UNIVERSITAT ROVIRA i VIRGILI

DEGREE IN BIOCHEMISTRY AND MOLECULAR BIOLOGY

FINAL DEGREE PROJECT

Epidemiology and antimicrobial susceptibility of *Neisseria gonorrhoeae* in the region of Tarragona, 2021-2022

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This project has been elaborated from the results obtained in my curricular practices carried out in the ICS Clinical Laboratory of the Joan XXIII Hospital, supervised by María Dolores Guerrero Torres.

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ABBREVIATIONS

AMR: Antimicrobial resistance

AZM: Azithromycin

BASSH: British Association for Sexual Health and HIV

CDC: Centers for Disease Control

CEEISCAT: *Centre d'Estudis Epidemiológics sobre les Infeccions de Transmissió Sexual i Sida de Catalunya*

CFX: Cefixime

CIP: Ciprofloxacin

CTA: Cysteine trypticase agar

CTX: Ceftriaxone

DGI: Disseminated gonococcal infection

DIF: Direct immunofluorescence

DNA: Deoxyribonucleic acid

DOX: Doxycycline

ECDC: European Center for Disease prevention and Control

ECOFF: Epidemiological cut-off

EEA: European Economic Area

ESC: Extended-spectrum cephalosporins

EU: European Union

EUCAST: European Committee on Antimicrobial Susceptibility Testing

FDA: Food and Drug Administration

HBV: Hepatitis B virus

HIV: Human immunodeficiency virus

HSV-2: Herpes simplex virus 2

I: Intermediate

im: Intramuscularly

IQR: Interquartile range

iv: Intravenous

LVX: Levofloxacin

MALDI-TOF: Matrix-Assisted Laser Desorption/Ionization Time of Flight

MDR: Multidrug-resistant

MIC: Minimum inhibitory concentration

MSM: Men who have sex with men

MSW: Men who have sex with women

MTZ: Metronidazole

NAAT: Nucleic acid amplification tests

NG: *Neisseria gonorrhoeae*

PCR: Polymerase chain reaction

PEN: Penicillin

PID: Pelvic inflammatory disease

PMN: Polymorphonuclear

po: Orally

PPV: Positive predictive value

R: Resistant

RCUT: Rapid carbohydrate utilization test

RENAVE: *Red Nacional de Vigilancia Epidemiológica*

RNA: Ribonucleic acid

S: Susceptible

SD: Single dose

STI: Sexually transmitted infection

WHO: World Health Organization

WSM: Women who have sex with men

XDR: Extensively drug-resistant

1. ABSTRACT

Neisseria gonorrhoeae (NG) is the etiologic agent of gonorrhea, the second most prevalent sexually transmitted infection (STI) and one of the most susceptible to becoming untreatable. In this research, a retrospective study was carried out on the sociodemographic, behavioral and clinical characteristics of 111 patients with gonorrhea in the Tarragona region, in addition to studying the antimicrobial resistance (AMR) of the isolated strains and comparing the use of PCR and microbiological culture as a diagnostic test. The study showed that PCR is used more than culture, that the characteristic profile of patients is a male between 24-34 years of age with symptomatic genital gonorrhea, and that only 2.8% of the isolates were susceptibility to all the tested antimicrobials. Epidemiological surveillance and AMR studies facilitate the adoption of measures that stop the appearance of multi-resistant strains.

1. RESUMEN

Neisseria gonorrhoeae (NG) es el agente etiológico de la gonorrea, la segunda infección de transmisión sexual (ITS) más prevalente y una de las más susceptibles a convertirse en intratable. En este trabajo se realiza un estudio retrospectivo sobre las características sociodemográficas, conductuales y clínicas de 111 pacientes con gonococia en el área de Tarragona, además de estudiar las resistencias antimicrobianas (AMR) de las cepas aisladas y comparar el uso de la PCR y el cultivo microbiológico como prueba diagnóstica. El estudio reflejó que se utiliza más la PCR que el cultivo, que el perfil característico de los pacientes es el varón de entre 24-34 años con gonococia genital sintomática y que sólo el 2.8% de los aislamientos fueron sensibles a todos los antimicrobianos testados. La vigilancia epidemiológica y los estudios de AMR facilitan la adopción de medidas que frenan la aparición de cepas multirresistentes.

2. INTRODUCTION

2.1 HISTORICAL EVOLUTION

Neisseria gonorrhoeae, a bacterium known as gonococcus, is the etiological agent of gonorrhea, a strictly human STI. Nowadays, gonorrhea is considered the second most prevalent bacterial STI globally (1).

Although exactly when it originated is unknown, gonorrhea has affected humans for thousands of years. In fact, there are references to this disease in ancient Egyptian, Roman, Greek and Chinese literature, as well as in the Old Testament of the Bible (Leviticus 15:1-3).

The term gonorrhea comes from the Greek *γονόρροια,* which translates as seminal flow, and which arises in the second century from the erroneous interpretation of Galen of Pergamum, a Greek philosopher and physician, of the purulent urethral discharge that was produced during infection (2). The Romans were also familiar with gonorrhea and believed that it originated in Greece, which is why they described it as “the disease of the Greeks”.

During the Middle Ages, the disease became an epidemic on the European continent. In this context, in 1378, the infection began to be cited as “the clap”, an expression that probably descends from the name of the old Parisian district Les Clapiers where prostitutes were housed (3).

The microorganism causing the infection was described in 1879 by Dr. Albert Neisser during his microscopic studies of stained smears from samples of purulent exudates from individuals with urethritis and *ophthalmia neonatorum*. Neisser described the organisms identified as micrococci due to their spherical shape (4), which is currently known that they were gonococcal diplococci.

The *in vitro* isolation of the bacterium was achieved for the first time in 1882 thanks to the cultures of Leistikow and Löffler. However, it was not until 3 years later, in 1885, when Bumm achieved pure gonococcal cultures and was able to demonstrate the etiological relationship of the infection by inoculating healthy volunteers. Finally, in that same year, Trevisan definitively coined the name *Neisseria gonorrhoeae* for this bacterium in honor of Neisser (5–7).

During the preantimicrobial era, treatment of gonorrhea consisted mainly of abstaining from alcohol and sexual activity, leading a healthy lifestyle and receiving systemic cures based on traditional balsams, urethral irrigations, chemical compounds (arsenic, antimony, mercury…) and hyperthermia (3).

Sulfonamides, discovered by Gerhard Domagk in 1935, were the first antibiotics used to treat gonorrhea. For a long time, gonococcal infections were easy to treat with the different therapeutic agents that were emerging (penicillin, quinolones and macrolides, mainly) but, successively, strains resistant to all of them have appeared. Due to high resistance to antimicrobials, *Neisseria gonorrhoeae* is currently highly susceptible to becoming an intractable infection (8). Considering that the World Health Organization (WHO) estimated about 82.4 million new cases of gonorrhea in 2020 (9), the development of multi-resistant (MDR) and extensively resistant (XDR) gonococci represents a major problem for public health that can lead to an increase serious complications and increased transmission of other STIs such as HIV (8).

For this reason, it is essential to develop programs that detect and prevent the emergence and spread of drug resistance and adequately control the infection. To facilitate the adoption of effective measures against multi-resistant strains, the WHO promotes the "Global action plan to control the spread and impact of antimicrobial resistance in *Neisseria gonorrhoeae*" and, in addition, considers gonococcus as a "Priority 2" pathogen in the "Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery and Development of New Antibiotics" (8–11).

2.2 *NEISSERIA GONORRHOEAE* DESCRIPTION

2.2.1 MICROBIOLOGICAL CHARACTERISTICS

The bacterium *Neisseria gonorrhoeae* belongs to the genus *Neisseria*, family *Neisseriaceae*, order *Neisseriales*, class *Beta Proteobacteria*, phylum *Proteobacteria*. Today more than 30 species included in the genus *Neisseria* are known, of all of them 11 are capable of colonizing humans, but only 2 are considered pathogenic: *Neisseria gonorrhoeae* and *Neisseria meningitidis*.

Specifically, *N. gonorrhoeae* is a Gram-negative coccus with a size between 0,6 and 1 µm in diameter. In the same way as the rest of the bacteria, it reproduces asexually by binary division. However, in the case of *N. gonorrhoeae*, this division is not complete since the septa of each of the daughter cells that originate do not separate completely, leaving the contiguous sides flattened. For this reason, gonococci group in pairs (diplococci) with an appearance like coffee beans. Its morphology, together with its intracellular location within neutrophils, contributes to its identification under light microscopy (12–15).

Neisseria gonorrhoeae presents a cell wall typical of Gram-negative bacteria, made up of a peptidoglycan layer and an outer membrane that includes complex polysaccharides bound to lipids and proteins. Unlike *N. mengiditis*, *N. gonorrhoeae* does not contain a capsule. In addition, it is also characterized by being immobile, containing neither spores nor flagella and by including on their cell surface pili and outer membrane proteins.

Biochemically, the gonococcus is defined as an aerobic or facultative anaerobic chemoorganotrophic microorganism. That is, it is a bacterium that obtains energy through the oxidation of organic compounds both in the presence and in the absence of oxygen, if nitrite is administered as the final electron acceptor in an anaerobic situation (16). All species belonging to the genus *Neisseria* are oxidases (they have cytochrome c oxidase) and catalases (ability to transform hydrogen peroxide into oxygen) positive. NG differs from the rest of the species due to its metabolic capacity to transform only glucose, but not maltose, sucrose, lactose, fructose or mannose (12).

The culture of NG is complicated due to its high susceptibility to environmental conditions. The bacterium undergoes autolysis when exposed to air, desiccation, ultraviolet light, extreme temperatures, alkaline or acid conditions, silver salts, and phenol. Additionally, all strains have demanding nutritional requirements and need cysteine, glucose, pyruvate or lactate as a carbon source in addition to specific growth factors such as vitamins, amino acids, nucleotides or minerals, among others. The most widely used culture medium is Thayer-Martin, enriched with a medium based on chocolate agar, nutritional supplements, hemoglobin and different antibiotics (14–16).

Optimum growth conditions occur at a temperature between 35°C-37°C, atmosphere 3-5% CO_2 and pH 7.2-7.6. Colonies can be observed after 24-48 hours, but due to its rapid autolysis, viability is lost after 48 hours (15,16).

2.2.2 VIRULENCE FACTORS

Throughout evolution, bacteria have evolved characteristics that allow them to invade host organisms, adhere to them through surface receptors, infect them, and evade the defense mechanisms of the immune system. Any molecular, structural or functional attribute of a pathogenic microorganism that allows or favors the invasion, growth and/or survival of the infection on the host is considered a virulence factor (17).

Like many other Gram-negative pathogens, *Neisseria gonorrhoeae* also possesses multiple virulence factors, including type IV pili, Por protein, Opa protein, Rmp protein, lipooligosaccharides, peptidoglycans, and IgA protease. Furthermore, antigenic shift of the various virulence factors occurs in 1 in 10^3 gonococci, a rapid rate of change for bacteria. It is this fact that allows *N. gonorrhoeae* to effectively evade the host's immune system (18).

Neisseria gonorrhoeae is a strictly human pathogenic microorganism that is not capable of surviving outside the host. Despite this, it can exist both extracellularly and intracellularly in hosts, so most of its genes encode elements involved in bacterial colonization and survival. Transmission is usually caused by sexual contact with an infected person, although it is not the only possible path of transmission (19).

Gonococcal infection can be divided into different stages: adhesion to the mucosa, invasion of epithelial cells, and colonization of tissues. The resulting infection may occur only at a local level or may spread, and cause disseminated infection.

2.3 CLINICAL SIGNS AND SYMPTOMS

Gonorrhea is transmitted mostly through sexual contact or perinatal contact from mother to child. The mucous membranes that can be affected are those of the urethra, cervix, rectum, oropharynx, and conjunctiva (20–22). The physical symptoms and signs vary depending on the anatomical location of the infection and whether there is coinfection with other pathogens, although many cases may be asymptomatic.

2.3.1 UROGENITAL INFECTION

Urogenital tract infections are the most common, especially in adults. They regularly present coinfection with other STIs such as *Chlamydia trachomatis* and facilitate the transmission and acquisition of HIV (22).

2.3.1.1 Men

Gonococcal infection in men mainly causes acute urethritis with symptoms of purulent urethral discharge (in more than 80% of cases) and dysuria (in more than 50% of cases). Asymptomatic urethral infection in men is very rare, it is estimated that less than 10% of infections are of this type. The incubation period varies between 2-8 days after exposure. (20).

If the urogenital infection is not treated and eliminated, it can ascend to the upper genital tract and cause severe reproductive complications that result in infertility, such as epididymitis or prostatitis (21).

2.3.1.2 Woman

In the case of women, the predominant symptom associated with gonorrhea is cervicitis, which occurs 5 to 10 days after exposure. However, the manifestations are generally mild and can mimic acute cystitis or vaginitis. Female endocervical and urethral infection includes leucorrhea in 50% of cases, abdominal pain in 25% of cases, dysuria in 10-15% of cases, and occasionally intermenstrual bleeding or menorrhagia. Unlike men, urogenital gonococcal infection in women is frequently asymptomatic (>50%) (20,22).

If not treated correctly, the infection can also ascend to the upper genital tract and cause pelvic inflammatory disease (PID), tubal factor infertility, and ectopic pregnancies. In pregnant women, gonorrhea has been linked to chorioamnionitis, premature rupture of membranes, spontaneous abortion, preterm delivery, low birth weight, and perinatal mortality (23).

2.3.2 ANORECTAL INFECTION

Rectal infections, in both men and women, are usually asymptomatic (>50%). Yet, in the rest of the cases it presents as a proctitis whose signs and symptoms are mostly anal discharge, perianal/anal pain or discomfort, tenesmus or rectal bleeding. This type of infection often occurs in men who have sex with men (MSM), although depending on the sexual practice, it can occur in people of both sexes. (20,22).

2.3.3 PHARYNGEAL INFECTION

Pharyngeal gonorrhea is transmitted through unprotected oral sex. In the same way as anorectal infections, pharyngeal infection is commonly asymptomatic in most cases (>90%), both in men and women. It is estimated that 20% of women with urogenital gonorrhea are coinfected with pharyngeal gonorrhea. Signs and symptoms include sore throat, erythema, oropharyngeal exudates, and lymphadenopathy (20,22).

Pharyngeal gonococcal infection is one of the reservoirs where a greater horizontal transfer of genes occurs between species of saprophytic microorganisms, therefore it plays a fundamental role in obtaining resistance genes (3).

2.3.4 CONJUNCTIVA INFECTION

N. gonorrhoeae can also affect the conjunctiva and cause conjunctivitis. This infection can occur in adults but mainly occurs in newborns (*ophthalmia neonatorum*) who are infected by their mothers as they pass through the birth canal. Gonococcal conjunctivitis has an incubation period of approximately 6 days and is characterized by pain, photophobia, and purulent discharge. If not treated promptly, it can lead to perforation of the cornea and blindness (21–23).

2.3.5 DISSEMINATED INFECTION

Disseminated gonococcal infection (DGI) or gonococcal bacteremia is a rare complication arising from hematogenous dissemination of *N. gonorrhoeae* from primary mucosal infection. It affects 0.4-3% of patients and is usually more common in areas with a high prevalence and incidence rate of gonorrhea (20,22).

Patients with DGI may present with nonspecific symptoms such as fever, malaise, or myalgia. However, the most common manifestations associated with DGI are synovial and cutaneous, including septic arthritis, asymmetric arthropathies, polyarthralgia, tenosynovitis, painful or painless petechiae, macules, papules, pustules, abscesses, vesicles, vasculitis, fasciitis, cellulitis, among others. The progression of the disease can lead to other serious complications such as perihepatitis, osteomyelitis, meningitis or endocarditis (22,24).

2.3.6 INFECTION IN CHILDREN

Gonococcal infection in childhood is classified based on clinical, moral and legal aspects into neonatal infection and postnatal infection.

Neonatal gonococcal infection occurs, as mentioned above, by exposure during childbirth to infected genital secretions from the mother. The most frequent clinical manifestation is *ophthalmia neonatorum*, although it can include other complications, for example, sepsis, meningitis and arthritis. Other symptoms also included are: pharyngitis, rhinitis and pneumonia. Babies can also develop scalp infections or abscesses from open wounds (22).

After the neonatal period, any STI suggests the possibility of sexual abuse. Even in some cases, urogenital infection in girls can be accidentally acquired in unhygienic environments, gonococcal infection in preadolescents is probably an indication of sexual abuse. Vaginitis is the clinical manifestation typical in prepubertal girls, while urethritis is the most in boys. The physical signs are expressed in the same way as in adults and, similarly, anorectal and pharyngeal infections go unnoticed as they are mostly asymptomatic (22).

2.4 DIAGNOSIS

Because *N. gonorrhoeae* is highly susceptible to environmental conditions, especially in culture, it is essential that the collection, transport and storage processes are correct and that there is a minimum delay between them to increase the viability of the microorganism and, therefore, the diagnostic performance.

The diagnosis of gonorrhea is established through the detection of NG or its genetic material in genital or extragenital samples employing microscopy, culture or nucleic acid amplification tests (NAATs).

2.4.1 SPECIMEN COLLECTION, TRANSPORT AND STORAGE

The choice of the specimen and the collection method depends on the diagnostic technique used in the laboratory, as well as the age, sex, clinical manifestations and sexual practices of the patient (15).

All specimens should be collected with Dacron or rayon swabs before any antibiotic treatment is administered. Moreover, the use of antiseptics, analgesics, lubricants, cotton swabs (contain fatty acids) and cellulose swabs (contain sulfur compounds) should be avoided because all these compounds are toxic and inhibit the growth of NG (15).

Generally, the specimen most used in women for the detection of NG by culture and microscopy is endocervical exudate, while for diagnosis using NAATs the best specimen is vaginal or endocervical exudates. In the case of heterosexual men, the ideal specimen for culture and microscopy is the urethral exudate and for NAATs, urine. In MSM and men and women with indicative clinical signs of anal and/or oral sexual practices, additional samples should be collected from the rectum and pharynx. Likewise, in case of suspicion of DGI, blood and joint fluid samples should also be taken (25).

After taking the sample, the ideal would be to immediately inoculate it into the culture medium to preserve the viability of the gonococcus. The culture should be carried out within 6 hours after sample collection, since after that time the viability of NG decreases significantly (15,25).

2.4.2 MICROSCOPY

In direct microscopy of the specimens, 2 different staining methods can be used: staining with methylene blue or Gram staining, the latter being the most widely used. In methylene blue staining, all bacteria are stained blue, while Gram staining allows differentiating between Gram-negative and Gram-positive bacteria.

The typical microscopic pattern of gonococci is intracellular storage in pairs (diplococci). In symptomatic men, microscopic observation (1000x) of more than 2 polymorphonuclears (PMNs) cells per field and intracellular Gram-negative diplococci allows a diagnosis of gonococcal urethritis to be made with high sensitivity (90-95%) and specificity (97-99%) (3,20,25,26).

This detection test is inexpensive, simple, provides rapid results, and has high sensitivity (90-95%) and specificity (97-99%) for diagnosis in symptomatic men with urethral discharge. However, in asymptomatic patients, endocervical specimens and extragenital specimens, the sensitivity and specificity of the test are extremely low and negative results do not exclude a possible infection, which is why it is not recommended as a diagnostic method.

In addition, microscopy in extragenital specimens is not recommended due to the presence of other bacteria of the saprophytic flora, especially other *Neisseria* species, with morphology like NG that compromises the microscopic result. It should be noted, on the other hand, that this method does not provide any antimicrobial resistance (AMR) data (3,20,25,26).

2.4.3 CULTURE AND IDENTIFICATION

2.4.3.1 Culture of *N. gonorrhoeae*

Culture is a specific and relatively sensitive diagnostic test appropriate for endocervical, urethral, rectal, oropharyngeal, and conjunctival specimens, but not for urine or vaginal specimens. Cause of the nutritional and environmental requirements of *N. gonorrhoeae*, the reliability of a culture for the detection of gonorrhea can be affected by various factors: anatomical location of the sample, specimen collection technique, transport conditions and duration, composition and quality of the culture medium and inoculation and incubation conditions (25).

Referring to what was previously described, gonococci are very strict pathogens, both nutritionally and environmentally. They do not tolerate desiccation and should be inoculated immediately after sample collection into non-selective and selective culture media containing antimicrobials (vancomycin, colistin, trimethoprim lactate, nystatin, or amphotericin B) that inhibit the growth of other commensal bacteria and fungi. The culture medium, both non-selective and selective, must be enriched, like chocolate agar, in vitamins, amino acids, nucleotides, iron and other growth factors. The most widely used laboratory media for the routine diagnosis of gonorrhea are Thayer-Martin, modified Thayern-Martin, Martin Lewis, and New York City, which differ from each other in the antimicrobial agents they contain. Once they have been inoculated, the plates should be incubated at a temperature of 35-37°C in a moist atmosphere enriched in CO_2 (3-5%) for approximately 48h (15).

Over the years, culture has been considered the gold standard method for the isolation and identification of *N. gonorrhoeae* and is the only established method that allows full AMR testing, essential for detecting and monitoring antimicrobial resistance. Furthermore, if necessary, culture also provides the possibility of carrying out bacterial typing studies and is the recommended method for persistent infections or suspicion of therapeutic failure. Despite this, it is a slow test and requires specimen collection, transport and storage-specific conditions, as well as a very strict culture methodology to obtain adequate sensitivity and specificity (3).

2.4.3.2 Presumptive identification

After culture, the presence of NG is studied by combining several detection methods. One of them is the presumptive identification, based on the observation of the morphology and appearance of the colony on the plate (shiny greyish color), the microscopic study of the Gram stain (Gram-negative diplococci) and the catalase and oxidase tests positive**.**

2.4.3.3 Confirmatory identification tests

To confirm the identification of *N. gonorrhoeae* and to rule out other closely related species, such as *N. meningiditis*, *N. lactamica* and other *Neisseria*, especially in extragenital samples, confirmatory identification tests are performed, which can be biochemical, immunological or based on the spectrometry of masses (25).

I. Biochemical tests

N. gonorrhoeae can be differentiated from other species by its ability to exclusively metabolize glucose (or dextrose) and no other sugars. Traditionally, detection of this unique pattern of carbohydrate fermentation is performed by inoculating pure cultures onto different cysteine trypticase agar (CTA) medium, each supplemented with a 1% different sugar and phenol red as a pH indicator. After approximately 24 hours, if the bacteria can metabolize the sugar in question, the production of acid because of fermentation will lead to a pH change in the culture medium resulting in a color change of the indicator from red to yellow (15,25). In the case of NG, a color change is expected only on CTA agar medium supplemented with glucose.

This method has been replaced by other tests based on the same principle that are commercially available and are faster, more specific (99%) and more sensitive (100%), such as the rapid carbohydrate utilization test (RCUT). The RCUT is a nongrowth-dependent method for the detection of acid production from carbohydrates by *Neisseria* species. In addition, some of the commercial tests include not only acid production tests, but also tests for other biochemical characteristics (such as enzyme production), including DNase and nitrate reduction (15).

Another type of biochemical test used for the identification of *N. gonorrhoeae* is the detection of enzymes that hydrolyze chromogenic substrates (compounds that contain a color-forming group). These substrates are commercially available to detect the presence of a multitude of enzymes, including glycosidases, peptidases, phosphatases or esterases, among others. For the identification of NG, the enzymes β-galactosidase, γ-glutamylaminopeptidase, and prolyl-hydroxyprolyl aminopeptidase should be detected. However, its exclusive use is not recommended since other *Neisseria* species can give false positives and, in addition, there are also gonococcal strains that do not show prolyl-hydroxyprolyl aminopeptidase activity and can give false negatives. (25).

None of the biochemical tests for the confirmatory identification of NG are 100% sensitive and specific, so tests based on other principles may be required to resolve doubtful results (15).

II. Immunological tests

Immunological methods used for confirmatory identification of gonococcus are direct immunofluorescence (DIF) and coagglutination. Both are based on the binding of monoclonal antibodies to the gonococcal membrane protein PorB. These assays can be performed directly on the colonies on the plate and do not require a pure subculture, making them faster than biochemical identification tests.

The most popular commercial kit for the coagglutination test is the Phadebact Monoclonal GC (MKL Diagnostics) which contains staphylococcal cells coated with a mixture of monoclonal antibodies directed against the gonococcal protein PorB. When the antibodies mix with the porin, agglutination occurs, visible thanks to a blue-colored reagent that facilitates reading. GonoGen II (BioConnections) uses a similar panel of antibodies except that they are attached to metal particles instead of staphylococcal cells (15,25).

As an immunofluorescent method, there is the MicroTrak *N. gonorrhoeae* test (Trinity Biotech) that contains monoclonal antibodies labeled with fluorescein isothiocyanate specifically directed to the Por proteins of the gonococcal outer membrane. In this way, when observing a positive culture for NG under the fluorescence microscope, the diplococci show a fluorescent green stain (15,25,26).

III. MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time of Flight)

MALDI-TOF is a technique based on mass spectrometry that permits us to quickly and easily identify bacteria based on their protein profile.

The mass spectrum resulting from this test act as a fingerprint that allows specific identification of the microorganism in question. Although this method has been established to have a positive predictive value (PPV) of 99.3% for the detection of NG, the results should be interpreted with caution for NG and other *Neisseria* species when isolated from extragenital samples (26).

2.4.4 NUCLEIC ACID AMPLIFICATION TESTS

Confirmation of *N. gonorrhoeae* can also be achieved from the identification of its genetic material (DNA or RNA) through molecular tests such as NAATs, which are used for identification from culture or, more frequently, to perform the diagnosis directly from the sample.

NAATs are the most sensitive and specific diagnostic tests. Compared with culture, molecular techniques, apart from being more sensitive, are easier and faster to perform, could be automated, detect non-viable gonococci, and are therefore less demanding regarding the collection, transport, and storage conditions of the specimen and can be realized with a wider range of specimens, including non-invasive and self-collected samples from the patient. The guidelines recommend urine as a specimen for molecular tests, in the case of men, and vaginal or endocervical exudates, in the case of women.

In another way, NAATs are recommended for both asymptomatic and symptomatic individuals and, even though not all tests are validated by the Food and Drug Administration (FDA), they are more sensitive than culture for extragenital specimens (rectal and pharyngeal). Besides, many of the commercial NAATs are included for the simultaneous identification of multiple pathogens (*N. gonorrhoeae*, *C. trachomatis*, *Mycoplasma genitallium*, among others) in a single reaction, which increases the performance of this type of test (3,20,25,26).

Regardless, there are also disadvantages associated with NAATs. It should be considered that the diagnostic accuracy of the technique may be affected by genetic variations and genomic plasticity of NG. It has been corroborated that the loss or modification of the target regions to whom the tests are directed reduces the sensitivity, as well as can decrease the specificity with the appearance of cross-reactions with other *Neisseria* species present in the common flora. Furthermore, the sensitivity and specificity vary according to the type of amplification technique. Other disadvantages of diagnosis from

NAATs are the high cost of equipment and reagents and the impossibility of performing AMR tests (3,20,25,26).

The first molecular tests developed were non-amplified nucleic acid hybridization assays, such as PACE 2 (Gen-Probe). Currently, the main commercial NAATs are based on the most widely used polymerase chain reaction (PCR), transcription-mediated amplification and strand displacement amplification.

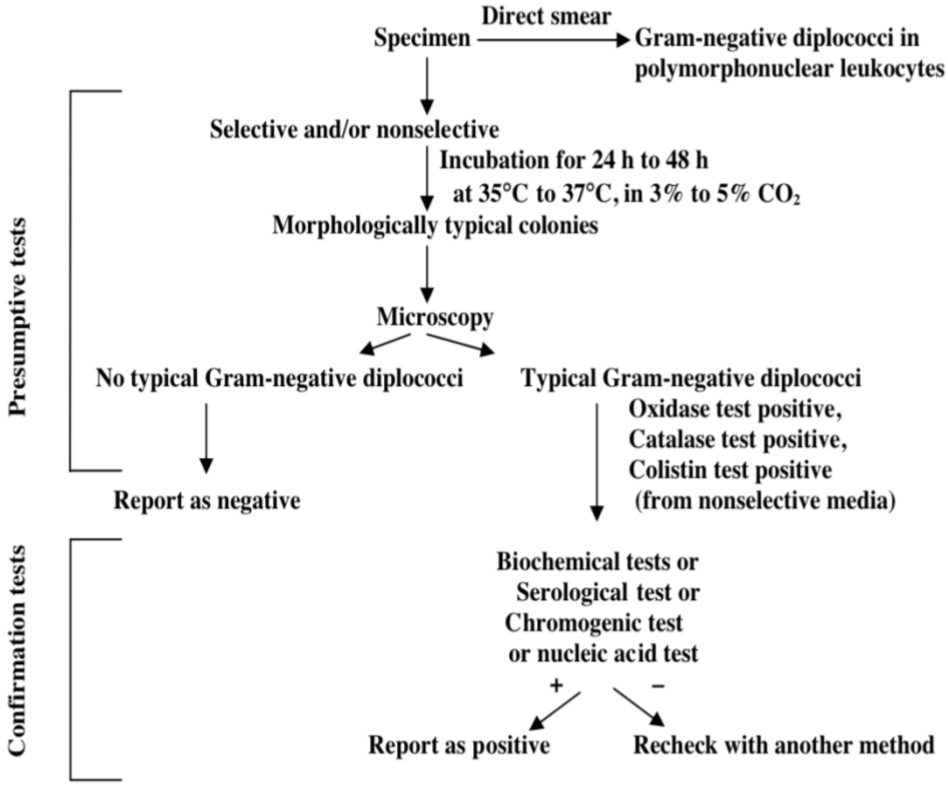


Figure 1 - Algorithm for culture and identification of Neisseria gonorrhoeae (15).

2.5 TREATMENT AND ANTIMICROBIAL SUSCEPTIBILITY

2.5.1 HISTORY OF TREATMENT AND EVOLUTION OF RESISTANCES

Since the introduction of sulfonamides as the first antimicrobial agent for the treatment of gonorrhea in the first half of the 20th century, *N. gonorrhoeae* has demonstrated its continued ability to develop resistance to all antibiotics that were introduced. Figure 2 (8) illustrates in a timeline the evolution of antibiotic treatments against gonorrhea, as well as the appearance of resistance to these agents over time.

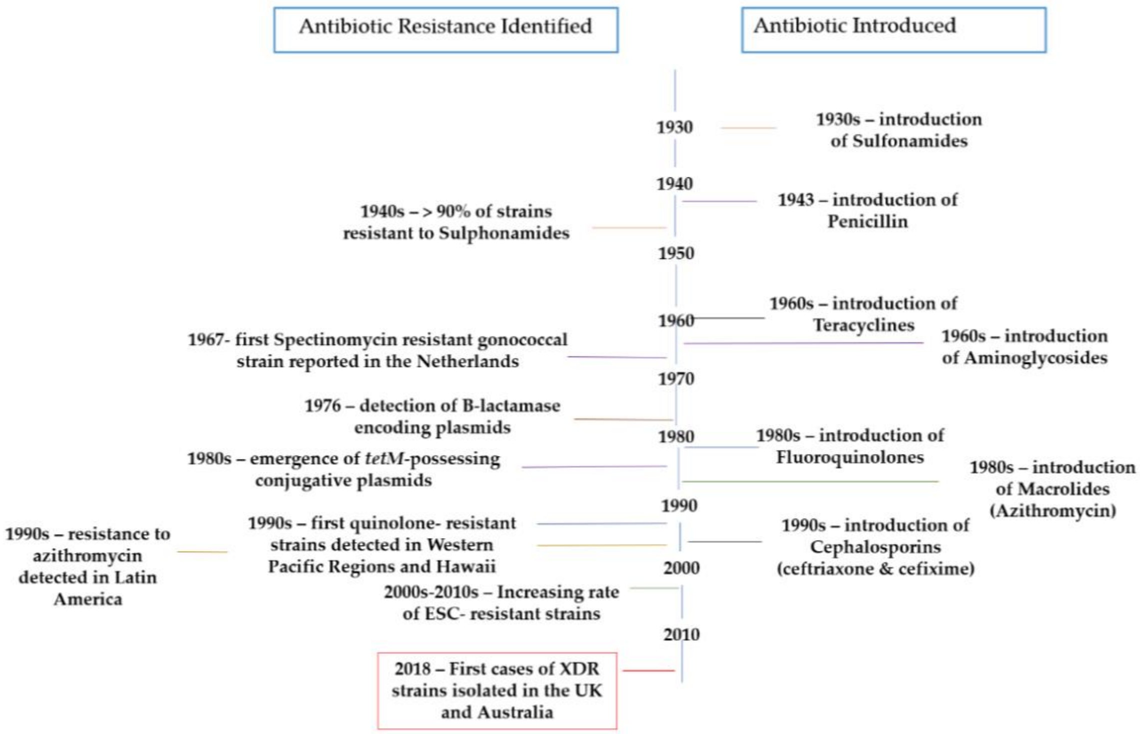


Figure 2 - Timeline of the introduction of treatments used against gonorrhea (right) and the first reports of resistance (left).

I. SULFONAMIDES

In 1935, after Gerhard Domagk's discovery, sulfonamides became the first antimicrobial used for the treatment of gonorrhea. Although, initially, they were able to cure 80-90% of cases, resistant gonococcal strains began to appear, causing therapeutic failure. During the following years, sulfonamides were replaced by other related agents, such as sulfapyridine or sulfathiazole. Even so, by the end of the 1940s, practically 90% of gonococcal isolates were resistant to sulfonamides *in vitro*. Despite this, especially in low-resource countries, sulfonamides (sulfamethoxazole) continued to be used in combination with trimethoprim for decades (3).

I. PENICILLIN

Penicillin (PEN), accidentally discovered in 1928 by Alexander Fleming, was introduced as an antimicrobial treatment for gonorrhea in 1943, mainly in cases of treatment failure with sulfonamides. Penicillin quickly supplanted sulfonamides as the first-line treatment of gonorrhea. In the first years, penicillin, in low doses, cured more than 95% of cases. However, over time, gonococcal strains began to accumulate chromosomal resistance determinants that led to a progressive increase in penicillin doses required to obtain appropriate cure rates. In 1946, the first cases of gonorrhea resistant to high doses of penicillin were detected and, during the following decades, the proportion increased.

Because of the appearance of resistance mediated by plasmid and chromosomal determinants, penicillin was no longer used as a first-line antimicrobial in the late 1980s. Prsently, gonococcal strains with resistance mediated by plasmids and/or chromosomal mechanisms to penicillin are common worldwide (3,27).

II. TETRACYCLINES

Tetracyclines were introduced as a therapeutic option for gonorrhea in the 1950s, especially in patients allergic to penicillin. As with penicillin, the gradual accumulation of chromosomal and plasmid resistance determinants caused the tetracycline minimum inhibitory concentration (MIC) for gonorrhea to increase over time. The first tetracycline-resistant gonococcal strains were reported in the United States in 1985 and now are widespread globally (3,27).

III. SPECTINOMYCIN

Spectinomycin was synthesized and commercialized as a specific treatment for gonorrhea in the early 1960s, primarily for cases of high-level resistance to penicillin. In 1967, the existence of a gonococcal strain resistant to spectinomycin was reported for the first time and, in the 1980s, it was no longer used as a first-line empirical treatment. Although, currently, is exceedingly rare to find gonococcal strains with high-level resistance to spectinomycin, this antibiotic is not available in many countries because it is feared that resistance will be rapidly selected among gonococcal strains. Likewise, this drug reaches suboptimal concentrations for the treatment of pharyngeal gonorrhea, with an efficacy of around 80% (3).

IV. QUINOLONES

Synthetic quinolones were discovered in the 1960s as a by-product of the manufacture of chloroquine. Nalidixic acid is the predecessor compound of all fluoroquinolones. In the 1980s, fluoroquinolones (especially ciprofloxacin) were widely used to treat gonorrhea. In the first place, low doses were recommended but, with the appearance of resistance, these doses were increased. Around 1990, some Western Pacific Asian countries abandoned fluoroquinolones as first-line treatment for gonococcal infection, but it was not until the mid-2000s that they ceased to be used without exception in most states. Nowadays, the prevalence of gonococcal strains resistant to fluoroquinolones is high worldwide (3).

V. MACROLIDES

Until the development of azithromycin (AZM) in the 1980s, no macrolide was sufficiently effective against gonorrhea. Yet, in the mid to late 1990s, a decline in susceptibility and resistance to azithromycin began to be reported in Latin America. Subsequently, this resistance arose in other areas of the world, especially in those where there was a high use of azithromycin. As in the case of spectinomycin, azithromycin is not recommended as monotherapy for fear of a rapid selection of resistance. Still, azithromycin is used as a dual therapy for gonorrhea in most treatments (3).

VI. CEPHALOSPORINS

The cephalosporins most generally recommended and used for gonococcal treatment are extended-spectrum cephalosporins (ESC) or third-generation cephalosporins: injectable ceftriaxone (CTX) and oral cefixime (CFM). Nevertheless, due to the lack of availability of cefixime, some countries have also used other oral cephalosporins such as cefuroxime, cefditoren, cefpodoxime and ceftibuten (3,21).

Since the 1990s, ESC-resistant gonococcal strains have begun to emerge, first in Japan and later throughout the world. The fact that ceftriaxone was not approved as a treatment in Japan until the late 1990s led to the use of many oral cephalosporins in suboptimal doses as monotherapy. Consequently, this could have led to subinhibitory therapeutic doses and thus to the selection of strains resistant to cephalosporins. Over time, this has led to a progressive increase in the MICs of cefixime and ceftriaxone, leading to resistance and therapeutic failure (3).

In 1995, the first cefixime-resistant gonococcal strain was isolated in Kanagawa, Japan. From 1999 to 2001, eight treatment failures were reported with cefixime (200mg) and four more were documented with a prolonged regimen of cefixime (200mg/6h 3 days) in 2002 and 2003. In 2006, Japan excluded all oral ESCs from treatment guidelines (21).

Over the last two decades, gonococcal strains with resistance to ESCs have spread globally, which added to the existence of resistance to other categories of antimicrobials, has given rise to MDR and XDR gonococci. MDR strains are defined as those that present resistance to 1 of the currently recommended therapies (cephalosporins or azithromycin) plus resistance to at least 2 other antimicrobials (penicillin, ciprofloxacin, tetracycline, erythromycin, among others). Instead, XDR strains are defined as those that present resistance to 2 of the currently recommended therapies (cephalosporins and azithromycin) plus resistance to at least 2 other antimicrobials (penicillin, ciprofloxacin, tetracycline, erythromycin, among others).

In 2010, the entire scientific community was alerted to the first isolations of XDR strains to all ESCs. The first strains of N. gonorrhea XDR resistant to ESC, and the rest of the antimicrobials against gonorrhea, were identified in Kyoto (Japan), Quimper (France) and Catalonia (Spain) (21).

Considering that ceftriaxone is the last option for empirical first-line monotherapy against gonorrhea, the appearance of XDR gonococci could usher in a new era where gonococcal infection become untreatable (3).

2.5.2 ANTIMICROBIAL SUSCEPTIBILITY TESTING

The study of antimicrobial susceptibility has 2 fundamental objectives: to guide the clinician in choosing the best antibiotic treatment and to monitor the evolution of AMR. Susceptibility can be determined by phenotypic or genotypic methods. Phenotypic methods are based on the *in vitro* activity of an antibiotic against a microorganism, while genotypic methods detect the genes and mutations involved in antimicrobial resistance. In clinical practice, the antimicrobial susceptibility of NG is detected exclusively by culture-based phenotypic methods, which should be performed starting from a pure culture of *N. gonorrhoeae* of about 24 hours and seeded on a non-selective medium, such as chocolate agar (24).

With antibiograms, it is possible to obtain qualitative results that indicate whether the bacterium is susceptible or resistant to an antibiotic, or quantitative results that determine the MIC of the antibiotic capable of inhibiting bacterial growth. There are three culture-based tests to determine antimicrobial susceptibility: agar dilution, E-test, and disk diffusion. The first two methods are quantitative, while disk diffusion is a qualitative assay (28).

Agar dilution is the gold standard method recommended by the WHO for the determination of MIC. In this test, the bacteria are cultivated in a GC agar medium, to which a defined concentration of the antibiotic to be tested is added. The idea is to culture the microorganism in various plates with increasing concentrations of antibiotic so that, the lowest concentration that inhibits bacterial growth corresponds to the MIC value. However, even though it is considered the gold standard method, agar dilution is not a suitable process for many strains and is too laborious to perform routinely in clinical practice, for this reason, it is reserved mainly for reference laboratories. Correlated with agar dilution, but much simpler, the most frequently used quantitative method for MIC determination is the epsilometer or E-test (26).

In E-test, are used reactive strips made of plastic or non-porous paper that have a predefined gradient of antibiotic concentrations that allow one to determine on a scale the MIC of an antibiotic. The process is based on applying the strip above the plate at the time of inoculation. After incubation, the antibiotic diffuses into the medium, inhibiting bacterial growth in an elliptical-shaped. The value obtained from the intersection of the ellipse with the MIC value scale in the strip corresponds to the MIC of the antibiotic for the strain isolated in the culture.

Apart from quantitative determination, antimicrobial susceptibility can also be defined qualitatively by the Kirby-Bauer method or disk diffusion assay. The process consists of depositing blotting paper discs, which contain defined concentrations of antibiotic, on the surface of the plate, previously inoculated with the microorganism. After incubation, the antibiotic diffuses into the culture medium and inhibits growth, forming an inhibition halo around the disc. The size of the diameter of the halo allows for classifying the bacteria, according to different interpretation criteria, as susceptible (S), intermediate (I) or resistant (R). This assay is only recommended when the MIC cannot be determined by any of the above methods, and therapeutically relevant results need to be confirmed by other methods (26).

As some strains of NG carry a plasmid that codes for TEM-type β-lactamase, an enzyme that contains a high-level resistance to penicillin, to quickly detect the presence of this resistance, the nitrocefin chromogenic test is performed (26).

Two scientific societies oversee establishing the criteria for the interpretation of the antibiogram, allowing classify the microorganisms into 3 categories according to their clinical response to treatment: S, I or R. These societies are the Clinical and Laboratory Standards Institute (CLSI) and the European Antimicrobial Susceptibility Testing Committee (EUCAST).

Traditionally, a microorganism was defined as clinically susceptible by a level of antimicrobial activity associated with a high probability of therapeutic success; as intermediate if the therapeutic effect was uncertain; and as resistant if there was a high probability of therapeutic failure. However, in the year 2019, EUCAST redefined these terms emphasizing the relationship of the clinical category and the level of drug exposure, printed mainly to the definition of category I. The new categories are redefined as:

- <u>Susceptible, standard dosing regimen (S)</u>: when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent.
- <u>Susceptible, increased exposure (I):</u> when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.
- <u>Resistant (R):</u> when there is a high likelihood of therapeutic failure, even when there is increased exposure (29).

2.5.3 CURRENT TREATMENT

Treatment is defined as a clinical intervention aimed at correcting the symptoms or underlying causes responsible for causing a health problem. The main objective of gonococcal treatment is to eliminate the infection to avoid complications and reduce the transmission of the disease. The current treatment recommendations of the European Clinical Guidelines (2020) are summarized in Table 1 (20).

Table 1 - Treatment recommendations according to the 2020 European Clinical Guide; im, intramuscularly; po, orally; SD, single dose.

Recommended treatment	Alternative treatment
Uncomplicated gonococcal infection of the urethra, cervix and rectum	
Ceftriaxone 1g im SD + Azithromycin 2g po SD	• Spectinomycin 2g im SD + Azithromycin 2g po SD. • Ciprofloxacin 500mg po SD. • Gentamicin 240 mg im SD + Azithromycin 2g po SD. • Cefixime 400mg po SD + Azithromycin 2g po SD. • Ceftriaxone 1g im SD. • Azithromycin 2g po SD.
Oropharyngeal gonococcal infection	
Ceftriaxone 1g im SD + Azithromycin 2g po SD	• Ceftriaxone 1g im SD. • Ciprofloxacin 500mg po SD.
Gonococcal infection resistant to ESCs or therapeutic failures	
• Ceftriaxone 1g im SD + Azithromycin 2g po SD. • Gentamicin 240mg im SD + Azithromycin 2g po SD. • Spectinomycin 2g im SD + Azithromycin 2g po SD (except pharyngeal infection). • Ertapenem 1g im/24h 3 days.	
Other gonococcal infections in adults	
Conjunctivitis	
Ceftriaxone 1g im SD	

+ Azithromycin 2g po SD	
Epididymo-orchitis	
Ceftriaxone 500mg im SD + Doxycycline 100mg/12h po 10-14 days.	• Ofloxacin 200mg/12h po 14 days. • Levofloxacin 500mg/24h po 10 days.
PID	
Ceftriaxone 500mg im SD + Doxycycline 100mg/12h po 14 days + Metronidazole 500mg/12h po 14 days.	
DGI	
• Ceftriaxone 1g/24h iv or im 7 days. • Cefotaxime 1g 8h iv 7 days. • Spectinomycin 2g/12h im 7 days. (Switch to po after 1-2 days of improvement: cefixime 400mg/12h po or ciprofloxacin 500mg/12h po).	
Other gonococcal infections in children	
Ophthalmia neonatorum	
Ceftriaxone 25-50mg/kg iv or im SD (max. 125mg).	

Once the treatment has been administered, it is advisable to carry out a microbiological control to ensure the elimination of the infection and stop the chain of transmission. This is known as a cure test and, although it is recommended for any anatomical location, it is essential to perform it in cases of pharyngeal infection, persistence of symptoms, administration of alternative or second-line treatments, suspicion of non-adherence to treatment, suspicion of reinfection and in regions with high rates of antimicrobial resistance. The cure test can be performed using NAATs 3 weeks after treatment or by bacterial culture after, at least, 3 days.

2.5.4 FUTURE PROSPECTS FOR TREATMENT

Because of the extraordinary capacity to develop resistance to all the antimicrobials that have been introduced for its treatment, the incidence and morbidity of gonococcal infection are increasing more and more. In 2012, the Centers for Disease Control (CDC) classified *N. gonorrhoeae* as a superbug that could become untreatable soon. In addition, the WHO has also classified it as a "Priority 2" pathogen in the "Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery and Development of New Antibiotics" thus highlighting the importance of developing new treatments.

At present, the development of new therapies against resistant gonococci is focused on 3 objectives: the combination of existing antibiotics, the development of new antibiotics and the development of a vaccine.

In cases of resistance to cephalosporins or failure of first-line treatments, clinical guidelines recommend the combination of antibiotics such as gentamicin together with azithromycin. Other alternatives that can be considered are the combination of antibiotics commonly used in other types of infections, such as sitafloxacin, prescribed for respiratory infections, or delafloxacin, aimed at treating skin and soft tissue infections (8,30).

Nonetheless, the long-term solution includes developing new antibiotics from antimicrobial families other than those already included in the guidelines to delay the emergence of resistance as much as possible. Some of the new antibiotics that are in clinical trials are solithromycin, zoliflodacin, and gepotidacin (8).

However, taking into consideration the ability of NG to acquire resistance to all antibiotics that are introduced as a treatment, the ideal solution would be to develop a vaccine against this pathogen. To date, all attempts to create an effective vaccine have been unsuccessful. Of all, only four vaccines have progressed to clinical trials (whole cell, partially autolyzed, pili-based, and porin-based), although none have provided immune protection (24,30).

The first reference to a possible immunity against gonorrhea is collected in a retrospective study carried out in New Zealand involving the MeNZB vaccine against *N. meningitidis,* composed of outer membrane vesicles. This research reflects how vaccinated patients have a lower probability of contracting gonorrhea compared to unvaccinated. This finding provides experimental evidence that this type of vaccine could offer cross-protection by an unknown mechanism, which opens new avenues for research and development of vaccines against *N. gonorrhoeae* (16,24).

2.6 EPIDEMIOLOGY

In 2020, the WHO estimated that there were 82.4 million new cases of gonorrhea among individuals aged 15-49 worldwide, with a global incidence rate of 19 cases per 1,000 women and 23 cases per 1,000 men. Most of the cases were reported in the African Region and the Western Pacific Region (9).

Even so, the global incidence of gonorrhea, like that of other STIs, is difficult to quantify, since not all countries have case reporting systems.

2.6.1 EUROPE

According to the European Center for Disease prevention and Control (ECDC), in 2019 a total of 117,881 cases of gonorrhea were reported in 28 countries belonging to the European Union (EU) or the European Economic Area (EEA), an increase of 16.9% compared to 2018. Since 2010, confirmed gonorrhea cases have been increasing continuously (Figure 3) (31).

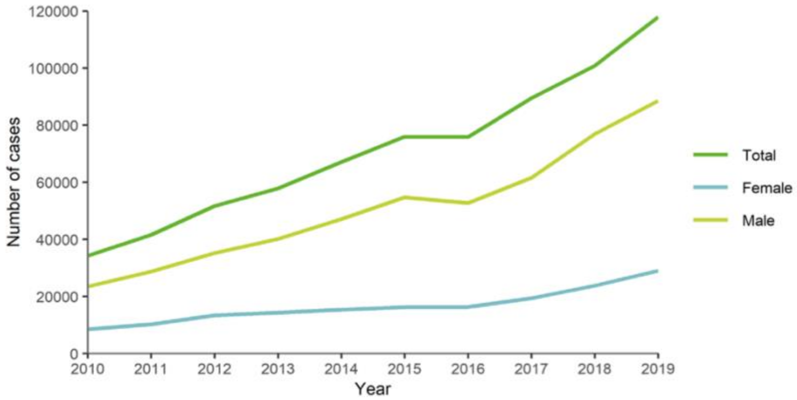


Figure 3 - Number of confirmed cases of gonorrhea by sex and year in the EU/EEA, 2010-2019.

The incidence rate was 31.6 cases per 100,000 population in those countries with case notification and surveillance systems. The highest notification rates were observed in the United Kingdom (116 per 100,000 population), Ireland (57 per 100,000 population), Denmark (38 per 100,000 population), Iceland (34 per 100,000 population), Malta (33 per 100,000 population), Norway (32 per 100,000 population), and Sweden (32 per 100,000 population). The lowest notification rates (<1 case per 100,000 population) were observed in Bulgaria, Croatia, Cyprus, Poland, and Romania (Figure 4) (31).

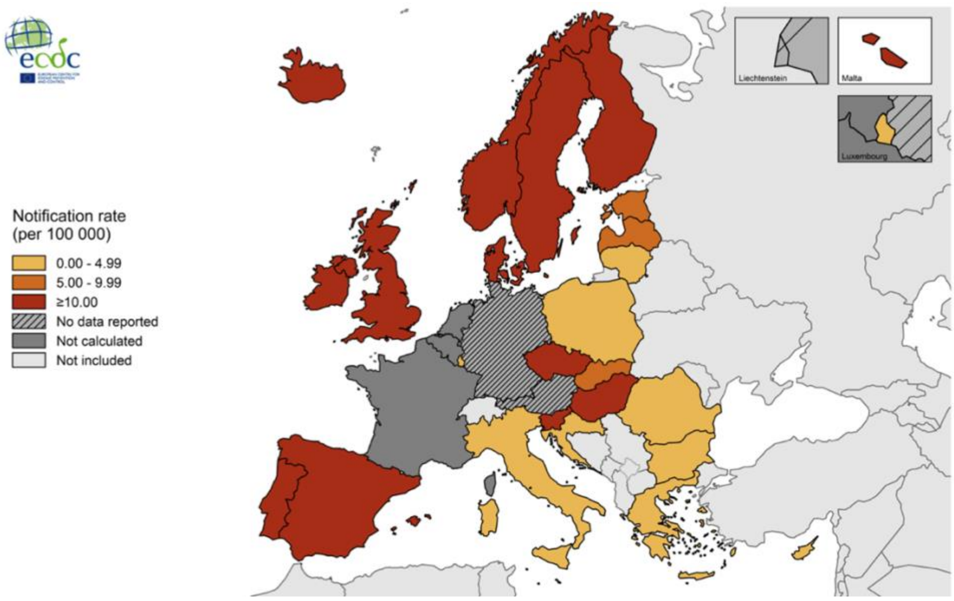


Figure 4 - Distribution of confirmed cases of gonorrhea per 100,000 population by country, EU/EEA, 2019.

By sex, the incidence rate was 48 cases per 100,000 population for men (88,537 cases) and 16 per 100,000 population for women (28,948 cases). The highest proportion of reported cases occurred in the age groups 25-34 years (37% of cases) and 15-24 years (35% of cases). The highest rates by age and sex occurred among men between the ages of 25 and 34, with 150 cases per 100,000 inhabitants (Figure 5) (31).

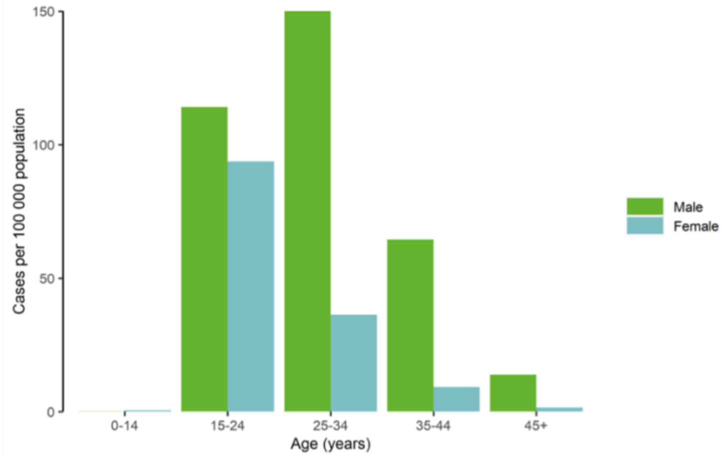


Figure 5 - Distribution of confirmed gonorrhea cases per 100,000 population, by age and sex, EU/EEA, 2019.

54% of all cases occurred among MSM, 37% among heterosexuals, and 9% reported as unknown. The cases diagnosed in MSM represented 74% of the male cases diagnosed. In patients with known serostatus, 12% were seropositive (31).

2.6.2 SPAIN

In Spain, population epidemiological information on STIs that must be declared is provided by the *Red Nacional de Vigilancia Epidemiológica* (RENAVE).

In 2021, 15,338 cases of gonococcal infection were reported in Spain, with an incidence rate of 32.41 cases per 100,000 population, higher than every year since 1995 (Figure 6) (32).

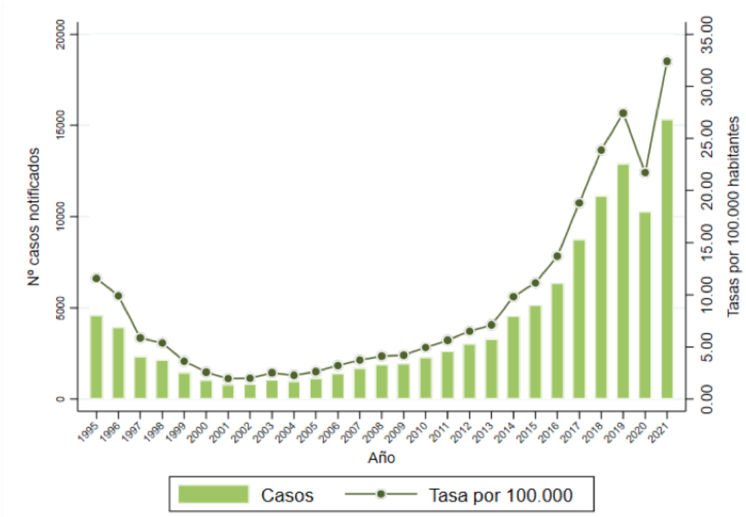


Figure 6 - Incidence of gonococcal infection. Number of cases and rates per 100,000 inhabitants. Spain, 1995-2021.

The highest rates were registered in Catalonia (86.90), Madrid (51.53) and the Balearic Islands (36.90). The lowest were reported in Extremadura (1.71), Asturias (5.65) and Castilla La Mancha (5.66). Ceuta and Melilla did not report cases in 2021 (Figure 7) (32).

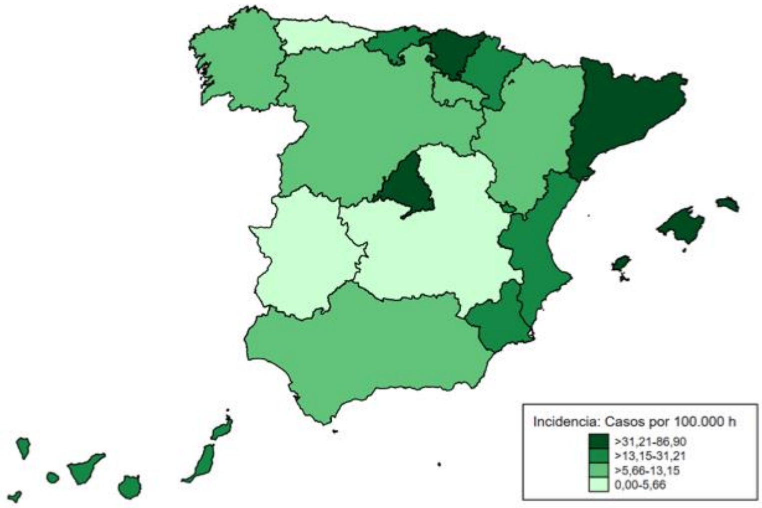


Figure 7 - Incidence of gonococcal infection by Autonomous Community, 2021. Rates per 100,000 population.

The rates in men were higher than those in women (56.07 and 10.79 per 100,000 population, respectively). By age, the highest rates occurred in the 25-34 years old group (119.95), followed by the 20-24 years old group (110.02). The average age at diagnosis was 31 years (IQR: 25-39).

By age and sex (Figure 8), the highest rates in men were observed between 25 and 34 years (208.89 cases per 100,000) and in women between 20 and 24 years (62.78 per 100,000) (32).

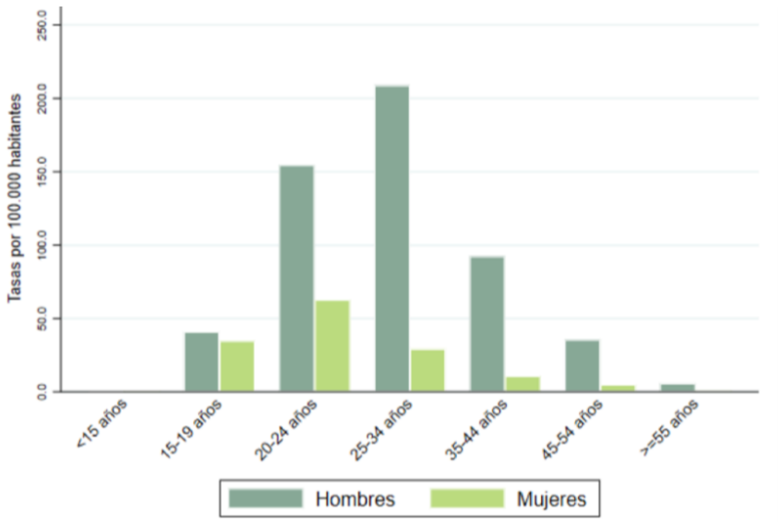


Figure 8 - Gonococcal infection. Incidence rates by age and sex group, 2021.

Of the total reported cases, 22.4% corresponded to homosexual men, 5.1% to heterosexual men, 8.3% to heterosexual women, 55.7% to men with unspecified sexual transmission and 8.4% to women with unspecified sexual transmission. Regarding the human immunodeficiency virus (HIV), 1.5% of the cases were positive (32).

2.6.3 CATALONIA

According to the *Centre d'Estudis Epidemiológics sobre les Infeccions de Transmissió Sexual i Sida de Catalunya* (CEEISCAT) in 2021 a total of 6,674 cases were reported in Catalonia. The incidence of gonorrhea increased by 44.1% compared to 2020, and with an average annual increase of 32.7% compared to the last 10 years, going from an incidence rate of 8.9 cases per 100,000 population in 2012 to 86.2 cases per 100,000 in 2021 (Figure 9) (33).

The highest incidence occurred in Barcelona Health Region (118.3) and the lowest in the Alt Pirineu and Aran Health Region (13.4) (Figure 10) (33).

Figure 9 - Evolution of the rate of gonorrhea cases per 100,000 population, Catalonia 2012-2021.

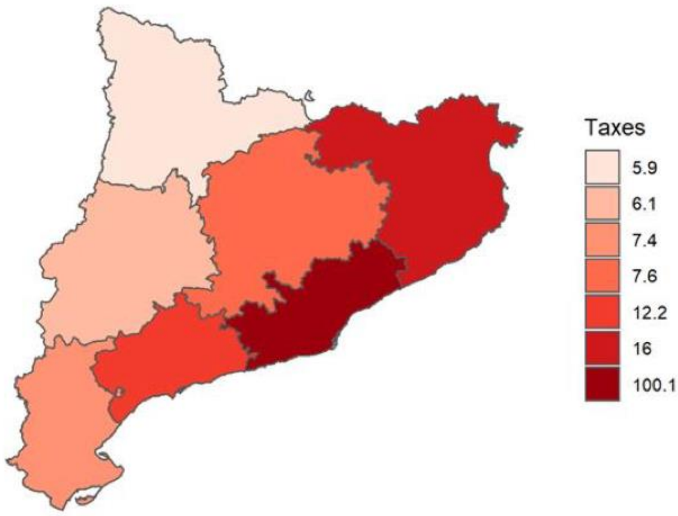


Figure 10 - Distribution of the incidence rate per 100,000 inhabitants by Health Region, Catalonia 2021.

The incidence per 100,000 population was 146.1 cases in men compared to 28.6 cases in women. By age, the highest number of reported cases occurred in the age group of 30-39 years in men and 20-29 years in women (Figure 11). The average age of reported cases was 33 years (33).

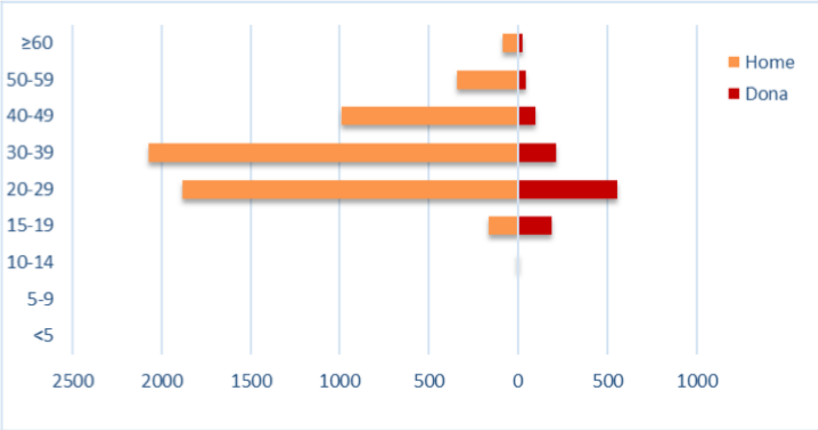


Figure 11 - Distribution of the number of gonorrhea cases by age and sex group, Catalonia 2021.

The transmission group more affected was MSM (58.1%) followed by heterosexual men and women (14% and 27.1% respectively). HIV coinfection was detected in 9.1% of cases. Of the total cases of gonorrhea, 17.2% were asymptomatic and 22.2% had presented a previous STI in the year before diagnosis (33).

2.7 RISK FACTORS

In the same way as in the rest of STIs, gonorrhea is transmitted and maintained by the core groups. Core group is a concept based on the epidemiology of STIs that refers to the groups that, to a greater extent, are responsible for maintaining the transmission of sexual infections in the population. Traditionally, it includes people with repeat infections, individuals with a high number of sexual partners, and people in high-risk occupations, such as prostitution (34).

The main risk factors of patients with gonorrhea are:

- Male gender
- Age <25 years old (22)
- Homosexual, bisexual and other MSM (22,23)
- Transgender (23)
- Racial, ethnic minorities and indigenous populations (23)
- Sex workers (22,23)
- Multiple sexual partners (22)

- History of gonorrhea or other STIs (22)
- Coinfections with other STIs
- Recreational drug use (22)
- Unprotected sexual contact (22)

Understanding, detecting, and defining the core groups within the population is essential to understand the transmission networks and bringing out effective prevention programs that curb the growing prevalence of gonorrhea. In addition, it is also crucial to study the evolution of AMR to update antimicrobial treatment guidelines.

3. OBJECTIVES

The main objective of this Final Degree Project is to obtain knowledge about gonococcal infection, its epidemiology and the antibiotic susceptibility profile of *Neisseria gonorrhoeae* strains isolated in culture during the period 2021-2022 in *Laboratori Clínic ICS Camp de Tarragona-Hospital Universitari Joan XXIII.* To achieve this purpose, the specific objectives are described below:

1) Describe the sociodemographic, behavioral, and clinical characteristics of patients with gonococcal infection in the study population.
2) Study the antimicrobial susceptibility profile of *N. gonorrhoeae* isolates to penicillin, ceftriaxone, cefixime, ciprofloxacin and azithromycin, interpreting the results following the cut-off points established by EUCAST.
3) Compare the diagnostic tools demanded by the requesting doctors, culture and/or PCR, to carry out the etiological diagnosis of gonorrhea.

4. MATERIAL AND METHODS

4.1 STUDY DESIGN

A retrospective study of *Neisseria gonorrhoeae*-positive patients registered in 2021-2022 in *Laboratori Clínic ICS Camp de Tarragona-Hospital Universitari Joan XXIII* is carried out.

For the search of bibliographical references, has been used the "PubMed" database, operating as keywords in the simple search *Neisseria gonorrhoeae*, as well as advanced searches combining the previous term with history, treatment, evolution, description, diagnosis, laboratory, structure, epidemiology or resistance, mainly.

4.2 PATIENTS CHARACTERISTICS

The study comprises sociodemographic, clinical, and behavioral characteristics of the patients from whom *N. gonorrhoeae* isolates were obtained in culture. The data collection has been taken out through the review of the patient's medical records. At no time has there been any treatment with patients, and the treatment of said data has been carried out in a coded manner. The variables included in the study are:

- <u>Sociodemographic variables:</u>
  - Age.
  - Gender: male or female.
- <u>Behavioral variables:</u>
  - Sexual orientation: MSM, MSW, WSM.
- <u>Clinical variables:</u>
  - Sample date.
  - Type of specimen.
  - Symptoms: yes or no. If so, the type of symptoms.
  - Coinfections with other STIs: yes or no. If so, the concomitant pathogen.
  - Pregnancy: yes or no.
  - STIs in the last year: yes or no.
  - Treatment.
  - Cure test: yes or no. If so, the date of control and result.
  - NG PCR request: yes or no. If so, PCR result.

4.3 *N. GONORRHOEAE* IDENTIFICATION

4.3.1 CULTURE AND IDENTIFICATION BY MALDI-TOF

The samples received in the laboratory were inoculated on chocolate agar and Thayer-Martin agar plates (BioMérieux). Subsequently, they were incubated for 48-72 hours at a temperature of 35-37°C and in a humid atmosphere with 5% CO_2, checking them daily.

In the presence of colonies that were compatible with NG due to size, shape, and appearance, presumptive identification was made using the positive oxidase test (Oxoid). All isolates were definitively identified using MALDI-TOF (Bruker Daltonics) following the manufacturer's instructions.

4.3.2 PCR MULTIPLEX

Samples were processed by multiplex PCR (Allplex™ STI Essential Assay, Seegenethat simultaneously detects the presence of DNA of *Chlamydia trachomatis*, *Neisseria gonorrhoeae, Trichomonas vaginalis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Ureaplasma parvum.*

4.4 ANTIMICROBIAL RESISTANCE TEST

The phenotypic study of antibiotic susceptibility of the isolated strains against penicillin, ceftriaxone, cefixime, ciprofloxacin, and azithromycin was performed using the E-test gradient diffusion strip method (BioMérieux) on chocolate agar. For the interpretation of the results, the cut-off points established by EUCAST in 2021 were followed (Table 2).

In each of the isolated strains, the presence of β-lactamase is also verified utilizing a chromogenic test (BBL Cefinase).

Table 2 – Cut-off points EUCAST 2021. [a] Beta-lactamase assay must always be performed, if it is positive, resistant penicillin will be reported. [b] EUCAST has not determined clinical breakpoints for azithromycin, so epidemiological breakpoints have been used that separate the wild population from isolates with some resistance mechanism (ECOFF, epidemiological cut-off). ECOFF for azithromycin is MIC>1g/L.

Antibiotic	**EUCAST 2021 MIC (mg/L)**	
	Susceptible	Resistant
Penicillin[a]	≤ 0.06	>1
Ceftriaxone	≤ 0.125	>0.125
Cefixime	≤ 0.125	>0.125
Ciprofloxacin	≤ 0.03	>0.06
Azithromycin[b]	≤1	>1

5. RESULTS

5.1 SOCIODEMOGRAPHIC CHARACTERISTICS

During the period from January 2021 to December 2022, a total of 111 patients with gonococcal infection were registered, whose main sociodemographic characteristics are summarized in Table 3.

Table 3 - Sociodemographic characteristics of the patients included in the study (n: number; IQR: interquartile range).

Sociodemographic characteristics (n=111)		
Gender, n (%)	Women	14 (12.6)
	Men	97 (87.4)
Average age, years (IQR)		30 (22-38.5)
Age group, n (%)	13-23	31 (27.9)
	24-34	45 (40.5)
	35-45	25 (22.5)
	46-54	10 (9.1)

As Table 3 shows, 87.4% of gonorrhea-infected patients were men and 12.6% were women. The age ranges from 13 to 54 years, with the average age being 30 years (IQR=22-38.5). The most affected age group is between 24 and 34 years, representing 40.5% of the subjects.

When stratifying the data according to sex, both men (IQR=22-39) and women (IQR=24-35.75) present an average age of 30 years.

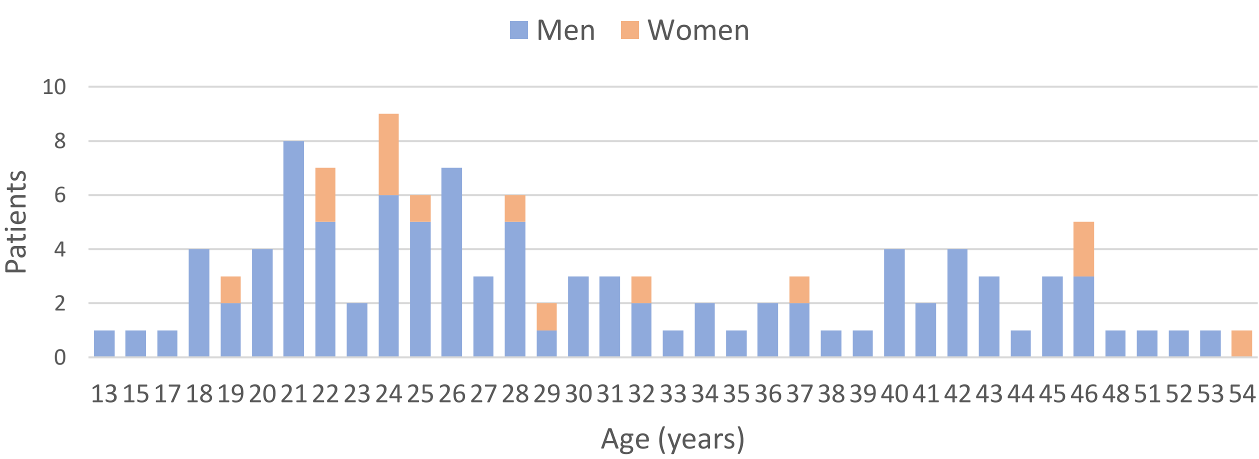


Figure 12 - Distribution age of patients.

5.2 BEHAVIORAL CHARACTERISTICS

With respect to behavioral variables, in this study, only sexual orientation was recorded. Table 4 describes the data collected about this aspect.

Table 4 - Behavioral characteristics of patients included in the study (n: number).

Behavioral characteristics (n=111)		
Sexual orientation, n (%)	MSM	12 (10.8)
	MSW	18 (16.2)
	WSM	7 (6.3)
	Unknown	74 (66.7)

Of the 111 patients included in the study, the sexual orientation of only 37 (33.3%) subjects was known. Regarding these 37 individuals, 12 (32.4%) are men who have sex with men, 18 (48.6%) are men who have sex with women, and 7 (18.9%) are women who have sex with men. Therefore, 100% of the women, with evidence of their sexual orientation, were heterosexual while, in the case of men, 60% were heterosexual and 40% homosexual. Figure 13 collects the data described.

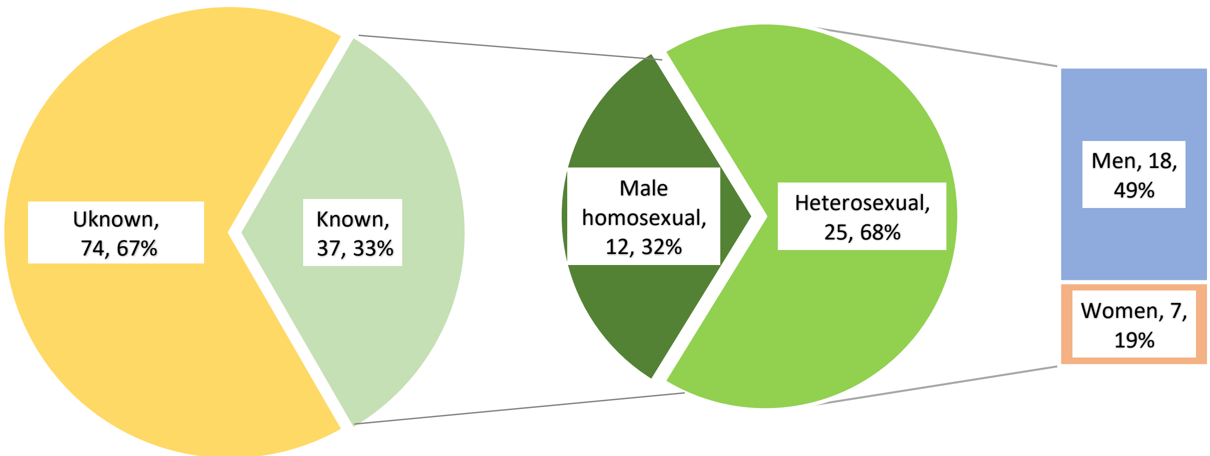


Figure 13 – Sexual orientation of patients.

5.3 CLINICAL CHARACTERISTICS

Figure 14 reflects the number of specimens from patients with gonococcal infection registered in each of the months of 2021 and 2022. In 2021, the number of cases was 34 and in 2022 was 77, an increase of 126.5%. The highest incidence took place in November 2022 (n=12) while in January 2022 no case of positive culture for gonorrhea was recorded.

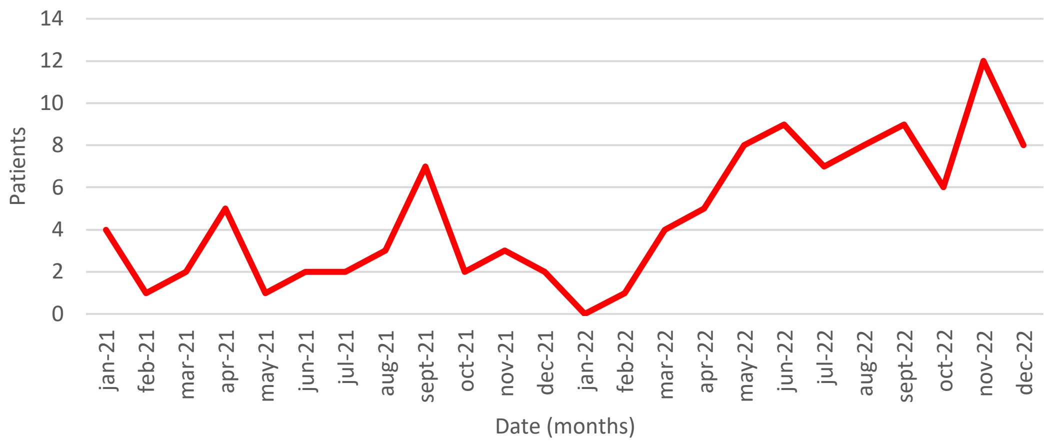


Figure 14 –Date of registered samples.

Of the 111 gonococcal isolates, 91 (81.9%) came from urethral discharge, 12 (10.8%) from vaginal exudates, 2 (1.8%) from balanopreputial exudate, 2 (1.8%) from endocervical exudate, 1 (0.9%) pharyngeal exudate, 1 (0.9%) rectal smear, 1 (0.9%) conjunctival exudate, and 1 (0.9%) pus from an abscess periurethral. Figure 15 represents the isolates of *N. gonorrhoeae* in function on the type of specimen.

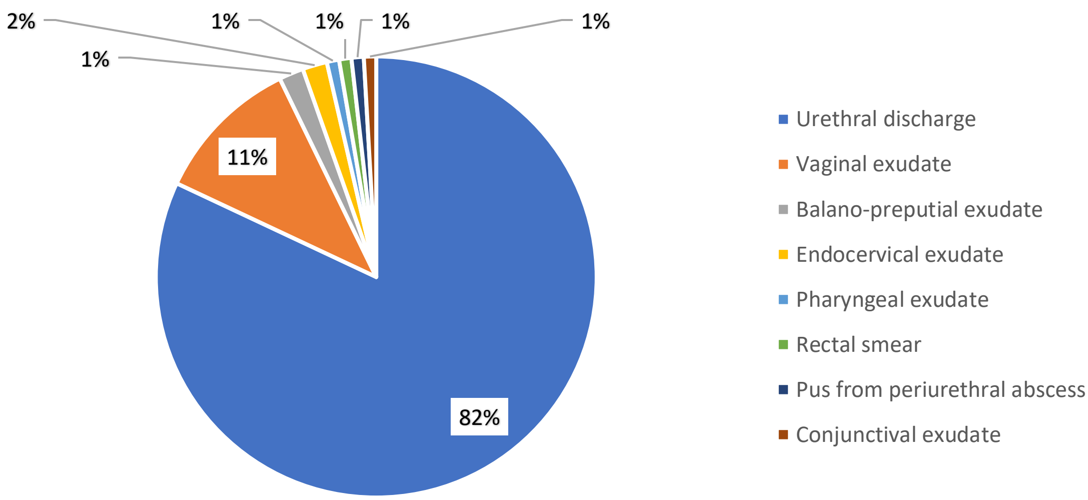


Figure 15 - Distribution of N. gonorrhoeae isolates according to the type of specimen.

The proportion of genital specimens (97.3%, n=108) was notably higher than that of extragenital specimens (2.7%, n=3).

Of the samples from men (n=97), 96.9% were genital (93.8% urethral exudates, 2% balano-preputial exudates and 1% pus from periurethral abscess) and 3.1% extragenital (conjunctival, pharyngeal and rectal smear). In the case of the samples collected from women (n=14), 100% were genital samples (85.7% vaginal exudates and 4.3% endocervical exudates). Figure 16 shows the distribution of the gonococcal isolates according to the type of specimen and the sex of the patient.

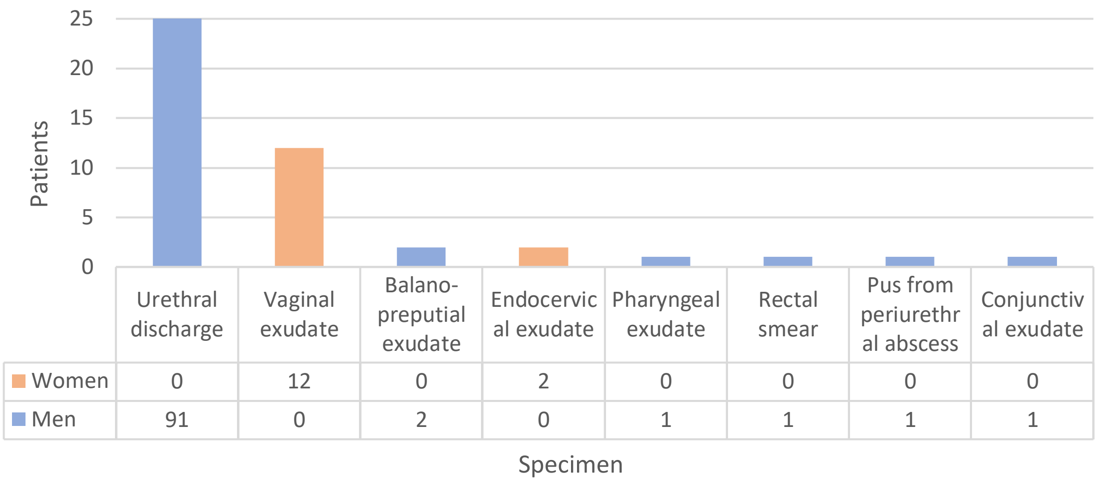


Figure 16 - Distribution of N. gonorrhoeae isolates according to the type of specimen and sex.

Concerning symptoms, 96.4% (n=107) of patients infected with NG presented some type of symptom, while only 3.4% (n=4) were asymptomatic. Samples from asymptomatic subjects were recollected from genital locations, 3 from men (urethra) and 1 from women (vagina).

The main symptoms suffered by patients with gonorrhea are listed in Table 5.

Table 5 – Symptoms of study patients.

Gonococcal infection symptoms		
Infection	**Symptoms**	**Number of patients** (%)
Conjunctivitis (n=1)	Purulent eye discharge	1 (100)
Rectal infection (n=1)	Purulent discharge	1 (100)
	Rectal tenesmus	1 (100)
Pharyngitis (n=1)	Purulent discharge	1 (100)
Cervicitis (n=3)	Leucorrhea	3 (100)
	Dysuria	2 (66.6)
	Pollakiuria	2 (66.6)
	Vulvitis	1 (33.3)
	Dyspareunia	2 (33.3)
Vaginitis (n=6)	Vulvitis	6 (100)
	Dysuria	4 (66.6)
	Vulvar nodules	1 (16.7)
	Leucorrhea	4 (66.6)
	Pollakiuria	4 (66.6)
PID (n=6)	Abdominal pain	6 (100)

	Stinging	2 (33.3)
	Leucorrhea	3 (50)
	Vulvitis	1 (16.7)
	Tub ovarian abscess	1 (16.7)
	Pollakiuria	1 (16.7)
	Dyspareunia	1 (16.7)
Urethritis (n=89)	Dysuria	58 (75.3)
	Purulent urethral discharge	77 (88.5)
	Suprapubic pain	2 (2.3)
	Stinging	5 (5.7)
	Hematuria	2 (2.3)
	Pollakiuria	4 (4.6)

On the other hand, it should be noted that none of the women included in the study were pregnant at the time of infection.

Simultaneously to the gonococcal infection, 28.8% (n=32) of the patients had some other concomitant infection. Of the 32 coinfected subjects, most (22.5%, n=25) were affected by 1 single pathogen, the predominant one being *C. trachomatis* (Table 6). Respecting HIV serostatus, 6.3% (n=7) of patients were seropositive, all cases belonging to men. 57% (n=8) of the women with gonococcal infection were affected by bacterial vaginosis and 14% (n=2) by candidiasis (Figure 19).

Table 6 - Description of concomitant STIs to N. gonorrhoeae.

Coinfections (%)		
Coinfection	Positive	32 (28.8)
	Negative	79 (71.2)
1 pathogen		25 (22.5)
Chlamydia trachomatis		8 (7.2)
Bacterial vaginosis		6 (5.4)
HIV		5 (4.5)
Treponema pallidum (syphilis)		3 (2.7)
Candida albicans		2 (1.8)
Ureaplasma urealyticum		1 (0.9)
2 pathogens		7 (6.3)
HIV + *C. trachomatis*		2 (1.8)

C. trachomatis + Bacterial vaginosis	2 (1.8)
C. albicans + VHS-2	1 (0.9)
C. albicans + *C. glabrata*	1 (0.9)
Treponema pallidum + HBV	1 (0.9)

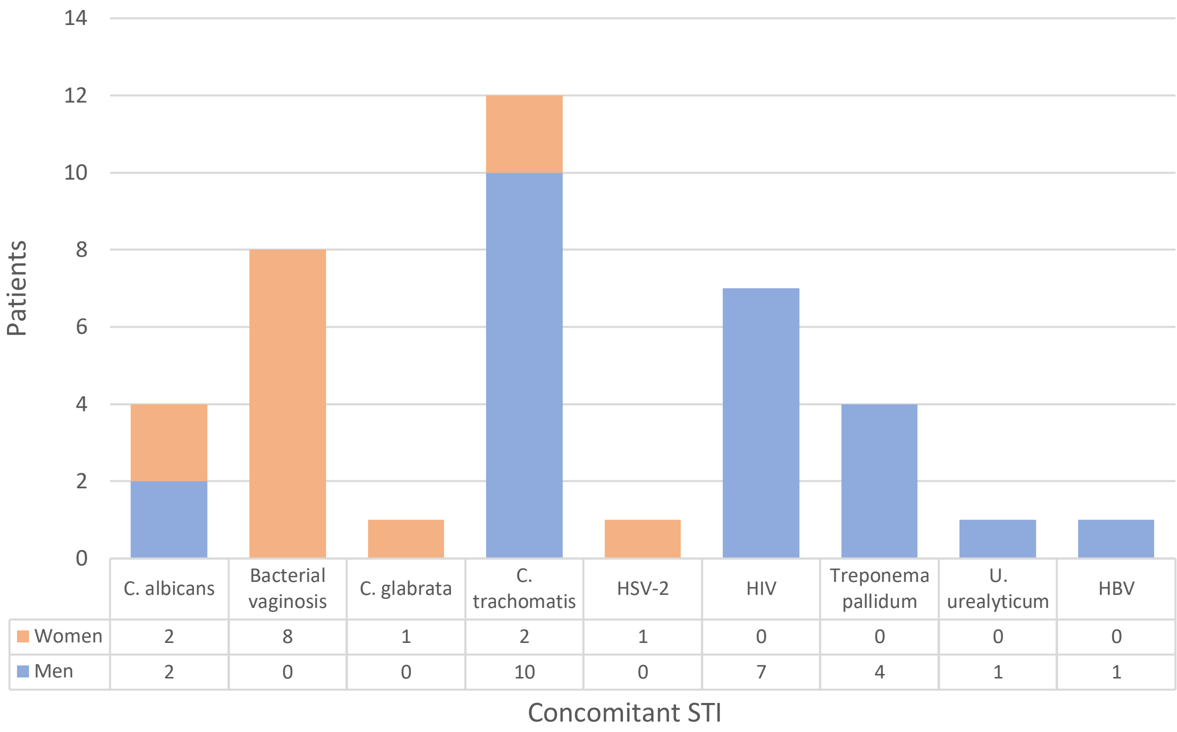


Figure 17 - Distribution of concomitant STIs to N. gonorrhoeae according to sex.

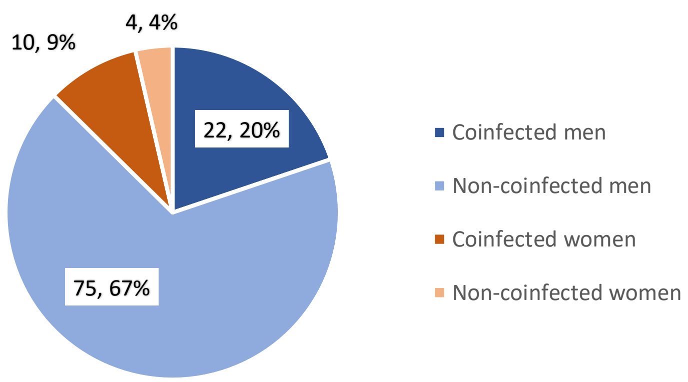


Figure 18 - Distribution of coinfected patients according to sex.

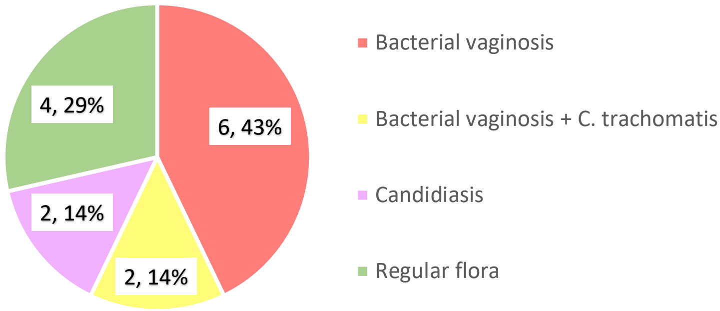


Figure 19 - Dysbacteriosis in women with gonococcal infection.

During the year before the diagnosis of gonococcal infection, there were only 6 individuals (5.4%), 4 men and 2 women, diagnosed with some STI.

Regarding antimicrobial treatment, 100% of patients with gonorrhea received treatment. Approximately half (n=47, 42.3%) were treated with dual therapy, azithromycin plus ceftriaxone, recommended by the 2020 European Clinical Guideline; the remaining half mostly received dual therapy of ceftriaxone plus doxycycline (n=35, 31.5%) or single treatment with ceftriaxone (n=12, 10.8%), which is what is recommended, on the other hand, by the British Association for Sexual Health and HIV (BASHH) Guidelines of 2018 and the CDC Guideline in 2021. Table 7 summarizes the treatments received by patients with gonococcal infection.

Table 7 - Treatment received by patients with gonococcal infection. CTX: Ceftriaxone; DOX: Doxycycline; MTZ: Metronidazole; AZM: Azithromycin; LVX: Levofloxacin; CFM: Cefixime; CIP: Ciprofloxacin.

Treatment of patients with gonorrhea (n=111)	
Monotherapy n (%)	17 (15.3)
CTX	
CTX 2g iv SD	1
CTX 1g im SD	9
CTX 500mg im SD	1
CTX 250mg im SD	1
DOX	
DOX 100mg/12h 7 days	1
AZT	
AZT 2g po SD	2
AZT 500mg po SD	1
CIP	
CIP 250mg/12 horas 7 days	1

Dual therapy, n (%)	89 (80.2)
CTX + AZT	
CTX 2g iv SD + AZT 1g po SD	1
CTX 1g iv SD 3 days + AZT 1g po MD	1
CTX 1g im SD + AZT 1g po SD	17
CTX 1g im SD + AZT 500mg po SD	1
CTX 1g im SD + AZT 500mg/24h 3 days	1
CTX 500mg im SD + AZT 1g po SD	20
CTX 500mg im SD + AZT 2g po SD	1
CTX 500mg im SD + AZT 500mg po SD	3
CTX 250mg im SD + AZT 1g po SD	2
CTX + DOX	
CTX 1g im SD + DOX 100mg/12h 7 days	25
CTX 500mg im SD + DOX 100mg/12h 7 days	7
CTX 250mg im SD + DOX 100mg/12h 14 days	3
CTX + MTZ	
CTX 500mg im SD + MTZ 500mg/12h 7 days	1
CTX + LVX	
CTX 500mg im SD + LVX 500mg/12h 7 days	1
CTX + CIP	
CTX 1g im SD + CIP 250mg/12h 7 days	1
CTX 500mg im SD + CIP 500mg/12h 7 days	1
AZT + DOX	
AZT 1g po SD + DOX 100mg/12h 7 days	1
AZT + CFM	
AZT 2g po SD + CFM 400mg/12h 7 days	1
AZT + CIP	
AZT 500mg po SD + CIP 500mg/12h 7 days	1
Triple therapy, n (%)	5 (4.5)
CTX + DOX + MTZ	
CTX 2g iv SD + DOX 100mg/12h 7 days+ MTZ 500mg/8h	1
CTX 500mg im SD + DOX 100mg/12h 7 days+ MTZ 500mg/12h 14 days	1
CTX + DOX + AZT	
CTX 1g im SD + DOX 100mg/12h 7 days+ AZT 1g po SD	1

CTX + AZT + CFM	
CTX 1g im SD + AZT 1g po SD + CFM 200mg/12h 10 days	1
AZT + DOX + CIP	
AZT 500mg po SD + DOX 100mg/12h 7 days+ CIP 500mg/12h 7 days	1

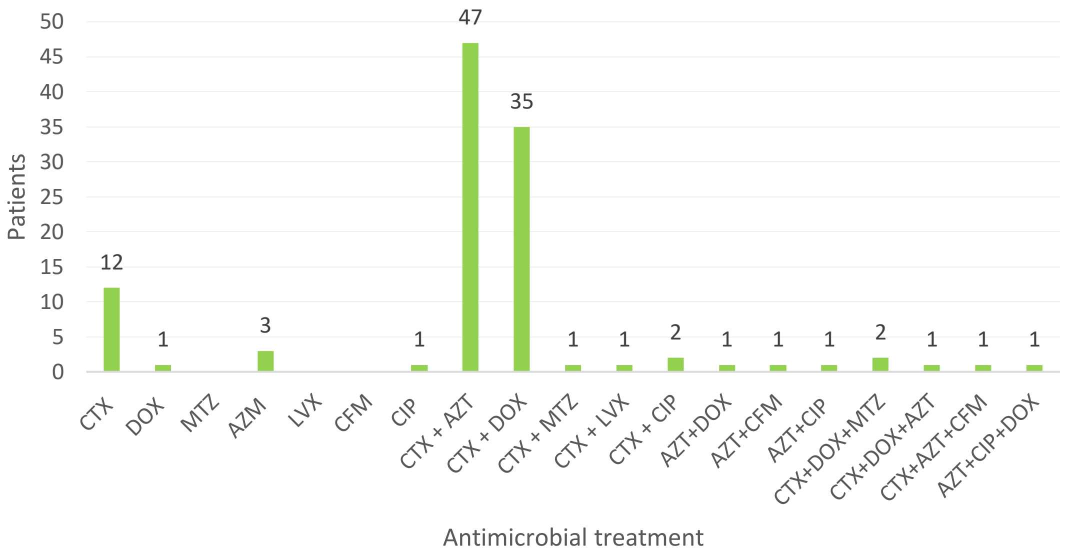


Figure 20 – Distribution of antimicrobial treatment received by patients.

The cure test was realized in 20.7% of the cases (n=23) with an average of 36.6 days (IQR: 17.5-44.5; range: 6-103). The test result was negative in 86.9% (n=20) of the cases and positive in 13.1% (n=3), with an average of 34.3 days. Patients with a positive cure test had been treated with monotherapy of CTX 1g im SD, CTX 500mg im SD plus AZT 1g po SD and CTX 1g im SD plus DOX 100mg/12h for 7 days, respectively.

On the other way, in 2022, in patients with a positive gonococcal culture (n=77), the solicitant doctors also requested a PCR test in 71.4% of cases (n=55), which gave a positive result in 72.7% of the samples (n=40). In another order, of patients with positive PCR for NG in 2022 (n=265), only 40 (15.1%) also presented positive cultures for NG.

5.4 ANTIMICROBIAL SUSCEPTIBILITY

Of the 111 *N. gonorrhoeae* isolates, only 105 were viable to effectuate the antibiograms. The percentages of antimicrobial susceptibility obtained are shown in Table 8 and the distribution is in Figure 21.

Table 8 - Antimicrobial susceptibility of N. gonorrhoeae isolates. S: Susceptible; I: Intermediate; R: Resistant.

Antibiotic	EUCAST		
	S (%)	I (%)	R (%)
Penicillin	4.8	84.8	10.4
Cefixime	100	-	-
Ceftriaxone	100	-	-
Ciprofloxacin	34.3		65.7
Azithromycin	98.1		1.9

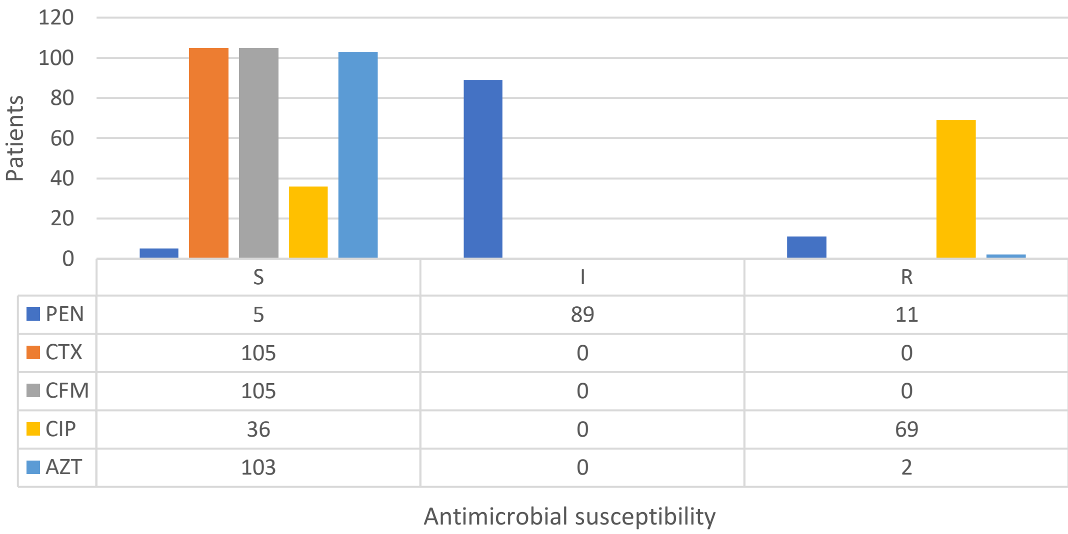


Figure 21 - Distribution of gonococcal isolates antimicrobial susceptibility (S, I, R).

Globally, only 2.8% (n=3) of the 105 isolates were susceptible to all the antibiotics tested, while 29.5% (n=31) presented intermediate susceptibility to penicillin and susceptibility to the rest of the antimicrobials. Almost 2 thirds of the strains (67.6%, n=71) presented some type of resistance; 57.1% (n=60) to a single antimicrobial, especially ciprofloxacin, and 10.4% (n=11) to 2 antibiotics, mostly penicillin and ciprofloxacin. Neither MDR nor XDR gonococcal strains were detected since azithromycin-resistant isolates were sensitive to both cephalosporins (ceftriaxone and cefixime) (Table 9).

Table 9 -Distribution of N. gonorrhoeae strains with resistance to different antimicrobials.

	Resistant	**Intermediate**	**n (%)**
Resistance 1 antibiotic (n=60)	Ciprofloxacin		2 (1.9%)
	Ciprofloxacin	Penicillin	57 (54.3%)
	Azithromycin	Penicillin	1 (0.9%)

Resistance 2 antibiotics (n=11)	Penicillin + Azithromycin		1 (0.9%)
	Penicillin + Ciprofloxacin		10 (9.5%)

5.4.1 PENICILLIN

In the case of penicillin, 4.8% (n=5) of the isolates were susceptible, 84.8% (n=89) presented intermediate susceptibility and 10.4% (n=11) were resistant. (Figure 22). All resistant isolates are due to the production of beta-lactamases.

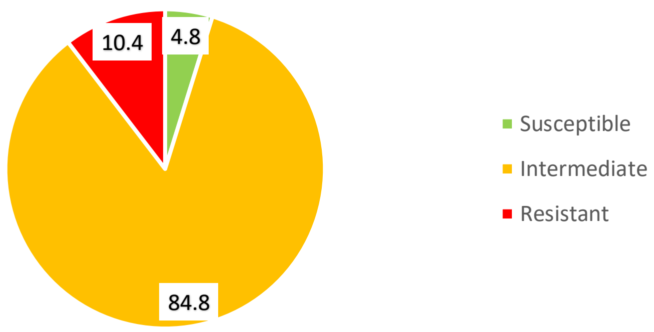


Figure 22 – Susceptibility to penicillin.

The distribution of the MIC to penicillin in the gonococcal isolates can be seen in Figure 23. The MIC ranges were between <0.016 mg/L and 64 mg/L, with an MIC_{50} of 0.25 mg/L and MIC_{90} of 0.75 mg/L.

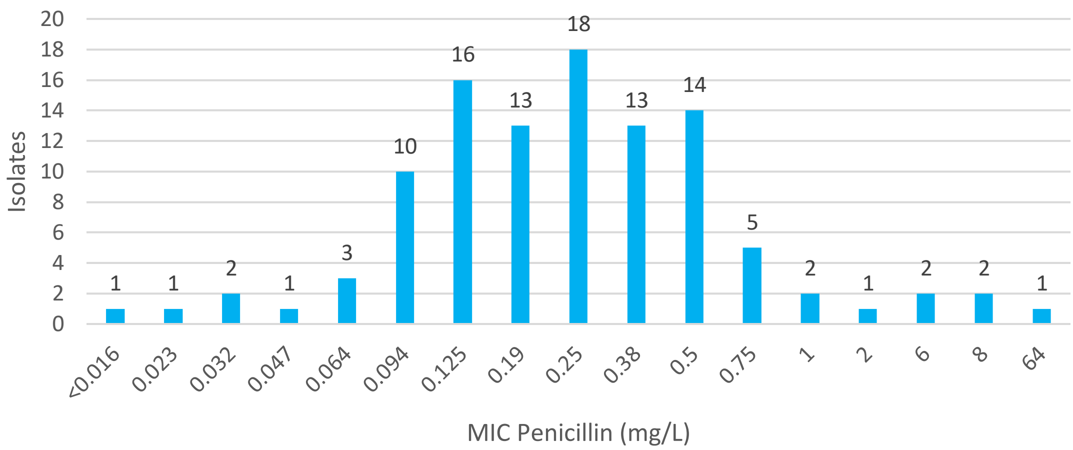
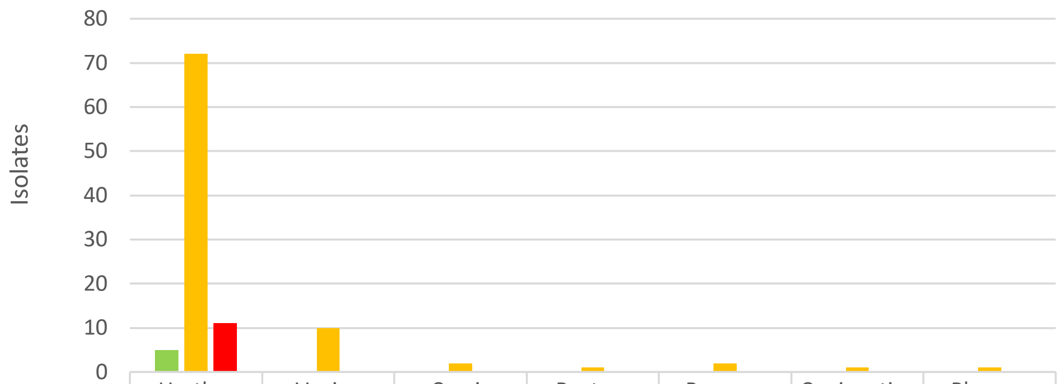


Figure 23 - Distribution of the MIC for penicillin of the 105 isolates of N. gonorrhoeae.

Figure 24 shows the data on susceptibility to penicillin of the gonococcal strains as a function of the anatomical location of the isolate. All penicillin-susceptible and resistant strains proceeded from the urethra. In the rest of the locations, the susceptibility of the strains was intermediate.



	Urethra	Vagina	Cervix	Rectum	Prepuce	Conjunctiva	Pharynx
Susceptible	5	0	0	0	0	0	0
Intermediate	72	10	2	1	2	1	1
Resistant	11	0	0	0	0	0	0

Anatomical location

Figure 24 - Distribution of antimicrobial susceptibility to penicillin according to the location of the isolate.

5.4.2 CEPHALOSPORINS

In this study, all gonococcal isolates were found to be susceptible to both ceftriaxone and cefixime.

The MIC distribution range for ceftriaxone ranged between MIC values <=0.016 mg/L and 0.037 mg/L (Figure 25). Both the MIC$_{50}$ and MIC$_{90}$ were <=0.016 mg/L for ceftriaxone. Instead, in the case of cefixime, the MIC range ranged from <=0.016 mg/L to 0.094 mg/L, with MIC$_{50}$ and MIC$_{90}$ of <=0.016 mg/L and 0.032 mg/L, respectively (Figure 26).

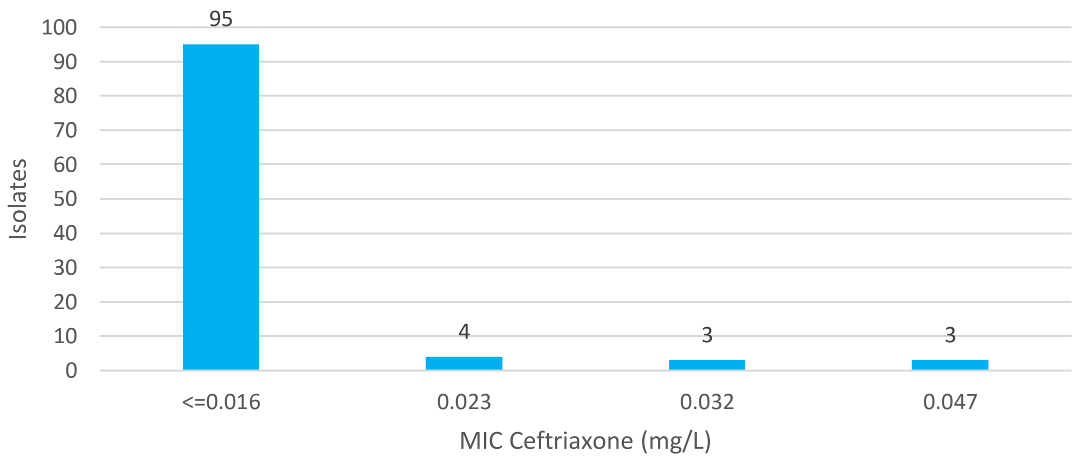


Figure 25 - Distribution of the MIC for ceftriaxone of the 105 isolates of N. gonorrhoeae.

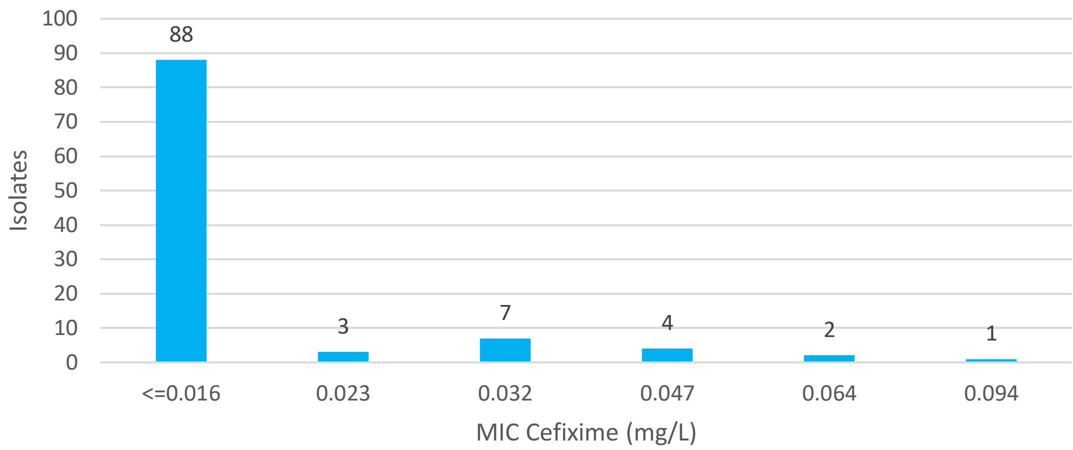


Figure 26 - Distribution of the MIC for cefixime of the 105 isolates of N. gonorrhoeae.

5.4.3 AZITHROMYCIN

As regards azithromycin, 1.9% (n=2) of the studied strains presented a MIC within the resistant category (MIC>1g/mL). The rest of the isolates were susceptible (Figure 27). The MIC ranges were between <0.016 mg/L and >256 mg/L, with MIC_{50} and MIC_{90} of 0.38 mg/L and 1 mg/L, respectively. The distribution of the MICs of azithromycin is shown in Figure 28. The anatomical location of the resistant isolates was the urethra.

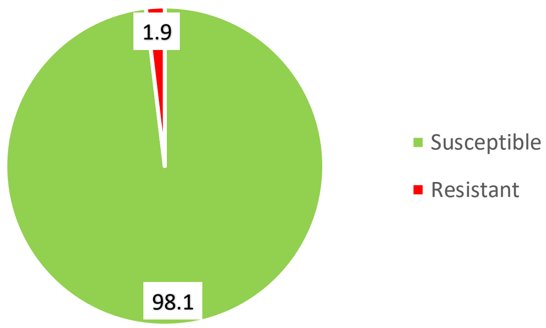


Figure 27 - Percentage of susceptibility and resistance to azithromycin.

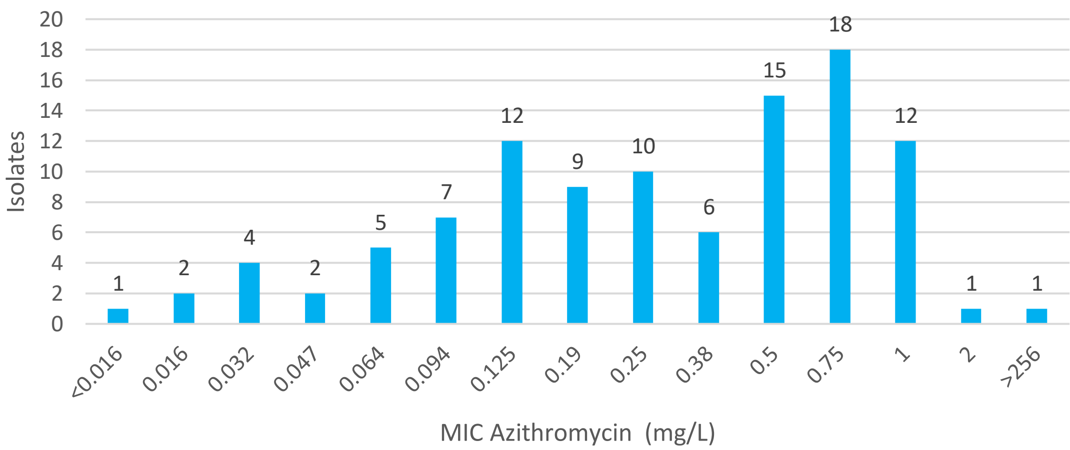


Figure 28 - Distribution of the MIC for azithromycin of the 105 isolates of N. gonorrhoeae.

5.4.4 CIPROFLOXACIN

Considering ciprofloxacin, a total of 65.7% and 34.3% of resistant and susceptible strains were registered, respectively (Figure 29). The distribution range of the MICs was from <=0.002 mg/L to >32 mg/L, with a MIC_{50} of 2 mg/L and MIC_{90} of 8 mg/L (Figure 30).

100% of susceptible strains to ciprofloxacin came from genital specimens (urethral, vaginal and endocervical exudates), while, in the case of extragenital samples, only resistant strains were isolated (Figure 31).

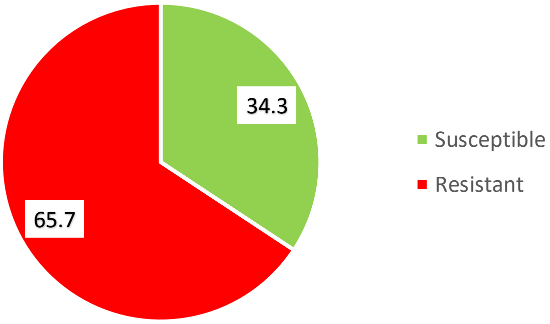


Figure 29 - Percentage of susceptibility and resistance to ciprofloxacin.

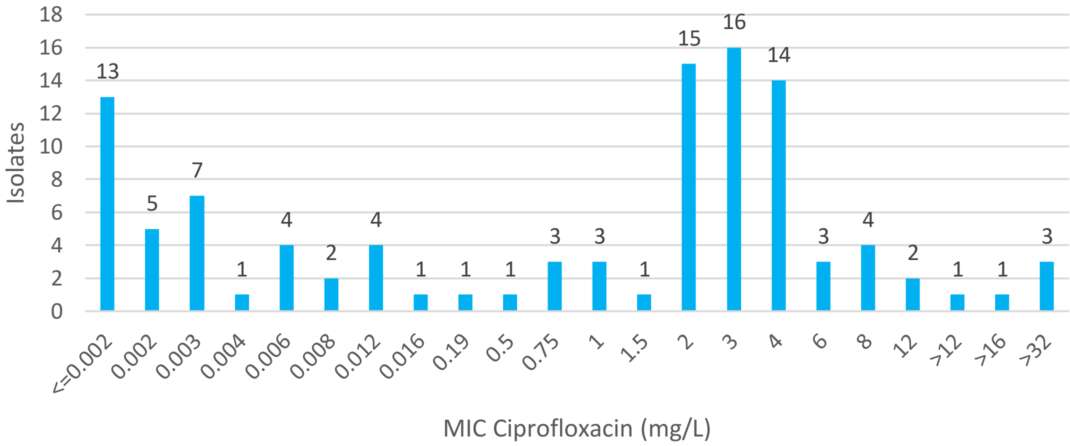
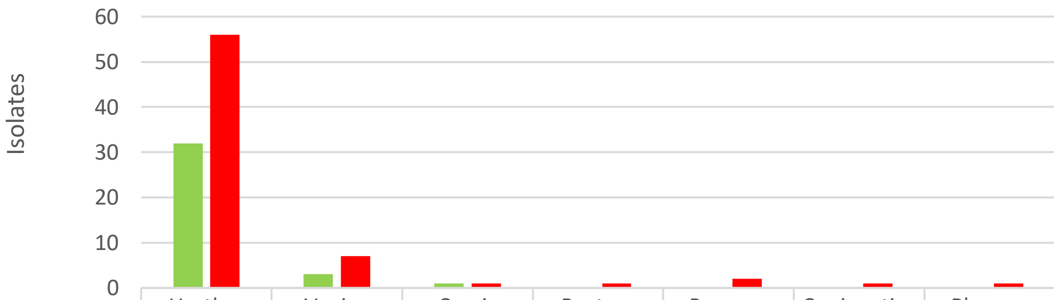


Figure 30 - Distribution of the MIC for ciprofloxacin of the 105 isolates of N. gonorrhoeae.



	Urethra	Vagina	Cervix	Rectum	Prepuce	Conjunctiva	Pharynx
Susceptible	32	3	1	0	0	0	0
Resistant	56	7	1	1	2	1	1

Anatomic location

Figure 31 - Distribution of antimicrobial susceptibility to ciprofloxacin according to the location of the isolate.

6. DISCUSSION

6.1 SOCIODEMOGRAPHIC CHARACTERISTICS

Sociodemographic characteristics of the patients included in this study reflect that there is a great difference between the number of diagnoses of gonorrhea in men and women, since 86.4% of the infected individuals were men compared to 12.6% who were women. These data agree with the rates of gonococcal infection that are collected at regional, national or European levels, which are always higher for the male gender (31–33). Usually, women suffer from asymptomatic infections, which is why they do not go to receive health care, are not diagnosed, and, therefore, their case is not registered, which would explain the bias in the number of cases towards the male gender. In this sense, to obtain clearer and more objective data on the number of women who contract gonorrhea, some organizations such as the CDC recommend screening sexually active women under 25 years of age (35).

Regarding age, the average age at diagnosis of gonococcal infection (30 years) does not vary significantly when compared with the Catalan (33 years) or Spanish (31 years) average (32,33). On the other hand, the most affected group was between 24 and 34 years old (40.5%) followed by those between 13 and 23 years old (27.9%), a window that coincides with the most affected age ranges according to the different epidemiological surveillance departments (31–33). The percentage of diagnoses among the young population, mostly due to unprotected sexual relations, is the most represented, so prevention measures should focus on this aspect.

6.2 BEHAVIORAL CHARACTERISTICS

The main limitation of this project when studying sexual orientation is that in a high percentage of patients (66.7%) the information is not available, which makes data analysis and comparison difficult.

Of the total number of cases, the transmission group most affected were heterosexual men (16.2%) followed by homosexual men (10.8%) and heterosexual women (6.3%). Unlike the data collected by RENAVE and CEEISCAT (32,33), in our study, there is a higher proportion of infected heterosexual men and MSM is not the most affected transmission group. The lack of information on the sexual orientation of most of the patients could be one of the reasons why the data do not coincide with those of the Catalan and Spanish average. Despite this, it is clear that the MSM population is a core group vulnerable to gonococcal infection.

6.3 CLINICAL CHARACTERISTICS

During the last 3 decades, the incidence of gonorrhea has been increasing year after year (32), a fact that is demonstrated by the increase in cases that occurred in 2022 (n=77) compared to 2021 (n=34). However, the median annual increase in the last 10 years in Catalonia is around 32.7% (33), a much lower percentage than that obtained in this study (126.5%). The disparity between these data is due to the pandemic situation caused by the coronavirus that drove many other infections and diseases to remain invisible during 2020 and 2021, either because people went to health centers to a lesser extent or because the data did not properly record.

As stated in the 2021 RENAVE annual report (32), most of the isolates included in our study came from genital samples, especially urethral samples. In this case, the proportion of extragenital samples (2.7%) was minimal compared to the genital ones (97.3%). In the same way that occurs with gonococcal infection in women, the low number of extragenital samples is because extragenital gonorrhea is usually asymptomatic, so patients do not require assistance and their cases are not studied.

Coinfection with some other concomitant pathogen was recorded in 28.8% of cases, with a predominance of bacterial infection by *C. trachomatis*. Of all subjects, 6.3% were HIV positive. In addition, 5.4% had some STI in the year before the diagnosis of gonococcal infection. Both the percentage of cases with positive serostatus and previous STIs is lower than that published by CEEISCAT, which is 9.1% for HIV coinfection and 22.2% for a previous STI (33), surely due to the lower Number of cases with which you work.

On the other way, given the association of gonococcal infection with the sexual transmission of HIV, knowing the patients who potentially have a higher risk of acquiring HIV makes it possible to carry out targeted prevention protocols and thus reduce the risk. Likewise, since gonorrhea usually has a short incubation period, it can be used as an indicator of risky sexual practices that facilitate the transmission of STIs, which would allow better monitoring of risky cases and the early initiation of treatments, if necessary.

Concerning antimicrobial treatment, 100% of the study patients received pharmacological treatment. In 91.9% of the cases, ceftriaxone was received as treatment, with or without the combination with other antimicrobials. This reflects that cephalosporins, especially ceftriaxone, are the antimicrobial agent of choice when treating gonococcal infections, as indicated by the guidelines.

Nevertheless, even though the European Clinical Guidelines recommend dual therapy of ceftriaxone plus azithromycin for the treatment of gonorrhea, more than half of the patients included in the study (57.7%) did not follow these recommendations. In these cases, dual treatment of ceftriaxone plus doxycycline was mostly administered, which is the treatment of choice when there is coinfection with chlamydia, or, to a lesser extent, monotherapy with ceftriaxone, which is recommended by the BASHH in 2018 or the CDC in 2021 (35,36). In addition, among the patients who did receive dual therapy of ceftriaxone plus azithromycin, the doses recommended in 2020 by the European Clinical Guidelines (ceftriaxone 1g im SD plus azithromycin 2g po SD) were not followed in any case, surely because these doses were recommended considering the possible gonococci resistant to third generation cephalosporins existence. Since all the gonococci isolates in the study were susceptible to cephalosporins, treatments with lower doses were equally effective. Even so, it can be observed how, in general, there was a great disparity both in the treatments and in the doses, which reflects a lack of consensus and knowledge among professionals when it comes to treating gonococcal infection.

As for the cure test, it was realized in 20.7% of the cases after an average of 36.6 days. The test result was positive in 13.1% of the patients in a range of 28-41 days post-diagnosis. To guarantee that the treatment has been effective, it is recommended that microbiological controls be carried out over a period of 3 weeks post-treatment if NAATs are used or after at least 3 days per culture. Although in our study the 3 positive cure tests were performed within the recommended period, we cannot affirm that they are due to therapeutic failures because we do not have the molecular typing of these strains, nor the behavioral data of these patients (sexual abstinence or protected practices during this time) and, possibly, since the gonococci were sensitive to ceftriaxone, it is most likely due to either reinfection, if sexual partners were not treated, or new episodes.

Besides, in 2022, a total of 265 positive PCR tests for NG were obtained, more than triple the number of cultures positive for gonococcus, which reflects that nucleic acid amplification techniques, in this specific case PCR, is the test of choice by physicians for the diagnosis of gonococcus. Likewise, this is also demonstrated if the number of cases with positive culture (n=77) that also had PCR requested (71.4%) and those that did not (28.6%) are compared. Even though PCR is a sensitive and faster technique than culture, both requests must be sent for the diagnosis of NG, since culture allows us to monitor the rates of resistance to antimicrobials and in this way, see changes in the local epidemiology of our study population.

6.4 ANTIMICROBIAL SUSCEPTIBILITY

One of the objectives of this project is to study the antibiotic susceptibility of the isolated gonococcal strains to detect the antimicrobial resistance that is currently faced in the Tarragona region.

In this sense, our study has shown that in 2021 and 2022 no strain of MDR or XDR gonococcus was detected. In addition, low resistance rates are maintained in the study population for all tested antimicrobials (penicillin, ceftriaxone, cefixime and azithromycin) except for ciprofloxacin. However, in the case of penicillin, most of the strains were resistant because they could produce beta-lactamases.

Further, the results suggest that there are no significant differences between the susceptibility of strains isolated from genital and extragenital areas, except for ciprofloxacin, which only seems to be susceptible to genital gonococcal infections.

It should be noted that 28.6% of the strains show a MIC for azithromycin of between 0.75 mg/L and 1 mg/L, figures close to the limit of resistance so that in a short time it is likely that the proportion of azithromycin-resistant gonococcal strains will increase.

As in the rest of the European countries, high rates of susceptibility to third-generation cephalosporins are maintained, although some countries report some isolates resistant to ceftriaxone (37).

7. CONCLUSIONS

As a conclusion of the project, it is extracted that the characteristic profile of patients with gonococcal infection is a man between 24 and 34 years old, therefore infection control and prevention programs should preferably be aimed at them. Affected women usually have an average age of 30 years old and are asymptomatic, so the global incidence of gonococcal infection in women is unknown and it would be necessary, as indicated by the guidelines, to carry out screening in sexually active women under 25 years old.

Despite the exceptional situation of the pandemic produced by COVID-19, the incidence of gonorrhea has increased enormously from 2021 to 2022.

Genital gonorrhea is the most frequent type of gonorrhea and is mainly symptomatic, especially in men. Consequently, to break the chain of transmission, it is important to make the patient understand the importance of notifying their last sexual couples and informing them about the danger of unprotected sexual practices. On the other hand, even though the percentage of extragenital gonorrhea among patients is lower, it does not usually manifest itself, which is why, when an STI is suspected, it is very important to carry out a detailed history of condom use in relation to the type of sexual practices and, if appropriate, take pharyngeal and rectal samples.

The prevalence of other STIs concomitant to the diagnosis of gonorrhea corresponds to almost a third of the subjects, with *C. trachomatis* infection predominating. In addition, 5% of the patients were affected by some STI during the year before diagnosis. Taken together, this highlights the importance of screening patients diagnosed with gonorrhea for other STIs and carrying out regular follow-ups.

The antibiotic of choice to treat gonococcal infection is ceftriaxone, individually or in combination with other antimicrobials. Even so, there is a great discrepancy between the therapies and doses recommended by health professionals.

When comparing NAATs with microbiological culture for the diagnosis of NG, doctors request PCR tests to a much greater extent.

Neither MDR nor XDR gonococcal strains were detected in this study, and virtually all of them were susceptible to ceftriaxone, cefixime, and azithromycin. Resistance is concentrated for ciprofloxacin and, to a lesser extent, penicillin. Neither differences have been observed between the susceptibility of strains isolated from genital and extragenital areas, apart from ciprofloxacin. Given the data presented, ceftriaxone continues to be a good treatment option, being able to be used in monotherapy and avoiding dual therapy with azithromycin to prevent the appearance of resistance in other bacterial STIs.

Continuous epidemiological surveillance and antimicrobial susceptibility programs are essential to detect changes in the population and provide reliable data to adapt local treatment guidelines and thus be able to detect possible antimicrobial resistance that could affect public health.

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