftriaxone and cefixime) and azithromycin. These findings
are consistent with previous studies conducted in Kenya that reported ciprofloxacin resistance levels of 53.2% (Philippe et al., 2012) and other parts of the world. Although recommendations to change the treatment regime have so far been published monitoring these resistance gonococcal strains remains imperative.

*Neisseria gonorrhoea* has several mechanisms of resisting the action of these important drugs for example; ciprofloxacin resistance mechanisms have been mainly due to simultaneous chromosomal mutations targeting chromosomal genes that are responsible for bacterial replication. Penicillin resistance is due to the alteration or mutation to key gene locus like *penA, penB, ponA, pem* and *mtr* and the production of penicillin binding proteins (PBP) that alter the structure of the drug (Johnson et al., 1975, Gill et al., 1998). All the *N. gonorrhoea* strains from this population were highly susceptible to cefixime, ceftriaxone, spectinomycin and gentamicin, that means the ESC are still effective in the treatment of local gonococcal strains in Kenya. All *N. gonorrhoea* isolates from men presenting with urethral discharges were susceptible to cefixime and ceftriaxone. This finding therefore makes these classes of antibiotics still effective in the management of local gonococcal infection in Kenya.

This study also reports the emergence of some gonococcal strains that are resistant to azithromycin which is a drug currently used as an oral single 2g dose first line therapy for the management of gonorrhoea. Azithromycin resistance was observed in 3/53 (5.6%) resistance and 9/53 (16.9%) intermediate susceptibility for men that presented with urethral discharge, while from the pharynx and urethral anatomical sites of asymptomatic MSM; 1(25%) strain was resistant and 1(25%) intermediate susceptibility. This developing resistance has already been reported in several other regions like England and Brazil (22.9%); (Chisholm et al., 2009). In a study conducted in Nanjing-China, high level azithromycin resistance was associated with the presence of the A214G mutation that was present in all the four alleles of the 23SrRNA (Chuan Wan et al., 2018). Azithromycin has also been widely used in the syndromic treatment of chlamydia infections in STI clinics (Chisholm et al., 2009) could
have contributed to the emerging high level resistance being shown now in some *N. gonorrhoea* strains as a result of cross resistance. A study conducted by Venessa and workers associated azithromycin resistant gonococci strains as result of co-evolvement with reduced susceptibility to cephalosporin group of antibiotics where azithromycin resistant gonococci strains were responsible with specific mutations at the 23rRNA gene (Venessa *et al.*, 2014).

More interesting were the two *N. gonorrhoea* recovered from the urethra that demonstrated high resistance to cefixime and ceftriaxone (MIC ≥2.0µg/l). The first participant had a history of previous drug use and had been treated with 2g of tinindazole without success. Then two weeks later a combination of both clindamycin and clotrimazole pessaries 200mg for two weeks was used for one week. The second participant had been treated with doxycycline and clotrimaxone pessaries 100mg, after two days 2g tinindaxole and doxycycline 100mg for one week was administered. Both participants were then managed using a combination of ceftriaxone 250mg and cefixime 400mg start dose. After two weeks a repeat culture to confirm test of cure was done and there was no growth in both cases.

Cephalosporins MIC creep have been reported in a study conducted in Kenya young uncircumcised men in Kisumu (Supriya *et al.*, 2009). It would have been important to isolate the genes responsible for resistance to the different antibiotics and also understand if this is the same strain that exhibits XDR that was first isolated in Japan (H041) or the F89 strain isolated in France or if a new gonococcal strain discovered in this region (Unemo *et al.*, 2011). *Neisseria gonorrhoea* strains exhibiting XDR have been isolated in CSW with verified treatment failures reported in Sweden, France and Norway (Unemo *et al.*, 2011, Ohnishi *et al.*, 2011, Camara *et al.*, 2012) and it would be interesting to examine if these strains have the same genetic composition.

Spectinomycin remains an effective option for the treatment of gonorrhoea as demonstrated in this current study where all gonococcal strains isolated highly susceptible to all gonococcal
strains tested. Verified resistance to spectinomycin is exceedingly rare worldwide for example; in the US the Gonococcal Isolate Surveillance Project (GISP) and European gonococcal antimicrobial surveillance program did not identify a spectinomycin resistant strain of gonococci (Workowski et al., 2008) during a five years study survey. This antibacterial agent has the disadvantage of limited anatomical treatment coverage as it can only be used for pharyngeal gonococcal infections only but not urethral and rectal gonorrhoea due to limited distribution in the host (Workowski et al., 2008). Spectinomycin has also the disadvantage of high cost and route of administration is intramuscularly, plus it is known to easily become resisted by gonococci when used frequently and routinely (Workowski et al., 2008). Spectinomycin although demonstrated maximum susceptibility to the local gonococcal strains its efficacy has not been determined in Kenya.

No resistance to gentamicin was reported in this study. In this study only 3 (5.6%) N. gonorrhoea strains were isolated from men that presented with urethral discharges showed intermediate susceptibility to gentamicin which contradicts to a study conducted in Europe where majority (83%) of the isolates had intermediate susceptibility to gentamicin (Chisholm et al., 2011). The findings of the European study were similar to a study carryout in Malawi (Chisholm et al., 2011) where most of the gonococcal isolates also had intermediate susceptibility to this drug. Gentamicin as demonstrated in many studies on drug susceptibility profile suggests that this drug may be the best alternative to be use for management of local gonococcal infections in many developing countries to its less expensive cost and availability. Nevertheless; the correlation between in vitro susceptibility and clinical outcomes has not yet been established for gentamicin. Only one study determined the clinical efficacy of gentamicin in the treatment of N. gonorrhoeae two years after its use in Malawi (Chisholm et al., 2011). Preliminary findings from a recent American study examining the effectiveness of combination of gentamicin with azithromycin suggested high efficacy of this regimen (Kirkcaldy et al., 2014). However, this study did not determine the efficacy of the
individual antibiotics, or efficacy of gentamicin for extra-genital infections. The latest CDC STD treatment guidelines recommend dual treatment with single doses of gentamicin plus azithromycin in suspected treatment failures cases with the recommended regimen (ceftriaxone plus azithromycin). However, all these potential treatment regimens require comprehensive in vitro and in vivo evaluations and recommendations to be made based on clinical trials, not only in vitro results.

Our results did not find a significant level of antibiotic resistance levels between the various antibiotics tested for the various anatomical sites (urethra, throat and rectal) of asymptomatic MSM. This was attributed by the low isolation rate of *N. gonorrhoeae* especially urethra, throat and rectum of asymptomatic MSM. The WHO recommends the use of oral cefixime and a macrolide for the treatment of rectal and urethral gonorrhoeae infections and injectable ceftriaxole for pharyngeal gonococcal infections. It would have been interesting to establish this association and determine if there is any possibility of cross resistance between different classes of antibiotics used to manage gonorrhoea or if the same classes of antibiotics could be used to treat gonorrhoea irrespective of site of infection.

This study did not detect high level resistance to the ESC especially in the gonococcal isolates recovered from men that presented with urethral discharge. There is a need to identify and evaluate possible future treatment options in view of concerns about emergence of resistance to ESCs. The ECS, gentamicin and spectinomycin were very effective and are relatively safe antibiotics which have shown high in vivo efficacy in Malawi and other parts of the world where that have extensively been used to treat gonorrhoea (Chisholm *et al.,* 2011, Unemo *et al.,* 2012 ). ESC resistant gonococci are unlikely to exhibit cross-resistance to gentamicin as cephalosporins act on bacterial cell wall synthesis whereas gentamicin disrupts protein synthesis. It was suggested that just a single intramuscular injection of gentamicin antibiotic could pave way for outpatient management and reduces the risk of
vestibular and renal toxicity seen with high drug concentrations for longer period for other infections (Kirkcaldy et al., 2014).

The local gonococcal isolates exhibited no resistance to gentamicin. This drug has been reported to have a high in vitro susceptibility against gonococcal MDR strains and also to strains that exhibit decreased susceptibility to currently recommended ESCs. This suggests that gentamicin has the potential for future use as a treatment of MDR gonococcal infections. However, this study lacks generalization due to its limited sample size and duration of study more studies need to be conducted to confirm antibiotic resistance trends at both local and national level.

5.2 Conclusions

i. *Neisseria gonorrhoea* was prevalent in symptomatic male patients presenting with urethral discharges and men who have sex with men are potential carriers of gonococcal strains in this region.

ii. The antimicrobial agents (penicillin, tetracycline and ciprofloxacin) previously used for the management of gonococcal infections showed high resistance levels and cannot be used for the management of gonorrhoea in this region.

iii. There was no significant level of resistance between the various antibiotics tested at the various anatomical sites of MSM.

iv. The current treatment regime that involves injectable ceftriaxone or oral cefixime with azithromycin recommended by CDC since 2003 is highly effective in the treatment of local gonococcal infections in Kenya although some resistance to azithromycin has started to emerge.
5.3 Recommendations

i. There should be continuous surveillance programs of *N. gonorrhoea* to determine susceptibility patterns targeting risk taking sexual groups in Kenya. This should help in gathering more data to be used in determining if there are differences in antimicrobial susceptibility profile of *N. gonorrhoea* strains recovered from urethral discharge samples from the other anatomical sites (anal and pharyngeal) that are difficult to sample. Generally, culture should form part of the diagnostic pathway of gonorrhoea followed by antibiotic susceptibility testing with MIC determination. This will provide valuable continued monitoring of antibiotic resistance development in strains of *N. gonorrhoea* circulating in Kenya.

ii. Further molecular analysis of all *N. gonorrhoea* isolates that exhibit resistance or increased MIC to the third generation cephalosporin should be performed to ascertain if the 2 gonococcal strains have similarity to those that were isolated in other parts of the world.

iii. There should be a stronger focus on screening of both anorectal and pharyngeal gonorrhoea from the MSM population since these sites are the potential reservoirs for resistant gonococcal strains, timely treatment so as to reduce new cases of acquired gonococcal from spreading into the general community.
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APPENDICES

Appendix A: Consent Form (English Version)

My name is Pole Lewa and I am an MSc student from Kenyatta University. I am conducting a study on “Antibiotic susceptibility profile of Neisseria gonorrhoea isolated from men attending a sexually transmitted infection clinic in Nairobi, County”. The information will be used by the Ministry of Health to understand changing patterns of drug resistance currently used to treat gonorrhoea and hence change local treatment guidelines on management of gonococcal infections in Kenya.

Procedure to be followed

Participation in this study will require that I ask questions and examine you to screen for symptoms of gonorrhoea of the urethra, anal and pharynx. Some specimens will be taken from you for further tests. I will record the information from you in a questionnaire.

You have the right to refuse participation in this study. You will get the same care and medical treatment whether you agree to join the study or not and your decision will not change the care you will receive from the clinic today or that you will get from any other clinic at any other time.

Please remember that participation in the study is voluntary. You may ask questions related to the study at any time.

You may refuse to respond to any questions and you may stop an interview at any time. You may also stop being in the study at any time without any consequences to the services you receive from this clinic or any other organization now or in future.

Discomfort and risks

If you take part in this study, the risk to you is very slight. Most of the study questions are general in nature, but there are some that are personal and may make you feel uncomfortable.

You are free to refuse to answer any questions. However, in order to obtain good results from
the study, it is important that you attempt to all questions if possible. If you give throat, rectal and urethral swab, the risks to you are small. You may get some slight bruising, cough, irritation or slight bleeding which may stop without being treated, please contact our study staff or health worker. All research team members are trained to protect your privacy and all information you share will be kept secret. If you experience prolonged signs of bleeding or coughing after leaving the clinic contact the study staff immediately using the telephone numbers provided to you or report to the nearest heath facility for immediate treatment.

**Benefits**

During the discussion of your results, you will receive HIV risk reduction counselling to understand that acquiring an STI would likely predispose you from acquiring and transmitting HIV infection. If you participate in this study you will help us to learn how to provide effective treatment to gonococcal infection and reduce the transmission of gonococcal strains. You will be investigated and treated for gonorrhoea for free. There is no cost to you for being in the study. You will receive Ksh.500 for cost of travel to the study site.

**Care and Protection**

We do not expect you to be harmed from taking part in this study. If you are harmed because of taking part in this study, you will be given treatment (including emergency treatment) at no cost to you. If the study clinic cannot give the treatment you need, they will refer you to a clinic where treatment will be given at no charge to you. GASP, the sponsor of this study, will cover the costs of reasonable medical expenses for injuries that result from taking part in this study. No other form of compensation is available from GASP.

**Confidentiality**
Everything we talk about including your results will be kept secret to the extent allowed by law. All study procedures will be conducted in private, to the extent possible. To protect your privacy, we will use a code number to identify you and all information about you, including your samples that we will collect. All volunteer information will be stored in locked file cabinets with restricted access. Data collection and administrative forms, laboratory specimens, and other study related reports will be identified only by a coded number in order to maintain volunteer confidentiality. Your name or any other facts that might point to you will not appear when we present this study or publish its results. All records that contain names or other personal identifiers, such as locator information and informed consent documents, will be stored separately from study records, which are identified only by a code number. All databases will be secured with password protected access systems. Forms, lists, logbooks, appointment books, and any other documentation that link volunteer ID numbers to other identifying information will be stored in a separate, locked cabinet with access limited to certain staff members.

Community consideration

KAVI-ICR has a team of community workers that work with various community stakeholders that have a responsibility of reviewing study protocol, mobilise and advocate for study participant recruitment processes. The various community stakeholders include:

   a) Community Advisory Board (CAB) members which is diverse in its representation and deals with community advocacy.

   b) Peer leaders that deal with community mobilization for study participants.

   c) Community networks like Community Based Organizations, Community Faith Based Organization and County Health Facilities that champion for right of study participant.

Contact information
If you have any questions you may contact Mr. Pole Lewa on 0722811884 and/or Dr. Anthony Kebira on 0735757560 or Kenyatta University Ethical Review Committee Secretariat on chairman.kuerc@ku.ac.ke, secretary@ku.ac.ke, ercku2008@gmail.com.

**Participant statement**

The above information regarding my participation in the study is clear to me. I have been given a chance to ask questions and my questions have been answered to my satisfaction. My participation in this study is entirely voluntary. I understand that my records will be kept private and that I can leave the study at any time. I understand that I will still get the same care and medical treatment whether I decide to leave the study or not and my decision will not change the care I will receive from the clinic today or I will get from any other time.

Name of participant ………………………………………………………………………………………………………

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

Signature or Thumb print Date

**Investigator statement**

I, the undersigned, have explained to the volunteer in a language he understands the procedures to be followed in the study and the risks and benefits involved.

Name of Interviewer ………………………………………………………………………………………………………

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

Interviewer signature Date
Witness: (If participant was not able to read the consent the Consent Information Sheet and Informed Consent Document).

I affirm that the informed Consent Document has been read to the participant, and he/she understands the study and I have witnessed the participant’s consent to participation.

Name…………………………………………… ………………………

Signature ………………………………….. Date ……………………………………
APPENDIX B: FOMU YA KIBALI (KISWAHILI VERSION)

Jina langu ni Pole Lewa na mimi ni mwanafunzi wa MSC kutoka Chuo Kikuu cha Kenyatta. Ninafanya utafiti juu ya "Ushauri wa kuzuia maambukizi ya antibiotic ya gonorrhea ya Neisseria pekee kutoka kwa wanaume wanaohudhuria kliniki ya maambukizi ya ngono huko Nairobi, kata". Taarifa itatumiwa na Wizara ya Afya kuelewa mabadiliko ya mwelekeo wa upinzani wa madawa ya kulevya ambao hutumia kuti gonorrea na hivyo kubadilisha miongozo ya matibabu ya ndani juu ya usimamizi wa maambukizo ya gonococcal nchini Kenya.

Utaratibu wa kufuatiwa
Kushiriki katika utafiti huu itahitaji kwamba mimi kuulize maswali na kukuchunguza ili uonyeshe dalili za ugonjwa wa uharibifu wa urethra, anal na pharynx. Vipimo vingine vitachukuliwa kutoka kwako kwa ajili ya vipimo vingi. Nitaandika rekodi kutoka kwenu katika dodoso.

Una haki ya kukataa kushiriki katika utafiti huu. Utapata huduma sawa na matibabu kama unakubali kujinga na utafiti au lau na uamuzi wako hautabadilika huduma utakayopata kutoka kliniki leo au kwamba utapata kutoka kliniki nyingine yoyote wakati wowote mwingine.

Tafadhali kumbuka kwamba kushiriki katika utafiti ni kwa hiari. Unaweza kuuliza maswali kuhusiana na utafiti wakati wowote.

Unaweza kukataa kujibu maswali yoyote na unaweza kusimamisha mahojiano wakati wowote. Unaweza pia kuacha kuwa katika utafiti wakati wowote bila matooke yoyote kwa huduma unazopokea kutoka kliniki hii au shirika lingine lolote sasa au baadaye.
Usumbufu na hatari


Faida

Wakati wa majadiliano ya matokeo yako, utapokea ushauri wa kupunguza uwezekano wa VVU kuelewa kuwa STI itakuwa uwezekano kwa kutoke wa kusuliza maambukizi ya VVU. Ikiwa ushiriki katika utafiti huu utatusaidia kupata jinsi ya kutoa tiba bora kwa maambukizo ya gonococcal na kupunguza maambukizi ya magonjwa ya gonococcal. Utapokea na kutibiwa kwa ajili ya kijiko kwa kwa kwa bora. Hakuna gharama kwako kwa kuwa katika utafiti. Utapokea Ksh.500 kwa gharama za usafiri kwenye tovuti ya utafiti.

Huduma na Ulinzi

Hatutarajii uharibiwe kutokana na kushiriki katika utafiti huu. Ikiwa umeshiriki kwa sababu ya kushiriki katika utafiti huu, utapewa matibabu (ikiwa ni pamoja na matibabu ya dharura) kwa gharama yoyote kwako. Ikiwa kliniki ya utafiti haiwezi kutoa matibabu unayohitaji, watawapeleka kwenye kliniki ambapo matibabu yatapewa bila malipo kwako. GASP, mdhamini wa utafiti huu, atafungua gharama za gharama za matibabu za kutosha kwa ajili ya
majeraha ambayo husababisha kushiriki katika utafiti huu. Hakuna aina nyingine ya fidia inapatikana kutoka GASP.

**Huduma na Ulinzi**

Hatutarajii uharibiwe kutokana na kushiriki katika utafiti huu. Ikiwa umeathiriwa kwa sababu ya kushiriki katika utafiti huu, utapewa matibabu (ikiwa ni pamoja na matibabu ya dharura) kwa gharama yoyote kwako. Ikiwa kliniki ya utafiti haiwezi kutoa matibabu unayohitaji, watawapeleka kwenye kliniki ambapo matibabu yatabewa bila malipo kwako. GASP, mdhamini wa utafiti huu, atafungwa gharama za gharama za matibabu za kutosha kwa ajili ya majeraha ambayo husababisha kushiriki katika utafiti huu. Hakuna aina nyingine ya fidia inapatikana kutoka GASP.

**Usiri**

Kila kitu tunachozungumzia kuhusu ikiwa ni pamoja na matokeo yako kitasimamishwa kwa kiasi cha kuruhusiwa na sheria. Taratibu zote za utafiti zitafanyika kwa faragha, iwezekanavyo. Ili kulinda faragha yako, tutatumia namba ya nambari ili kukutambua na habari zote kuhusu wewe, ikiwa ni pamoja na sampuli zako ambazo tutakusanya. Maelezo ya kujitolea yote yatahifadhiwa kwenye makabati ya faili yaliyofungwa na upatikanaji wa udhibiti. Mkusanyiko wa data na fomu za utawala, vilelezo vya maabara, na ripoti nyingine zinazohusiana na utafiti zitatambuliwa kwa nambari tu iliyoifadhiwa ili kudumisha siri ya kujitolea. Jina lako au mambo mengine yoyote ambayo yanaweza kukuelezea hayatatokea tunapowasilisha utafiti huu au kuchapisha matokeo yake. Rekodi zote zilizo na majina au vitambulisho vingine vya kibinafsi, kama taarifa ya locator na nyaraka za idhini ya taarifa, zitashifadhiwa tofauti na rekodi za utafiti, ambazo zinajulikana tu kwa namba ya nambari. Takwimu zote zitahifadhiwa na mifumo ya upatikanaji wa nenosiri. Fomu, orodha, vitabu vya vitabu, vitabu vya uteuzi, na nyaraka zingine zinazounganisha nambari za ID ya kujitolea
kwa habari nyingine za kutambua zitashifadhiwa katika baraza la mawaziri lililofungwa, na ufikiaji unaohitajika kwa wafanyakazi fulani.

**Kuzingatia jamii**

Kaimu-ICR ina timu ya wafanyakazi wa jamii ambayo hufanya kazi na wadu mbalimbali wa jamii ambao wana jukumu la kuchunguza itifaki ya utafiti, kuhamasisha na kuteetea mchakato wa kuajiri wa washiriki. Washirika mbalimbali wa jumuiya ni pamoja na:

a) Wanachama wa Bodi ya Ushauri wa Jamii (CAB) ambao ni tofauti na uwakilishi wake na huu hufanya kazi na wadau mbalimbali wa jamii.

b) Viongozi wa rika ambao hufanya kazi na wanda wadu mbalimbali wa jumuiya kwa washiriki wa kujifunza.

c) Mitandao ya Jumuiya kama Mashirika ya Jamii, Shirikisho la Imani ya Jumuiya na Vifaa vya Afya vya kata ambazo hufanya haki ya mshiriki wa kujifunza.

**Maelezo ya mawasiliano**

Ikiwa una maswali yoyote unaweza kuwasiliana na Mheshimiwa Pole Lewa mnamo 0722811884 na / au Daktari Anthony Kebira juu ya 0735757560 au Sekretarieti ya Kamati ya Ukaguzi wa Maadili ya Chuo Kikuu cha Kenyatta juu ya mwenyekiti.kuerc@ku.ac.ke, katibu@ku.ac.ke, ercku2008 @ gmail.com.

**Taarifa ya washiriki**

Jina la mshiriki ....................................................................................................................................

...........................................................................................................................................
...........................................................................................................................................

Saini au Nyaraka kuchapisha Tarehe

Taarifa ya uchunguzi

Mimi, aliyechaguliwa, ameeleza kwa kujitolea kwa lugha ambayo anaelewa taratibu za kufuatiwa katika utafiti na hatari na faida zinazohusika.

Jina la Mhojiwaji ................................................................................................................................

...........................................................................................................................................
...........................................................................................................................................

Msaidizi wa saini Tarehe
APPENDIX C: QUESTIONNAIRE
STUDY TITLE: ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF NEISSERIA GONORRHOEAE ISOLATED FROM URETHRAL DISCHARGES AND MEN WHO HAVE SEX WITH MEN IN NAIROBI

Date of Interview ______/____/20____

Code of the participant: ________________________

Visit Type: First [ ] Revisit [ ]

SECTION A: SOCIODEMOGRAPHIC CHARACTERISTICS

1. Date of birth ___/___/___ (Tarehe ya kuzaliwa)

2. Age of patient (Miaka) ________________________

3. Where do you live? (Unaishi wapi?)
   County (Jimbo) ___________________ (Village/Estate/Kijiji) _______

4. Occupation (Kazi uifanyanyayo)
   [1] Student Mwanafunzi
   [2] Casual labourer Kibarua
   [3] Self-employed Kujiajiri kibinamfisi
   [4] Salaried employment Kazi ya kujiajiri
   [5] Unemployed Sijaajiriwa
SECTION B: HISTORY OF SYMPTOMS

5. At what age did you have your first sexual intercourse?  
(Ulikuwa na umri wa miaka mingapi wakati uliposhiriki ngono kwa mara ya kwanza?).

6. Have you had a new sexual partner in the last three (3) months?  
(Je, umekuwa na mpenzi mpya ambaye umeshiriki naye ngono katika miezi mitatu (3) iliopita?)

   YES  NDIO
   NO   APANA

7. How many sexual partners have you had in the past three (3) months?  
(Je, umeshiriki ngono na watu wangapi katika miezi mitatu iliopita?).

   0   3
   1   4
   2   >4

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

   YES  NDIO
   NO   APANA

9. How many of your sex partners were male?  
(Je, niwangangapi kati ya wapenzi wako walikuwa wanaume?)

   0   3
10. In the last one (1) month, have you had receptive (bottom) anal sex, that is, where your sex partner put his penis in your anus? (Je, kwa mwezi moja (1) ulipita, mpenzi wako ameshashiriki ngono kwa kutumia sehemu yakö ya yuma, yaani mpenzi wako kuuingiza uume wake kwa mkundu wako?)

YES

NDIO

NO

APANA

11. In the last one (1) month, have you had insertive (top) oral sex that is put your penis in your sex partner’s month? (Je, Katika mwezi mmoja (1) uliopita, umekuwa na ngono ya kuungilia (juu) ya mdomo, ambayo inaweka uume wako katika mwezi wa mpenzi wako?)

YES

NDIO

NO

APANA

12. In the last one (1) month, have you had both receptive (bottom), that is anal sex and/or insertive (top) with your partner while having sexual intercourse? (Je, wewe kwa muda wa mwezi moja (1) wewe na mpezi wako ameshawahi ingiza uume wake kwa sehemu yakö ya Yuma na pia kwa mdomo wakati mulipokwa munashiriki ngono?)

YES

NDIO

NO

APANA

13. Have you and your sexual partners been treated for a sexually transmitted infection (STI) in the last three (3) months? (Je, mmoja wenu kati yako
na mpenzi/wapenzi wako ambaye ametibiwa ugonjwa wa zinaa katika miezi mitatu (3) iliyopita?

YES  NDIO

NO  APANA

14. Is there presence of discharges upon examination in the following areas? (Je, kuna kunapatikana usaha katika sehemu ya uume, mkundu na koo wakati wa uchunguzi

Penis (Uume)  YES (NDIO)  NO (APANA)

Anua (Mkundu)  YES (NDIO)  NO (APANA)

Throat (Koo)  YES (NDIO)  NO (APANA)

Participant study number …………………………………………………………………………………………………………

Name of Interviewer ………………………………………………………………………………………………………

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

Interviewer signature  Date