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Frequency and Susceptibility Pattern of *Neisseria gonorrhoeae* among Symptomatic Patients in Alriyadh Private Hospital, Saudi Arabia

التردد ونمط الحساسية للنيسيريا البنية لدي المرضي ذوي الاعراض بمستشفى الرياض الخاصّ، المملكة العربية السعودية

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الآية الكريمة

قال تعالي: (أَمَّن هُوَ قَانِتٌ آنَاءَ اللَّيْلِ سَاجِدًا وَقَائِمًا يَحْذَرُ الْآخِرَةَ وَيَرْجُو رَحْمَةَ رَبِّهِ قُلْ هَلْ يَسْتَوِي الَّذِينَ يَعْلَمُونَ وَالَّذِينَ لَا يَعْلَمُونَ إِنَّمَا يَتَذَكَّرُ أُولُو الْأَلْبَابِ).

سورة الزمر : الآية (9)

DEDICATION

To my lovely parents, brothers , sisters and husband

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First of all, I am gratefull to Allah for giving me strength to achieve this study. I special thanks to **Dr. Hind Haidar** for her countless hours of reflecting, reading, encouraging, and most of all patience throughout the entire process to achieve this study. I wish to thank my friends how supported me .

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ABSTRACT

Neisseria gonorrhoeae (*N. gonorrhoeae*) is the etiological agent for the strictly human sexually transmitted disease (Gonorrhea). Gonorrhea lead to severe complication such as sterility. This study was a descriptive cross-sectional study conducted to detect the frequency and susceptibility pattern of *N. gonorrhoeae* among symptomatic patients in Alriyadh Private Hospital, Saudi Arabia (SA) during the period from January 2018 to April 2019.

Ninety-three (n=93) symptomatic patients were included in this study, males were 91 and females were 2. Swab specimens were cultured on Modified Thayer-Martin agar (MTM) and chocolate blood agar (CBA), isolated organisms were identified by Vitek 2 Compact system and antimicrobial susceptibility pattern was performed by disc diffusion method. Data were analyzed by (SPSS) software program.

Out of 93 specimens 50 (53.8%) of the patients showed positive growth of *N. gonorrhoeae*. Male patients with positive growth of *N. gonorrhoeae* were 49/93(52.7%) and female was 1/93(1.1%). Single patients with positive growth of *N. gonorrhoeae* 31/93 (33.3%) while married patients were 19/93 (20.4%) , multiple sex partners with positive growth of *N. gonorrhoeae* were 9/93 (9.7%) and patients had no multiple sex partners were 41/93 (44.1%). Ten 10/93 (10.8%) patients with history of *N. gonorrhoeae* infection showed positive growth of *N. gonorrhoeae* while 40/93 (43.0%) with no history.

According to age group the majority of positive growth of *N. gonorrhoeae* belong to 17-27 years 27/93 (29.0%) followed by 28-38 years were 19/93 (20.4%), 39-49 years were 3/93 (3.2%) and >60 years were 1/93 (1.1%). There were no correlation between *N. gonorrhoeae* infection and risk factors including age, sex, history of STD, antimicrobial treatment, multiple sex partners and marital status, *p*. value > 0.05.

In conclusion the frequency of *N. gonorrhoeae* among symptomatic patients is very high, most of them in age group of 17-27 years. No correlation between *N. gonorrhoeae* infection and risk factors.

ملخص الأطروحة

النيسريا البنية (المكورات البنية) هي العامل المسبب لمرض السيلان الذي ينتقل عن طريق الاتصال الجنسي . السيلان يؤدي الى مضاعفات شديده مثل العقم . اجريت هذه الدراسة الوصفية للكشف عن تردد ونمط الحساسية من للنيسريا البنية لدى المرضى الذين يعانون من اعراض في مستشفى الرياض الخاص ،المملكة العربية السعودية خلال الفترة من يناير 2018 الى ابريل 2019.

تضمنت هذه الدراسة 93 من المرضى ذوي الأعراض. عينات المسحات زرعت في ثاير مارتن اجار واجار الشوكولاتة الدموي .عزل والتعرف على البكتريا عن طريق جهاز vitek 2 Compact system ونمط الحساسية عن طريق نشر الأقراص للحساسية.

حللت البيانات عن طريق الحزمة الإحصائية لإصدار برنامج العلوم الاجتماعية SPSS. من بين 93 عينه وجدنا 93/50(53,8%) من المرضى اظهروا نمو إيجابيا للنيسريا البنيه .المرضى الذكور بنمو إيجابي للنيسريا البنيه كانوا 93/49 (52.7%) والاناث 93/1 (1.1%). المرضى العازبين بنمو إيجابي للنيسريا البنيه 93/31 (33.3%) بينما المتزوجون 93/19 (20.4%)، متعددي شركاء الجنس بنمو إيجابي للنيسريا البنيه كانوا 93/9 (9.7) والمرضى غير متعددي شركاء الجنس كانوا 93/41 (44.1%).عشره 93/10(10.8%) من المرضى الذين لديهم أصابه سابقه للنيسريا البنيه اظهروا نتيجة ايجابيه للنيسريا البنيه بينما 93/.4(43.0%) الذين لم يصابوا من قبل.

وفقا للفئات العمرية كانت أغلب النتائج موجبة النمو93/27 (29.0%) تنتمي للفئه العمريه من 17-27 سنه، تليها الفئه 28-38 سنه 93/19 (20.4%)، الفئه 39 -49 سنه كانوا 93/3(3.2%) والفئه 60< سنه 93/1(1.1%). لا توجد علاقه إحصائية بين إصابة النيسريا البنيه وعوامل الخطر المتضمنة الجنس، تاريخ مرض منقول جنسيا، العلاج بالمضادات الحيوية، متعددي شركاء الجنس والحالة الزوجية، P. value > 0.05

خلصت هذه الدراسة الى ان تردد النيسريا البنيه في المرضى ذوي الأعراض عالي ، و معظمهم من الفئه العمرية 17-27 سنه .و لا توجد علاقه بين الإصابة بالنيسريا البنيه وعوامل الخطر.

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LIST OF ABBREVIATIONS

CBA	Chocolate blood agar
CDC	Center for Disease control
GC	Gonococci
HIV	Human immunodeficiency virus
MTM	Modified Thayer-Martin
NAAT	Nucleic acid amplification test
N. gonorrhoeae	*Neisseria gonorrhoeae*
PCR	Polymerase chain reaction
PID	Pelvic inflammatory disease
SA	Saudi Arabia
STDs	Sexually transmitted diseases
STIs	Sexually transmitted infections

CHAPTER ONE
INTRODUCTION

1.1. Introduction

Sexually transmitted diseases (STDs) includes a series of syndromes caused by pathogens that can be acquired by sexual intercourse or sexual activity(Siracusano *et al.*,2014). They can cause life-threatening complications if left untreated, STDs also represent a socioeconomic problem, deriving in treatment costs of tremendous proportions. Despite a substantial progress in diagnosis, treatment and prevention, the incidence of many common STDs is increasing, and STDs continue to represent a global public health problem and a major cause for morbidity and mortality (Carmona-Gutierrez *et al*, 2016).

Gonorrhea is a sexually transmitted disease caused by the bacteria *N. gonorrhoeae* for which humans are the only natural host (Hailemariam *et al.*, 2013). It causes an infection in the lower genital tract which can result in upper genital tract complications (Miranda *et al*, 2017) as wil as the infection of the anterior urinary tract (urethritis)(Cheesbrough, 2006). Infections with *N. gonorrhoeae* most commonly begin at the cervix in females, which marks the dividing line between the lower reproductive tract (vagina, ectocervix) and the upper reproductive tract (uterus, fallopian tubes, ovaries, and endometrium) (Lenz and Dillard, 2018). Gonorrhea spread during sexual contact and spread during delivery(Peña-Martí and Comunián-Carrasco, 2018). The estimated global prevalence of gonorrhea 0.8% (0.6–1.0) (Newman *et al.*, 2015).The main Outcome of the prevalences depend on the general young adult population, and by age, self-reported race/ethnicity, and geographic region of current residence, Antimicrobial resistance in *N. gonorrhoeae* is a major public health concern(Miller *et al*.,2004).

The most common site of *N. gonorrhoeae* infection is the urogenital tract. For male patient *N.gonorrhoeae* causes epididymitis, dysuria. (Miller, 2006). Gonococci (GC) is the etiology of symptomatic urethritis in men and association with sexual behaviors (Vigneswaran *et al*, 2016). The main clinical feature of acute gonorrhea is neutrophilic influx that is unable to clear infection. Women of reproductive age are predominantly at risk for serious sequelae of gonorrhea, including pelvic inflammatory disease (PID), ectopic pregnancy, and infertility (Stevens and Criss, 2018).

Gonorrhea if remained untreated infections can lead to complications, In men, gonorrhea cause testicular and prostate infections and infertility (Bala *et al.*, 2011).In women, it causes serious complications such as PID, ectopic pregnancy, infertility, epididymitis, gonococcemia, and disseminated gonococcal infection, the most important challenge today is the emergence of multidrug-resistant gonorrhea (Skerlev and Čulav-Košćak, 2014).

1.2. Rationale

The most common infectious etiologies of urethritis is *N.gonorrhoeae* (Vigneswaran *et al*., 2016). Gonorrhea is a major global health concern (Stevens and Criss, 2018). It is a strict human pathogen responsible for more than 100 million new STDs worldwide each year (Gala *et al*, 2018). It has demonstrated rapid changes in its susceptibility patterns over the years (Piszczek *et al*, 2015). Due to the global emergence of antibiotic resistance, the Center for Disease Control (CDC) recently listed *N. gonorrhoeae* as an urgent threat to public health. No vaccine is available of the disease (Gala *et al*, 2018).

Data on STDs in SA and other Islamic countries are limited. Gonococcal urethritis was the most commonly reported sexually transmitted infections (STIs) in SA. The incidence of STDs in SA is limited, appropriate preventive strategies that conform to the Islamic rules and values are essential and should be of highest priority for policymakers because of the potential of such infections to spread particularly among the youth. STIs are one of the most under-recognized health problems worldwide. Many people with these infections do not have symptoms and remain undiagnosed. Diseases that are diagnosed are frequently not reported and counted. Most of the published data on the prevalence and incidence of STDs come from developed countries (Madani, 2006). Transmission of *N. gonorrhoeae* among infected men and their female sex partners was spread directed from men to women(Lin *et al*, 1998).

Data about *N.gonorrhoeae* infection in SA are limited, so, this research will highlight the frequency and susceptibility pattern of this organism to prevent its complication.

1.3. Objectives

1.3.1. General Objective

To detect the frequency and susceptibility pattern of *Neisseria gonorrhoeae* among symptomatic patients in Alriyadh Private Hospital, Saudi Arabia.

1.3.2. Specific objectives

1. To isolate and identify *N. gonorrhoeae* using VITEK compact system.
2. To determine antimicrobial susceptibility testing by Clinical Laboratory Standards Institute (CLSI) disc diffusion methods.
3. To determine the frequency of *N. gonorrhoeae* among symptomatic patients.
4. To correlate between *N. gonorrhoeae* and risk factors including age, sex, history of STD, antimicrobial treatment, multiple sexual partners and marital status.

CHAPTER TWO
LITERATURE REVIEW

2. Literature review

2.1. Genital tract infections definition

Reproductive tract infections include endogenous infections, iatrogenic infections and STIs (Muual and Geubbels ,2006).

2.2. STDs

STIs are caused by a wide variety of bacteria, viruses, and parasites that are communicated from one human being to another primarily by vaginal, anal, or oral sexual contact (Wagenlehner *et al*., 2016)

2.2.1. Types of STDs

STIs presenting with genital, anal, perianal, or oral ulcers: Herpes simplex virus infection, Syphilis caused by *Treponema pallidum*, Urethritis, Cervicitis and Trichomonas infection. STIs presenting with genital warts: Human papillomaviruses, human immunodeficiency virus caused by HIV virus and Hepatitis C infections cause by Hepatitis C virus (Wagenlehner *et al*., 2016).

2.3. Gonorrhea

Gonorrhea is a sexually transmitted disease caused by the bacteria *N. gonorrhoeae* for which humans are the only natural host. The causative organism is highly adapted to the genital tract and causing asymptomatic and undetected infection in females in which acquisition of gonococcal infection late in pregnancy can adversely affect labor and delivery as well as the well-being of the fetus (Hailemariam *et al*, 2013).

2.3.1. Types of gonorrhea

Infections with *N. gonorrhoeae* most commonly begin at the cervix in females, which marks the dividing line between the lower reproductive tract (vagina, ectocervix) and the upper reproductive tract (uterus, fallopian tubes, ovaries, and endometrium)(Lenz and Dillard, 2018). *N. gonorrhoeae* acquired at multiple mucosal sites in the lower genital tract, including the urethra, cervix, Bartholin's and Skene's glands, the anorectal canal, pharynx, and conjunctiva. It can spread to the upper genital tract, abdominal cavity and other systemic sites (Walker and Sweet, 2011).It cause cervicitis (Young and Argáez, 2017). It is the most common causes of urethritis in men (Vigneswaran *et al*., 2016). PID is characterized by infection and inflammation of the upper genital tract in women: the uterus, fallopian tubes and/or ovaries. *N.*

gonorrhoeae was the most isolated pathogen(Mitchell and Prabhu, 2013). Endometritis the infection and inflammation of uterine lining, is a frequent and morbid condition among young women, *N. gonorrhoeae* associated with endometritis(Haggerty *et al.*,2004). Hematogenic spread cause the gonococcal infection to the joints and skin (Al-Madboly and Gheida, 2017).

2.3.2. Symptoms of gonorrhea

Urethritis is a disease characterized by urethral inflammation and is a common presentation of several STIs, Symptoms of urethritis include discharge, dysuria, urinary frequency, pruritus and/or irritation with micturition (Vigneswaran *et al.*, 2016) mucoid and mucopurulent. Signs of urethral discharge on examination can also be present in persons without symptoms (Workowski and Bolan, 2015). Urethral discharge occur in both male and female infected with *N.gonorrhoeae* (Tille, 2017). Males, adolescents and young adults continue to represent the majority of gonorrhea cases. Research is needed to better understand the current trends in gonorrhea infection in order to maintain, evaluate and improve primary and secondary STI prevention activities (Choudhri *et al*., 2018). The majority of infected men show symptoms(Hubbard, 2010). symptomatic responses to infection are unique to men and women. In women, gonococcal infections are asymptomatic 50 to 80% of the time or are accompanied by nonspecific symptoms such as vaginal discharge (Nudel *et al*., 2018) lower abdominal discomfort, and dyspareunia. The lack of discernible symptoms(Alirol *et al*,2017) abnormal uterine bleeding, or rectal pain, The urethra and cervix are the most frequently affected anatomical sites, followed by anal and pharyngeal areas (Piszczek *et al*.,2015). While most infected men remain asymptomatic, those who develop symptoms often show robust inflammation characterized by purulent urethral discharge accompanied by large numbers of polymorphonuclear leukocytes (PMNs) (Nudel *et al*,2018). penile discharge, red or swollen urethra, and tenderness in the testes .Males may not display symptoms for up to 1 month after infection. The characteristics of the urethral discharge may vary from cloudy to clear and is therefore an unreliable indicator for gonococcal urethritis in males, pain on urination, sore throat, abdominal pain, fever, and painful sexual intercourse (Tille, 2017). itch, testicular or rectal pain (Piszczek *et al*.,2015), The gonorrhea organisms can spread (disseminate) from an initial local site into the blood and cause infection of other organs. Symptoms of disseminated gonococcal infection include rash, fever, joint pain, infection of joints, and inflammation of tendons, the

inner lining of the heart (endocarditis), and the membranes covering the brain and spinal cord (meningitis) (Peña-Martí and Comunián-Carrasco, 2018).

2.3.3. Transmission incidence

Gonorrhea frequently spread during sexual contact (i.e. vaginal intercourse, oral sex, or anal sex and from a pregnant woman to her baby during delivery(Peña-Martí and Comunián-Carrasco, 2018). Asymptomatic patients may serve as a reservoir of infection to their partners(Bala *et al*., 2011). Female sex workers working in high risk to have for *N. gonorrhoeae* infection and transmit the disease (Luo *et al.*,2015).

2.3.4. Prevalence of gonorrhea

The estimated global prevalence of gonorrhea 0.8% (0.6–1.0%), among men, estimated gonorrhea prevalence was 0.6% (0.4–0.9%), estimated new cases of gonorrhea 78 million (Newman *et al*, 2015). Regional and global 95% uncertainty intervals (UIs) was generated using the delta method, The 2016 global prevalence of gonorrhea estimates in women was 0.9% (95% UI: 0.7–1.1), in men was 0.7% (95% UI: 0.5–1.1), Total estimated incident cases 86.9 million (95% UI: 58.6–123.4 million) gonorrhoea cases(Rowley *et al.*, 2019).

2.3.5. Causative agents

2.3.5.1. *N. gonorrhoeae*

The causative agent of gonnorrhea is a bacterium termed *N. gonorrhoeae*. This pathogen is Gram-negative non-motile aerobic diplococcus. It is fastidious microorganism, which requires incubation in the presence of CO_2. Multiple virulence factors, which strongly participate in the pathogenesis of gonococcal infections skin (Al-Madboly and Gheida, 2017).

2.3.6. Virulence

Pili constitute one of the most important virulence factors, because they mediate attachment to mucosal cell surfaces and are antiphagocytic. piliated GC are usually virulent, whereas nonpiliated strains are a virulent. Two virulence factors in the cell wall are endotoxin (lipooligosachride, LOS) and the outer membrane proteins. The organism's IgA protease can hydrolyze secretary IgA, which could otherwise block attachment to the mucosa. GC have no capsules. The main host defenses against GC are antibodies (IgA and IgG), complement, and neutrophils. Antibody mediated opsonization and killing within phagocytes occur, but repeated gonococcal infections

are common, primarily as a result of antigenic changes of pili and the outer membrane proteins (Levinson, 2012).

2.3.7. Pathogenesis

GC like meningococci, cause disease only in humans. The organism is usually transmitted sexually; newborns can be infected during birth. Because GC is quite sensitive to dehydration and cool conditions. sexual transmission favors its survival. Gonorrhea is usually symptomatic in men but often asymptomatic in women. genital tract infections are the most common source of the organism, but anorectal and pharyngeal infections are important sources as well. GC infect primarily the mucosal surfaces (e.g., the urethra and vagina), but dissemination occurs. certain strains of GC cause disseminated infections more frequently than others. The most important feature of these strains is their resistance to being killed by antibodies and complement. The mechanism of this serum resistance is uncertain, but the presence of porin protein (protein A) in the cell wall, which inactivates the C3b component of complement, appears to play an important role. The occurrence of disseminated infection is function not only of strain of GC but also of the effectiveness of the host defenses. persons with a deficiency of the late-acting complement components (C6-C9) are at risk for disseminated infections, as are women during menses and pregnancy. disseminated infections usually arise from asymptomatic infections, including that local inflammation may deter dissemination proteins (Levinson, 2012).

2.3.8. Laboratory Diagnosis

2.3.8.1. Specimens

Specimens for culture should be collected from infected sources (cervix, urethra, rectum, or throat). Swabs or exudates are acceptable specimens, and should be kept at room temperature prior to processing (Hubbard,2010).

2.3.8.2. Specimen preservation

Specimens should be inoculated immediately to appropriate enriched and selective media. If this is not possible, special transport media are available for shipment of specimens to the Clinical microbiology laboratory. One such transport system is the JEMBEC system with CO2 generating tablets (Engelkirk and Engelkirk, 2008).

2.3.8.3. Gram Stain

Swab specimens should be rolled (not rubbed) onto glass microscope slides to preserve the integrity of any PMNs present in the specimen. For specimens from men, the Gram stain is considered positive if, and only if, intracellular Gram-negative

diplococci Observing PMNs and only extracellular Gram-negative diplococci is considered equivocal for gonorrhea, but is often considered a significant enough finding to warrant initiation of treatment (Engelkirk and Engelkirk, 2008). Gram stain of urethral secretions that demonstrates polymorphonuclear leukocytes with intracellular Gram negative diplococci can be considered diagnostic for infection with *N. gonorrhoeae* in symptomatic men .Because of lower sensitivity, a negative Gram stain should not be considered sufficient for ruling out infection in asymptomatic men (Workowski and Bolan, 2015).

2.3.8.4. Cultivation

N. gonorrhoeae typically does not grow on sheep Blood agar, the medium of choice for cultivation is CBA because CBA supports the growth of many other organisms found as commensals in specimens collected for the recovery of GC, a selective medium is necessary. Commonly used selective media are : Thyer-Martin , modified Thyer-Martin , Martin-Lewis , New York city , GC-LECT media. These media all contain vancomycin and colistin to inhibit Gram- positive and Gram-negative bacteria respectively,along vwith antifungal agent to suppress the growth of yeast. Trimethoprim is used in most of these media to prevent growth of the swarming of *Proteus spp*. To recover vancomycin sensitive strains of GC many laboratories include a CBA plate as a primary plating medium (Mahom *et al*.,2008).

2.3.8.5. Incubation Conditions and Duration

Inoculated plates should be incubated at 35º in a 3% to 5% CO2 atmosphere. Incubation is accomplished by use of a CO_2 incubator, CO_2 generating pouch, or a candle extinction jar. Scented or colored candles may be inhibitory to the GC, so only white wax candles are used in the candle extinction jar (Mahom *et al.*,2008).

2.3.8.6. Colonial morphology

After incubation for 24hrs in a moist aerobic environment enriched with 5-10%CO_2, colonies on Modified New York City (MNYC) medium arte small (1.mm), gray and convex; after 48hrs the colonies are larger (1.5-2.5mm), sometimes with a crenated margin and opaque raised centre. On Thyer-Martin medium growth is slower; although colonies are similar to those on MNYC medium they are usually smaller (Collee *et al*.,2011).

2.3.8.7. Biochemical tests

The GC is oxidase positive and utilizes glucose but not maltose, sucrose, lactose or fructose. The rapid carbohydrate utilization test; measures preformed enzymes and provides a quicker and more reliable identification than conventional growth-dependent sugar tests using solid or semi-solid media(Collee *et al.*,2011).

2.3.8.8. Serological tests

The low sensitivity and specificity of existing serological tests, and the persistence of antibody due to past infection, limit their value in clinical practice. Serological tests are not suitable for screening for gonococcal infection and should not be used in this way to diagnose or exclude gonorrhea(Collee *et al.*,2011).

2.3.8.8.1. Coagglutination tests

Performed on primary culture and confirmed results can be obtained one day earlier than tests that require subculturing from primary culture plates. The cell suspensions are boiled and combined with monoclonal antibodies that detect heat-stable epitopes on the PorI outer membrane protein and detection reagent. Coagglutination with test reagents indicates a positive result (Ng and Martin, 2005).

2.3.8.8.2. Fluorescent antibody test

The fluorescein isothiocyanate-labelled monoclonal antibody in the MicroTrak *N. gonorrhoeae* culture confirmation test reacts specifically with *N. gonorrhoeae*. Positive cultures of *N. gonorrhoeae* show apple-green fluorescent staining of the kidney shaped diplococci (Ng and Martin, 2005).

2.3.8.9. molecular technique for detection of *N. gonorrhoeae* from urine and urogential specimens specimens

A variety of molecular diagnostic procedures are available to diagnose gonorrhea using urogenital specimens and urine. These include both nucleic acid amplification tests (NAATs) (e.g., polymerase chain reaction (PCR), transcription mediated amplification, and strand displacement amplification tests) and nucleic acid hybridization tests (Engelkirk and Engelkirk, 2008). Nucleic acid tests permit the rapid and sensitive detection of *N. gonorrhoeae* from clinical samples without the requirement of bacterial viability. They have been in use since the early 1990s and can be categorized in nucleic acid hybridization assays and NAATs (Versalovic *et al.*,2011).

2.3. 8.9.1. Nucleic acid methods for confirmation of culture

Many methods are available for detection of *N. gonorrhoeae* nucleic acids including PCR, Ligase chain reaction, Strand displacement amplification system and nucleic acid sequence-based amplification. Specific primers for PCR-based detection methods are the most sensitive, although specificity depends on the choice of primers. The use of primers to target the cryptic plasmid is not recommended in areas where there is a high prevalence of proline-, citrulline- and uracil-requiring strains, which are typically plasmidless. Commercial kits are available but are more expensive when used as confirmation tests (Ng and Martin, 2005).

2.3. 8.9. 2. Nucleic Acid Amplification Tests

All main commercial NAATs developed to date use multiplex NAATs, targeting both *N. gonorrhoeae* and *Chlamydia trachomatis*(Versalovic *et al.*,2011).These include the following tests method: Roche Amplicor CT/NG test, manufacturer by Roche diagnostics. The second method is Roche Cobas Amplicor CT/NG test BD probe ET, which manufacturer by Roche Diagnostics and BD diagnostic system. The third is Gene-probe AptimaGC and Gene-probe Aptima Combo2, manufacturer by Gene-Probe. The last method type is Abbott Real Time CT/NG, manufacturer by Abbott Molecular(Mahom *et al*.,2008).

2.3.8.9.3. Hybridization Assays

The two commercially available hybridization assays include Digene CT/GCDual ID HC2(HC2;Qiagen) and Gen-Probe Pace 2 (P2;Gen-ProbeINC., San Diego,CA),which use RNA probes targeting genomic DNA and DNA probes targeting rRNA, respectively. The detection method of the RNA-DNA hybrids in theHC2 assay involves antibody-mediated recognition of the hybrids andsubsequent binding of alkaline phosphatase-conjugated antibodies, which act on a chemiluminescent substrate. Signal amplification results from multiple alkaline phosphatase molecules beingattached to conjugated antibody, of which several bind to a single captured hybrid. In the P2 assay the DNA probes are labeled with a chemoluminescent substance, which is quantified after separation of the stable DNA-RNA hybrid from nonhybridization probe. The sensitivity of hybridization tests is probably higher than that of culture(Versalovic *et al.,* 2011).

2.3.8.9.4. DNA probe system

DNA probe system (AccuProbe, Gen-Probe, USA) has been developed for culture confirmation. The AccuProbe system uses a chemiluminescent-labelled, single-

stranded DNA probe that is complementary to the ribosomal RNA (rRNA) of *N. gonorrhoeae*. The DNA/RNA hybrid from a positive culture is detected using a luminometer. In comparison with rapid acid production and coagglutination tests, a DNA probe test is more specific and sensitive (Ng and Martin, 2005).

2.3.9. Sex risk factor

Men were more likely to have gonorrhea, but rates equilibrated by 1996 and have remained similar since then. In 2009, the gonorrhea rate among women was higher than the rate among men (Walker and Sweet, 2011).Study done in Australia, the largest increase in notification between 2007 and 2012 was observed in both men and women in new south wales (2.9- and 3.7-fold greater in 2012 than 2007,respectively) and Victoria (2.4- and 2.7- fold greater in greater in 2012 than 2007, respectively), men in the Australian capital territory and women in Queensland. The highest notification rates remained in indigenous people in the northern territory and western Australia, and particularly in women, although rates may have decreased over the study period changes in age and sex distribution, antimicrobial resistance and patterns of exposure and acquisition were neglible (Roberts-Witteveen *et al*., 2014).

Another study in Canada, they found males had consistently higher rates than did females (70.2 per 100,000 versus 40.6 per 100,000 in 2015) and faster rising rates (85.2% versus 39.5% in 2010–2015) (Choudhri *et al.*,2018).

2.3.10. Complications

Gonorrhea is an easily curable STI, but if remained undetected, untreated infections and co-infections can lead to complications, In men, gonorrhea cause testicular and prostate infections and infertility (Bala *et al.*, 2011). It can lead to epididymitis, reduced fertility, and urethral stricture(Alirol *et al.*,2017). In women, it cause serious complications such as PID, ectopic pregnancy, infertility, epididymitis, gonococcemia, and disseminated gonococcal infection,the most important challenge today is the emergence of multidrug-resistant gonorrhea (Skerlev and Čulav-Košćak, 2014), tubal factor infertility, adverse pregnancy outcomes in females(Bala *et al.*, 2011).Also it can cause problems for both the pregnant woman and her baby. It is associated with preterm delivery, pre-labour rupture of the membranes, low birthweight, and inflammation of the endometritis after giving birth. Babies can infected during birth, this can result in eye infections (ophthalmia neonatorum - an eye infection contracted during birth) as the baby passes through the birth

canal(Peña-Martí *et al.,* 2018). It is increase the risk of both transmission and acquisition of HIV (Bala *et al.*, 2011).

2.3.11. Treatment

The ideal treatment regimen for gonorrhea should cure at least 95% of infections. An antibiotic to which more than 5% of *N. gonorrhoeae* strains exhibit resistance should not be used(Piszczek *et al*,2015). Recommended therapy for treatment of *N. gonorrhoeae* includes ceftriaxone and azithromycin. Penicillin resistance by betalactamase production is common; fluoroquinolone and tetracycline resistance is increasing. Reduced susceptibility to oral cephalosporins (e.g., cefixime) is being increasingly observed (Tille, 2017).

Canadian guidelines recommend either intramuscular (IM) ceftriaxone or oral cefixime as the preferred antibiotic of choice. In contrast, the CDC and organizations in other countries advocate only parenteral cephalosporins as first-line therapy. (Piszczek *et al*,2015).

In another study conducted, A recent study examines the synergistic effect of this combination along with gentamicin combined with five other antimicrobials (cefixime, ceftriaxone, spectinomycin, azithromycin, moxifloxacin, and ertapenem) The study concludes that gentamicin in combination with ertapenem or cefixime could be introduced as new antimicrobial dual therapy as combinations showed maximum efficacy and synergism against GC strains (Suay-García and Pérez-Gracia, 2018).

In study done in Japan by Furuya· and Tanaka (2009), they found a high prevalence of *N. gonorrhoeae* isolates resistant to antimicrobial agents. Ciprofloxacin was 73.4% in 2006 and it was still so high, tetracycline was 38.5% in 2006 and that of isolates resistant to penicillin G (PCG) was 17.5%. First-line therapy for gonococcal infections, only three parenteral regimens of single doses of ceftriaxone, cefodizime or spectinomycin are recommended by the Japanese Society for Sexually Transmitted Diseases. The cefixime+azithromycin combination demonstrated greater synergy than other combinations.

2.3.12. Antimicrobial resistant

When treatment failures are minimized, the potential spread of resistant disease is also reduced. The guidelines address the increasing resistance to cephalosporins, with recommendations varying based on the geographic region. The mechanism for resistance was largely due to alterations in the *penA, porB1b* and *mtrR* genes, which

diminish b-lactam binding to the cell wall, decrease permeability of cephalosporins and increase drug efflux from the cell, respectively (Piszczek *et al*,2015).

Study done in Uganda (2019), Low level of antimicrobial susceptibility to cefuroxime (50%) followed by erythromycin and gentamycin both at 25% was observed. An alarming resistance to ceftriaxone and ciprofloxacin at 100% followed by penicillin 75% was exhibited by the colonies (Peace *et al.*,2019).

In study of Martin and his colleges in Canada (2019) between 2012 to 2016, the proportion of multi drug-resistant GC increased from 6.2% to 8.9% and a total of 19 cases of extensively drug-resistant GC were identified (0.1%, 19/18,768). The prop55ortion of isolates with decreased susceptibility to cephalosporins declined between 2012 and 2016 from 5.9% to 2.0% while azithromycin resistance increased from 0.8% to 7.2% in the same period .

According to WHO, there is an urgent need to update treatment recommendations for gonococcal infections to respond to changing antimicrobial resistance (AMR) patterns of *N. gonorrhoeae*. High-level resistance to previously recommended quinolones is widespread and decreased susceptibility to the extended-spectrum (third-generation) cephalosporins, another recommended first-line treatment in the 2003 guidelines, is increasing and several countries have reported treatment failures (WHO,2016).

In study of Martin and his colleges in Canada (2015) since 2009, there has been a rise in antibiotic-resistant *N. gonorrhoeae*. In 2013, 24.3% of the isolates were resistant to erythromycin, 18.9% were resistant to penicillin, 33.0% were resistant to tetracycline, and 29.3% were resistant to ciprofloxacin. The percentage of isolates with decreased susceptibility to ceftriaxone and/or cefixime was 3.9% in 2013. The proportion of azithromycin resistant *N. gonorrhoeae* isolates increased from 0.4% in 2009 to 1.2% in 2013(Martin *et al*.,2015).

2.8.13. Control and prevention

Education has been inversely correlated to behavioral risk-taking associated with the acquisition of STIs in adolescents (Walker and Sweet,2011). The condom offers maximum protection(more than 90%) against *N.gonorrhoea*e (Marfatia *et al.*, 2015). To minimize gonorrhea transmission, persons treated for gonorrhea should be instructed to abstain from sexual activity for 7 days after treatment and until all sex partners are adequately treated. All persons who receive a diagnosis of gonorrhea should be tested for other STDs, including chlamydia, syphilis, and HIV(Workowski and Bolan,2015).

2.3.13.1. Vaccination

Vaccine development for *N. gonorrhoeae* has been problematic, but recent progress in the field has provided new hope that a gonococcal vaccine may be feasible. Several new vaccine antigens have been characterized in various models of infection. The first potential vaccine-induced protection against gonorrhea in humans has been reported, with decreased rates of gonorrhea described among individuals vaccinated with the *Neisseria meningitidis* serogroup B vaccine (Edwards *et al*, 2018). Geographic clustering of gonococcal infections is associated with minority ethnic groups, low socioeconomic status and lack of education(Piszczek *et al.*, 2015).

2.3.14. Previous studies

In study done in SA, a total of 39049 STIs were reported to the Ministry of Health. Reported STIs included gonococcal urethritis (5547 infections, 14.2%), The average annual incidence of STIs per 100,000 population for Saudis and non-Saudis, was 4.2 for gonorrhea (Madani, 2006).

In study done in Dubai, United Arab Emirate, 201 female patients aged 16–80 years were enrolled in 2010. The prevalence of cervical infection with *N. gonorrhoeae* was 5.5% in 11 patients (Mehrabani *et al*.,2014).

In another study conducted in Kuwait, 8539 women were screened, 69.5% were Kuwaitis while 30.5% were non-Kuwaitis. About 51. 3% were aged ≤40 years. The overall prevalence *N. gonorrhoeae* was 1.5% (Al-Sweih *et al*., 2011).

Study done in Uganda (2019), They found the prevalence of *N. gonorrhoeae* was 4.9% and the high prevalence rates among the young age group (15-25 years)was 7.7% (Peace *et al.*,2019).

In another study in Kenya (2016), 197 women attending the reproductive health clinic in Kenyatta National Hospital consented to participate in the study. The prevalence of *N. gonorrhoeae* was 8%. participants found to have *N. gonorrhoeae* (37%) were aged between 30-39 years. Married participants with *N. gonorrhoeae* were (14/16, 87%)(Nzioka,2016).

In study of Kafi and his colleges (2009) in Sudan, a total of 338 women with age ranging from 15 to 69 year in a suburban Sudanese community were randomly selected and studied, observed that gonorrhea was detected in 1.2% of the subjects(Kafi *et al.*,2009).

In study done Ethiopia, They found the prevalence of *N. gonorrhoeae* was 20.8% (Geremew *et al.*,2017).

In another study conducted in Canada, 19,845 cases of gonorrhea were reported corresponding to a rate of 55.4 cases per 100,000 population and a 65.4% increase from 2010 (33.5 cases per 100,000 population). Rates among adults 60 years and older increased faster than rates among younger people. The highest rates were among those 15–29 years of age. The Northwest Territories, Nunavut and Yukon had the highest gonorrhea rates in 2015(Choudhri *et al.*,2018).

Study done in UK (2012), the diagnosis rates for gonorrhea among adults aged 20 to 24 years were 249 per 100,000 for men and 140 per 100,000 for women (Creighton, 2014).

In study of Rostami and his colleges (2017) in central Iran, 420 volunteers were screened , 277 (65.9%) had genital signs/symptoms. Five specimens (1.2%) in Thayer-Martin culture and 17 (4.1%) in real-time PCR were identified as *N.gonorrhoeae* (Rostami *et al.*, 2017).

CHAPTER THREE
MATERIALS AND METHODS

3. Materials and methods

3.1. Study design

This was a descriptive cross-sectional study conducted to determine *N.gonorrhoeae* among symptomatic patients.

3.2. Study area

This study was done in in Al-Riyadh State (Kingdom of Saudi Arabia) in the own laboratory of Private Hospital.

3.3. Study duration

The study was conducted during the period from January 2018 to April 2019.

3.4. Study population

All urethritis male and cervicitis female patients into urology outpatient clinic in Private Hospital in Al-Riyadh state were participated in this study.

3.5. Inclusion criteria

All patients suffering from STIs symptoms(urethritis and cervicitis). Male and female either under treatment or not were included in this study.

3.6. Exclusion criteria

Patients who refused to participate to this study and patients have no sign and symptoms.

3.7. Sample size

A total of ninety-three (n=93) patients were included in this study.

3.8. Sampling technique

The study based on non-probability convenience sampling technique. Samples were taken from attended agreed patients**.**

3.9. Ethical consideration

Approval to conduct this study was obtained from the College Ethical Committee, Sudan University of Science and Technology and hospital administration. Verbal consent was taken from each participant after explaining the objectives of this study and take permission and agreement from responsible.

3.10. Data collection

A structured questionnaire (appendix 1) was used for the collection of both qualitative and quantitative data. Which include demographic information such as risk factors including age, sex, history of STD, antimicrobial treatment, the sign and symptoms,history of illegal relationship and marital status.

3.11. Cultural methods

3.11.1. Specimens collection

Urethral swab specimens were collected from male patients. The exudate from the urethra was collected using a clean, sterile swab. Cervical swab was collected from female patients. All specimens were collected by physician and received to the laboratory within 5 minutes or should be kept into Amies transport medium **(**appendix2) (Cheesbrough, 2006).

3.11.2. Direct microscopical examination

Direct Gram's stain (see appendix 3) smear was prepared by spreading the swab evenly on a clean slide and was fixed with 95% methanol for 2 minutes. The fixed smear was covered with crystal violet stain for 30-60 seconds then rapidly washed off with clean tap water. All the water was tipped off and cover the smear with Lugol's iodine for 30-60 seconds and washed off with clean water. Then the smear was rapidly decolorized (few seconds) with acetone- alcohol and immediately cleaned with water. Then the smear was covered with neutral red stain for 1-2 minutes. The neutral red was washed off with clean water, the slide was wiped clean and left to air-dry. The dried smear was examined microscopically, first with the 40x objective to check the staining and to see the distribution of material, and then with the oil immersion objective (X100) to report the bacteria **(**appendix 4) Presence of pus cells intracellular and extraceullar with Gram negative diplococcic indicate *N. gonorrhoeae*(Cheesbrough, 2006).

3.11.3. Inoculation and incubation of specimens

All specimens were inoculated in MTM agar (appendix 5) is an enriched and selective medium for the isolation of *N.gonorrhoeae* and CBA (appendix 6) by looping out technique. All specimens should be inoculated to media immediately after specimen collection for optimal organism recovery and incubated at 37 ºC for up to 48 hours at 5-10% CO_2 enriched atmosphere(Cheesbrough, 2006).

3.11.4. Identification of the growth

Agar plates were examined for growth after 24 hours, if negative or scanty growth the plates should incubated again for 48 hours. *N. gonorrhoeae* colonies gave translucent ,gray, glistening and smooth consistency on MTM (appendix 7) and CBA (Cheesbrough, 2006).

3.11.5. Indirect Gram's stain

Any suspected colonies were subjected to Gram's stain .Smear was prepared by emulsifying a small portion of bacterial colony in a drop of normal saline and spread evenly on a clean slide and allowed to air-dry. The smear was fixed by rapidly pass the slide smear uppermost three times through the flame of a spirit lamp or pilot flame of a Bunsen burner. *N.gonorrhoeae* showed Gram's negative diplococci kidney shape (Cheesbrough, 2006).

3.11.6. Biochemical tests

3.11.6.1. Oxidase test

This test used to determine the presence of bacterial cytochrome oxidase by using the oxidation of the substrate tetramethyl-p-phenylenediamine dihydrochloride to indophenols. Two or 3 drops of freshly prepared oxidase reagent was placed in a piece of filter paper in a clean petri dish. A colony of the tested organism was remove using a piece of stick or glass rod and smeared it on the filter paper. Look for the development of a blue-purple colour within 10 seconds. *N. gonorrhoeae* was oxidase positive (Cheesbrough, 2006).

3.11.7. Identification of *N. gonorrhoeae* by Vitek 2 Compact system

Vitek 2 Compact system (bioMérieux, France) was used for full Identification for *N. gonorrhoeae* according to Vitek 2 Compact system manual (appendix 8).

3.11.7.1. Preparation of suspension

VITEK 2 DensiCHEK Plus (appendix 9) was used to check suspension density , the tube of saline was Placed in the DensiCheck Plus and rotate one full turn. The reading was 0.0. Polystyrene tube (12mm x 75mm) was filled with 3 ml of (0.45% to 0.5% NaCl, pH 4.5 to 7.0) sterile saline. A homogeneous suspension of pure culture in the saline was prepared. Then, Insert tube into the optical block of the DensiCheck Plus making and confirmed it was seated at the bottom. The tube was rotated one complete revolution within 2 seconds and checked the display for density reading. Acceptable readings: 3 for *N. gonorrhoeae* according to Vitek 2 Compact system manual according to Vitek 2 Compact system manual.

3.11.7.2. Processing Test Cards

Placed the tube with NH card in the cassette. Filled in a cassette worksheet with the test card and specimen information for the cassette. Placed the test cards and specimen test tubes in their appropriate slots. The cassette was loaded into the Filler Station. Then, the cassette was transfered to the Vitek 2 Compact cassette loading station within 10 minutes. Load and Go Method used to enter the information from the cassette worksheet into the Setup Tests Post Entry according to Vitek 2 Compact system manual.

3.11.7.3. Principle of Vitek 2 Compact system for NH card

A vacuum chamber incubated at 35.5°C. The 64-well NH card contains 30 biochemical tests in the following categories: 11 glycosidase and peptidase tests, 10 acidification tests, 5 alkalinization tests, and 4 miscellaneous tests. An intermittent reading every 15 min allowed for identification after 6 hours (Valenza *et al*.,2007).

3.11.7.4. Result

The results were generated by a computer-assisted algorithm of the VITEK 2 system. Identification results were obtained after a 6 hours incubation of the NH cards inside the VITEK 2 system (Valenza *et al*., 2007).

3.11.7.5. Reporting of the result

Neisseria gonorrhea was isolated after 48 hours incubation at 37°C.

3.11.8. Antimicrobial testing

3.11.8.1. Preparation of inoculum

Colony suspension was prepared , equivalent to a 0.5 McFarland standard prepared in 0.9% phosphate-buffered saline, pH 7, colonies used from an overnight (20- to 24-hour) CBA plate incubated in 5% CO2 by sterile disposable loop (CLSI, 2018).

3.11.8.2. Antimicrobial discs

Antibiotics tested were cefotaxime (30mg), ciprofloxacin (5mg), ampicillin (10mg) , ceftriaxone(30mg), tetracycline(30mg), Were obtained from (CLSI, 2018).

3.11.8.3. Seeding of the plates

The plates were inoculated by dipping a sterile swab into the inoculum standardized to match the 0.5 McFarland turbidity standard, equivalent to 1.5 _ 108 CFU/mL.. The excess inoculum was removed by pressing and rotating the swab firmly against the side of the tube above the level of the liquid Streak the swab all over the surface of the medium three times, rotating the plate through an angle of 60° C after each Application, Finally, pass the swab round the edge of the agar surface. Leave the

inoculum to dry for a few minutes at room temperature with the lid closed (Cheesbrough, 2006).

3.11.8.4. Application of antimicrobial discs

The antimicrobial discs were placed on the inoculated plates by using a pair of sterile forceps, the discs were spaced evenly, approximately 15 mm from the edge of the plate, and I disc placed in the centre of the plate. Each disc should be gently pressed down to ensure even contact with the medium, then the plates should be placed in an incubator at 35 °C within 30 minutes of preparation for 24 hours.

test a maximum 4 disks on a 100-mm plate. For some agents, eg, fluoroquinolones or cephalosporins, only 2 to 3 disks may be tested per plate (CLSI, 2018).

3.11.8.5. Incubation

36°C ± 1°C (do not exceed 37°C); 5% CO2 for 20–24 hours (90) (CLSI, 2018).

3.11.8.6. Reading of the zones of inhibition

After overnight incubation, measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (appendix 10). Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth (CLSI, 2018).

3.11.8.7. Interpretation of the results

The zones size of each antimicrobial interpretative table was reported by using the on CLSI Table 2F. Zone Diameter for *N. gonorrhoeae* (CLSI, 2018).

3.11.8.8. Testing organisms for quality control

Quality control was performed to measure the effectiveness of antimicrobial agents by using a control *N. gonorrhoeae* ATCC®* 49226 When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges (CLSI, 2018).

3.12. Data analysis

Processing and analysis of data were carried out by means of the statistical package for the social sciences (SPSS) software program version IBM SPSS Statistics 25. A descriptive statistic frequencies were calculated, Chi-squire test was used to compare between qualitative variables. *P*. value was adjusted to be ≤ 0.05.

CHAPTER FOUR

RESULTS

A total of ninety three (n=93) patients suffering from STDs symptoms were included in this study males were 91 (97.8%) and females were 2 (2.2%) table (4.1), with age range from 17-62 years (with mean age 28.63years, SD ± 7.907. Ninety three 93/93 of the patients enrolled in this study were suffering from STDs symptoms.

Age group was classified to five age groups 17-27 years were 47/93 (50.5%) of the patients, 28-38 years were 37/93 (39.8%) and 7/93 (7.5%)were aged between 39-49 years and 50-60 years were 1/93 (1.1%) and 1/93 (1.1%) were aged >60 years. According to marriage status single patients were 54/93 (58.1%), married patients were 39/93 (41.9%) **.** Patients had multiple sex partners were 12/93 (12.9%) , 81/93 (87.1%) did not have multiple sex partners. Eighteen18/93 (19.4%) of the patients had a history of *N. gonorrhoeae* while 75/93 (80.6%) of the patients had no history of *N. gonorrhoeae*. Three 3 (3.2%) of the patients under gonorrhea treatment, 90 (96.8%) of the patients not under treatment (table 4.1).

Fifty 50/93 (53.8%) of the patients showed positive growth of *N. gonorrhoeae,* while 43/93(46.2%) of them were negative (Figure 4.1).

Out of 93 specimen males showed positive growth of *N. gonorrhoeae* were 49/93(52.7%) and female was 1/93(1.1%). No statistical significant difference was found between sex and *N. gonorrhoeae,* P. value = 0.914.

Single patients positive growth of *N. gonorrhoeae* 31/93 (33.3%) while married patients were 19/93 (20.4%).This result showed no statistical significant difference between marital status and growth of *N. gonorrhoeae,* P. value = 0.4

Patients had multiple sex partners with positive growth of *N. gonorrhoeae* were 9/93 (9.7%) and patients had no multiple sex partners were 41/93 (44.1%), no statistical significant difference between multiple sex partners and growth. P. value = 0.1

Ten 10/93 (10.8%) patients with history of gonorrhea infection showed positive growth of *N. gonorrhoeae* while 40/93 (43.0%) with no history .Statistical insignificant difference between positive growth of *N. gonorrhoeae* and history of gonorrhea was found. P value = 0.8 and as well as treatment P value = 0.05

According to age group the majority of positive growth of *N. gonorrhoeae* belong to 17-27 years were 27/93 (29.0%) followed by 28-38 years were 19/93 (20.4%), 39-49 years were 3/93 (3.2%) and >60 years were 1/93 (1.1%), Insignificant statistical

difference was found between age group and growth. P value = 0. 609 (Table 4.2). Susceptibility profile of the *N. gonorrhoeae* isolates revealed that all the isolates were sensitive to third generation cephalosprine 50(100%) (Ceftriaxone and cefotaxime). Moreover, all the isolates were resistant to penicillin 0(0%) and tracycline 0(0%) while 2/50(4.0%) of the isolates were sensitive to ampicillin and 1/50(2.0%) to ciprofloxacin as shown in table (4.3).

Table (4.1) : Distribution of demographic data among the study population

Variables	Frequency	Percent
Sex		
male	91	97.8%
Female	2	2.2%
Total	93	100%
Multiple partners		
Yes	12	12.9%
No	81	87.1%
Total	93	100%
History		
Yes	18	19.4%
No	75	80.6%
Total	93	100%
Treatment		
Yes	3	3.2%
No	90	96.8%
Total	93	100%
Marital status		
Single	54	58.1%
Married	39	41.9%
Total	93	100%
Age group		
17-27 years	47	50.5%
28-38 years	37	39.8%
39-49 years	7	7.5%
50-60 years	1	1.1%
>60 years	1	1.1%
Total	93	100.0%

Table (4.2): The association between of gonorrhea and risk factors

Variables	Growth of *N. gonorrhoeae*		Total	*P.* value
	Positive No%	Negative No%		
Sex				
male	49(52.7%)	42(45.2%)	91(97.8%)	0.914
Female	1(1.1%)	1(1.1%)	2(2.2%)	
Marital status				
Single	31(33.3%)	23(24.7%)	58.1%)(54	0.407
Married	19(20.4%)	20(21.5%)	39(41.9%)	
Multiple sex partners				
Yes	9(9.7%)	3(3.2%)	12(12.9%)	0.114
No	41(44.1%)	40(43.0%)	81(87.1%)	
History				
Yes	10(10.8%)	8(8.6%)	18(19.4%)	0.865
No	40(43.0%)	35(37.6%)	75(80.6%)	
Treatment				
Yes	0(0.0%)	3(3.2%)	3(3.2%)	0.058
No	50(53.8%)	40(43.0%)	90(96.8%)	
Age group				
17-27years	27(29.0%)	20(21.5%)	47(50.5%)	
28-38 years	19(20.4%)	18(19.4%)	37(39.8%)	
39-49 years	3(3.2%)	4(4.3%)	7(7.5%)	0.609
50-60 years	0(0.0%)	1(1.1%)	1(1.1%)	
>60 years	1(1.1%)	0(0.0%)	1(1.1%)	

Table (4.3): Antibiotic susceptibility pattern of *N. gonorrhoeae*

Antibiotic	Sensitive	Resistance
Ceftriaxone	50(100.0%)	0(0.0%)
Cefotaxime	50(100.0%)	0(0.0%)
Penicillin	0(0.0%)	50(100.0%)
Ampicillin	2(4.0%)	48(96.0%)
tetracycline	0(0.0%)	50(100.0%)
ciprofloxacin	1(2.0%)	49(98.0%)

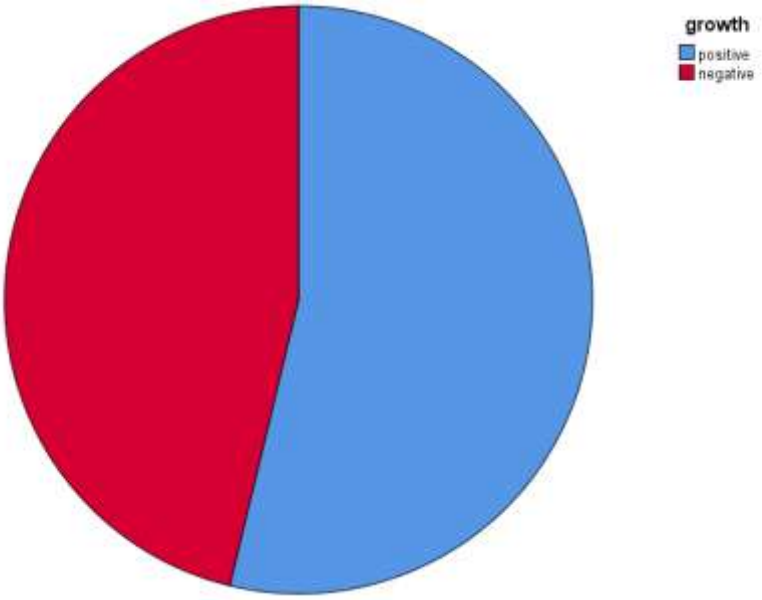

Figure (4.1): Frequency of positive *N. gonorrhoeae* among the study population

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. Discussion

N. gonorrhoeae is a bacterial sexually transmitted pathogen that most commonly infects the lower genital tract, the cervix in women, and anterior urethra in men (Lovet and Duncan, 2018). Uuntreated infection lead to severe complications such as infertility (Unemo and Sikor, 2017).

The current study found that 50/93 (53.8%) of the patients showed positive growth of *N. gonorrhoeae* , lower findings were observed by Mahmoud *et al* (2017) they found 5 (1.2%) in Thayer-Martin culture. Lower findings observed by Geremew *et al* (2017) in Ethiopia found 20.8% *N. gonorrhoeae* .The percentage of positive result was high may be due to the patients were male and symptomatic.

In the current study out of 93 specimen males showed positive growth of *N. gonorrhoeae* were 49/93(52.7%) the same finding was observed by Budkaew and his colleagues (2019) who found 159/ 358 (54.47%) male tested were positive for gonorrhea.

In this study female were positive growth for *N. gonorrhoeae* 1/93 (1.1%) , Slightly similar findings were observed by Wong *et al* (2015) (0.9%).

In the current study most of the patient were single 31/93 (33.3%) different results were reported by Hailemariam *et al* (2013) they found most of the study subjects, 118 (54.9%) were married. May be due to strict Islamic role regarding married people the percentage of single population with positive result was high.

In this study 9/93 (9.7%) of patients had multiple sex partners with positive growth of *N. gonorrhoeae* although Pillay and his college (2018) found the multiple sex partners will had higher risk of becoming infected.

The current study showed 10/93 (10.8%) patients with history of *N. gonorrhoeae* infection had positive growth , Slightly higher findings were observed by Bautista *et al* (2017) who found 14.4% of the women had at least one repeat infection and 13.7% of the men .

In this study all isolates were resistant to penicillin and tracycline similar to Owusu *et al* (2018) who found increasing resistance to penicillin and tetracycline.

In this study all the isolated *N. gonorrhoeae* were sensitive to third generation cephalosprine (Ceftriaxone and cefotaxime) this finding match the Canadian guidelines recommend either intramuscular (IM) ceftriaxone or oral cefixime as the preferred antibiotic of choice (Piszczek *et al*,2015).

In this study the most frequency of infection in the age group 17-27 years 27(29.0%) , slightly similar findings reported by Owusu *et al* (2018) who found 15–49 years was the most frequent age group infected with *N. gonorrhoeae.* Slightly similar finding was observed by Hailemariam and his colleagues (2013) in which 5/11 (45.5%) in age group of 20–24 years were positive for *N. gonorrhoeae.*

5.2. Conclusion

The frequency of *N. gonorrhoeae* in this study was high . There is no association between sex, age, marital status, multiple sex partners, history of gonorrhea, treatment and *N. gonorrhoeae* growth.

All *N. gonorrhoeae* isolates showed high susceptibility pattern to third generation cephalosporin (Ceftriaxone and cefotaxime) and resist to penicillin and tetracycline.

5.3.Recommendations

Research is needed to better understand the current trends in gonorrhea infection in order to maintain, evaluate and improve primary and secondary STI prevention activities.

Because gonorrhea is often asymptomatic in women, screening is critical for the identification of infection and the prevention or limitation of upper genital tract spread, and horizontal and vertical transmission.

The necessity of establishing national guidelines and/or screening program utilising improved technique for the detection *N. gonorrhoeae* among high risk groups in the kingdom.

Further studies are needed to measure the adverse reproductive outcomes associated with STIs in SA. There is need for point of care STI screening services to be introduced in clinics.

Antibiotic sensitivity monitoring for STIs should be instituted. A National policy on the rational use of antibiotics for the management of STIs should be Formulated. Many females do not like to go to clinics because of the cultural herting in SA . Doctors should requested all possible STDs cases for gonorrhea in order to prevent misdiagnosis.

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Appendices

Appendix I

Sudan University of Science and Technology

College of Graduate Studies

Questionnaire

Frequency and Susceptibility Pattern of *Neisseria gonorrhoeae* among Symptomatic Patients in Alriyadh Private Hospital, Saudi Arabia

No……. Date……

1/Age:……

2/Patient's status:

Inpatient (….) Outpatient(….)

3/Marital status: Not married (….) married (….)

4/ Multiple sex partners ? Yes (….) No (….)

5/ History of STD? Yes (….) No (….)

6/ Treatment? Yes (….) No (….)

Spesify ……..

7 / Symptoms?

urethral discharge (….) dysuria (….),frequency of urination (….), irritation (….), penile discharge (….), red or swollen urethra (….), pain on urination (….), itch (….), testicular or rectal pain (….), vaginal discharge (….)

8/Results:

I/ Direct Gram's stain……

II/ Culture……..

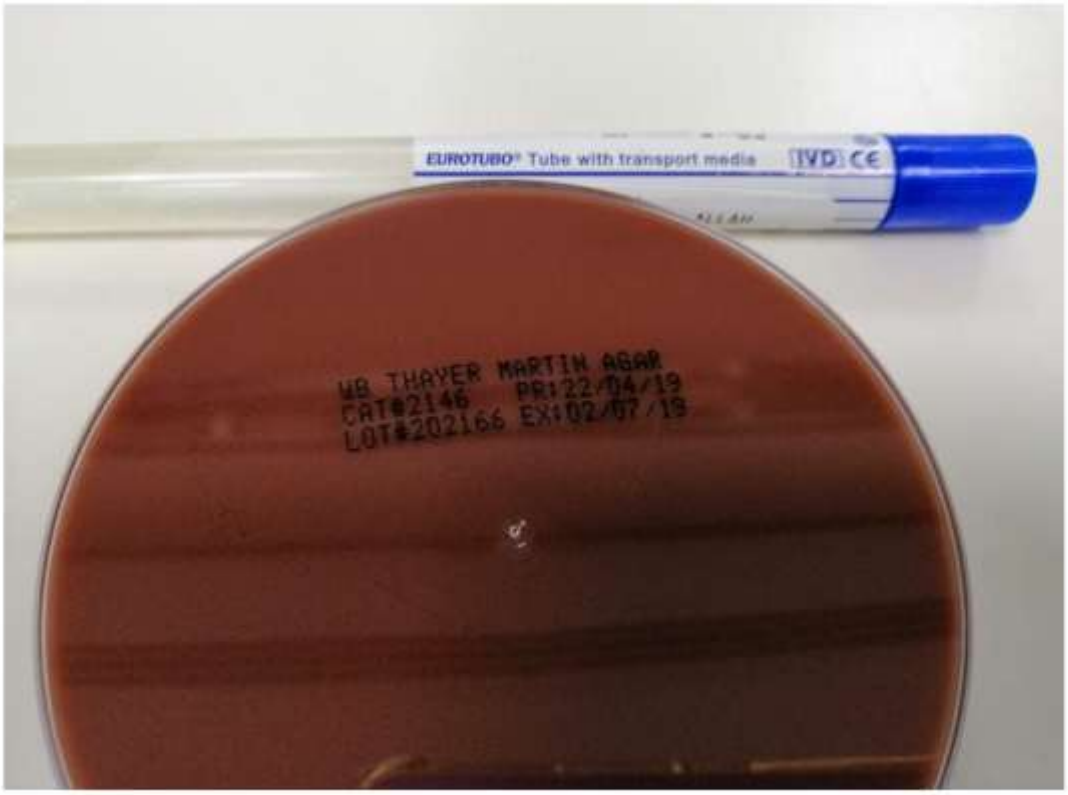
III/ Suseptibility Test

Sensitive……

Intermediatly…….

Resistant……

Appendix 2



Color plate (1): Amies transport medium

Appendix 3

Gram's stain

Crystal violet ……20 gm

Ammonium oxalate …….99gm

Ethanol……..95 gm

Distil water……..……..1 L

Potassium iodine ……20gm

Iodine……..10 gm

Distilled water…….……1 L

Acetone –alcohol decolorizer:

Acetone ……..500 ml

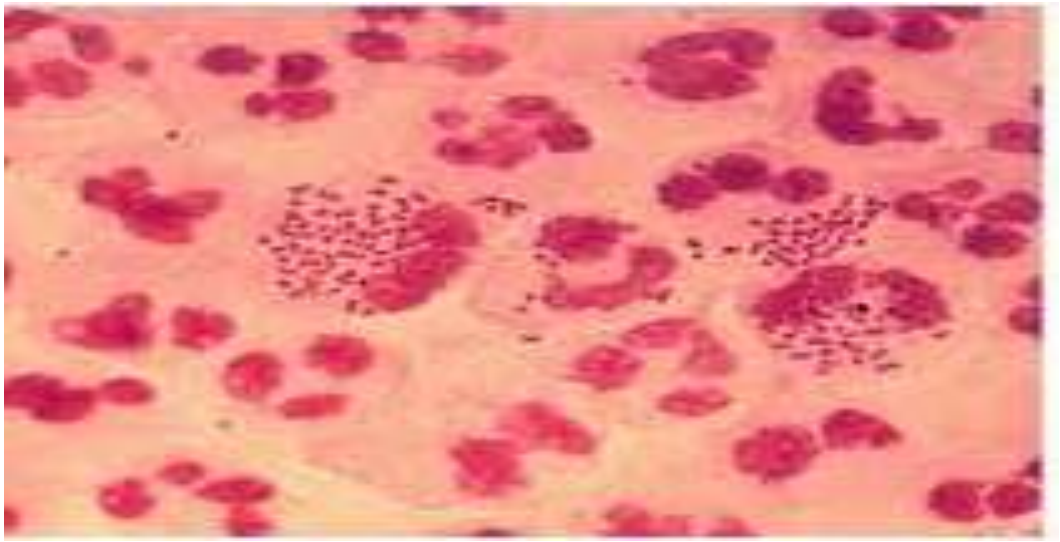
Ethanol or methanol absolute ……465 ml

Distilled water…….……..25 ml

Saffranin……..……..0.54 g

Distilled water……..…….100 ml

Appendix 4



Color plate (2): *N. gonorrhoeae* on Gram stain

Appendix 5

Preparation of Thayer Martin Medium

Composition**

Peptone, special…….. 23.000 Gms / Litre

Starch……. 1.000 Gms / Litre

Sodium chloride……. 5.000 Gms / Litre

Agar…… 13.000 Gms / Litre

pH…….. 7.0±0.2

Directions

Suspend 21.0 grams in 450 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45°C. Aseptically add 50 ml of sterile lysed blood and rehydrated contents of one vial of Vitamino Growth Supplement (FD025) and V.C.N Supplement (FD023) or V.C.N.T Supplement (FD024). If desired GC Supplement with Antibiotics (FD021) can be used as a single supplement. Mix well before pouring into sterile Petri plates. If Hemoglobin (FD022) is used suspend 21.0 grams of Thayer Martin Medium Base in 250 ml distilled water.Heat to boiling to dissolve the medium completely. Prepare 250 ml of 2% hemoglobin solution. Sterilize separately by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45°C. Mix both and add the supplements as above.

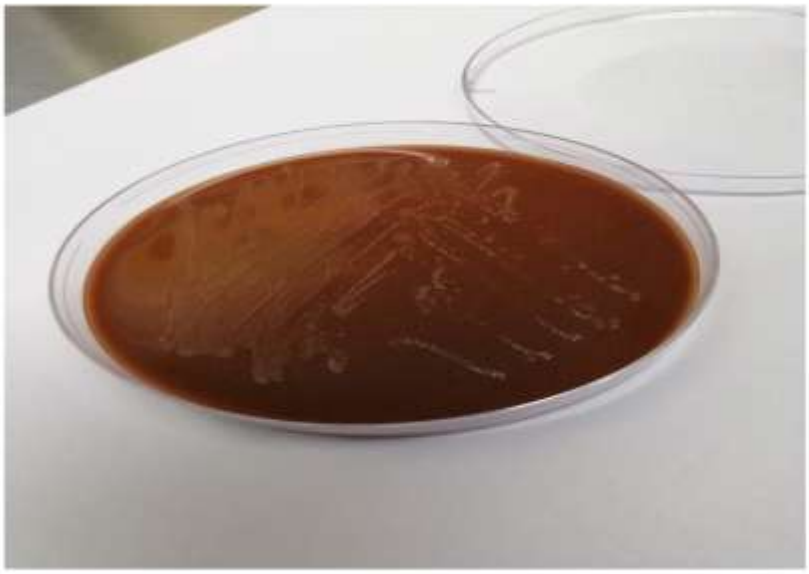
Appendix 6

Preparation of Chocolate Agar

Heat lyse a volume of horse or sheep blood that is 5% of the total volume of media being prepared very slowly to 56oC in a water bath. Dispense 20 ml in to 15x100mm Petri dishes. Allow the media to solidify and condensation to dry.

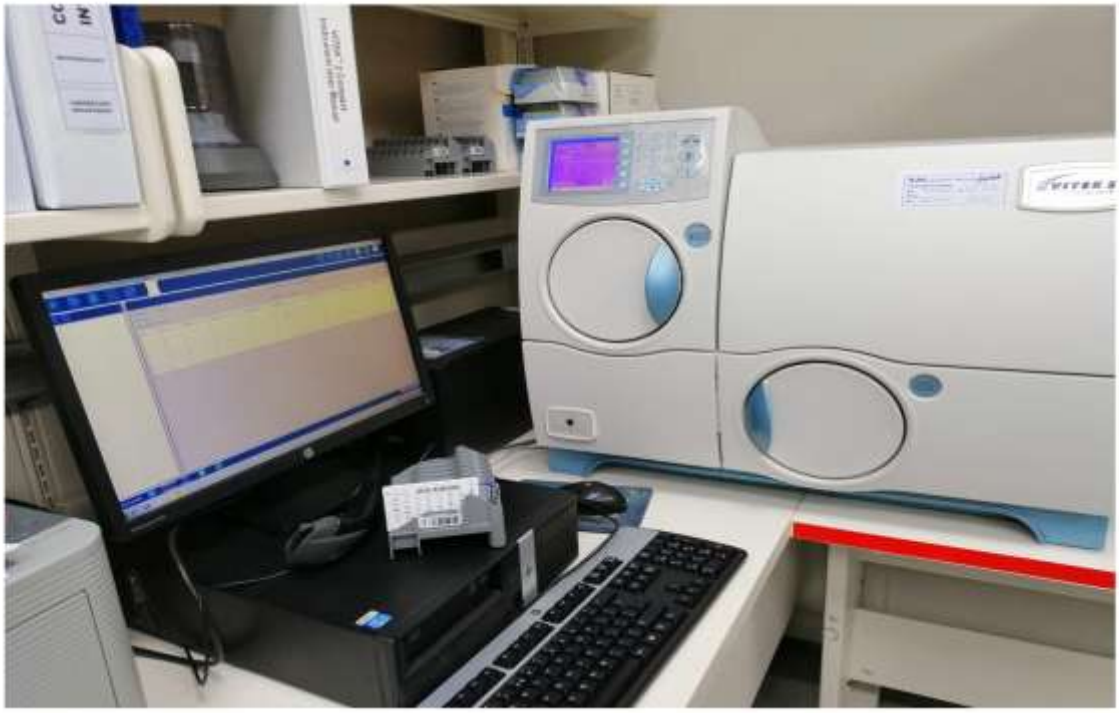
Place the plates in sterile plastic bags and store at 4oC until use. As a sterility test incubate an un inoculated plate for 48 hours at 35 – 37 o C with 5% Co2 (or in a candle- jar).

Appendix 7



Color plate (3): Growth of *N. gonorrhoeae*

Appendix 8

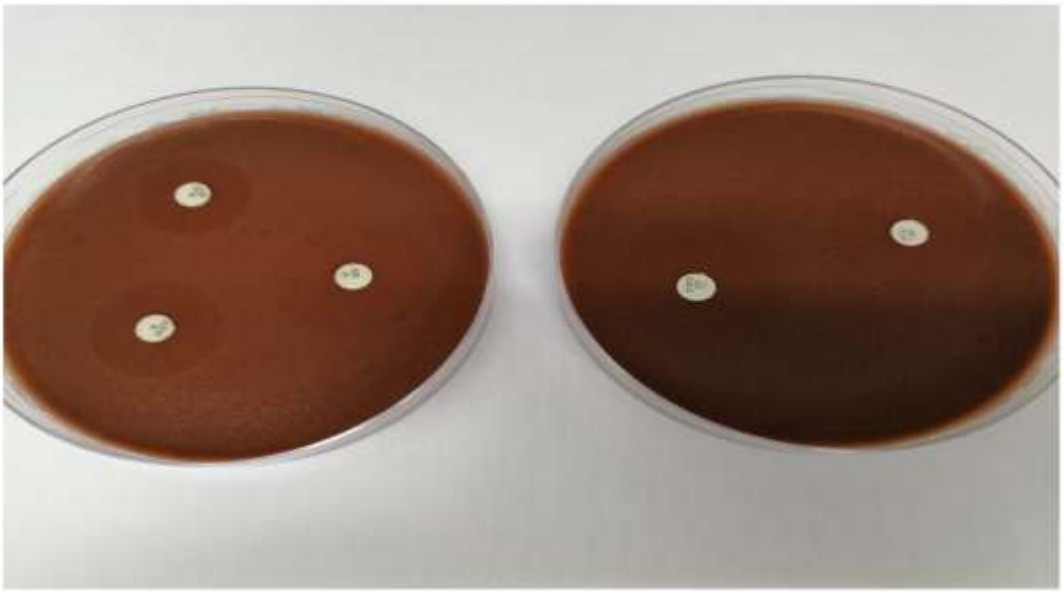


Color plate (4): Vitek 2 Compact system

Appendix 9

Color plate (5): VITEK 2 DensiCHEK Plus

Appendix 10



Color plate (6): Antimicrobial susceptibility test of *N. gonorrhoeae*