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DIAGNOSTICS AND CHARACTERIZATION OF *NEISSERIA GONORRHOEAE* INFECTION IN THI-QAR PROVINCE

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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MOHAMMED JASIM THAJEEL AAL-EZIREJ

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DIAGNOSTICS AND CHARACTERIZATION OF *NEISSERIA GONORRHOEAE* INFECTION IN THI-QAR PROVINCE

By Mohammed Jasim Thajeel AAL-EZIREJ

January 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

Advisor : Asst. Prof. Dr. Okan ÜRKER
Co-Advisor : Asst. Prof. Dr. Ashraf Fadhil JOMAH

Examining Committee Members:

Chairman : Asst. Prof. Dr. Okan ÜRKER
Biology
Çankırı Karatekin University

Member : Asst. Prof. Dr. Bahadır AKMAN
Department of Agricultural Sciences
Iğdır University

Member : Asst. Prof. Dr. Emin BOZKURT
Biology
Çankırı Karatekin University

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. İbrahim ÇİFTÇİ
Director of Graduate School

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Mohammed Jasim Thajeel AAL-EZIREJ

ABSTRACT

DIAGNOSTICS AND CHARACTERIZATION OF *NEISSERIA GONORRHOEAE* INFECTION IN THI-QAR PROVINCE

Mohammed Jasim Thajeel AAL-EZIREJ

Master of Science in Biology

Advisor: Asst. Prof. Dr. Okan ÜRKER

Co-Advisor: Asst. Prof. Dr. Ashraf Fadhil JOMAH

January 2023

The aim of this research is conducted to examine the *Neisseria gonorrhoeae* and detection some indicators of *Neisseria gonorrhoeae* infection from patients in Thi-Qar province and comparison of conventional versus molecular methods as related to the detection of *Neisseria gonorrhoeae*. The research was conducted in Nasiriyah City/Dhi-Qar Governorate, Iraq. 100 samples were collected from male and female patients suspected to have gonorrhea (urethral discharge with dysuria) from different regions in Nasiriyah City (75 males and 25 females, aged 18-45 years). were involved and a special form of information was filled in for each patient. direct swabs were taken from patients who attended Nasiriyah Hospitals Clinics and private laboratories. Those cases that were caused by gonococci were diagnosed by locating the *Neisseria gonorrhoeae* bacteria in the samples. The results of the microscopic examination gave 95 samples of *N. gonorrheae* out of the total samples of 100, after staining them with gram stain, as the cells appear under the microscope Gram negative intracellular diplococci (G.N.I.D) within polynuclear leukocytes, after performing gram stain chocolate agar was utilized as an enriched media for each sample our results from cultivating it on chocolate showed that 95 samples (95.0%) also were positive out of the total samples of 100 samples, but after replanting it on the culture medium modified Thayer martin media revealed that 90 (90.0%) out of 100 positives. All isolates that showed culture and microscopic characteristics identical to those of *Neisseria gonorrhoeae* were subjected to biochemical tests to confirm the diagnosis. 90 *N. gonorrhoeae* isolates were chosen for genetic testing in this research in order to confirm the results of microscopy and culturing, The findings

of the molecular diagnostic of the bacteria that cause gonorrhea using particular primers that were specific for the *Orf1* gene indicated that 82 (91.11 %) of samples, out of a total of 90, were positive for the bacterium. We tested the susceptibility of several *Neisseria* isolates. resistance to penicillin and tetracycline was quite widespread (91.11% and 97.78%, respectively), and this research also discovered that 83.33% of *N. gonorrhoeae* were resistant to at least one class of antibiotic. gonorrhoeae were resistant to ciprofloxacin, however, ceftriaxone and spectinomycin were effective against one hundred percent of the isolates tested.

2023, 66 pages

Keywords: *Neisseria gonorrhoeae*, Gonorrhea, Virulence factors

ÖZET

THI-QAR İLİNDEKİ *NEISSERIA GONORRHOEAE* ENFEKSİYONUN TEŞHİSİ VE KARAKTERİZASYONU

Mohammed Jasim Thajeel AAL-EZIREJ
Biyoloji, Yüksek Lisans
Tez Danışmanı: Dr. Öğr. Üyesi Okan ÜRKER
Eş Danışman: Dr. Öğr. Üyesi Ashraf Fadhil JOMAH
Ocak 2023

Bu araştırmanın amacı, Thi-Qar ilindeki hastalardan *Neisseria gonorrhoeae* ve *Neisseria gonorrhoeae* enfeksiyonunun bazı göstergelerini tespit etmek ve *Neisseria gonorrhoeae*'nin tespiti ile ilgili olarak geleneksel ve moleküler yöntemLerin karşılaştırılmasını yapmaktır. Araştırma Nasiriyah City/Dhi-Qar Governorate, Irak'ta gerçekleştirilmiştir. Nasiriye Şehrinin farklı bölgelerinden gonore (dizüri ile birlikte üretral akıntı) olduğundan şüphelenilen erkek ve kadın hastalardan (75 erkek ve 25 kadın, 18-45 yaş arası) 100 örnek toplanmış ve her hasta için özel bir bilgi formu doldurulmuştur. Nasiriye Hastaneleri Kliniklerine ve özel laboratuvarlara başvuran hastalardan doğrudan sürüntü örnekleri alınmıştır. Örneklerde *Neisseria gonorrhoeae* bakterisi tespit edilerek gonokokların neden olduğu vakalar teşhis edilmiştir. Mikroskobik inceleme sonucunda toplam 100 örnekten 95'inde N. gonorrheae tespit edilmiştir. Gram boyası ile boyandıktan sonra, hücreler mikroskop altında polinükleer lökositler içinde Gram negatif hücre içi diplokoklar (G.N.I.D) olarak görünmektedir. Gram boyaması yapıldıktan sonra her numune için zenginleştirilmiş besiyeri olarak çikolata agar kullanıldı ve ekim sonuçlarımız toplam 100 numuneden 95 numunenin (%95,0) pozitif olduğunu gösterdi. Ancak, modifiye edilmiş Thayer martin besiyerine tekrar ekildikten sonra 100 pozitif örnekten 90'ının (%90,0) pozitif olduğu görülmüştür. *Neisseria gonorrhoeae* ile aynı kültür ve mikroskobik özellikleri gösteren tüm izolatlar, tanıyı doğrulamak için biyokimyasal testlere tabi tutulmuştur. Doksan *N. gonorrhoeae* izolatı, mikroskopi ve kültür sonuçlarını doğrulamak amacıyla bu araştırmada genetik testler için seçilmiştir, *Orf1* genine spesifik özel primerler kullanılarak gonoreye neden

olan bakterilerin moleküler tanısına ilişkin bulgular, toplam 90 örnekten 82'sinin (%91,11) bakteri için pozitif olduğunu göstermiştir. Penisilin ve tetrasikline karşı direnç oldukça yaygın olan çeşitli N. izolatlarının duyarlılığı test edilmiştir (sırasıyla %91,11 ve %97,78) ve bu araştırma ayrıca *N. gonorrhoeae*'nin %83,33'ünün en az bir antibiyotik sınıfına dirençli olduğunu ortaya çıkarmıştır. Gonorrhoeae siprofloksasine dirençlidir, ancak seftriakson ve spektinomisin test edilen izolatların yüzde yüzüne karşı etkilidir.

2023, 66 sayfa

Anahtar Kelimeler: *Neisseria gonorrhoeae*, Gonore, Virülans faktörleri

PREFACE AND ACKNOWLEDGEMENTS

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Mohammed Jasim Thajeel AAL-EZIREJ
Çankırı-2023

CONTENTS

LIST OF SYMBOLS

%	Percentage
≤	Greater or equal to
≥	Less than or equal to
°C	Degree celsius
µg	Microgram
bp	Base pair
cm	Centimeter
g	Grams
Ib	Dollars per pound
L	Liter
M	Molar
mg	Milligram
min	Minute
mL	Mililiter
mM	Milimolar
mm	Millimeter
pH	Power of hydrogen
pmol	Picomole
Psi	Pounds per square inch
U	Unit
V	Volume
β	Beta
µL	Microliter
µm	Micrometer

LIST OF ABBREVIATIONS

ACF	Colonization factor antigen
AST	Antibacterial susceptibility test
BHI	Brain heart infusion
CDC	Center for disease control
CIP	Ciprofloxacin
CRO	Ceftriaxone
CSWs	Commercial sex workers
DGI	Disseminated gonococcal infection
Fc	Fragment, crystallizable
Fcb	Fragment, antigen binding
G.N.I.D	Gram negative interacellular diplococcic
LOS	lipopolysaccharide
MSM	Men who have sex with men
NAD	Nicotine adenine dinucleotide
NCCL	National committee for clinical laboratory
PCR	Polymerase chain reaction
PEN	Penicillin
pH	Power of hydrogen
PI	Protein
PID	Pelvic inflammatory disease
PII	Protein II
PIII	Protein III
PMNs	Polymorphonuclear cells
Rmp	Reduction-modifiable proteins
STI	Sexually transmitted illness
STM	Spectinomycin
Tbp	Transferrin binding proteins
TET	Tetracycline
WHO	World health organization

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1. INTRODUCTION

Gonorrhea is a sexually transmitted infection (STI) that is still a serious problem for public health on a worldwide scale. The infectious agent that causes gonorrhea is *Neisseria gonorrhoeae*, which is also known as the gonococcus. The monitoring of clinical strains of *N. gonorrhoeae* conducted by the World Health Organization (WHO) has uncovered strains that are resistant to the majority of the medicines that are currently in use. This highlights the immediate danger of widespread gonorrhea that cannot be treated. The World Health Organization has developed treatment guidelines with the dual goals of eradicating the organism and stopping the future spread of gonorrhea that is resistant to antimicrobials (Carmona-Gutierrez *et al.* 2016). Gonorrhea, which has an annual incidence of over 78 million cases worldwide, uncontrolled transmission, and limited treatment options in both low-income countries and poorer communities in developed countries, will cause an increase in the incidence of infection and complications from infection if it is not treated. This is due to the fact that gonorrhea has an annual incidence of over 78 million cases worldwide (Carmona-Gutierrez *et al.* 2016, Unemo *et al.* 2016, Newman 2015).

Although the vaginal mucosa is where *N. gonorrhoeae* is most often found to colonize, the organism is also capable of colonizing the anal, ocular, and nasopharyngeal mucosa (Lee *et al.* 2002, Danby *et al.* 2016). Pathology is often the consequence of damage brought on by the activation of innate immune responses at the sites of colonization since *N. gonorrhoeae* does not express potent exotoxins. In women, if an infection in the ascending genital tract goes untreated, it may lead to a variety of issues, including pelvic inflammatory disease, infertility, and ectopic pregnancy (Hupp 2011). It is also possible for mothers to pass on the condition of newborn blindness to their offspring while they are still in the womb. If left untreated, infection with *N. gonorrhoeae* may progress to a disseminated gonococcal infection, which has the potential to cause infectious arthritis and endocarditis.

Neisseria gonorrhoeae and *Neisseria meningitidis*, generally known as the meningococcus, are the two pathogenic species belonging to the Neisseria genus. *Neisseria meningitidis* is the most common bacterial cause of bacterial meningitis. *N. gonorrhoeae* is a member of the genus Neisseria (Hoffman and Weber 2009). In addition, there are at least eight commensal non-pathogenic Neisseria species that make up a major portion of the human nasal and oropharyngeal flora (Marri 2012). Other Neisseria species may colonize a variety of non-human mammalian and non-mammalian hosts, including non-human primates, dogs, cats, herbivorous mammals, dolphins, birds, and insects. Non-human primates are also mammalian hosts that other Neisseria species can colonize (Liu *et al.* 2015). Phylogenetic investigations suggest that *N. gonorrhoeae* and *N. meningitidis* descended from the same ancestor but have since diverged into two different lineages that generally reside in two discrete habitats. These niches are the vaginal mucosa and the nasopharyngeal mucosa, respectively (Bratcher *et al.* 2014, Joseph *et al.* 2011, Maiden 2008). *N. gonorrhoeae* cannot survive being dehydrated or being exposed to temperatures that are outside of what is normal for them. This is in contrast to *N. meningitidis*, which can endure being separated from its human host for extended periods of time and spread through respiratory droplet transmission. The events that led to the evolution of two separate organisms that are highly similar in their core genome and physiology but cause markedly different diseases in different locations. It is sometimes difficult to identify colonization factors from the virulence factors that are essential to elicit host harm since both commensal and pathogenic Neisseria spp. occupy the same environments. Because of this, it may be challenging to distinguish between the two (Bratcher *et al.* 2014).

1.1 Aims of the Study

The importance of the thesis included the molecular biochemical test of *Neisseria gonorrhoeae.*

2. LITERATURE REVIEW

2.1 Gonorrhoea

The most significant factor that causes gonorrhea to cause epidemics is its short incubation period and ability to spread from an infected person to a healthy person via the reproductive system. Gonorrhea is one of the most common diseases in the world and is one of the most infectious and endemic diseases that cause epidemics (Morgan and Decker 2016). The primary cause of sexually transmitted diseases and gonorrhea worldwide is the bacterium Neisseria gonorrhoaea. When it comes to sexually transmitted bacterial infections, gonorrhea comes in second place behind chlamydia (Figure 2.1). It could spread throughout the body, harming the heart valves and joints (Handsfield *et al.* 2005).

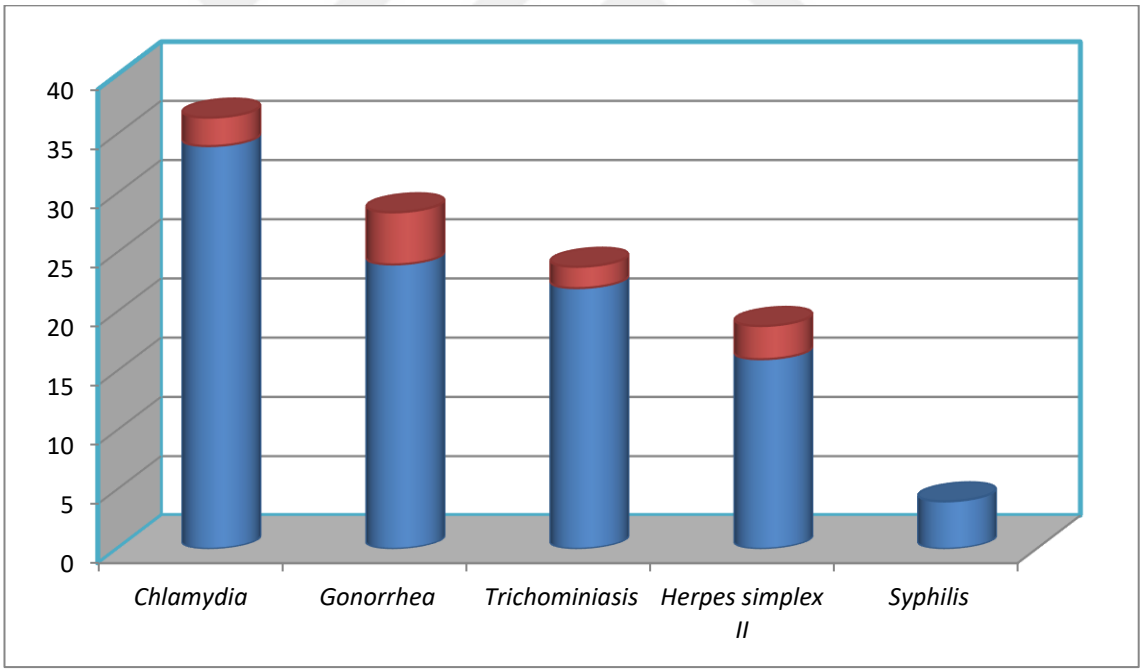

Figure 2.1 Global estimates of sexually transmitted diseases (CDC 2007)

Gonorrhea is one of the first recognized sexually transmitted illnesses (STIs). Regarding its precise origin, there is considerable disagreement, although the general assumption is that the sickness has existed since ancient times (Jose *et al.* 2020). The Chinese emperor

Huang Ti (2600 BC) mentioned an illness resembling gonorrhea in his handbook (Lee and Ladizinski 2012). It is thought that the reference to "a seed problem" in the Book of Leviticus in the Old Testament and the advised measures allude to this sickness (Jose *et al.* 2020). Hippocrates (460–375 BC) referred to gonorrhea as "strangury" and believed it was caused by "Venus' delights". Celsus (25 BC–50 AD) had a thorough understanding of gonorrhea and its consequences. He used to catheterize urethral stricture patients (Gruber *et al.* 2015). Galen (131–200 AD), a Greek physician, invented the word "gonorrhea" and defined it as "an undesired discharge of sperm" (gono: seed, rhea: flow) (Morgan and Decker 2016).

Because the symptoms of the illness are similar to the first symptoms of syphilis, I originally thought that gonorrhea was one of the signs of syphilis. This belief persisted until 1838, when the vaccination of men with pus established that gonorrhea and syphilis are two separate diseases (Qvist 1977).

In 1879, Albert Ludwig Sigesmund Neisser identified the pathogen *Neisseria gonorrhoeae*. His findings were first published in 1882 (Neisser 1932). In 1882 and 1885, Leistikow and Bumm were successful in cultivating the organism in a culture medium. By inoculating the organism into the male urethra, the latter was able to create the classic symptoms and indications of infection. In 1906, Muller and Oppenheim introduced the gonococcal complement fixation test, which was an additional accomplishment (Jose *et al.* 2020).

2.2 Modes of Transmission

People infected with gonorrhea are the only source of infection because the germs are transmitted to a healthy person through sexual contact, so gonorrhea is considered an STD (Sexually Transmitted Disease). In some cases, gonorrhea infection is transmitted by touching the infected person, by rubbing against the affected area, or by using wet clothes and toilet seats contaminated with the gonorrhea germ. Among the factors affecting infection with gonorrhea are the rate of sexual contact and the number of sex partners (Winn *et al.* 2006).

Gonorrhea may affect newborn babies as a result of direct infection from a mother infected with gonorrhea during the birth process, causing Gonococcal ophthalmia, a condition known as congenital ophthalmia unless preventive measures are taken. Infants born to women are infected with gonorrhea, as it affects the skin of the cornea and causes bacterial keratitis and ulceration shown in Figure 2.2. If not treated promptly, it leads to vision loss (Woods 2005, Malhotra *et al.* 1998).

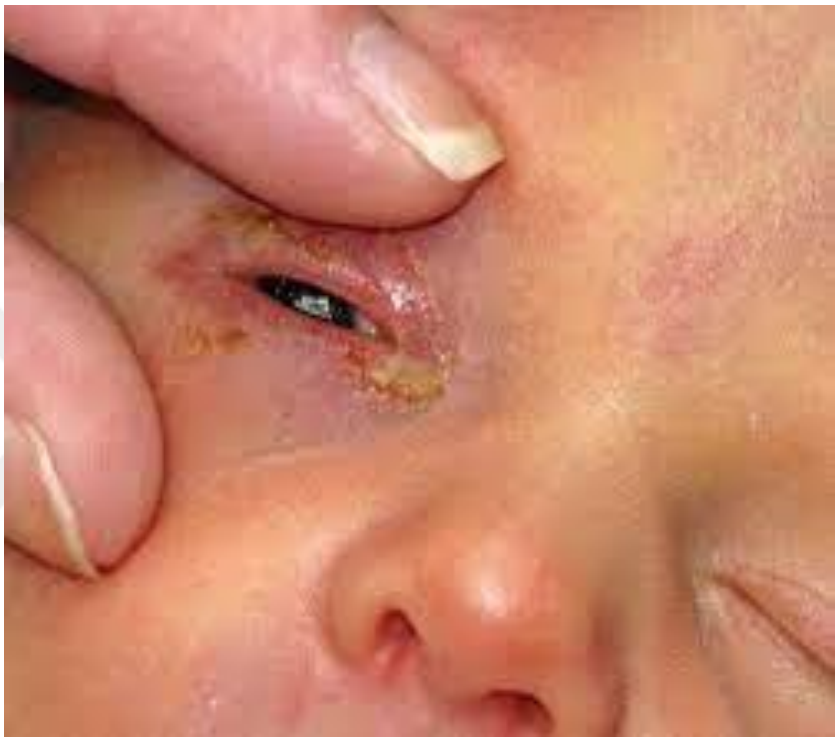

Figure 2.2 A infant with Gonococcal ophthalmia (Woods 2005)

2.3 Overview of *Neisseria gonorrhoeae*

Neisseria gonorrhoeae is a bacterial pathogen that causes gonorrhea, a sexually transmitted illness. *N. gonorrhoeae* has preyed on people since antiquity, and ancient Greek, Biblical, and Chinese manuscripts make reference to it (Edwards and Apicella 2004). The ancient Greek physician Galen coined the term "gonorrhea," which means "flow of seed." The bacteria was found by Neisser in 1879 (Edwards and Apicella 2004). Prior to the discovery of efficient antibiotic treatments for *N. gonorrhoeae*, conventional treatment regimens included urethral irrigation, abstention from alcohol and sexual

activity, rest, and systemic therapy with different balsams (Oriel 1994). Successfully cultivated *N. gonorrhoeae,* and with the invention of Gram staining (Gram 1884) and the carbohydrate oxidation test, the diagnosis of gonorrhea greatly improved. The discovery of sulphonamides for the treatment of gonorrhea (Oriel 1994) and, subsequently, penicillin provided optimism for the eradication of gonorrhea. As it turns out, *N. gonorrhoeae* has a remarkable capacity to adapt and withstand any antibiotic treatment, and it continues to be a global public health and STI issue. New phenotypic and genotypic information on *N. gonorrhoeae* has clarified the bacterium's capacity to avoid the human immune system and environmental dangers such as antimicrobials. Gonorrhea will continue to exist.

2.4 Clinical Manifestations of Gonococcal Infection

2.4.1 Men

Men with urethral gonorrhea frequently experience painful urination and/or urethral discharge two to five days after infection (Mayor *et al.* 2012). In the absence of therapy, the majority of urethral infections heal within a few weeks (Hook and Handsfield 2008). Epididymitis, which may cause testicular or scrotal discomfort, is one of the possible problems for males. Rarely, epididymitis might result in male infertility. While asymptomatic gonorrhea is less frequent in males than in women, many men suffer from it, thereby increasing their risk of problems if they neglect to seek treatment (Hook and Handsfield 2008)

2.4.2 Women

Women's endocervical canal is the site of urogenital infections, which are often asymptomatic. When they do, symptoms often appear 10 days after exposure. Painful urination, excessive vaginal discharge, or midcycle vaginal hemorrhage are examples of symptoms (Hook and Handsfield 2008). However, signs and symptoms are often modest and might be confused with vaginal or bladder infections. Gonococcal infection in women

may result in significant problems even in silent infections. Pelvic inflammatory disease (PID) may develop if the infection extends to the uterus or fallopian tubes. PID may lead to internal abscesses, severe pelvic pain, and damage to the fallopian tubes that increase the chance of ectopic pregnancy or infertility (Soper 2010). Gonorrhea during pregnancy is especially dangerous since an infected mother might infect her unborn child while giving birth. This may cause the infant's eyes to get infected, which might end in blindness (Woods 2005). Another severe possible gonorrhea consequence in infants is diffused gonococcal infection (DGI), which often develops one to four weeks after delivery (Woods 2005).

2.4.3 Men and women

Both sexes are susceptible to pharyngeal and rectal infections, but men who have intercourse with other males are more likely to get these conditions (MSM) (Hook and Handsfield 2008). Rectal infections may not cause any symptoms at all or they may cause bowel motions that are unpleasant, sore, discharge, and/or bleed. Although pharyngeal infection usually has no symptoms, it may nonetheless result in a sore throat. Untreated gonorrhea may result in DGI, which can be fatal in both men and women. Chlamydia and gonorrhea are often co-infected in men and women with gonorrhea (Lyss *et al.* 2003). Additionally, research has shown that gonorrhea and other STI infection promotes HIV transmission (Johnson and Lewis 2008).

2.5 Epidemiology of *Neisseria gonorrhoeae*

Epidemiological factors are concentrated in the occurrence and spread of gonorrhea infection with factors related to the germ itself, such as the presence of cilia, outer membrane, cell proteins, biochemical growth requirements, pathological severity, and sensitivity to anti-life agents, and with factors belonging to the main host, which is the human being, such as age, gender, race, sexual behaviors, social determinants, and body immunity, and these factors vary according to the body. According to the nature of the geographical area (Sullivan *et al.* 2011).

Gonorrhea is one of the most prevalent sexual diseases, and this disease is associated with high rates of infection and the social and economic consequences of this. This remains a public health problem around the world, as it affects hundreds of millions annually. Many reports indicate that the incidence of it is steadily increasing in several regions of the world, especially in the last two decades (Tapsall 2009).

Farrar *et al.* (1997) estimated that three million new cases of gonorrhea occur annually in the United Kingdom. Gross and Tyring (2011) indicated that the rate of this disease in the United States amounted to 600.000 cases annually in 1996 and that 85% of these recorded cases occurred in Hispanic and African Americans. Infection of adolescent girls with gonorrhea is five times more than others.

One of the reasons that led to the recent spread of gonorrhea is the emergence of cases of infection of the rectum, outlet, and pharynx with Neisseria gonorrhea, and these unexpected cases of gonorrhea have epidemiological importance in the spread of the disease, especially among homosexual males, as well as the increase in cases of no symptoms for asymptomatic gonorrhea in men and women (Workowski *et al.* 2008).

A number of studies indicated that women carry the bacterium Neisseria gonorrhea, and they do not show symptoms of the disease, as they are considered a reservoir of transmission of the bacterium in the community (Sweet and Gibbs 2002). Another reason is the emergence of a case of gonorrhea of penicillin-resistant strains, and this infection led to the difficulty of treatment and thus to an increase in its spread (Kirkcaldy *et al.* 2011).

Dunkelberg *et al.* (1970) found that the incidence of gonorrhea in women attending an inpatient clinic for sexually transmitted diseases in the US state of Georgia was 40%, while the incidence of Trichomonas vaginalis was 57% and Haemophilus vaginalis was 31%. Ridgway and Oriel (1977) found that the incidence of gonorrhea in women attending a hospital in London was 19%, and chlamydia in 24% out of 1323 women. Edwards *et al.* (1978) conducted an epidemiological study at Paul-Ramsey Hospital in Minnesota, USA, during the years 1970-1977, in which 6464 pregnant women were

examined, and it was found that the rate of gonorrhea in women was 18%. Tabrizi *et al.* (1997) found that the incidence of gonorrhea in women attending STI inpatient clinics in the Northern Territory of Australia was 11%, while the incidence of Trichomonas vaginalis and chlamydia was 16% and 5%, respectively. A study was conducted in the city of Plovid in Bulgaria on 156 women, their ages ranged between 15-45 years, and the incidence of gonorrhea was 5% (Tchoudomirova *et al.* 1998). In 2005, the World Health Organization (WHO 2005) conducted a global assessment of the incidence of gonorrhea, as shown in Figure 2.3.

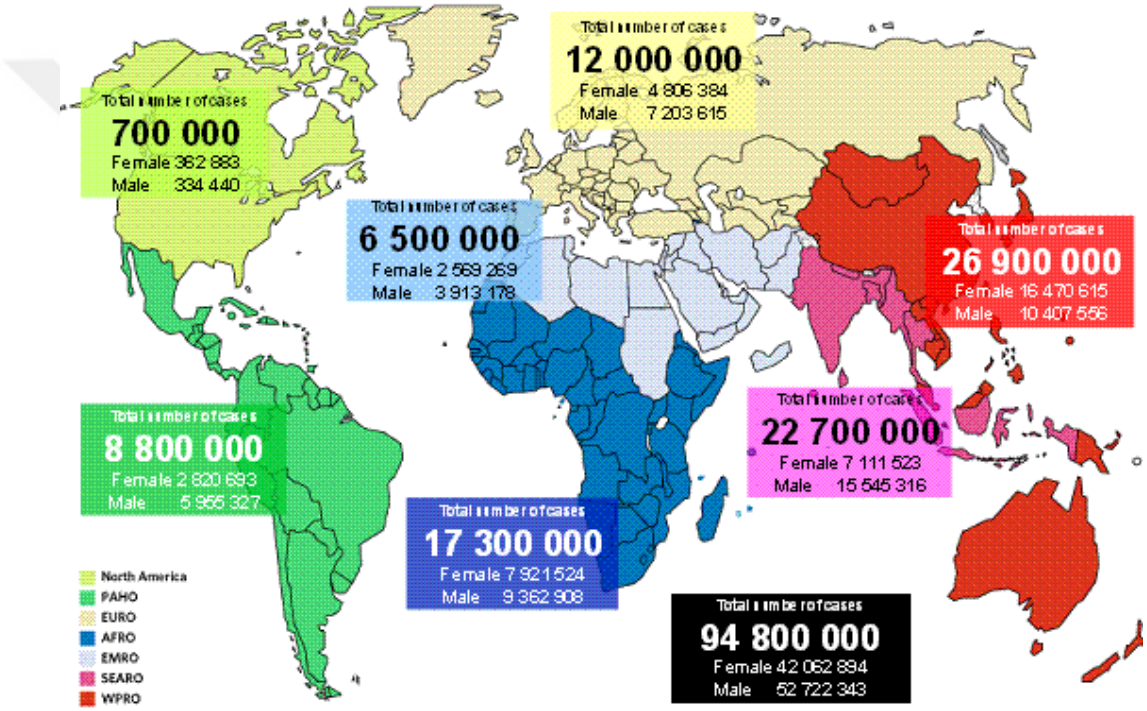

Figure 2.3 Global estimatid incidence of gonorrhoeae (WHO 2005)

2.6 Classification of *Neisseria gonorrhoeae*

Neisseria gonorrhoeae is classified under Kingdom: Bacteria, Phylum: Proteobacteria. Classification within a phylum depends on the internal organization of the organism rather than on its external form. The Proteobacteria phylum is the largest group of bacteria and contains different types of pathogenic bacteria such as Helicobacter, Vibrio, Salmonella, and Escherichia, and its classification is based on the ribosomal RNA (rRNA)

sequence. rRNA sequence N. The genus Neisseria and type gonorrhoeae of gonorrhoeae are members of the family Neisseriaceae and the class Beta Proteobacteria, respectively, and the order Neisseriales as in Table 2.1 (Winn *et al.* 2006, Elias *et al.* 2011).

Table 2.1 Classification of *N. gonorrhoeae* (Elias *et al.* 2011)

Classification	
Kingdom	Bacteria
Phylum	Proteobacteria
Class	Beta Proteobacteria
Order	Neisseriales
Family	Neisseriaceae
Genus	*Neisseria*
Species	*gonorrhoeae*

2.7 Characteristics of *Neisseria gonorrhoeae*

Neisseria gonorrhea is a Gram-negative cocci. It is characterized by its oval or spherical shape, with a diameter ranging from 1.0-0.6 μm. It is arranged in pairs. Its cells are Kidney or bean-shaped (Figure 2.4). They are often found with or within Polymorphonuclear cells (PMNs) (Cullimore 2000, Todar 2005).

Figure 2.4 *Neisseria gonorrhoeae* bacteria under an electron microscope (Todar 2005)

Gonorrhea is a non-motile bacteria that is not spores, and the temperature range for its growth is very narrow. The optimum temperature for its growth is 35-37°C and its pH 7.4. Adding 5-10% of carbon dioxide increases its growth (Freingold and Peacocke 1999).

N. gonorrhoeae are very fastidious to the requirements of growth factors, and these bacteria need enriched media such as Thayer-Martin medium and Chocolate medium. The amino acids they need are arginine and hypoxanthine. The nitrogenous base (Uracil) and its many other requirements in culture media include: hemoglobin and nicotine adenine dinucleotide (NAD), and yeast extract and other additives are needed for the growth and isolation of the organism (Janda and Knapp 2003).

The gonorrhea cocci ferment sugar glucose and produce only acid and are positive for the catalase and oxidase tests (Farrar *et al.* 1997). *N. gonorrhoeae* are sensitive to environmental conditions such as temperature extremes, ultraviolet exposure, pH, or drying. Drying kills these bacteria within 1-2 hours, but they can survive for several hours on clothing, body fluids, and other surfaces, and have the ability to survive for 6 to 7 weeks in dried pus (Martin *et al.* 2007).

N. gonorrhoeae appear after an incubation period of 24-18 hours in the form of round and smooth colonies with a diameter of 1-0.5 μm opaque and gray to white in color, and after 48 hours the colonies are larger in size (2-3 μm) (Cullimore 2000, Todar 2005).

2.8 Pathogenesis and Virulence Factors of *Neisseria gonorrhoeae*

N. gonorrhoeae are parasitic bacteria on the human as all attempts made to cultivate and grow these bacteria in other animals have failed. In order for Neisseria gonorrhea to cause infection, it must first attach to the mucous membranes lining the genital tract, and then bind to and penetrate into the columnar epithelial cells (Du *et al.* 2005). The most important virulence factor of these bacteria are their ability to change their surface antigens outside and inside the body, thus being able to overcome the immune system, in

addition to containing cilia (pilli) that help them to adhere to mucosal surfaces and surface proteins such as pro, rpm and lipopolysaccharide (LOS) (Quillin and Seifert 2018). The strains secrete the enzyme Pencillinase, which prevents the binding of penicillin and then bacteria become resistant to it and other virulence factors (Liao *et al.* 2008) (Figure 2.5).

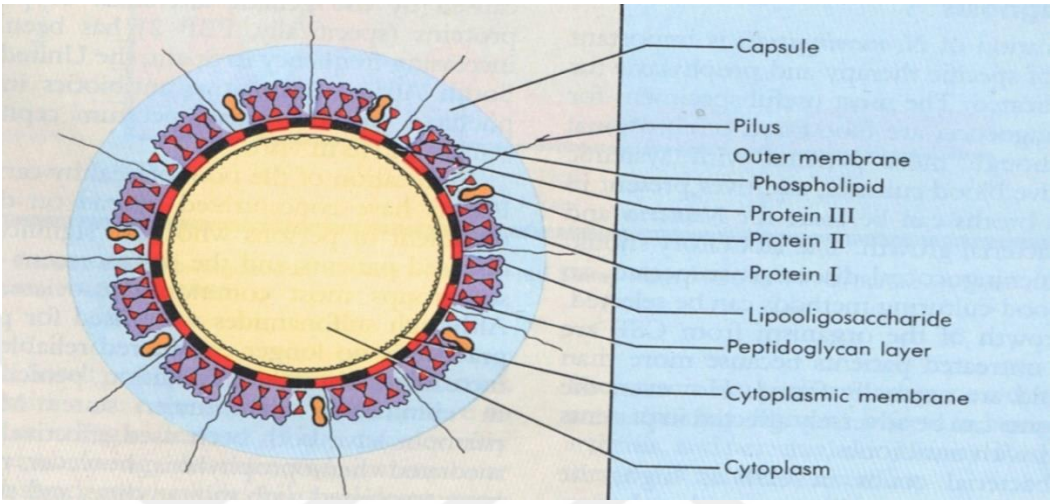

Figure 2.5 The virulence factors of *Neisseria gonorrhoeae* (Liao *et al.* 2008)

2.8.1 Pili

They are semi-rigid filamentous growths found on the surface of bacterial cells (mostly Gram-negative bacteria, spheroids, and Bacillus, rarely found in Gram-positive bacteria). The pili consist of repeating micro-unit pilin, which consists of about 165 amino acids. It is composed of a protein called pilin, numbering from 10 to several hundred, shorter in length than flagella and less in diameter, and they cannot be seen with an optical microscope and can be observed with an electron microscope (Brooks *et al.* 2004). Pili generally have different functions (Proft and Baker 2009):

a. Adhesion to most surfaces (cellular and acellular).
b. Formation of surface films from growth on liquid environments that may help to increase the growth rate in static farms.
c. A catalyst for infection (Virulence factor) Pathogenic bacteria with filaments can adhere to cell surfaces as they multiply and excrete toxins or penetrate membranes.

Cilia molecules in *N. gonorrhoeae* can be immunologically divided into three imaginary regions (Brooks *et al.* 2004):

i. constant region and consists of a high proportion of hydrophobic amino acids (the amino acids do not differ in them).

ii. The central semivariable region consists of many types of amino acids, contributes to adhesion to host cells, and has little immune response.

iii. The highly variable region is found at the carboxylic end, which has the ability to replace amino acids, and from 1 to 4 the genetic codes can be inserted or deleted.

Neisseria gonorrhoeae bacteria move by type IV pili and work to adhere to the surface of the host organism. This type of cilia moves the bacteria by sticking to the surface, and, and gives a signal to move the cell back and drag it forward (Jarrell *et al.* 2009, Mattick 2002). *N. gonorrhoeae* can pull 100.000 times more than their own weight, and this phenomenon is the most powerful biological engine known to date. It is believed that it has a role in protecting bacteria from the process of phagocytosis (Tobiason and Seifert 2001, Jarrell *et al.* 2009).

2.8.2 Lipooligosaccharide (LOS)

N. gonorrhoeae have the typical outer membrane of Gram-negative bacteria that consists of proteins, phospholipids and lipopolysaccharide LPS. The latter is distinct from LPS in other bacteria because it has a very branched base structure of oligosaccharides and does not contain repeating O-antigen units. For these reasons, LPS in *Neisseria gonorrhoeae* is called lipooligosaccharide LOS (Zhu *et al.* 2001). Bacteria secrete or form structures in the outer membrane called blebs, which are bubble-like growths that appear in the outer membrane during the period of its growth. injury (Hartley *et al.* 2011).

In *Neisseria gonorrhoeae*, the molecular weight of LOS ranges from 3000-7000 Daltons (Brooks *et al.* 2004) and its structure is similar to that of glycosphingolipids found in human cells. LOS inhibits anti-viral activity and phagocytosis by multinucleated cells and

its complement, and as a result, leads to failure of immune system recognition of bacteria (Van Vliet *et al.* 2009).

2.8.3 Protein I (PI or Por protein)

The first protein has a molecular weight of 32-36 kDa (Heckels 1981). It is concentrated in the outer membrane and is the most abundant protein, with a percentage of 60% of its presence, and it consists of a single polypeptide chain. The first protein is a porous complex with a third protein (protein III) called porins and is found in the outer membrane of bacteria, and it crosses the cell membrane and acts as a pore. Bacteria allow the passage of hydrophilic solutes and negative molecules (anions), and it is not like transmembrane carrier proteins because it is a large molecule and works as channels, which are specialized to differentiate between types of molecules (Chen and Seifert 2013).

Depending on the structure variation, the first molecule of PI is divided into two groups IA and IB and PIAs are smaller than PIBs (Unemo *et al.* 2011, Liao *et al.* 2008, Olesky *et al.* 2006).

The first protein is similar to porins in Escherichia coli in molecular size and pore formation of the same size. The molecules of the first protein move easily from the pathogenic Neisseria to the adjacent intact membranes in the host organism. as the molecules of the first protein move from the outer membrane of Neisseria to the cytoplasmic membranes of human cells, and this process is the first step of the Neisseria invasion. There is a relationship between the molecular weight of the first protein and the type of injury. The first protein with low molecular weight is resistant to complement and is present in disseminated infection. As for the first protein with high molecular weight, it is present in genital infections with symptoms (Chen and Seifert 2013).

2.8.4 Protein II PII or Opa protein

Neisseria gonorrhoeae produces another outer membrane protein called Opa proteins (PII), which is composed of subunits with a molecular weight ranging from 24 to 32 kDa. Description of the protein in the opaque colony is not found in the same strain of returning transparent colony, so the protein is called Opa protein (Brooks *et al.* 2004).

Expression of PII in Neisseria strains may undergo antigenic changes during infection, and this variation is thought to contribute to the evasion of the immune response and may be partly responsible for recurrent gonococcal infection and militarization and colonization elsewhere in the host. The function of the second protein is to attach to epithelial cells and pleomorphic white blood cells (Gray-Owen 2003).

Variations in the second protein may be important in determining the different stages of gonococcal infection although the second protein is one of the virulent factors of *Neisseria gonorrhoeae* the cocci that form colonies that do not produce the second protein are more pathogenic (Cole and Jerse 2009, Sadarangani *et al.* 2011).

2.8.5 Protein III (Rmp)

Other proteins discovered in the outer membrane of *Neisseria gonorrhoeae* include Reduction-modifiable Proteins having a molecular weight of around 33 kDa. Protein III is antigenically stable and not prone to antigenic change in all gonococci. These proteins form a complex with Por and LOS (Brooks *et al.* 2004) and have comparable gene sequences to the OmpA protein in Escherichia coli. In the case of infection with gonococcus or Escherichia coli bacteria, Protein III stimulates the formation of antibodies, and when these immune bodies are present in the blood, they combine with the exposed parts of the protein and block the places where the immune bodies unite to kill the bacteria, hence the name blocking antibodies.

2.8.6 Transferrin binding proteins (Tbp)

Most types of microorganisms need iron as a basic element for growth and other vital processes, including RNA synthesis and electron transfer (Briat 1992). *N. gonorrhoeae* obtain iron from direct contact with Iron-binding proteins, including transferrins, which are glycoproteins responsible for regulating the level of free iron in biological fluids. In order for bacteria to obtain iron from host transferrins, they must contain proteins with special receptors there are two types of proteins in *N. gonorrhoeae*, TbpA and TbpB, which stimulate the special receptors in *N. gonorrhoeae* proteins in low iron concentrations and extract iron directly from transferrin and lactoferrin respectively, and these proteins can extract iron (DeRocco and Cornelissen 2007).

2.8.7 IgA1 protease

It is an enzyme that cleaves the peptide bonds of the IgA1 antibody in the hinge region between the Fc region (Fragment, crystallizable) and the Fab region (Fragment, antigen binding). Cocci and some pathogenic microorganisms such as Haemophilus influenza and Streptococcus pneumonia secrete the IgA1protease enzyme to destroy the structure and function of the IgA1 antibody, thus eliminating an important aspect of immunity (Mistry and Stockley 2006).

The IgA antibody is found in mucosal areas such as the intestines, respiratory tracts and urinary tracts, and can directly contact the foreign body in the mucosa to prevent the colonization of the mucous membranes (Mistry and Stockley 2006).

The cleavage of the IgA antibody by IgA1 Protease leads to the separation of the Fab region, which is the region that contains the antigen binding region, from the Fc region, which is the end region of the antibody responsible for interaction with cell surface receptors, and as a result, the pathogen will contact the region of IgA1 Fab and thus evade from the immune system (Almogren *et al.* 2003).

2.9 Diagnosis

Gram stain and culture were the traditional procedures used to detect gonorrhea; however, more recently developed diagnostic methods based on polymerase chain reaction (PCR) are becoming more used (Ljubin-Sternak and Meštrović 2014, Barry and Klausner 2009). Those patients who do not respond to the first therapy should have a culture done to evaluate their antibiotic sensitivity (Deguchi *et al.* 2010).

It is advised that tests for screening and diagnosis of gonorrhea infection employ polymerase chain reaction, also known as nucleic acid amplification, in order to detect genes that are specific to *N. gonorrhoeae*. In order to conduct these PCR-based tests, a sample of urine, urethral swabs, or cervical/vaginal swabs are required. Culture, which is the process of growing colonies of bacteria in order to isolate and identify them, and Gram stain, which is the process of staining bacterial cell walls in order to reveal morphology, are both additional methods that can be used to detect the presence of *N. gonorrhoeae* in all specimen types, with the exception of urine.

If Gram-negative, oxidase-positive diplococci are seen on a direct Gram stain of urethral pus (male genital infection), then the diagnosis of gonorrhea infection may be made without any further tests (Ng and Martin 2005). However, a direct Gram stain of cervical swabs is not effective in the case of female illness because the *N. gonorrhoeae* organisms are less concentrated in these samples than they are in male samples. Because Gram-negative diplococci that are normally found in the vaginal flora are unable to be differentiated from *N. gonorrhoeae*, the likelihood of receiving a false positive result is enhanced. Consequently, cervical swabs need to be cultured according to the conditions outlined before. In order to confirm the diagnosis, a culture of a cervical or vaginal swab specimen must produce oxidase-positive, Gram-negative diplococci. When it comes to diagnosing infections of the mouth, rectum, eyes, blood, or joints, culture is particularly helpful since PCR-based diagnostics are not yet well established in all laboratories (Ng and Martin 2005). In addition to this, culture may be used for the assessment of antibiotic sensitivity, the failure of therapy, and epidemiological objectives (outbreaks, surveillance) (Ng and Martin 2005).

Cultures should be taken from every feasible mucosal location in individuals who have a suspected case of disseminated gonococcal infection (DGI) (e.g., pharynx, cervix, urethra, rectum). In addition to this, you need to get three different blood culture sets. In situations of septic arthritis, synovial fluid should be collected for analysis (Bignell and Jensen 2009).

All individuals who get a positive gonorrhea test result should also be screened for other sexually transmitted illnesses such as chlamydia, syphilis, and the human immunodeficiency virus (Deguchi *et al.* 2010). According to a number of studies, the percentage of young individuals with gonorrhea who also have chlamydia co-infection ranges from 46 to 54% (Kahn *et al.* 2005, Dicker *et al.* 2003). People in the United States between the ages of 14 and 39 who have gonorrhea are almost twice more likely to also have a chlamydial infection, with 46% having both infections (Datta *et al.* 2007). Because of this, tests for gonorrhea and chlamydia are often performed together. There is a fivefold increase in the risk of HIV transmission among those who have been diagnosed with gonorrhea infection. During a bout of gonorrhea, HIV-positive individuals who are afflicted have an increased risk of shedding HIV and transmitting the virus to partners who are not infected with the virus (Ryan and Ray 2004).

2.10 Antimicrobial Treatment and Resistance in *Neisseria gonorrhoeae*

Antimicrobial therapy and resistance Many antimicrobials, including sulphonamides, penicillins, tetracyclines, aminoglycosides, macrolides, cephalosporins, and fluoroquinolones, are naturally effective against *N. gonorrhoeae*. The susceptibility of the bacteria and the location of the illness should both be considered when selecting an antibiotic treatment. Antimicrobial resistance in *N. gonorrhoeae* Treatment that is effective against gonococcal infection is not only an essential component of attempts to restrict the spread of illness but also the only means to completely eradicate the disease. Regrettably, the number of available treatment choices has decreased over the course of time due to the development of resistance to each new class of antibiotics (Unemo and Shafer 2011). The development of AMR in *N. gonorrhoeae* may occur either via plasmid-mediated processes or through mutations that occur on chromosomes, or both. Plasmid-

mediated pathways, on the other hand, provide resistance to tetracycline and/or penicillin, but accumulating chromosomal mutations may lead to either single-drug or multidrug resistance. The Western Pacific Region of the World Health Organization (WHO) is considered to be the birthplace of the vast majority of the antibiotic-resistant *N. gonorrhoeae* strains that have since spread across the world (Unemo and Nicholas 2012).

The criteria put forward by the World Health Organization state that in order for an antibiotic to be approved, it must have a cure rate of at least 95%. To put it another way, when the incidence of treatment failure for a particular antibiotic reaches 5% or greater, the use of that antibiotic is no longer advised. *N. gonorrhoeae* has quickly evolved resistance to every class of medications that have been used for treatment since sulphonamides were first introduced in 1936, despite the fact that regular monitoring and periodically revised treatment recommendations have been implemented (Unemo and Shafer 2014). By the middle of the 1940s, resistance to sulfa drugs was already a widespread problem. Around this time, penicillin was developed and shown to be very successful against gonorrhea. However, its efficacy began to decline within 10 to 15 years, and by 1989, it was no longer suggested as a therapeutic option (Unemo and Shafer 2014). Around the same time, resistance to tetracycline also removed it as a therapeutic option, when it had been used in the past for patients in whom penicillin was contraindicated. This happened in situations where tetracycline had previously been utilized (Unemo and Shafer 2014). In the 1990s, reports of resistance to the fluoroquinolone antibiotics like ciprofloxacin, which were also once very successful against gonorrhea, started to emerge; by 2007, these drugs were no longer considered viable treatment options (CDC 2007). Around the same period, there was also an increase in the prevalence of azithromycin resistance (Unemo and Shafer 2014). At this stage, the only monotherapeutic treatment choices that could be broadly suggested were cephalosporins of the third generation, which were still efficacious at rather low dosages (Unemo and Shafer 2014). In recent years, drug resistance to the recommended drugs' final line of defense has been observed in several regions (Unemo *et al.* 2010, Unemo *et al.* 2011). This has the potential to usher in an era of infections that cannot be treated (Unemo and Nicholas 2012), and it has prompted recommendations to use dual therapy (Unemo and Shafer 2014).

2.11 Preventive Measures

Methods for preventing gonorrhea and other gonococcal infections Education, counseling, diagnosis, treatment, and case-finding are all key components of a successful strategy for gonorrhea control, as are any number of other sexually transmitted illnesses. Gonorrhea is only one of many sexually transmitted diseases (STDs). The provision of health care services and the promotion of safer sexual practices are both crucial components. This is done with the goal of preventing illnesses that are transmitted sexually by encouraging healthier sexual habits. It has been shown that the use of condoms is an effective method for preventing the transmission of gonorrhea (Kenneth 2008). The guidelines for the treatment of STDs made by the CDC include a number of prophylactic measures against gonococcal infection. Some of these preventative measures include the following: I the teaching and counseling of individuals at risk on methods to prevent gonorrhea via changes in sexual practices including abstinence and reduction of sex partners, and the use of condoms during the sexual act; (ii) the identification of people who are infected but do not have any symptoms. I the teaching and counseling of people who are at risk for gonorrhea on strategies to prevent the disease by making adjustments in their sexual practices, such as abstention, a decrease in the number of sexual partners, and the use of condoms during sexual activity. People who are experiencing symptoms are not likely to seek diagnostic and treatment services. This can be overcome by conducting mass screenings of people who engage in high-risk sexual behaviors, such as commercial sex workers, medical sexual offenders, and people who have interactions. People who are experiencing symptoms are unlikely to seek diagnostic and treatment services. (iii) An established surveillance system that is outfitted with the people and logistics necessary to carry out an efficient diagnosis of the instances of gonorrhea is a necessary component for an effective diagnostic and treatment plan for infected individuals. This system must be equipped with the people and logistics necessary to carry out an efficient diagnosis of cases of gonorrhea. Evaluation, treatment, and counseling of sex partners of persons who are infected with gonorrhea can be implemented by partner notification by the infected persons seeking treatment. A well-organized antimicrobial surveillance system is essential for the development of an effective treatment regimen.

Gonorrhea is a disease that is shared between people through sexual contact and is considered to be a sexually transmitted disease (STD) (CDCP 2006).

3. MATERIALS AND METHODS

3.1 Material

3.1.1 Laboratory equipments

The laboratory equipments utilized at this work are listed in Table 3.1.

Table 3.1 Laboratory equipments, as well as the manufacturer and place of origin

Equipment	**Company/Country of Origin**
Incubator	Binder (Germany)
PCR thermal cycler	Bio Rad (USA)
Gel electrophoresis	Bio-rad-(Italy)
Water distiller	GFL(Germany)
Incubator	Memmert (Germany)
Bath water	GFL (Germany)
Oven	GallenKhamp (England)
Digital camera	Nikon (Japan)
Autoclave	Selecta (Spain)
Vortex	Whirlimixer (England)
Micropipettes	Slamed (USA)
Microscope	Olympus (England)
Microscope with digital camera	Olympus (England)
Transilluminator	UVP (USA)
pH-meter	Metrohm AG (Spain)
Centrifuge	Netheland (Germany)
Eppendorf bench centrifuge	HermLe (Germany)
Micro spin centrifuge	My Fugene (China)

3.1.2 Laboratory materials

The laboratory materials utilized in this research are listed in Table 3.2.

Table 3.2 Materials in the laboratory, company, and place of origin

Material name	**Company/Country of Origin**
Ethidi Ethidium bromide	Promega(USA)
Methyl red	BDH (England)
Bromophenol blue	promega (USA)
Agarose	promega (USA)
Pepton	BDH (England)
Tris-base	BDH (England)
Boric acid	BDH (England)
Crystall violet	BDH (England)
Sucrose	BDH (England)
DNA DNA extraction kit	Geneaid
Master mix PCR	Bioneer (Korea)
Formalin	BDH (England)
Vitamin Growth supplement	Himedia (India)
Ethanol	Fluka (Switzerland)
Isopropan	Fluka (Switzerland)
Glucose	BDH (England)
Glycerol	BDH (England)
Yeast Autolysate supplement	Himedia (India)
V.C.N.T. supplement	Himedia (India)
Hemoglobin Powder	Himedia (India)

3.1.3 Culture media

Various bacteriological culture media were utilized in this research, as mentioned in the Table 3.3.

Table 3.3 Bacterial culture media and their origin utilized in the research

Cultures	**Company/Country**
Modified Thayer Martin Agar	Himedia/Accumax (India)
Chocolate media	Himedia/Accumax (India)
Muller Hinton agar	Himedia/Accumax (India)
Nutrient broth medium	Difco (USA)
Brain-Heart infusion broth	Rasmi (India)

3.1.4 Antibiotic discs

According to the performance requirements for antimicrobial susceptibility testing, tested the sensitivity of isolates *N. gonorrhoeae* about 5 types of antibiotics by disk diffusion method the antibiotics and their interpretation values that were utilized in this research are listed in Table 3.4 (NCCLS 2000).

Table 3.4 Antibiotic discs, their symbol, concentration and interpretation value

Class	Sym.	Conc.	Company	Inhibition diameter (mm)		
				(S)	(I)	(R)
Ceftriaxone	CRO	30 µg	MAST Group (UK)	≥0.06	0.04	≤0.05
Spectinomycin	STM	100 µg	MAST Group (UK)	≥64.0	63.0	≤62.0
Ciprofloxacin	CIP	5 µg	MAST Group (UK)	≥0.06	-	≤0.05
Penicillin	PEN	10 µg	MAST Group (UK)	≥0.06	-	≤0.05
Tetracycline	TET	30 µg	MAST Group (UK)	≥0.5	0.04	≤0.03
(S: Sensitive, I: Intermediate, R: Resistant)						

3.1.5 Preparation of culture media

The following procedure was used to sterilize the culture medium in an autoclave at 121°C, 15 Ib/inch^2, for 15 min after it had been prepared in accordance with the manufacturer's instructions.

3.1.5.1 Chocolate hiveg agar base

45 grams of chocolate agar are suspended in 500 mL distilled water and heated until boiling to dissolve the medium completely then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and then cooled the medium to a temperature of 50°C, then added to the sterilized medium 2% of hemoglobin and vitamin growth or yeast autolysate supplement, then poured the medium into the dishes It was incubated at 37°C for 24 hours to ensure that it was not contaminated, and kept at 4°C until use (Lehmann 1999).

The preparation required addition of the following compounds:

A. Haemoglobin powder

10 grams of powder heamoglobin were taken and placed in a dry flask; then 500 mL of sterile distilled water is gradually added, and exposed to 121°C temperature for 15 minutes with continuous movement after each addition until completing the volume to 500 mL.

B. Vitamin growth supplement

This supplement consists of two vials. One vial contains vitamin B (0.1 mg), L-Glutamine (100mg), adenine sulphate (10mg), guanine hydrochloride (0.3 mg), P- amino benzoic acid (PABA) (0.13 mg), L-cysteine (11mg), NAD (coenzyme I) (2.5 mg), co carboxylase (1mg), ferric nitrate (0.2 mg), thiamine hydrochloride (0.03 mg), cysteine hydrochloride (259 mg) and dextrose (1 gm). The other vial contains dextrose (1gm) and distilled water (10mL). The contents of the first vial are dissolved in the contents of the second vial under sterile and antiseptic conditions. Then they are mixed well and transferred to the media, dissolved well, and subjected to a temperature of 45- 50°C under sterile condition.

C. Yeast autolysis supplement

The vial contents of the yeast autolysis supplement contain yeast autolysis (5 gm), dextrose (0.5 gm), and sodium bicarbonate (0.075 gm). Aseptically, the contents of the vial are moisturized with 10 mL of sterile distilled water, mixed well, then transferred to the media, dissolved well, and subjected to a temperature of 45-50°C under sterile condition.

3.1.5.2 Thayer martin agar modified

21 g of Thayer Martin medium base agar Modified was dissolved in an amount of distilled water, then the volume was completed to 450 milliliters with distilled water and sterilized by autoclave, after which the medium was cooled to a temperature of 45°C, then (2%) of hemoglobin and vitamin growth supplement were added to the sterile medium, as well as antibiotics (V.C.N.T. supplement), after which the medium was poured into dishes and incubated at a temperature of 37°C for 24 hours to ensure that it was not contaminated, and kept at a temperature of 4°C until use (MacFaddin 1985, Thayer and Martin 1966).

A. Base Antibiotic. V.C.N.T. vial

The V.C.N.T vial contain vancomycin (1.500 mg), colistin methane sulphonate (3.75 g), Nystatin (6250 Units) and trimethoprim (2.500 mg).

3.1.5.3 Brain heart infusion (BHI) broth

37 grams of brain heart infusion were added to 1 litter of D.W, mixed well and distributed into final containers and sterilized by autoclaving at 121°C for 15 minutes. According to manifacturi company.

3.1.5.4 Nutrient broth

13.0 g should be dissolved in 1 L of distilled water. Fill tubes with the mixture, and then autoclave for 15 min at 121°C at 15 psi pressure. These bacterial isolates are grown and preserved on this medium that has been supplemented with 15% glycerol.

3.1.5.5 Muller-Hinton agar medium (Muller-Hinton agar)

38 grams were suspended in 1000 milliliters of distilled water, which was then brought to a boil to fully dissolve the medium. It is used in anti-microbial susceptibility testing

after being autoclaved for 15 minutes at 15 lbs pressure and 121°C for 15 minutes to sterilize it (Mueller and Hinton 1941).

3.2 Methods

3.2.1 Sample's collection and identification

100 sampleswere collected from male and female from patients suspected to have gonorrhea (urethral discharge with dysuria) from different regions in Nasiriyah City between July 2022 and December 2022 throughout the research period were involved and a special form of information was filled out for each patient including name, age, sex, job and marital state, using questionnaires, direct swabs were taken from patients who attended Nasiriyah Teaching Hospital and Al-Hussein Teaching Hospital in Nasiriyah City/Dhi-Qar Governorate, Iraq, Clinics and private laboratories (APPENDIX 1).

3.2.2 Isolation of *Neisseria* bacteria

The samples were cultured immediately after collection on rich media chocolate agar for bacterial growth, and selective media modified Thayer martin agar (MTM) contact antibiotic to prevent the growth of all the types of bacteria except *N. gonorrhoeae*, and incubated at 37 degrees Celsius for 24 hours and at the same time cultured on chocolate media being an enriching medium that allows the growth of negative bacteria of the dye of gram, as well as it is a differentiating medium by which it is possible to differentiate between fermented and non-fermented bacteria of lactose sugar, incubated for 24 hours at a temperature of 37°C and the colonies that were distinguished by their gray color were transferred to another dish containing the same medium for the purpose of purification, and the replanting continued on this medium until pure colonies were obtained.

3.2.3 Diagnosis of bacterial isolates

Bacterial isolates were diagnosed based on culturological and microscopic characteristics and biochemical tests, according to what was mentioned in (Bignell and Jensen 2009) and as follows:

3.2.3.1 General culture characteristics

The bacteria were initially diagnosed by studying the general agricultural characteristics of the colonies growing on the culture media, then the apparent colony forms were studied and determined on the basis of color, shape and size, in addition to noting the lack of other general characteristics such as fermentation.

3.2.3.2 Microscopic characteristics

Microscopic examination of bacterial cells was carried out by staining them with Gram stain and examining them under the oil lens of a light microscope.

3.2.3.3 Biochemical tests

A number of diagnostic biochemical tests were used based on each of (Washington *et al.* 2006, Janda and Knapp 2003, Ng and Martin 2005):

Catalase production test: Put part of the bacterial growth on a glass slide, and a drop of hydrogen peroxide reagent was added to it. The appearance of bubbles is evidence of a positive test.

B. oxidase test: A part of a colony growing on the nutrient agar was taken and placed on a filter paper, then a drop of Paraphenylene diamine Hydrochloride Tetra methyl reagent was added to it. The color was dark purple, evidence that the isolate is positive for this test, as the positive result appears within 30-60 seconds.

Sugars fermentation test (glucose, lactose, sucrose and maltose): This assay was used to investigate the ability of bacteria to ferment these sugars, by inoculating the prepared sugars fermentation medium with bacterial isolates grown on chocolate agar medium. The positive result was observed by changing the color of the red detector to yellow, and the production of gas in the test tube, while the negative result was by retaining the red color of the detector and not producing gas in the test tube, within 24h at 37°C.

3.2.4 Antibacterial susceptibility test (AST)

3.2.4.1 Disk diffusion method

The disk diffusion susceptibility method was carried out according to the Kirby-Bauer method National Committee for Clinical Laboratory Standards (NCCLS 2000) The test is performed by following steps.

1. A bacterial broth culture was prepared by transferring a separated colony on 24 hour old blood agar plate culture to a tube containing 5 mL of sterile normal physiological saline and its turbidity was compared to the standard turbidity tube which gives (1.5×10^8 CFU/mL).

2. Using a sterile swab, 0.1 mL of broth culture was transferred and streaked over the surface of a Mueller-Hinton agar plate. Each time, the plates were rotated roughly 60 degrees to achieve uniform inoculum dispersion. The plates were permitted to dry at room temperature for 5 min.

3. The antibiotic discs were applied to the Mueller-Hinton agar plate using sterile forceps at a rate of 5-6 discs per plate. Overnight, the plates were incubated at 37°C.

4. The growth inhibition zones around each antibiotic disk are measured to the closest mm.

5. Zone sizes for each medication were evaluated using criteria established by the CLSI. These criteria were 'specific' for that sensitivity category (i.e. sensitive, intermediate, or resistant).

6. The zone diameters were compared with standard curves of NCCLS (NCCLS 2000).

3.2.5 Save isolates

The isolates were kept at -20°C after being grown in heart and brain liquid broth for 24 hours at 37°C, then added to it at a 1:1 ratio of sterile glycerol with a concentration of 40% (Farraj *et al.* 2010).

3.2.6 Molecular identification using PCR technique

90 *N. gonorrhoeae* isolates were used for molecular detection (PCR), and the selection of those isolates was based on microscopic examination, Culturing on rich media (chocolate agar), culturing on Modified Thayer martin media, and biochemical test. The Diagnosis was resorted to by PCR to confirm the results of the traditional diagnosis (microscopic examination and to rely on the specifications of bacteria on culture media and biochemical tests).

3.2.6.1 DNA extraction

The process of extracting DNA from bacterial cells suspended in a physiological solution was carried out from direct swabs and urine samples, according to what was mentioned in (Santos *et al.* 2003) using the extraction kit supplied by Geneaid Company according to the following steps:

(a) Pellet Cells

- The previously cultured bacteria cells were transferred to a 1.5mL eppendorf tube.
- Centrifuging these small tubes at a speed of 14,000-16,000 rpm for one minute at room temperature, after which the filtrate was disposed of.
- 200 μL of GT Buffer solution was added to the tubes to resuspend the cells by stirring with a pipette or using a vortex.

- The small tubes were incubated for 5 minutes at a room temperature of 25°C.

(b) Lysis step

- 200 µL of GT Buffer solution was added to each sample and mixed well using the mixer device for 5 seconds.
- The tubes were incubated at 70°C for 10 minutes, until almost clear decomposed samples were obtained. During incubation, they were stirred every three minutes. During this step, the Elution Buffer DNA solution was prepared and incubated at 70°C until used in the last step.

(c) DNA binding

- 200 µL of alcohol was added to each sample and agitated vigorously and when DNA precipitation occurred the agitating was stopped.
- Prepare the GD column in the collection tube.
- The entire contents of the sample, including the precipitate, were transferred to GD colum DNA.
- Centrifugal work at a speed of 14.000-16.000 revolutions / minute for 2 minutes and at room temperature.
- The filtrate in the collection tube has been disposed of.

(d) Washing

- Microliter of the first washing solution (W1 Buffer) to the DNA binding column.
- Centrifugal work at a speed of 14.000-16.000 rpm for 30 seconds at room temperature.
- The filtrate in the collection tube has been disposed of.
- 600 microliters of the second wash solution were added to which the ethanol solution was previously added.

- Centrifugal work at a speed of 14.000-16.000 rpm for 30 seconds at room temperature.
- The filtrate in the collection tube has been disposed of.
- Centrifuge for 3 minutes at 14.000-16.000 rpm and at room temperature to dry the DNA binding column.

(e) DNA elution

- Transfer the dried DNA GD column to a new 1.5 mL micro-tube.
- 100 µL of preheated Elution buffer (step two) or TE Buffer solution was added to the DNA binding column.
- Centrifugal work at a speed of 14.000-16.000 rpm for 30 seconds at room temperature.
- The result is pure DNA dissolved in 100 µL of Elution buffer.

3.2.6.2 Primers

Using the target gene as a template, the primer sequences were used to amplify the highly conserved region of the target gene from bacterial isolates. Primers were bought from a commercial supplier called Macrogen in Korea (Table 3.5). The packaging for all of the primers was lyophilized. As advised by the manufacturer, this product was dissolved in nuclease-free water to a final concentration of 100 pmol and stored in a deep freezer as a stock solution until it was required for PCR amplification. 90 µL of nuclease-free water was added to the stock solution of primer in order to bring the concentration up to 10 pmol/µL when 10 µL of the primer stock solution was added.

Table 3.5 Oligonucleotide primers of different genes used in this study

Gene	Sequence [5'-3']		Amplicon Size (bp)	Reference
Orf1	F R	**GTCCGCGTCGAAGGCCATAC** **CACATCTACGCGGCGGTCAG**	280	(Shahcheraghi *et al.* 2010)

3.2.6.3 PCR mixture preparation and thermal cycling protocol

The bacteria were diagnosed by PCR reaction using a primer designed for the *Orf1* gene the PCR reaction mixture and thermal cycler programs were given in Table 3.6 and Table 3.7, respectively. The master mix contains bacterial-derived DNA polymerase Taq, dNTPs, MgCl2, dNTP (dATP, dGTP, dCTP, dTTP), Tric-HCl (pH 9.0), KCl and reaction solution at optimum concentration for efficient amplification of DNA templates by PCR.

Table 3.6 Reaction volume and components of PCR

Components	**Volume Sample**
Master Mix	12.5 µL
Forward primer	3 µL
Reverse primer	3 µL
Nuclease Free Water	1.5 µL
DNA	5 µL
Total volume	25 µL

Table 3.7 PCR thermal cycler programs

Gene	**Initial denaturation**		**Denaturation**		**Annealing**		**Extension**		**Final extension**		**Cycle**
	˚C	**Time**	**˚C**	**Time**	**˚C**	**Time**	**˚C**	**Time**	**˚C**	**Time**	
Orf1	94	01:00	94	00:30	52	00:30	72	00:50	72	8:00	40

3.2.6.4 Electrophoresis of agarose gel

After being separated on an agarose gel at a concentration of 1.5% in TBE buffer with a pH of 8, the products were Ethidium bromide staining followed by UV photography. It was possible to observe the electrophoresis result thanks to the gel documentation system (Bartlett and Stirling 1998).

4. RESULTS AND DISCUSSION

4.1 Microscopic Examination (Gram Stain)

The results of the microscopic examination gave 95 samples as *N. gonorrheae* out of the total samples of 100, after staining them with gram stain, as the cells appear under the microscope Gram negative interacellular diplococcic (G.N.I.D) within polynuclear leukocytes (PMNs). Both (Chessbrough 2000, Haddow *et al.* 2004, Iser *et al.* 2005, Medenica and Pavlovi 2007) have indicated the shape of bacteria cells and their presence inside the polynuclear leukocytes (PMNs) and (Stohl *et al.* 2012) pointed out that although multinucleated cells possess a strong anti-bacterial arsenal through multiple killing mechanisms, gonococci have the ability to survive inside these cells, which indicates the development of gonococci. Among the difficulties in diagnosing bacteria microscopically is the lack of clarity within samples containing large numbers of multinucleated leukocytes, while it can be easily diagnosed in samples containing fewer numbers. The results were confirmed by (Issa *et al.* 2008), who demonstrated that 23 out of 38 patients were positive with gram stain in a study carried in Tikrit city during a period from October 2007 to the end of 2008.

However, this method is relatively insensitive and does not provide a definitive diagnosis for samples of asymptomatic men and women with injuries outside the genitals, as (Bignell and Jensen 2009) indicated the possibility of accepting the final diagnosis using a light microscope in symptomatic infections, and not accepting the diagnosis in cases of women and those without Symptoms (Workowski *et al.* 2008) also pointed out that microscopic examination is not accepted in infections without symptoms, and these sources are identical to the results of this study, as it showed the possibility of direct diagnosis of patients who have symptoms and the lack of positive results in cases of infected women or men who do not have symptoms.

4.2 Culturing

Microscopy-based diagnosis of *N. gonorrhoeae* revealed kidney-shaped, gram-negative intracellular diplococci. It is not enough to say it is *N. gonorrhoeae*; this needs to be verified through culture utilizing enriched medium and selective media for *N. gonorrhoeae* culturing. For this reason, after performing gram stain, chocolate agar was utilized as an enriched media for each sample our results from cultivating it on chocolate showed that 95 samples (95%) were positive out of the total samples of 100 samples; these findings are consistent with those made by (Issa *et al.* 2008).

In other hands the results confirmed that with gram stain from the growth the colonies were gray color, round shape and heavy growth using of selective media Modified Thayer- Martin for culturing of bacteria showed 90 cases positive (90%) out of the total samples of 100 samples. Because this culture medium is modified as it contains a group of antibiotics (V.C.N.T) to inhibit the growth of all types of bacteria except *N. gonorrhoeae*. The colonies appear gray, round, raised shiny, like the dew drops. This description is similar to the standard description of colonies on Thayer martin as shown in Figure 4.1 and Table 4.1 (Janda and Knapp 2003, Jawetz *et al.* 2001).

Table 4.1 Results of traditional and methods for detection of *Neisseria gonorrhoeae*

Type of test	**Total number of samples**	**No. (%)**	
		Positive	**Negative**
Gram Stain	100	95 (95.0%)	5 (5.0%)
Culture For Chocolate Agar	100	95 (95.0%)	5 (5.0%)
Culture For Selective Media Modified Thayer Agar	100	90 (90.0%)	10 (10.0%)

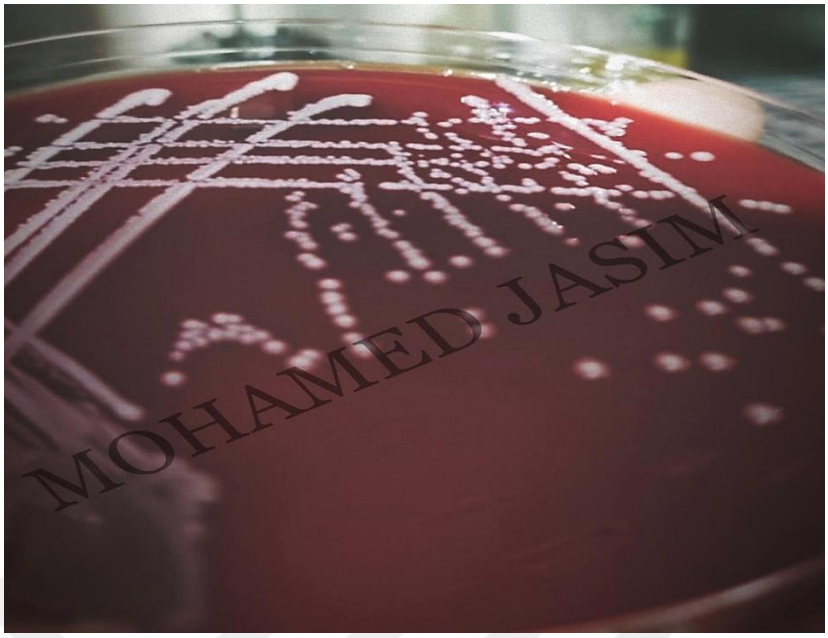

Figure 4.1 Brown colonies of Neisseria bacteria on Thayer-Martin medium

4.3 Biochemical Diagnostics

All of the 90 isolates that showed culture and microscopic characteristics identical to those of *Neisseria gonorrhoeae* were subjected to biochemical tests to confirm the diagnosis. Table 4.2 shows the biochemical tests that were approved for the diagnosis of *N. gonorrhoeae* isolates according to what was stated in (Janda and Knapp 2003, Ng and Martin 2005), it is clear from the obtained results that the affiliated bacterial isolates of *N. gonorrhoeae* are identical to the global diagnostic characteristics according to (Todar 2005), All isolates of *N. gonorrhoeae* gave a positive result for the oxidase assay, and this is because the isolates of *N. gonorrhoeae* are characterized by the presence of a terminal acceptor to O_2 such as cytochrome C that uses oxygen to produce energy, while it gave a positive result in the catalase assay, evidence of the appearance of air bubbles of O_2 produced by the enzyme catalase. Responsible for converting hydrogen peroxide to O_2 and H_2O, descriptions are in agreement with (Jawetz *et al.* 2001, Alsaedi 2013), and the isolates gave a negative result in the urease production assay because they were unable to produce the urease enzyme that hydrolyses urea to ammonia, and did not change the yellow color of the medium, when the movement was examined by stabbing the semi-

solid medium, it was observed in all isolates that the growth did not spread outside the limits of the stab, indicating that it was immobile.

Table 4.2 Biochemical tests characteristic of *Neisseria gonorrhoeae* bacteria

Test	Result
Gram stain	Negative
Oxidase test	Positive
Catalase test	Positive
Motility test	Negative
Glucose	Positive
Maltose	Negative
Sucrose	Negative
Lactose	Negative
Urease test	Negative

From the foregoing, it appears that the diagnosis by traditional methods, including biochemical tests, is slow and tiring, in addition to the fact that biochemical tests take a large space due to the large number of different media and reagents that may burden the laboratory worker, especially if the number of samples to be diagnosed is a lot, in addition to that it may lead to sample loss for several reasons, including the exposure of the sample to contamination due to the unavailability of sterile conditions and the expiration of the materials, and the diagnosis by traditional methods requires high accuracy, concentration and experience, and the diagnosis may depend on the personal opinion of the worker (Turton *et al.* 2008).

4.4 Relationship of Age and Infections with *Neisseria gonorrhoeae*

The result showed that the higher rate of infection was in 18- 24 years of age (72.22%) this may be due to highly sexual activity at these age groups compared to other age groups, followed by the age group between 25-29 years, with a rate of (17.78%), while the lowest percentage was in 30 -38 years of age (10.0%) while the age group between 39-45 years did not witness any infection with *N. gonorrhoeae* as shown in Table 4.3 and Figure 4.2. Angus and Kam (2013), reported that among patients with STD, nearly half of the cases occur in individuals with 18-24 years of age. The present data came in agreement with the findings of (Alsaedi 2013) who noticed a significant difference in age

group, when they detected the highest percentage (51.47%) of infection in age group, (18-24) and the lowest percentage of infection was in the age group from 30 to 39 while they indicated no detected incidence in 40-44 group, (Darawgha 2005) in Erbil obtained almost similar results, as the highest incidence of gonorrhea occurred in the age group 18-24 and 25-29 years (49.6% and 30.1%, respectively of the total positive samples), and no infection was recorded in the age group 44-40 years, these results are in complete agreement with our findings.

Table 4.3 Infection with *Neisseria gonorrhea* according to age group

Age group (year)	**Infection *N. gonorrheae***	
	No	**%**
18-24	65	72.22
25-29	16	17.78
30-38	9	10.0
39-45	0	0
Total	90	100

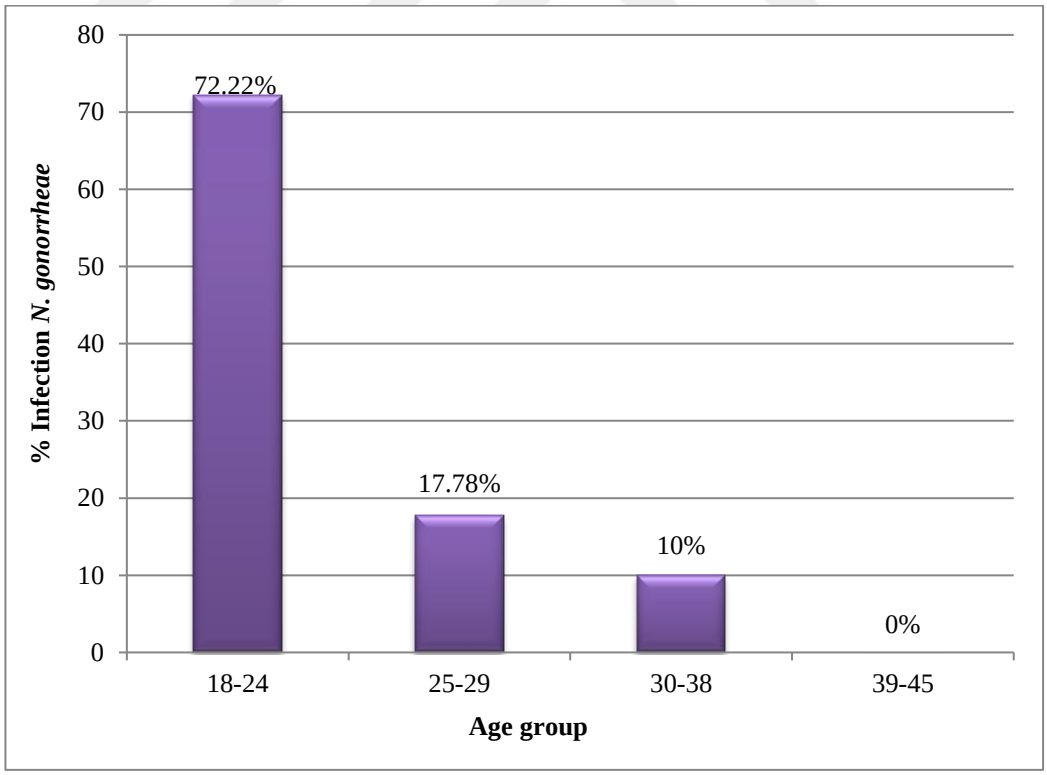

Figure 4.2 Distribution of *N. gonorrheae* infection according to the age group

4.5 The Relationship of Education Quality and Infections with *N. gonorrhoeae*

The relationship between educational level and the variance in the rates of infection with *Neisseria gonorrhoeae* bacteria. The results showed that the highest percentage of total infection occurred among the illiterate classes (53.33%), followed by the infection of those qualified with primary education (38.89%), then those qualified with secondary education (4.44%), and the lowest percentage (3.33%) were for those qualified with higher education. As the results were identical to the results of (Darawgha 2005), where the highest rate of gonorrhea infection was in uneducated women (51.8% of the total positive samples) compared to the lowest rate (3.6%) registered with qualifications in higher education, also a similar association was found in a study done by (Alsaedi 2013), who found that the higher percentage was in illiterate (51.25%) and the lowest was in qualified higher education (3.7%), and these results also agree with the conclusion of (Youssef 1996) in Baghdad, who indicated that the number of gonorrhea infections is inversely proportional to the culture of the individual. As for (Gibney *et al.* 2001) they recorded the highest infection rate (74.2%) among uneducated women, (De Lima Soares *et al.* 2003) indicated that the highest incidence of sexually transmitted diseases, including gonorrhea, was among the uneducated women (59.7%) and the lowest was (1.7%) among those with higher education qualifications (Table 4.4) (Figure 4.3).

Table 4.4 Patients with *N. gonorrhoeae* infection based on their level of schooling

Education	**Infection *N. gonorrheae***	
	No	**%**
Illiterate	48	53.33
Primary Education	35	38.89
Secondary Education	4	4.44
Higher Education	3	3.33
Total	90	100

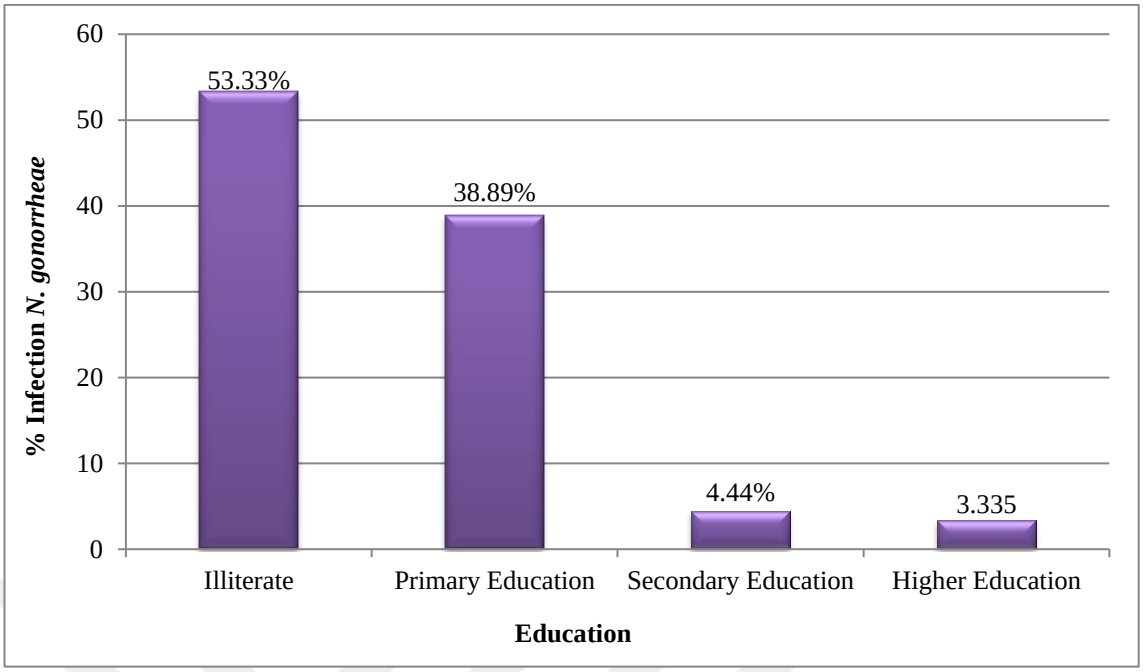

Figure 4.3 Patients with *N. gonorrhoeae* infection according to the education status

4.6 Relationship of Economic Status and Infections with *Neisseria gonorrhoeae*

The relationship of economic status with gonorrhea was studied, as it was noted that the highest rate of total infection was among patients with bad economic status (55.56%), followed by patients with medium economic status (31.11 percent), then patients with good economic status (8.89%). The lowest percentage (4.44%) was in patients with very good economic status (Figure 4.4) (Table 4.5). This study agrees with the findings of (Darawga 2005) that the highest infection rate was recorded among women with poor economic status (50% of the total positive samples) and the lowest rate (5.4%) among women with very good economic status. The results of the current study do not agree With the findings of (Youssef 1996) in Baghdad, which indicated that the number of gonorrhea infections is directly proportional to the level, while (De Lima Soares *et al.* 2003) indicated that the highest incidence of sexually transmitted diseases, including gonorrhea, was recorded among women with poor economic status, reaching 79% compared to 2.7% among women with good economic status. Also, researchers of the World Health Organization (WHO 2001) indicated that the poor classes More susceptible to gonorrhea, followed by the middle classes and then the rich classes.

Table 4.5 Patients with *N. gonorrhoeae* infection according to the economic status

Economic situation	Infection *N. gonorrheae*	
	No	**%**
Bad	50	55.56
Moderate	28	31.11
Good	8	8.89
very good	4	4.44
Total	90	100

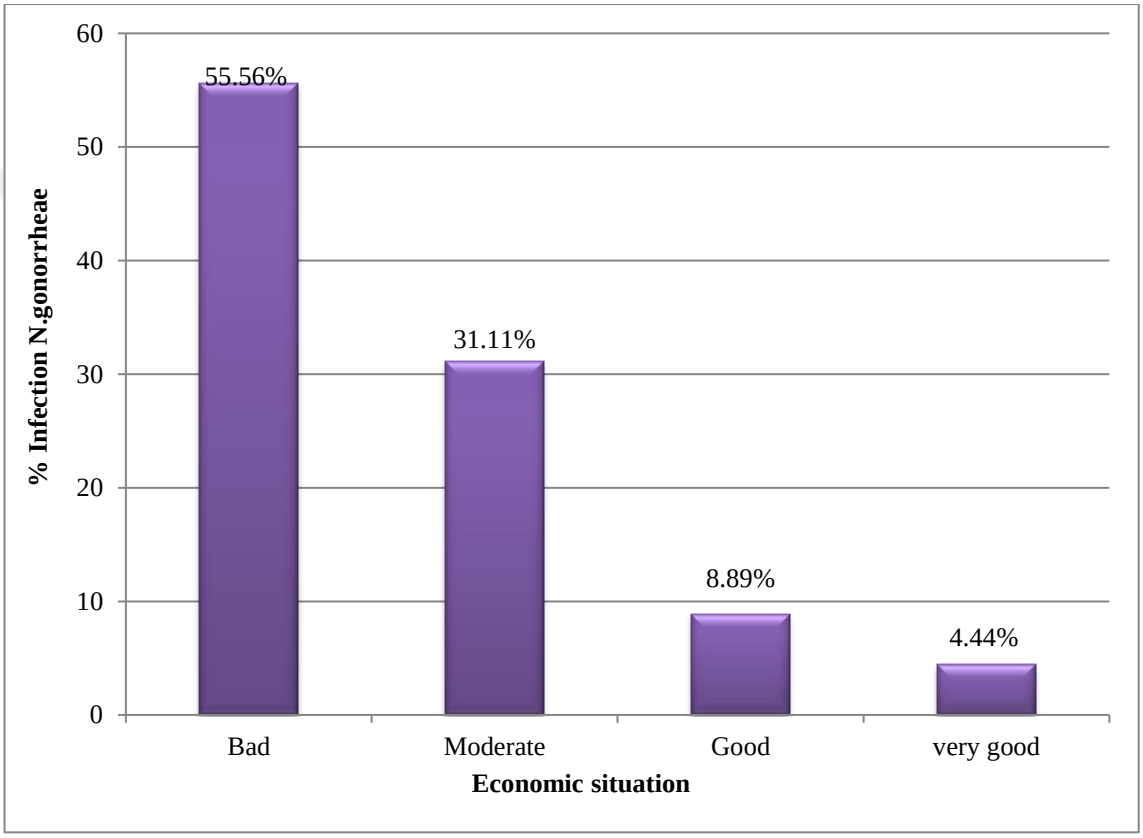

Figure 4.4 Distribution of the patients with *N. gonorrhoeae* infection according to the economic status

4.7 Antibiotic Sensitivity Test for *Neisseria gonorrhoeae*

In this research, we tested the effect of different antibiotics on all *N. gonorrhoeae* isolates. These isolates showed different sensitivity toward the antibiotics utilized in this research, as shown in Table 4.6 and in Figure 4.5, Figure 4.6 and Figure 4.7.

Table 4.6 Effect of different types of antibiotics on *N. gonorrhoeae*

Antibiotics	Sym.	Sensitive		Intermediate		Resistant	
		No	%	No	%	No.	%
Ceftriaxone	CRO	90	100	0	0	0	0
Spectinomycin	STM	90	100	0	0	0	0
Ciprofloxacin	CIP	4	4.44	11	12.22	75	83.33
Penicillin	PEN	0	0	8	8.89	82	91.11
Tetracycline	TET	0	0	2	2.22	88	97.78

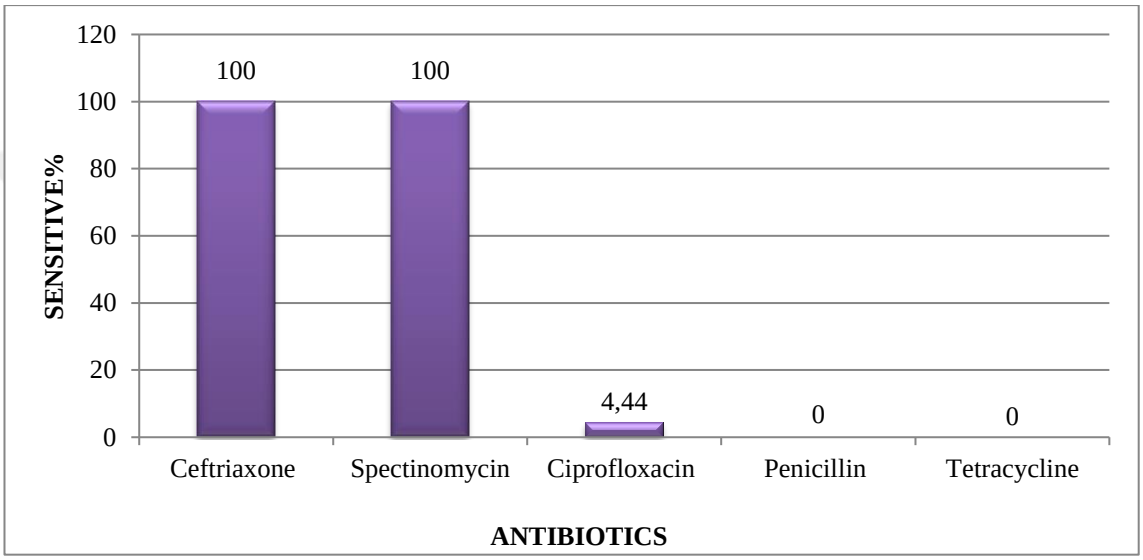

Figure 4.5 Sensitivity rate of *N. gonorrhoeae* isolates to different antibiotics

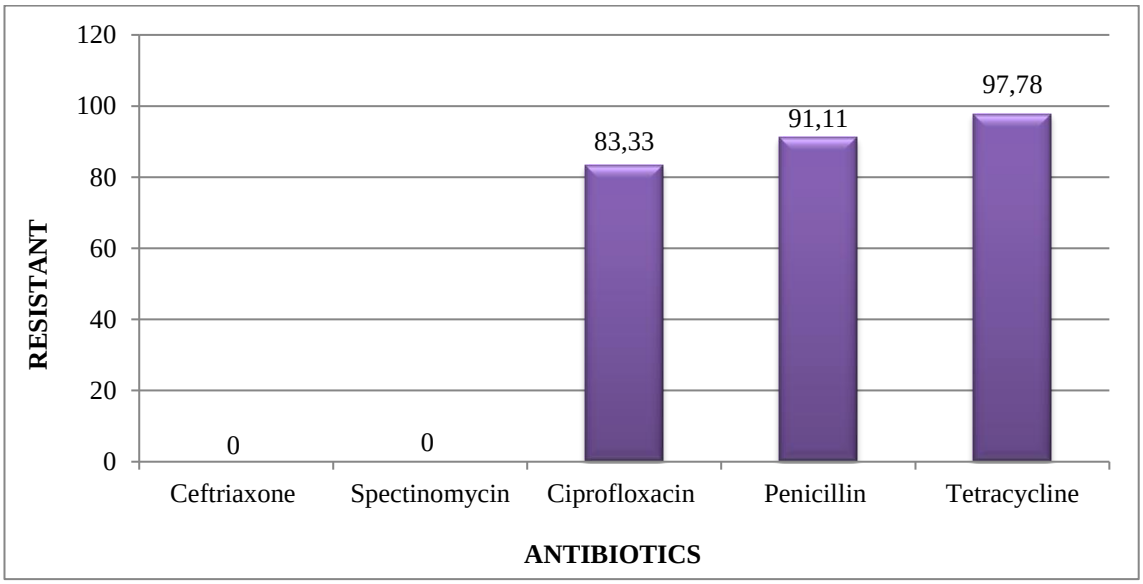

Figure 4.6 Resistant rate of *N. gonorrhoeae* isolates to different

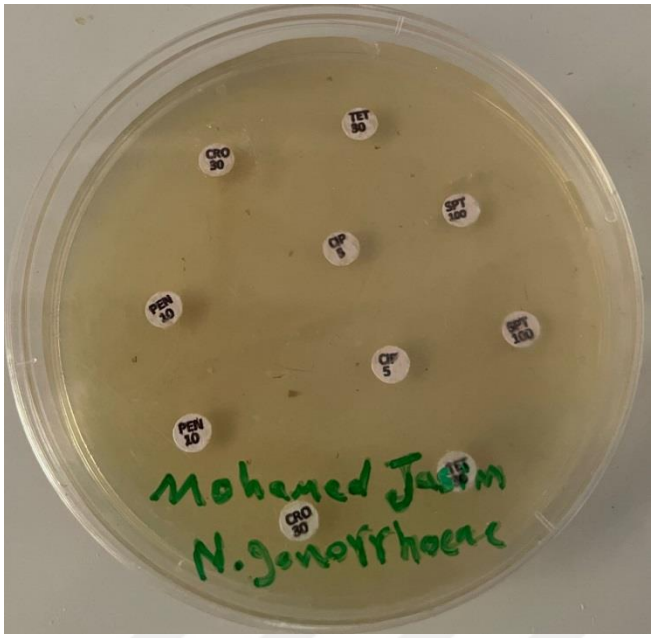

Figure 4.7 Diffusion of discs antibiotics on Mueller-Hinton agar medium

As expected, resistance to penicillin and tetracycline was very common (91.11% and 97.78%, respectively), this result was consistent with those mentioned in (Unemo *et al.* 2019, Wi *et al.* 2017), as the resistance of *N. gonorrhoeae* bacteria to penicillin and tetracycline (90.80% and 98.12%, respectively). These drugs have not been recommended for the treatment of gonococcal infection for many years because of the known high resistance rates amongst gonococci worldwide (Gross and Tyring 2011).

This study also found that 83.33% of *N. gonorrhoeae* were resistant to ciprofloxacin, with only 4.35% fully susceptible. This is a higher proportion than found in previous studies of smaller numbers of isolates from Asian Laos: reduced quinolone susceptibility rates were reported to be 20% in 2000–2001 (Sihavong *et al.* 2007), 75.9% in 2002 (WHO 2003), 65.5% in 2005 (WHO 2006), 33% in 2007 and 11% in 2008 (Tapsall *et al.* 2010). In another study in Asian Laos, ciprofloxacin resistance in *N. gonorrhoeae* was found to increase from 11.9% in 2000 to 69.5% in 2002 (Phantouamath *et al.* 2003). We suggest that *N. gonorrhoeae* from Laos should be considered likely to be quinolone-resistant unless proven otherwise. This is similar to the situation in Thailand (Srifeungfung *et al.* 2009), Vietnam (Olsen *et al.* 2013), Myanmar (Aung *et al.* 2013), China (Cao *et al.* 2015), Bangladesh (Khanam *et al.* 2016) and many other regions (Essack *et al.* 2017, CDC 2013,

Lahra 2013, ECDC 2010). Therefore, ciprofloxacin should no longer be used for treatment of gonococcal infection, This is consistent with the results obtained in our research.

The results also found that 100% of the *N. gonorrhoeae* isolates were sensitive to ceftriaxone and spectinomycin, this results was consistent with those mentioned in (Workowski and Bolan 2015), as the sensitivity of *N. gonorrhoeae* bacteria to ceftriaxone and spectinomycin (100%). In addition, ceftriaxone and spectinomycin were recommended for the treatment of infection with *N. gonorrhoeae* in pregnancy or in those who fail to respond to treatment with ciprofloxacin (Moodley *et al.* 2006).

4.8 Genetic Diagnosis of the Gene *Orf1*

90 *N. gonorrhoeae* isolates were chosen for genetic testing in this research in order to confirm the results of microscopy and culturing. The existence of the gene *Orf1* was screened using universal primer and the expected sizes for the gene fragments were 280 bp. the first step in adopting PCR protocol for gene amplification is to choose the DNA extraction procedure to get a target DNA which is suitable for applying in PCR experiment The results of this study indicated that DNA extraction kit procedure from isolated bacteria giving pure DNA fragments for all tested isolates in accordance with other researchers who preferred using direct and enrichment extraction procedure in clinical isolates when used as a template for PCR (Mahanta *et al.* 2011, Shahcheraghi *et al.* 2010). Results of the molecular diagnosis of the bacteria causing gonorrhea using specific primers that were specific for the *Orf1* gene, revealed that (91.11%) of samples (82 out of 90) were positive. there were 82 (91.11%) samples that had positive results (Figure 4.8).

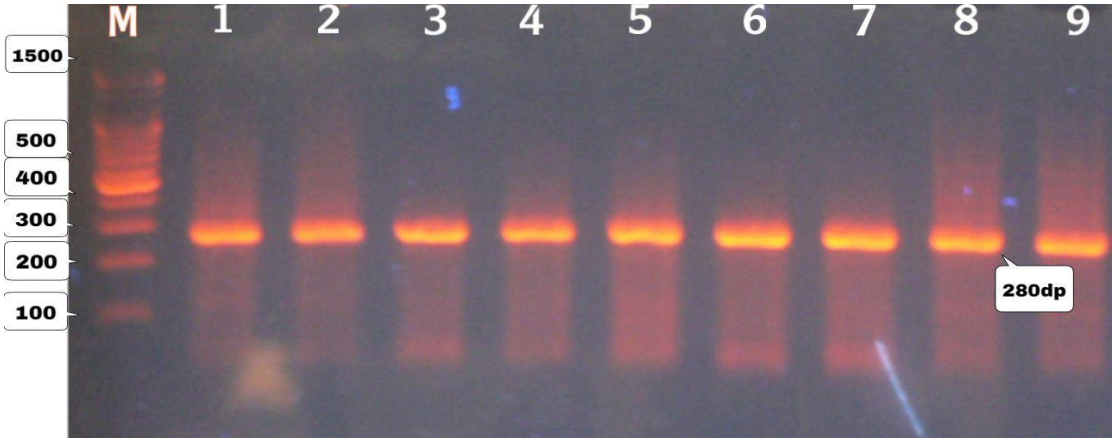

Figure 4.8 PCR results from *N. gonorrhoeae* isolates' extracted DNA that were amplified using *Orf1* gene primers are shown on an agarose gel stained with ethidium bromide. DNA molecular size marker Lane (M) (100-1500 bp ladder). The isolates in lane between 1 and 9

Among 90 positive samples which were diagnosed by traditional methods and analyzed for Conventional PCR detection of 16sRNA gene (260bp), there were 82 (91.11%) samples which had positive results (Table 4.7).

Table 4.7 Diagnosis by PCR of *N. gonorrhoeae* isolates

Test	Sample Patients	No %	
		Positive	Negative
PCR	90	82 (91.11)	8 (8.89)

Orf1 gene primers were used for the detection of *N. gonorrhoeae* in many researches, (Shahcheraghi *et al.* 2010). These primers were demonstrated to be a highly sensitive and specific biomarkers for the detection of *N. gonorrhoeae*. It is one of the specific targets to confirm infection, and positive amplification of N. gonorrhea specific DNA may be considered as a direct evidence of the presence of the pathogen (Farraj *et al.* 2010, Shahcheraghi *et al.* 2010). In a similar study performed by (Chaudhry *et al.* 2002) it was reported that *N. gonorrhoeae* was successfully isolated from urethral and cervical swabs of 379 patients in South India. Among 1100 endocervical swabs examined, 13 (1.18%) samples had positive results by using polymerase chain reaction (PCR) on *Neisseria gonorrhoeae* targeting CCPB gene (Hassanzadeh *et al.* 2013). Clear correlation appeared among PCR technique and culturing method and biochemical tests, since positive samples

gave positive amplification results with conventional PCR. When comparing the results of this study with other researchers (Mahanta *et al.* 2011, Alsaedi 2013), the present results agree with the results of (Alam *et al.* 2002) who reported that the PCR method is sensitive more than culture method in diagnosis of *N. gonorrhoeae*.

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

- The result showed that the high rate of infection was in 18-24 years of age (72.22%) this may be due to highly sexual activity in these age groups compared to other age groups.
- Education has an important role in reducing the spread of infection with *N. gonorrhoeae*, where the results showed that the highest percentage of total infection occurred among the illiterate classes (53.33%) and the lowest percentage (3.33%) was for those qualified with higher education.
- It was noted that the highest percentage of total infection was among patients with poor economic status (55.56%).
- Resistance to penicillin and tetracycline was very common (91.11% and 97.78%, respectively), this study also found that 83.33% of *N. gonorrhoeae* were resistant to ciprofloxacin, the results also found that 100% of the *N. gonorrhoeae* isolates were sensitive to ceftriaxone and spectinomycin
- Clear correlation appeared between PCR technique and culturing method and biochemical tests; when samples were positive with it gave positive amplification results with conventional PCR, but the PCR is more sensitive.

5.2 Recommendations

- Doing similar studies using large scale of patients and in great ethnic and population groups to prove these facts on the entire Iraqi population.
- Using the PCR reaction to diagnose various bacterial infections in Iraqi hospitals, as it is accurate in diagnosis, in addition to the possibility of examining a large number of samples simultaneously.
- Studying the other important genes which involve in pathogenesis and virulence of *N. gonorrhoeae*

- It is necessary to make a larger molecular study on antibiotic sensitivity of *N. gonorrhoeae* including more antibiotics.

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APPENDICES

APPENDIX 1. Ethics committee report

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APPENDIX 1. Ethics committee report (Continued)

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