3

Gonorrhea in Men Sex Men and Heterosexual Men

Ángela María Rosa Famiglietti and Susana Diana García
Faculty of Pharmacy and Biochemistry
University of Buenos Aires
Argentina

1. Introduction

Gonorrhea is a sexually transmitted infectious disease known since ancient times, with biblical (Old Testament) references. The etiologic agent is *Neisseria gonorrhoeae,* commonly called gonococcus. *N. gonorrhoeae* has accumulated mechanisms of antimicrobial resistance so that from the year 2007 it joined the list of multi-resistant, informally called "Superbugs" (Shafer *in Neisseria* Molecular Mechanisms of Pathogenesis, 2010).

1.1 General description

Neisseria gonorrhoeae was described by Neisser in 1879 and first cultivated in 1882 by Leistikow and Loeffler. At this time, members of the genus *Neisseria* are classified in the family *Neisseriaceae* with the genera *Kingella*, *Eikenella*, *Simonsiella*, and *Alysiella*. This family is now placed in the β-subgroup of the Phylum *Proteobacteria* (Janda and Gaydos *in* Manual of Clinical Microbiology, 2007).

N. gonorrhoeae is a gram-negative diplococci with adjacent sides flattened, like coffee beans, do not form spores, oxidizes dimethil or tetramethil-phenylenediamine (oxidase test positive) and catalase positive (superoxol test with 30% H_2O_2); not to reduce nitrites. It may grow optimally at 35-37ºC, but it's unable to grow at low temperature (22ºC). The most of gonococcus have an obligate requirement for CO_2 (5%) and humidity of 70-80%, it does not tolerate drying out. Clinical specimens should be collected with Dacron or rayon swabs. These swabs should be inoculated immediately on appropriate media. Calcium alginate may have inhibitory effect and cotton swabs contain fatty acids that inhibit the growth of gonococci. *N. gonorrhoeae* grows on selective media, modified Thayer Martin, as *Neisseria meningitidis.* Cysteine is an essential aminoacid for their growth. Some strains have specific requirements of certain amino acids, pyrimidines and purines as a result of defective or altered biosynthetic pathways. These particular nutritional requirements form the basis for a typing method for gonococci called auxotyping. *N. gonorrhoeae* differs from other species of the genus by oxidizing glucose and do not use maltose, sucrose, lactose or fructose in cysteine-tryptic digest semisolid agar-base medium (CTA) containing 1% carbohydrate and a phenol red pH indicator, or rapid carbohydrate test; not to reduce nitrites and their inability to grow at low temperatures (22ºC).

The use of enzyme substrates that demonstrate the presence of prolyl-hydroxyprolyl aminopeptidase and the abscense of β-galactosidase and γ-glutamyl aminopeptidase also to confirm the identification. Currently there are commercially available rapid tests to confirm the isolation of *N. gonorrhoeae* such as immune reactions by using fluorescent monoclonal antibodies or coagglutination tests, as well as molecular techniques using specific probes or specific primers **(**Janda and Gaydos *in* Manual of Clinical Microbiology, 2007).

1.2 Transmission and clinical manifestations

N. gonorrhoeae is an exclusive human pathogen and this is their only reservoir. The transmission is from person to person through sexual intercourse, so the primary infection in adults is installed in the genital area, anal and/ or pharynx, where there is susceptible epithelium: cubic or cylindrical not stratified.

In males, *N. gonorrhoeae* causes an acute urethritis with dysuria and urethral discharge. The incubation period averages 2 to 7 days. About 2.5 to 5 % of men are asyntomatic. Complications of untreated gonococcal urethritis are: epididymitis, periurethral abscesses, infections of the Tyson's glands and Cowper's glands, orchitis and prostatitis (Janda and Gaydos *in* Manual of Clinical Microbiology, 2007). In women, gonococcal infections cause cervicitis, only approximately half of which occur with symptoms and which can go on to cause pelvic inflammatory disease, ectopic pregnancies, and infertility. In addition, in both men and women exposed orally or anally, gonococcal infections can cause a predominantly asyntomatic pharyngitis or proctitis. Less commonly, *N. gonorrhoeae* can cause conjunctivitis, endocarditis, tenosynovitis, arthritis, meningitis, inflammation of the liver capsule and disseminated blood stream infections (Barry, 2009).

Conjunctival infection occurs in adult and neonatal by accidental inoculation or contamination in the birth canal and causes ophthalmia neonatorum, which if not treated properly can lead to blindness (Marrazo *in* Principle and Practice of Infections Disease, 2010).

Asymptomatic infection in women has been linked to the biofilm production, which includes nature complex mixes of exopolysaccharides, proteins and DNA. Bacteria living in biofilms are more resistant to biocides and antimicrobial agents. Also, the horizontal transfer of resistance genes is more likely and increases the spread of resistance genes (Apicella *in Neisseria* Molecular Mechanisms of Pathogenesis, 2010). Gonococcal infection may facilite transmission of human immunodeficiency virus (HIV), increasing the number of target cells for HIV in the inflammatory exudate present in symptomatic patients (Bala, 2010)

1.3 Diagnosis of gonococcal infections

Diagnosis of gonococcal infection has historically been a combination of clinical signs and symptoms plus the microbiologic study. The diagnosis of urethritis in men can be made by observing gram-negative intracellular diplococci associated with polymorphonuclear leukocites on a smear prepared from the urethral discharge. The Gram stain has a sensitivity of 90 to 95% and specificity of 95 to 100% for diagnosing genital gonorrhea in symptomatic men. In cervicitis, the sensitivity of this test is less than 50%, when compared with properly

performed culture. Culture on selective media, modified Thayer Martin, it allows recovery of *N. gonorrhoeae* from body sites harboring an endogenous bacterial flora.

At the last years, nucleic acid detection techniques allow direct detection of *N. gonorrheae* in clinical samples and do not require viable organisms. The assay may be divided into three types: (i) direct detection probe hybridization to the target nucleic acid with direct detection of the hybrid; (ii) nucleic acid amplification tests (NAATs); and (iii) amplified-signal probe test, which hybridize with nucleic acid and then amplify the signal of the probe. These assays are generally much more sensitive that culture and are highly specific for urogenital infections, however, depending on the assay used (e.g. PCR) some concerns have arisen about the specificity of these tests from other anatomic sites. The advantages of the test are specimens may be transportes from geographically distant areas and stored for several days prior to testing. The NAATs also allow use non-invasive specimen types such as urine and vaginal swabs. The disadvantages of using nonculture nucleic acid probe or amplification tests include unavailability of a viable isolate for antimicrobial susceptibility testing and the possibility of a positive test after treatment, since nucleic acids from organism may persist for a period of time after successful therapy (Barry, 2009).

1.4 Epidemiology of gonococcal infections

Gonococcal infection is more common among young persons, particularly those aged 15-24 years, persons with lower socio-economic status, men who have sex with men (MSM), illicit drug users, commercial workers an racial/ethnic minority groups (Barry, 2009).

Gonococcal infections are among the most common reportable infections around the World. In the Unite Stated, gonorrhea is consistently the second-most frequently reported notifiable infection with more than 350,000 reported in 2006 (Barry, 2009; CDC, 2006).

In the United States, some European countries and Australia, the incidence of gonorrhea decreased at the end of the 1.980s to mid of 1.990s it was mainly attributed to changes in sexual behaviour produced after the appearance of HIV and the introduction of fluoroquinolone treatment (Famiglietti, 2000; Griemberg, 1997; Trotter *in Neisseria* Molecular Mechanisms of Pathogenesis, 2010).

Gonococcal infection has increased over previous years, although not consistently in all countries. In the United States the incidence of gonorrhea in recent years shows an upward trend, while the rate of gonorrhea in 2009 decreased 10.5% respect 2008 (Diez, 2011).

The World Health Organization (WHO) estimated that, globally, there were 62 million new cases of gonorrhea in 1999, with 27 million (44%) in South and South-East Asia, 17 million (27%) in Sub-Saharan Africa and 7.5 million (12%) in Latin America and the Caribbean (WHO, 2001).

2. Currents problems

2.1 *Neisseria gonorrhoeae*: Antimicrobial resistance and clinic impact

The penicillin introduction by the end of 1940s led to the eradication of *N. gonorrhoeae* sulfonamides resistant and it was the antimicrobial agent that kept its effectiveness for nearly forty years (approximately 1945-1985), although it required increasing the dose in

response to the emergence of chromosomal resistance and the use of alternative drugs for the treatment of isolates penicillinase producers (PPNG), since its appearance in 1976 (Ashford, 1976). In the mid of 1980s the prevalence of these strains exceeded 40% for what should no longer be used for empiric treatment of gonorrhea. At that time, the percentage of isolates tetracycline resistant was high, greater than 50% (Famiglietti, 2001). To the high prevalence of isolates with chromosomal resistance to tetracycline (CMRNG), originated in 1970, joined the plasmid resistance (TRNG) which is mediated by the *tetM* determinant in conjugative plasmid that confers high level resistance (Morse, 1986).

The market introduction of fluoroquinolones in the middle of 1980s, was the ideal replacement of penicillin, not only be used as a single-dose and would be highly effective, but also has advantage on the route of administration of drug and fewer adverse effects.

In the United States, due to increased resistance to penicillin and tetracycline, the Centers for Disease Control and Prevention (CDC) recommended the use of extended- spectrum cephalosporin or a fluoroquinolone as firt line treatment for the uncomplicated gonococcal infection (CDC, 1993).

The use of highly effective fluoroquinolone against PPNG helped limit rapid spread of these strains (Janda and Gaydos *in* Manual of Clinical Microbiology, 2007). However, in 1986 resistance to enoxacin was reported in Netherlands and the first treatment failures to fluorquinolones were documented in Japan in 1.994. (Tapsall, 1992; Deguchi, 2010). Such isolates emerged in Argentina in 2000 (Fiorito, 2001).

At the last years, the emergence of *N. gonorrhoeae* resistant to fluoroquinolones reintated the global problem of the treatment and control of gonorrhea (Update CDC, 2007). The increase followed a kinetics up more often affecting men who have sex with men (MSM), but then also was extended to heterosexuals men (HET) Figure 1 (CDC,2004; García 2008). MSM constitute the most important sources of transmission to sexual contacts from extragenital infections (rectum and pharynx), which are often asymptomatic. The high incidence of these strains forced a change in the empirical treatment of gonorrhea by cephalosporins, initially in MSM and their contacts, and later in the heterosexual men (CDC, 2006, 2010).

About macrolides, since its introduction in 1952, erythromycin was used for the treatment of various infections with an acceptable degree of adverse effects. In 1975 it was recommended as treatment for gonorrhoea in pregnant women allergic to penicillin (U.S. Public Health Service, 1975). In 1977, it was observed a decline in the drug effectiveness (Brown, 1977).

Azithromycin is an azalide, structurally and pharmacologically related with erythromycin. It was recommended for the treatment of infection caused by *Chlamydia trachomatis*, a single dose of 1 g orally, while for gonorrhea is used in a higher dose (2g) (CDC, 2002).

Given that *N. gonorrhoeae*, easily develops resistance to antimicrobial agents and its rapid spread, the WHO and CDC recommend changing the treatment regimen when the prevalence of antimicrobial resistance is> 5% (WHO, 2003).

Currently, the CDC recommends for the treatment of uncomplicated gonorrhea and infections located in the cervix, urethra and rectum, ceftriaxone 250 mg IM in one dose or cefixime 400 mg associated with azithromycin 1g VO or doxycycline 100 mg / day for 7

days , VO (CDC, 2010). However there are regions where resistance to cefixime does not allow its use (Barry, 2009).

Azithromycin and doxycycline are intended to cover the presence of *Chlamydia trachomatis* non gonococcal urethritis . The recommended dose of azithromycin for treatment of gonorrhea is 2g VO to prevent the emergence of resistance (CDC, 2010).

About the antimicrobial agents which are recommended by the CDC, there were treatment failures with azithromycin when it was given in a single dose of 1g VO for uncomplicated gonorrhea, with isolates had MICs between 0.125 and 0.5 µg / ml (Zarantonelli , 1999; Ehret, 1996). Recently, in several countries are emerging of high-level resistance to azithromycin strains (Galarza 2009; Palmer, 2008; Chisholm, 2009; Starnino, 2009).

Ceftriaxone (third-generation cephalosporin, parenteral) retains its activity against *N. gonorrhoeae*, but recently have been appearing isolates with decreased sensitivity in various regions of the world (Martin, 2006; Barry, 2009, Tapsall, 2009; Chisholm, 2010; Deguchi, 2010; Garcia, 2010a).

3. Present study with review of literature

3.1 Incidence of gonorrhea

The STD Program of the National Hospital of Clinics, "José de San Martín" University of Buenos Aires, Argentina, deals with 80% patients from the city and 20% from the suburban areas. In a period of 10 years (2000-2009), was investigated the presence of *Neisseria gonorrhoeae* in 4273 male patients (52% MSM and 48% HET).

The average age of MSM was 33 years (range 14-75 years) and HET was 34 years (range 15-82 years). The incidence of gonorrhea was 10.3% and 9.5% in MSM and HET respectively ($p > 0.05$) and it was more frequent in men between 20 and 29 years old in both groups of patients.

In MSM urethral location was the most frequent (70%), followed by rectal (34%) and lastly the pharynx (17%). This increase in the incidence of gonorrhea in HET in 2008 and 2009 compared to 2007, was significant ($p < 0.05$) and has also been reported by other authors (Velicko, 2009). It could be due to increased in this population of *N. gonorrhoeae* fluoroquinolone resistant in this population, since it was initially installed in the MSM.

Fluctuations were observed in the annual incidence of gonorrhea in MSM and heterosexual men between 1995 and 2009, with a decrease at the end of the 1990s and beginning of the new millennium but then it continued to increase slowly in recent years, specially among heterosexual men.

The analysis of results was divided into two periods: 2000-04 (first period), the emergence of *N. gonorrhoeae* fluoroquinolone resistant in 2005 in our Hospital pointed the beginning of the second period: 2005-09 (second period). The Table 1 shows the incidence of *N. gonorrhoeae* from clinical specimens according to patient's sex habits.

The National Health Surveillance of the Ministry of Health of Argentina (SNVS) had registered 28.517 and 17.017 cases of gonorrhea in the periods 2000-4 and 2005-09 respectively.

Period	Heterosexual men			MSM				
	N°		Location	N°		Location		
	Patients	Positive (%)	Urethra	Patients	Positive (%)	Urethra	Rectum	Pharinx
2000-04	1259	100 (7.9)	100	1143	117 (10.2)	78	45	15
2005-09	802	95 (11.8)	94 Joined (1)	1069	111 (10.4)	81	33	24
Total	2061	195 (9.5)	195	2212	228 (10.3)	159	78	39

Table 1. Number and percentaje of gonorrhea cases in males according their sexual habits and location sites.

3.2 Antimicrobial resistance

All isolates were tested for beta lactamase by chromogenic cephalosporin Nitrocefin method (Cefinase BBL. Beckton Dickinson). Over 324 isolates of *N. gonorrhoeae* was determined the minimum inhibitory concentration (MIC) of several antimicrobial agents by the agar dilution method according to the recommendation of Clinical Laboratory Standard Iinternational (CLSI, 2009).

3.2.1 Penicillin

The mechanisms of resistance to penicillin are the production of β-lactamase (penicillinase), chromosomal resistance by altering PBP1 and 2, reduced income and increased efflux.

During periods 2000-04 and 2005-09 the global prevalence of *N. gonorrhoeae* β- Lactamase positive (PPNG) was 8.3% and 12.6%, and chromosomal resistance (CMRNG) was 4.2% and 23.7% respectively. A significant increase of *N. gonorrhoeae* with chromosomal resistance to penicillin was observed for both groups of patients ($p<0.05$) in the second period, the results are showed in table 2.

Períod/SH	total N° isolates	CMRNG%	PPNG%
2000-04			
MSM	117	6.8	10.2
HET	100	1.0	6
Total	**217**	**4.2**	**8.3**
2005-09			
MSM	111	33.3	7.2
HET	95	12.6	18.9
Total	**206**	**23.7**	**12.6**

Table 2. Prevalence of *Neisseria gonorrhoeae* resistant to penicillin

Whereas the number of isolates β- lactamase positive (PPNG) among MSM decreased ($p > 0.05$) between first and second periods, the prevalence of chromocomal resistance (CMRNG) to penicillin was significantly higher than HET ($p<0.05$). It could be related to the rectal location, since the environment is rich in fatty acids and hydrophobic molecules that can act as a positive selection factor favoring the emergence of resistant strains (Mtr phenotype) (García, 2011).

Resistance to penicillin founded is within the range of Argentine national data (Galarza, 2010).

3.2.2 Tetracycline

Plasmid tetracycline resistance is mediated by the *tet M* determinant (MIC ≥ 16 µg/ml) while chromosome type resistance involves the modification of ribosomal protein (S10-Val 57) (MIC ≥2 µg/ml) (Shaffer *in Neisseria* Molecular mechanisms of Pathogenesis, 2010).

The resistance was higher in the second period for both groups ($p>0.05$) (figure 1).

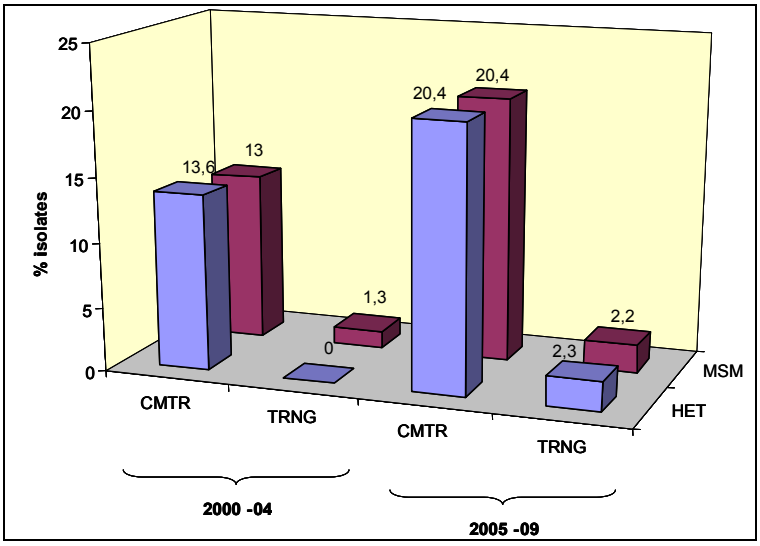


Fig. 1. Percentage of chromosomal and plasmid resistance to tetracycline.

Isolates with low-level resistance to tetracycline (MIC <16 μg / ml) had no cross-resistance to minocycline (CIM: 0062-4 μg / ml) (García, 2010a, 2011)

Tetracycline showed little activity. The plasmid resistance was uncommon (5 / 324), as in other regions of Argentina (Galarza, 2010b).

In figure 1 are shown the plasmid and chromosomal resistance to tetracycline in both groups of patients.

3.2.3 Spectinomycin

In 2005-09 period a 11% of the isolates showed intermediate sensitivity to spectinomycin (MIC = 64 μg / ml), 80% of cases from MSM (García, 2010a, 2011). However, the Argentina national data showing 100% sensitivity, but in South Africa was found 33.4% resistance to this antimicrobial (Govender, 2006).

Spectinomycin is effective for the treatment of anogenital gonorrhea but is not useful for pharyngeal infection. Besides *N. gonorrhoeae* can rapidly develop resistance of high-level resistance by a single mutation, it was documented in the U.S., Korea and United Kingdom (Reyn, 1973; Ashford, 1981; Barry, 2009).

3.2.4 Emerging of antimicrobial resistance

The emerging resistance of *N. gonorrhoeae* in the last decade is to erythromycin, azithromycin, fluoroquinolones and decreased sensitivity to ceftriaxone.

In *N. gonorrhoeae* were identified four efflux pumps: MtrCDE, FarAB, MacAB, and NorM that belong to the families RND, MF, ABC and MATE respectively. Only a few gonococci *mef* gene was detected (Shafer *in Neisseria* Molecular Mechanisms of Pathogenesis, 2010).

3.2.4.1 Resistance to erythromycin and azithromycin

Resistance to macrolides and azalides in *N. gonorrhoeae* may be due to different mechanisms: overexpression of efflux pumps, alteration of the target site, decreased outer membrane permeability and inactivation of the drug.

Of the efflux pumps, MtrCDE, MacAB and NorM recognized relevant antibiotics to treat gonorrhea. Protein efflux pump encoded by the *mef* gene can also recognize macrolides. The *mef* gene is located on a conjugative transposon, but has been found in a limited number of gonococci (Luna, 2002).

The MtrCDE-efflux pump can export erythromycin, tetracycline, penicillin, and hydrophobic compounds including fatty acids, bile salts, steroids and antibacterial peptides (Morse, 1982, Hagman, 1995), while the pump NorM exports quinolones (Rouquette-Loughlin, 2003) and MacAB pump exports macrolides (Rouquette-Loughlin, 2005)

The expression of genes encoding the bacterial efflux depends of several regulatory levels. The loss of regulatory systems can result in high levels of antimicrobial resistance.

The *mtrCDE* operon has three genes encoding each of the proteins that make up this bomb: MtrC (fusion protein) and MtrR (cytoplasmic membrane protein), MtrE (outer membrane protein).

The complex of genes *mtrCDE* represents a transcriptional unit is located 250 bp upstream and divergently transcribed respect to *mtrR* gene, which is the gene that encodes a regulatory protein MtrR (repressor) of *mtrCDE* transcription (Lucas, 1997).

The MtrR protein negatively regulates transcription of the *mtrCDE* operon, it binds to DNA in the region of 250 bp separating the genes *mtrR* and *mtrC*, a region where is the promoter of the operon transcription. In clinical isolates were found *mtrR* mutations that alter the repressor protein and its binding to DNA, resulting in decreased repression of the *mtrCDE* operon (Lucas, 1997).

These mutations are found in the region between residues 32 and 53 that make up the domain helix-turn-helix protein in the DNA binding and mutations "missense" causing A39T amino acid replacement (replacement of alanine by threonine in position 39) or G45D (replacement of glycine by aspartic acid at position 45) can increase the resistance to hydrophobic antimicrobial presumably because avoid the binding of the MtrR repressor protein to DNA up stream on *mtrCDE* . Another mutation described in clinical isolates is one that occurs in the middle of the coding sequence of *mtrR*: H105Y

(Replacement of histidine by tyrosine at position 105) or in the C-terminal domain that can impact the function of MtrR probably by altering the formation of multimer MtrR (Shafer, 1995; Warner, 2008).

There are differences in the level of resistance conferred by mutations, the "missense" mutations in the coding sequence typically result in low to intermediate level of resistance while mutations in the promoter are associated with high level of resistance (Warner, 2008).

Another mechanism of regulation of this efflux system is exercised by a region of inverted repeats of 13 bp in the *mtrR* gene promoter, it has a function as a transcriptional control element cis type. In laboratory mutants and clinical isolates was found that the deletion of one base pair A: T in this region causes an increased expression of *mtrCDE* operon and repression of *mtrR* gene *t*ranscription, it confers low-level resistance to tetracycline , erythromycin, penicillin and hydrophobic agents such as crystal violet, fecal lipids, bile salts, detergents (Eg: Triton X-100, nonoxynol-9) (Hagman, 1995) In these cases, erythromycin and azithromycin MICs were up to 2 μg / ml and 0.5 μg / ml respectively (Zarantorelli, 2001, Cousin, 2003).

Previous studies indicate that isolates of *N. gonorrhoeae* from MSM were more resistant compared to inhibition by a variety of structurally different hydrophobic compounds, including fecal lipids, bile salts, detergents (eg, Triton X-100) and hydrophobic antibiotics (eg: erythromycin). It has been attributed to mutations in the efflux system of multiple transferable resistance(Mtr) resistance to erythromycin and Triton X-100, it can serve as a phenotypic marker for resistance mediated by Mtr in *N. gonorrhoeae* (Morse, 1982; Zarantonelli, 1999).

There is clinical evidence to suggest that efflux pumps increase the capacity of gonococci to survive during human infection. First, *mtrR* mutants are isolated frequently from rectum of infected patients presumably because it environment is rich in hydrophobic compounds such as long chain fatty acids (Morse, 1982).

Another mechanism is described in gonococcal is the ribosomal rRNA methylation by methylases, thereby blocks the binding site of macrolides by methylation in 23 S rRNA in an adenosine residue at position 2058 (in *E.coli*), it site is the domain of the peptidyl transferase (Leclerq and Courvalin, 1991).

In *N. gonorrhoeae* have been identified enzymes rRNA methylases encoded by the genes *ermB*, *ermC*, *ermF* and recently *ermA (*Roberts, 1999; Chen, 2010).

The genes encoding rRNA methylase may be in conjugative transposons and they may confer to gonococci highly level of resistance to erythromycin (4-16 μg / ml) or decreased sensitivity to azithromycin (1 - 4 μg / ml) without mutations in *mtrR* (Roberts, 1999; Shafer, *in Neisseria* Molecular Mechanisms of Pathogenesis, 2010)

In recent years the study of strains highly resistant to azithromycin revealed another mechanism of resistance due to mutations in the gene encoding the "loop" domain V of 23S rRNA peptidyl transferase in the 50S subunit of the ribosome (Chisholm, 2010; Galarza, 2010a).

The outer membrane of gram-negative represents a significant permeability barrier to antibiotics, particularly hydrophobic compounds. Different degrees of truncation of LPS oligosaccharides (known as lipooligosaccharides [LOS] in *Neisseria*) can alter the entry of hydrophobic molecules (Lucas, 1995).

There was not agreement between the diffusion and dilution methods for erythromycin and azithromycin, which is why we could not recommend break points for diffusion test.

In our study, erythromycin resistance increased statistically significantly from first to second period for MSM 20.8% and 39.8% respectively ($p < 0.05$), while for the HET the difference was not significant, 22.9% and 25.0% respectively;($p > 0.05$).

The difference between MSM and HET was not statistically significant ($p > 0.05$). The activity of azithromycin was superior to erythromycin.

In the second period two isolates of *N. gonorrhoeae* with erythromycin MICs = 256 μg / ml were associated with azithromycin MICs of 4 and 16 μg / ml, the second strain also was resistant to tetracycline, penicillin and ciprofloxacin. These findings are consistent with those of other regions of Argentina and other countries (Galarza, 2009; Palmer, 2008; Starnino, 2009, Chisholm, 2010) and point to the emergence of high-level resistance to azithromycin, it has impact public health and would limit the drug as a treatment option for gonorrhea.

In the isolates with Mtr phenotype (MIC of Triton-X100> 2000 μg/ml), the most frequently mutation found was a deletion of a base pair A:T in the region of inverted repeats of 13 bp in the promoter region and mutations in the coding region of *mtrR* gene which could be associated with overexpression of MtrCDE pump efflux and subsequent macrolide resistance as other authors have shown (Morse, 1982; Zantorelli, 2001). It was the only mechanism of macrolide resistant found in these isolates (García, 2011).

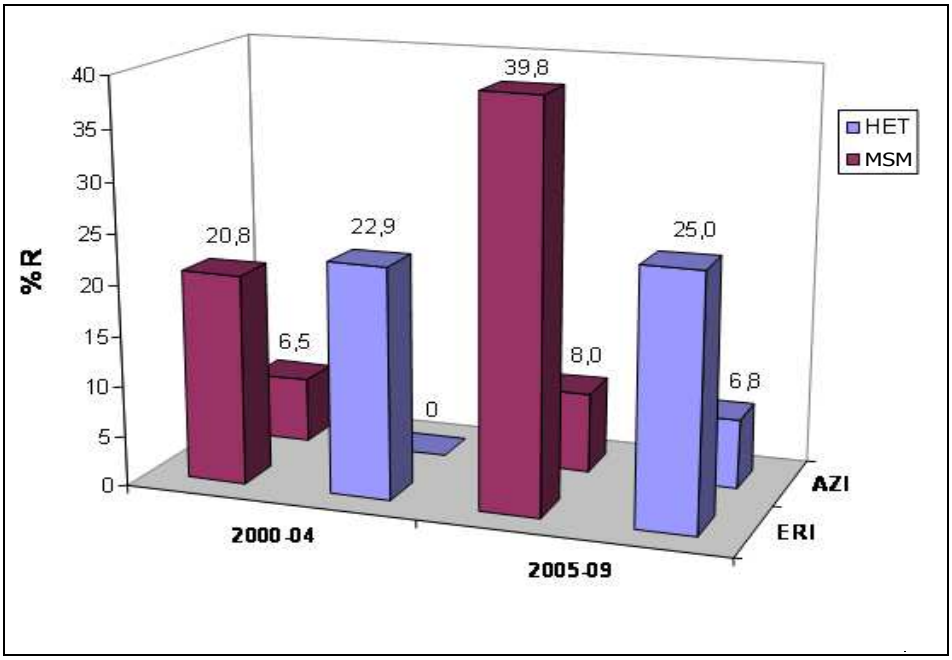


Fig. 2. Percentage of *N. gonrrhoeae* resistant to erythromycin and azithromycin.

In recent years there were communications about the finding of gonococci with high level of resistance to azithromycin (MIC ≥ 256 μg / ml) in many countries. The first isolation came from Argentina in 2001 (Galarza, 2009), and then in Europe: Scotland, 2004, England, Wales and Italy 2007 (Chisholm, 2009, 2010, Stamino, 2009).

The argentine isolate had a MIC > 2048 μg / ml and A2143G mutation was detected in the 4 allele. This mutation corresponds to A2059G, described previously in *Escherichia coli* but not in gonococci (Galarza, 2010).

The use of subtherapeutic doses of azithromycin may be the cause of the emergence of strains of *N. gonorrhoeae* resistant to the antimicrobial agents.

3.2.4.2 Resistance to fluoroquinolones

In gonococci the most frequently found mechanism is the alteration of the target site of fluoroquinolones: mutations in QRDR regions (Quinolone Resistance Determining Region) of genes *gyrA*, *gyrB*, *parC* and *parE* encoding topoisomerase enzymes that translate in changes in the amino acids sequence in GyrA and GyrB, subunits of topoisomerase II (DNA gyrase) and ParC, subunit of topoisomerase IV. Mutations in *gyrB* have little significance, since low-level confer resistance to nalidixic acid only, whereas, mutations in *gyrA* and *parC* genes, confer clinical resistance to the fluoroquinolones. Mutations in *parC* occur only in isolates that had at least one mutation in *gyrA* (Otero, 2002). Mutations in *parE* is not related to high-level resistance to quinolones.

Another mechanism is related to the decrease in cytoplasmic concentration of the drug, it can occur by overexpression of efflux pumps and reduced permeability.

The *mtrCDE* overexpression can confer low-level resistance to fluoroquinolones and hydrophobic compounds (Hagman, 1995).

Other efflux system detected in *N. gonorrhoeae* , like NorM of Vibrio parahaemolyticus tht belonged to the MATE transporter superfamily, it recognizes cationic compounds resulting in increased gonococci resistance to norfloxacin and ciprofloxacin (Rouquette-Loughlin, 2003).

The phenotype of low resistance may be due to decreased penetration of antibiotic through the bacterial cell membrane. The routes of penetration of quinolones through the bacterial cell wall are not completely defined, but hydrophilic molecules seem to diffuse through the outer membrane of gram-negative porin-type channels (Hooper *in* Infectious Diseases Principles and Practice, 2010).

In the Hospital of Clinics of the University of Buenos Aires were detected the first isolates fluoroquinolone resistant (QRNG) in 2005 and its frequency was increasing in the coming years especially in the MSM, this forced a change in empirical therapy later extended to heterosexual men (Garcia, 2008). We couldn't demonstrate epidemiological link among cases, excepting two. The percentage of QRNG according to the patient's sexual habits are shown in Figure 3.

Resistance to ciprofloxacin is extensive to other fluoroquinolone (norfloxacin, ofloxacin, gatifloxacin and lomefloxacin).

The resistance to fluoroquinolones was associated to multidrug resistance (García 2010b)

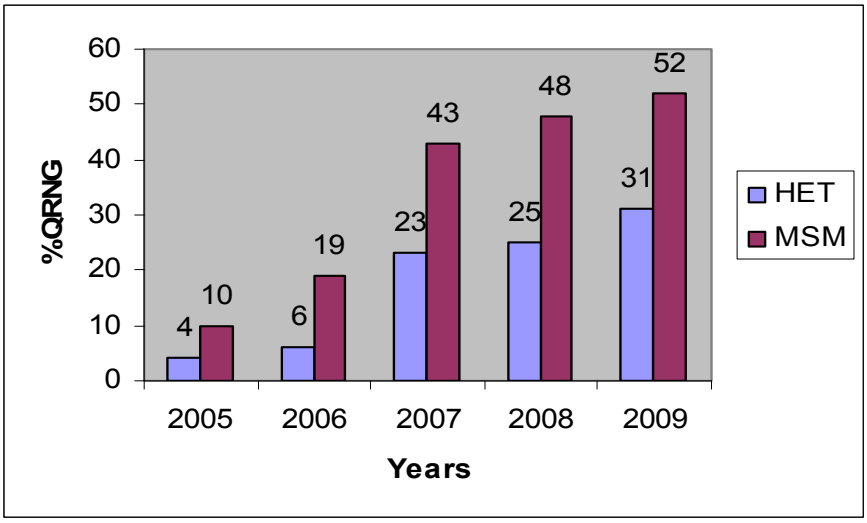


Fig. 3. *N. gonorrhoeae* fluoroquinolone resistant along the years.

In the QRNG the mechanism found was mutations in topoisomerases (subunits GyrA and ParC) (García, 2011).

3.2.4.3 Ceftriaxone decreased sensitivity

The genetic mechanisms that increase resistance to third generation cephalosporins in *N. gonorrhoeae* have not been fully elucidated, but much of that resistance is due to mosaic *penA* alleles and probably require alterations in PBP, decreased membrane permeability barrier and overexpression of efflux pumps.

N. gonorrhoeae has three PBPs, named 1, 2 and 3. The PBP2 has a 10 times higher affinity for penicillin G that PBP1 and is believed to be the site of increased binding to cephalosporins.

Some alterations in PBP2 were associated with resistance to β-lactams including cephalosporins. The most common mechanism associated with resistance to cefixime is altered PBP2 (Ameyama, 2002). Recently, *penA* alleles different from previously known strains appeared in increased resistance to third generation cephalosporins such as cefixime and ceftriaxone. These alleles encode at least 60 mutations in PBP2 compared to the wild strain and they have a mosaic gene organization appears to have been caused by recombination with commensal *Neisseria* genes such as *N. perflava*, *N. sicca, N. cinerea or N. flavescens* (Ameyama, 2002; Tanaka, 2006).

It was reported the isolation of *N. gonorrhoeae* with ceftriaxone MIC = 0.5 µg / ml had the mosaic PBP2, but also mutations in *ponA* (L421P), *penB* (A120 and A121) and *mtrR*. They hypothesized that the L421P substitution in the gene encoding PBP1 *ponA* may be important in conferring resistance to ceftriaxone. The possible importance of ponA L421P was subsequently supported by data Takahata in which strains with the substitution L421P was associated with increased cephalosporin MICs, compared with only transformants that had the mosaic PBP2 (Takahata, 2006). However, Nicholas found neither the presence or absence of *ponA* affected the MICs of cephalosporins (Nicholas, 2008).

This indicates that the mosaic is important but not worth enough to generate the high level of resistance to cefixime (Barry, 2009).

penB mutations, in the "loop" of the gene, reduces the permeability to hydrophilic antibiotics and is considered related to chromosomal resistance to penicillin, cephalosporins, tetracyclines and fluoroquinolones (Tanaka, 2006).

In our experience, ceftriaxone proved to be more active, however there is an emergence of strains with decreased sensitivity (0.125 to 0.25 µg / ml) and the increase of such isolates was notable especially in the year 2009. A 100% of the isolates were susceptible to ceftriaxone, but the 7.7% showed reduced susceptibility (MIC: 0.125 to 0.25 µg / ml) and did not express extended spectrum beta lactamase (βLEE) (García, 2010b)

This situation is similar to other countries, in Australia (2005) 1% of the isolates of *N. gonorrhoeae* had MIC ≥ 0.062 µg / ml; 12.1% in Amsterdam in 2008; 3% in Russia (2004) and elsewhere (Tanaka, 2006; Barry, 2009; Martin, 2006).

In Japan, cefixime therapy failures date back to 2000, it stopped to be used in 2006 and it was replaced by ceftriaxone and spectinomycin which are used as first-line drugs for empirical treatment of uncomplicated gonorrhea (Barry, 2009). In China, Hong Kong and

Taiwan were detected isolates with decreased sensitivity to ceftriaxone and treatment failures with ceftibuten. In Vietnam, Thailand and the Philippines have been isolated sporadically *N. gonorrhoeae* with ceftriaxone MICs of ≥ 0.5 µg / ml. In Europe and the U.S. were also found isolates of *N. gonorrhoeae* with ceftriaxone MIC ≥ 0.125 µg / ml. (Barry, 2009, Martin 2006).

In Argentina, the first report of isolates of *N. gonorrhoeae* with decreased sensitivity to ceftriaxone came from in the Hospital of Clinics, "José de San Martín", Universiy of Buenos Aires. The emergence of strains with reduced sensitivity to ceftriaxone is an alert to the possibility of treatment failure in a short period of time.

The combination of different mechanisms of resistance in the same strain, or affectation of multiple antimicrobial for developing a type of mechanism leads to the emergence of strains resistant to several antimicrobial agents simultaneously. Tapsall (2009) defined *N. gonorrhoeae* multi drug resistant (MDR-NG) when the isolate has resistance to a class of antimicrobial listed in category I: parenteral Cephalosporins / oral cephalosporins / Spectinomycin, plus the resistance of two or more classes of antimicrobial in category II:

penicillins/ fluoroquinolones/azithromycin/aminoglycosides/carbapenems.

Extensively-drug resistant *N. gonorrhoeae* (XDR-NG) include those resistant to two or more of the antibiotic classes in category I and three or more in category II.

In our study 4.3% (14/324) isolates of *N. gonorrhoeae* were categorized as multiresistant and 0.3% (1 / 324) resistant gonococci extensively according to the definitions of Tapsall (2009) (García, 2011).

4. Genetic relationship of isolates of *N. gonorrhoeae* ciprofloxacin resistant

The characterization of different isolates of *N. gonorrhoeae* provides the information necessary to understand the strains circulating in a community, identify chains of transmission, the temporal changes that may occur and the emergence and spread of antimicrobial resistance (Rahman, 2001).

The first methods used were based on phenotypic characteristics, the auxotipificación and serotyping; also the profile of antimicrobial resistance and type of plasmids contribute to the characterization of strains, but they do so limited, these techniques have less discrimination index for delineate strains of a species that genotyping assays.

Several molecular techniques have been applied to the study of *N. gonorrhoeae*, ribotyping, AP-PCR, REP-PCR, ARDRA, PCR-RFLP, sequencing of the genes *opa*, *TbpB*, NG-MAST, MLST. Pulsed-field gel electrophoresis (PFGE) is a standard technique for studying populations of many bacteria, but it would not be discriminating enough to distinguish the great diversity of genotypes expected in a recombinant nonclonal population as *N. gonorrhoeae* (O'Rourke, 1995).

However, in a study by Van Looveren, 1999, was found that the discriminative power of some typing methods for *N. gonorrhoeae*, defined as the method's ability to differentiate unrelated strains (Simpson Index) was higher for PFGE (SI:0.997).

The election of the technique depends of objective early or late evolution.

The most discriminative techniques are multilocus sequence typing (MLST), multiantigen sequence typing (NG-MAST), *porB*-based DNA sequence analysis and PCR-RFLP *opa.* To study isolates during short time periods in order to detected transmission chains, identify circulating strains community epidemics, e.g. the elected methods are *porB* sequence analysis and NG-MAST. Furthermore, isolates with identicalDNA sequencing types may be further subdivided by using bother high-resolution methods, such as PFGE and Opa typing.

The family of *opa* genes evolve rapidly in order to distinguish genetically unrelated strains and to demonstrate sexual contacts, as well as the persistence of strains in the community present in asymptomatic individuals.

opa gene analysis is sufficiently discriminative to detect short chains of transmission (García, 2011).

The analysis of different genotypes of *N. gonorrhoeae* over the years, in the population stutied, let we conclude that the increase in quinolone resistance was not due to the spread of a single strain.

Other molecular technique applicable for this purpose to *N. gonorrhoeae* are MLST and NG-MAST with discriminatory indices exceeding 0.9 (Tazi, 2010; Morris, 2009).

5. Conclusion

In conclusion, selective pressure exerted by antibiotics and the inappropriate use of them reflects the high number of *N. gonorrhoeae* fluoroquinolone resistant is circulating in our program area, resistance to erythromycin and azithromycin and the findings of decreased susceptibility to ceftriaxone and especinomicina.

An increase of *N. gonorrhoeae* fluoroquinolone resistant was detected initially in the MSM population that later spread to the HET and based the modification of empiric treatment of gonorrhea by ceftriaxone. Be alert to the emergence of gonococci with decreased susceptibility to ceftriaxone, which also presented simultaneous resistance to other agents and its association with MSM population.

Currently exist a large number of resistant strains of *N. gonorrhoeae* fluoroquinolone resistant circulating and most of them are not shared between HET and MSM.

6. Future perspectives

In view of the widespread emergence of resistant isolates and the multidrug resistance profile of *N. gonorrhoeae* also to emphasize or intensify prevention campaigns among young people should be evaluated strategies that can be applied in case of emergence of resistance to ceftriaxone. One of them is not yet tested other antibiotics prescribed for the treatment of gonorrhea in order to search for drugs up in case of emerging resistance to third generation parenteral cephalosporins. There is limited experience in the treatment of gonococcal infections with aminoglycosides, these drugs have been used in Asia and Africa. No kanamycin resistance was detected but it developed resistance to gentamicin when it is used in Malawi (Barry, 2009). In a surveillance study of resistance of *N.*

gonorrhoeae in Europe, 95% of 1366 isolates showed MIC of gentamicin in a narrow range: 4-8 ug / ml (Chisholm, 2010).

The frequency of tetracycline resistance is very high and resistance to azithromycin is more than 5% and circulating strains with high resistance, so that these drugs would not be appropriate to empirical treatment of gonorrhea.

Spectinomycin, is an aminocyclitol that shows good activity and appear to be among the possible treatment in case of resistance to ceftriaxone, but there are gonococci with intermediate sensitivity (Garcia, 2010; Barry, 2009). Furthermore, mutations in a single step are able to quickly generate resistance and it was checked when spectinomycin was used as treatment against PPNG strains. We should also take into account the lack of eradication of throat infection (Barry, 2009).

Tigecycline, glycylcycline derivative of tetracycline, showed in vitro activity against *N. gonorrhoeae* resistant to tetracycline, but there have been no clinical trials for infections caused by this organism. Rifampin may be an alternative but quickly generates resistance (Barry, 2009).

Other drugs that could be tested are Lactivicinas (LTV) that inhibits PBPs but their structure does not contain the β-lactam ring and thus is resistant to the action of β-lactamases, are active against clinical isolates of *Streptococcus pneumoniae* resistant to penicillin (Macheboeuf, 2007).

Medicinal plants like Ocimum sanctum were investigated as possible source of new active drugs against *N. gonorrhoeae*, after extraction and purification of its active ingredient "eugenol" tested its activity against *N. gonorrhoeae* and shown to have antimicrobial activity, in view of its efficacy and low toxicity can be a potential molecule to be developed for clinical application (Shoken, 2008).

Some authors suggest the design of drugs to site of action of efflux pumps as they are responsible for simultaneous resistance to several antimicrobial (Shafer *in Neisseria* Molecular Mechanisms of Pathogenesis, 2010).

Other strategies to use would be: I) Increase the dose of cephalosporin, ii) multi-dose cephalosporin regimens; III) microbiologically directed treatment, IV) association of antimicrobial agents, V) Rotate empirical treatments (Chisolmon, 2010).

The development a vaccine for *N. gonorrhoeae* would be effective tool to act from prevention, however attempts at developing it were difficult because of the antigenic diversity and variability, and the lack of an animal model. Research in these areas will play a large rol in the future.

On the other hand, is not only important to detect carriers but also testing antimicrobial susceptibility against the isolation of *N. gonorrhoeae* from each sites of infection.

7. Acknowledgment

We thank UBACyT B108 Project of University of Buenos Aires. Argentina.

8. References

Ameyama S, Onodera S, Takahata M, Minami S, Maki N, Endo K, Suzuki H & Oishi Y.(2002). Mosaic-like structure of penicillin-binding proteína 2 Gene (*pen*A) in clinical isolates of *Neisseria gonorrhoeae* with reduced susceptibility to cefixime. *Antimicrob Agents Chemother*, Vol. 46, Nº 12, (December, 2002), pp. 3744-3749, ISSN: 0066-4804.

Apicella MA, Falsetta M, Neil R & Steichen C. (2010). Gonococcal Biofilms, In: *Neisseria Molecular Mechanisms of Pathogenesis*, Attardo Genco C & Wetzler L, editors, pp. 55-60, Caister Academic Press Boston MA. USA, ISBN: 978-1-904455-51-6, Boston MA. USA.

Ashford WA, Golash RG & Henning VG. (1976). Penicillinase producing *Neisseria gonorrhoeae*. *Lancet*, Vol. 2, pp.:657-658, ISSN: 0140-6736.

Ashford WA, Potts DW & Adams HJU. (1981). Spectinomycin- resistant penicillinase-producing *Neisseria gonorrhoeae*. *Lancet*, Vol. 2, pp. 1035-1037, ISSN: 0140-6736.

Bala M & Sood Seema. (2010). Cephalosporin Resistance in *Neisseria gonorrhoeae. J Glob Infect Dis*, Vol. 2, Nº 3. pp. 284-290, ISSN: Print- 0974-777X.

Barry PM & Klausner J. (2009). The use of cephalosporins for gonorrhea: The impending problem of resistance. *Expert Opin Pharmacother*, Vol. 10, Nº 4, (March, 2009), pp. 555-577, ISSN: 1465-6566.

Brown ST, Pedersen IIB & Holmes KK. (1977). Comparison of erythromycin base and escolate in gonococcal urethritis. *Jama*, Vol. 238 (13), pp. 1371-1373, ISSN: 0098-7484.

Centers for Disease Control and Prevention. (1993). Sexually transmited disease treatment Guidelines. *Morb Mortal Weekly Rep*, Vol. 42, Nº (RR-14), pp. 1-101, ISSN: 0149-2195.

Centers for Disease Control and Prevention. (2002). Increases in fluorquinolone- resistant *Neisseria gonorrhoeae* - Hawaii and California 2001. *Morb Mortal Weekly Rep*, Vol. 51, pp. 1041-1044 , ISSN: 0149-2195.

Centers for Disease Control and Prevention. (2004). Increases in fluorquilonolone resistance *Neisseria gonorrhoeae* among men who have sex with men- US, 2003 and revised recomendations for gonorrhea treatment. *Morb Mortal Weekly Rep,* Vol. 53, Nº 16 pp.335-338, ISSN: 0149-2195.

Centers for Disease Control and Prevention (CDC). (2006). Sexually Transmitted Diseases Treatment Guidelines. *Morb Mortal Weekly Rep*, Vol. 55, Nº (RR-11), pp. 364-366, ISSN: 1545-8601.

Centers for Disease Control and Prevention. (2010). Sexually transmitted Diseases Treatment Guidelines. *Morb Mortal Weekly Rep*, Vol. 59, Nº (RR-12), (December, 2010), pp. 49-55, ISSN: 1545-8601.

Chen PL, Lee HC, Yan JJ, Hsieh YH, Lee NY, Ko NY, Lin CW, Chang CM & Wu CJ. (2010). High Prevalence of Mutations in Quinolone- resistance- determining Regions and *mtr*R Loci in Polyclonal *Neisseria gonorrhoeae* Isolates at Tertiary Hospital in Southern Taiwan. *J Formos Med Assoc*, Vol. 109, Nº 2, pp.120-127, ISSN: 0929-6646.

Chilsholm SA, Neal TJ, Alawattegama AB, Birley HDL, Howe RA & Ison CA. (2009). Emergence of high-level azithromycin resistance in *Neisseria gonorrhoeae* in England and Wales. *J Antimicrob Chemother*, Vol. 64, Nº 2, (May 2009), pp. 353-358, ISSN: 0305-7453.

Chisholm SA, Dave J & Iso C. (2010). High-Level azithromycin resistance occurs in *Neisseria gonorrhoeae* as a result of a single point mutation in the 23S rRNA genes. *Antimicrob Agents Chemother*, Vol.54, Nº 9, (September, 2010), pp. 3812-3816, ISSN: 0066-4804.

Clinical Laboratory Standards Institute. (CLSI). 2009. *Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically*, 19th Informational Suplement M07-A8, Vol. 29, Nº 3, (January 2009), pp.68-71. Wayne, Pa, USA, ISBN: 1—56238-690-5; ISSN: 0273-3099.

Cousin SL, Whittington WL & Roberts MC. (2003). Acquired macrolide resistance genes and the 1 bp deletion in the *mtrR* promoter in *Neisseria gonorrhoeae*. *J. Antimicrob Chemother*, Vol. 51, Nº 12 pp.131-133, ISSN: 0305-7453

Deguchi T, Nakane K, Yasuda M & Maeda S. (2010). Emergence and Spread of Drug Resistant *Neisseria gonorrhoeae*. *J Urol,* Vol. 184, (September, 2010), pp. 851-858, ISSN: 0066-4804.

Diez M & Díaz A. (2011). Infecciones de transmisión sexual: epidemiología y control. *Rev Esp Sanid Penit,* Vol 13; pp. 58-66, ISSN: 1575-0620.

Ehret JM, Nims LJ & Judson FN. (1996). A clinical isolation of *Nesisseria gonorrhoeae* with in vitro resistance to erytromycin and decreased susceptibility to azithromycin. *Sex Transm Dis*, Vol. 23, Nº 4, (July- August, 1996), pp. 270-272, ISSN: 0148-5717.

Famiglietti A, García S, de Mier C, Casco R, Vay C& de Torres R. (2000). *Neisseria gonorrhoeae* Drug Susceptibility in Buenos Aires, Argentina. *APUA Newsletter*, Vol. 18, Nº 4, pp. 1-4, ISSN: 1524-1424.

Famiglietti A, García S, de Mier C, Casco R, Belli L, Marcenac F, Vay C& de Torres R. (2001). Evolución of *Neisseria gonorrhoeae* drug susceptibility in Buenos Aires, Argentina 1985 1999. *Sex Transm Infect*, Vol. 77, Nº 2, (April, 2001), pp. 142 – 143, ISSN: 1368-4973.

Fiorito S, Galarza P, Pagano I, Oviedo C, Lanza A, Smayevsky J, Weltman G, Buscemi L & Sanjuán E .(2001). Emergence of high level ciprofloxacin resistant *Neisseria gonorrhoeae* strain in Buenos Aires, Argentina. *Sex Transm Dis*, Vol. 77, pp. 77, ISSN: 0148-5717.

Galarza PG, Alcalá B, SalcedoC, Fernández Canigia L, Buscemi L, Pagano I, Oviedo C& Vázquez JA. (2009). Emergence of high level azithromycin- resistant *Neisseria gonorrhoeae* strain isolated in Argentina. *Sex Transm Dis*, Vol. 36c, pp. 787-788, ISSN: 1368-4973.

Galarza PG, Abad R, Fernández Canigia L, Buscemi L, Pagano I & Oviedo C. (2010a). New Mutation in 23S rRNA Gene Associated with Level of Azithromycin resistance in *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother*, Vol. 54, Nº 4, (April, 2010) pp. 1652-1653.

Galarza P, Pagano I, Oviedo C, Reggiane S & RED ITS. (2010b). Vigilancia de la sensibilidad antimicrobiana de *Neisseria gonorrhoeae* en Argentina- PROVSAG 2009. XII Congreso Argentino de Microbiología, Resumen 28058. *Rev Argent Microbiol*, Vol. 42 (Supl 1), (October, 2010), pp. 256, ISSN: 0325-7541.

García S, Casco R, Perazzi B, De Mier C, Vay C & Famiglietti A. (2008). Resistencia de *Neisseria gonorrhoeae* a ciprofloxacina según hábitos sexuales. *Medicina (B. Aire*s), Vol. 68, pp. 358-362, ISSN: 0025-7680.

García SD, Casco R, De Mier C, Perazzi B, Yaya J, Vay C & Famiglietti A. (2010a). Vigilancia epidemiológica de las resistencias emergentes en *Neisseria gonorrhoeae.* Análisis de 5 años (2005-2009). 9° Congreso Internacional de Medicina Interna del Hospital de Clínicas. Resumen 64. *Medicina (B. Aires)* , Vol. 70 (Supl 1), (August, 2010), pp. 27, ISSN: 0025-7680.

García S, Casco R, Perazzi B, de Mier C, Yaya J, Vay C & Famiglietti A. (2010b). *Neisseria gonorrhoeae*: La era de la multiresistencia? XII Congreso Argentino de Microbiología. Resumen 625. *Rev Argent Microbiol*, Vol. 42 (Supl 1), (October, 2010), pp. 257, ISSN: 0325-7541.

García SD. (2011). Epidemiología y bases moleculares de la resistencia a los antimicrobianos en *Neisseria gonorrhoeae.* Doctoral Thesis. Faculty of Pharmacy and Biochemist, University of Buenos Aires. Buenos Aires, Argentina. 248 pp.

Govender S, Lebani T & Nell R. (2006). Antibiotic susceptibility patterns of *Neisseria gonorrhoeae* isolates in Porth Elizabeth. *S Afr Med J*, Vol. 96, Nº3; pp. 225-226, ISSN: 0256-9574.

Griemberg G, Pizzimenti MC, Famiglietti AMR, Belli L, Vay C, Garcia S, Cardinalli A, Costa M, Marcenac F& Casco R. (1997). El impacto del HIV sobre la incidencia de sífilis y gonorrea en un Hospital Universitario (1985 – 1994). *Medicina (B. Aires),* Vol. 57, Nº 11, pp. 1-6, ISSN: 0025-7680.

Hagman KF, Pan W, Spratt BG, Balthazar JT, Judd RC& Shafer WM. (1995). Resistance of *Neisseria gonorrhoeae* to antimicrobial hydrophobic agents is modulated by the *mtrRCDE* efflux system. *Microbiol*, Vol. 141, Nº 3, (November, 1994), pp. 611-622.

Hooper DC& Strahilelevitz. (2010). Quinolones, In: *Principles and Practice if Infectious Diseases*, Mandell G, Bennett JE & Dolin R (Eds.) 7th ed, Churchill Livingston, Elsevier Philadelphia PA, pp. 451-470, ISBN: 978-0-4430-6839-3.

Janda WM & Gaydos CA. (2007). *Neisseria,* In: *Manual of Clinical Microbiology*, 9th , Murray PR, Baron EJ, Jorgensen JH, Landry ML& Pfaller MA (Eds), pp. 601-620, Ed. Washington D. C., ASM Press, ISBN:-10:1-55581-371-2; ISBN: -13: 978-1-55581-371-0, Phiadelphia PA.

Leclercq R & Courvalin P. (1991). Bacterial resistance to macrolide, lincosamide and streptogramin antibiotics by target modification. *Antimicrob Agents Chemother*, Vol. 35, pp. 1267-1272, ISSN: 0066-4804.

Lucas CE, Hagman KE, Levin JC, Stein DC & Shafer WM. (1995). Importance of lipooligosaccharide structure in determining gonococcal resistance to hydrophobic antimicrobial agents resulting from the *mtr* efflux system. *Mol Microbiol*, Vol. 16, Nº 5, (June 1995), pp. 1001-1009, ISSN: 0305-7453.

Lucas CE, Balthazar JT, Hagman KE & Shafer WM. (1997). The MtrR repressor binds the DNA sequence between the *mtrR* and *mtrC* genes of *Neisseria gonorrhoeae*. *J Bacteriol*, Vol.179, Nº 13, (July, 1997), pp. 4123-4128, ISSN: 0021-9193.

Luna VA, Heiken M, Judge K, Ulep C, Van Kirk N, Luis H, Bernardo M, Leitao J & Roberts MC. (2002). Distribution of mef(A) in gram-positive bacteria from healthy Portuguese children. *Antimicrob Agents Chemother*, Vol. 46, Nº 8; pp. 2513-2517

Macheboeuf P, Fischer DS, Brown T, Zervosen A, Luxen A, Joris B, Dessen A & Schofield CJ. (2007). Structural and mechanistic basis of penicillin- binding protein inhibition by lactivicins. *Nature Chemical Biology*, Vol. 3, Nº 9, (September, 2007), pp. 565-569, ISSN: 1552-4450.

Martin IM, Hoffman S & Ison CA. (2006). European Surveillance of Sexually Transmitted Infections (ESSTI): The first combined antimicrobial susceptibility data for *Neisseria gonorrhoeae* in Western Europe. *J Antimicrob Chemother*, Vol. 58, Nº 3, (July,2006), pp. 587-593, ISSN: 0305-7453.

Marrazo JM, Handsfield H H& Sparling PF. (2010). *Neisseria gonorrhoeae*, In: *Principle and Practice of Infections Disease* 7 th ed, Mandell G, Bennett JE & Dolin R (Eds.) , Churchill Livingston, Elsevier, pp. 2242-2258, ISBN: 978-0-4430-6839-3, Philadelphia PA.

Morse SA, Lysko PG, Mc Farland L, Knapp JS, Sandstrom E, Critchlow C & Holmes KK. (1982).Gonococcal strains from homosexual men have outer membrane with reduced permeability to hydrophobic molecules. *Infect Inmun,* Vol. 37, Nº 2, (August, 1982) pp. 432-438.

Morse SA, Johnson SR, Biddle JW & Roberts MC. (1986). High –level tetracycline resistance in *Neisseria gonorrhoeae* is result of acquisition of streptococcal *tet M* determinant. *Antimicrob Agents Chemother*, Vol. 30, pp. 664-670.

Morris SR, Moore DF, Hannah PB, Wang SA, Wolfe J, Trees DL, Bolan G & Bauer HM. (2009). Strain Typing and Antimicrobial Resistance of Fluoroquinolone- Resistant *Neisseria gonorrhoeae* Causing a California Infection Outbreak. *J Clin Microbiol*, Vol. 47, Nº 9, (September, 2009), pp. 2944-2949.

Nicholas RA, Zhao S, Tomberg J, Unemo M & Davies C. (2008). Genetics of intermediate resistance to expanded-spectrum cephalosporins in *Neisseria gonorrhoeae*, Abstract P054. 16th International Pathogenic Neisseria Conference; Sep, Rotterdam, The Netherlands; pp.133. Available at: http://www.ipnc2008.org/

O'Rourker M, Ison CA, Renton AM & Spratt BG. (1995). Opa-typing: a high-resolution tool for studying the epidemiology of gonorrhoea. *Mol Microbiol* , Vol: 17, Nº 5, pp.865-875.

Otero L, Villar H, Vázquez JA & Vázquez F. (2002). *Neisseria gonorrhoeae* resistente a quinolonas: Un nuevo problema de salud pública en España. *Enferm Infecc Microbiol Clin*, Vol. 20, Nº 3. pp. 123-126, ISSN: 0213-005X.

Palmer HM, Young H, Winter A & Dave J. (2008). Emergence and spread of azithromycin-resistant *Neisseria gonorrhoeae* in Scotland. *J Antimicrob Chemother,* Vol. 62, Nº 3, (June,2008), pp. 490-494, ISSN: 0305-7453.

Rahman M, Alam A, Nessa K, Nahar S, Dutta DK, Yasmin L, Monira S, Sultan Z, Khan SA & Albert MJ. (2001). Treatment Failure with the Use of Ciprofloxacin for Gonorrhea Correlates with the Prevalence of Fluoroquinolone-Resistant *Neisseria gonorrhoeae* Strains in Bangladesh. *Clin Infect Dis*, Vol. 32, Nº 6, pp. 884-889, ISSN: 1058-4838.

Reyn A, Schmith H, Trier M & Bentzon MW. (1973). Spectinomycin hydrochloride Trobicin in the treatment of gonorrhea. Observation of resistant strains of *Neisseria gonorrhoeae*. *Br J Vener Dis,* Vol. 49, Nº1, pp. 54- 59.

Roberts MC, Chung WO, Roe D, Xia M, Marquez C, Borthagaray G, Whittington W & Holmes KK. (1999). Erythromycin-Resistant *Neisseria gonorrhoeae* and Oral Commensal *Neisseria* spp. Carry Know rRNA Methylase Genes. *Antimicrob Agents Chemother*, Vol. 43, Nº 6, (June, 1999) pp. 1367-1372, ISSN: 0162-0134.

Rouquette-Loughlin C, Dunham SA, Kuhn M, Balthazar JT & Shafer WM. (2003). The Nor M efflux pump of *Neisseria gonorrhoeae* and *Neisseria meningitidis* recognizes

antimicrobial cationic compounds. *J. Bacteriol*, Vol. 185, Nº 3, (February, 2003), pp. 1101-1106, ISSN: 0305-7453.

Rouquette-Loughlin CE, Balthazar JT & Shafer WM. (2005). Characterization of the MacA-MacB efflux system in *Neisseria gonorrhoeae*. *J Antimicrob Chemother*, Vol. 56, (September, 2005), pp. 856-860, ISSN: 0021-9193.

Shafer WM, Balthazar JT, Hagman KE & Morse SA. (1995). Missense mutations that alter the DNA- binding domain of the MtrR protein occur frecuently in rectal isolates of *Neisseria gonorrhoeae* that are resistant to faecal lipids. *Microbiol*, Vol. 41, pp. 907-911.

Shafer WM, Folster JP & Nicholas RA. (2010). Molecular Mechanisms of Antibiotic Resistance Expressed by the Pathogenic *Neisseriae, I*n: *Neisseria* Molecular Mechanisms of Pathogenesis, Genco CA, Wetzler L(Eds), pp. 245-268, Caister Academic Press, ISBN: 978-1-904455-51-6, Boston, MA. USA,

Starnino S, Stefanelli P & on behalf of the *Neisseria gonorrhoeae* Italian Study Group. (2009). Azithromycin- resistant *Neisseria gonorrhoeae* strains recentjy isolated in Italy. *J Antimicrob Chemother*, Vol. 63, Nº 6, (April, 2009), pp. 1200-04, ISSN: 0305-7453.

Shokeen P, Bala M, Singh M & Tandon V. (2008). In vitro activity of eugenol, an active component from Ocimum sanctum, against multiresistant and susceptible strains of *Neisseria gonorrhoeae*. *Int J Antimicrob Agents*, Vol. 32, (August, 2008), pp. 174-179, ISSN: 0924-8579.

Takahata S, Senju N, Osaki Y, Yoshida T & Ida T. (2006). Amino acid substitutions in mosaic penicillin-binding proteína 2 associated with reduced susceptibility to cefixime in clinical isolates of *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother*, Vol. 50, Nº 11, (November, 2006) pp. 3638-3645, ISSN: 0305-7453.

Tanaka M, Nakayama H, Huruya K, Konomi I, Irie S, Kanamaya A, Saika T & Kobayashi T. (2006). Analysis of mutations within multiple genes associated with resistance in a clinical isolate of *Neisseria gonorrhoeae* with reduced ceftriaxone susceptibility thatshows a multidrug-resistant phenotype. *Int J Antimicrob. Agents*, Vol. 27, pp. 20-26.

Tapsall JW, Schultz RR, Lovett R, Munro R. (1992). Failure of 500 mg ciprofloxacin therapy in male urethral gonorrhea. *Med J* Aust, Vol. 156, pp.143, ISSN: 1058-4838.

Tapsall JW (2009) *Neisseria gonorrhoeae* and emerging resistance to extended spectrum cephalosporins. Curr *Opin Infect Dis*, Vol. 22, Nº 1, (February, 2009) pp. 87-91.

Tazi L, Pérez-Losada M, Gu W, Yang Y, Xue L, Crandall KA & Viscidi RP. (2010). Population dynamics of *Neisseria gonorrhoeae* in Shanghai, China: a comparative study. *BMC Infect Dis* , Vol. 10, Nº 1, pp. 13, ISSN: 14712334.

Trotter C, Hughes G & Ison C. (2010). Epidemiology in the Vaccine Era. In: *Neisseria Molecular Mechanisms of Pathogenesis*, Attardo Genco C & Wetzler L, editors, pp. 227-243, Caister Academic Press Boston MA. USA, ISBN: 978-1-904455-51-6, Boston MA. USA.

Unemo M, Dillon JR. (2011). Review and International Recommendation of Methods for Typing *Neissseria gonorrhoeae* Isolates and Their Implications for Improved Knowledge of Gonococcal Epidemiology, treatment, and Biology. Clin Microbiol Rev, Vol 24, pp. 447-458

Update to CDC's sexually transmitted diseases treatment guidelines, 2006: Fluoroquinolones no longer recommend for treatment of gonococcal infections. (2007). *Mortal Weekly Rep*, Vol. 56, Nº 14, pp. 332-336, ISSN: 1545-861X.

US Public Health Service. (1975). Gonorrhea recommended treatment schedules. *Ann Intern Med*, Vol. 82, pp. 230-233.

Van Looveren M, Ison CA, Ieven M, Vandamme P, Martin IM, Vermeulen K, Renton A & Goosens H. (1999). Evaluation of the Discriminatory Power of Typing Methods for *Neisseria gonorrhoeae*. *J Clin Microbiol*, Vol. 37, Nº 7, pp.2183-2188.

Velicko I & Unemo M. (2009). Increase in reported gonorrhea cases in Sweden, 2001-2008. *Euro Surveill*, Vol. 14, Nº 34, pp24.

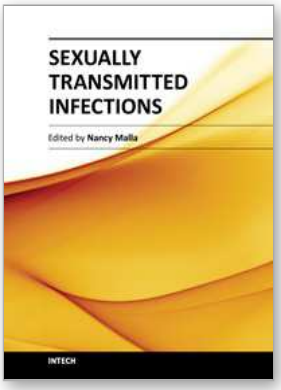
Warner DM, Shafer WM & Jerse AE. (2008). Clinically relevant mutations that cause derepression of the *Neisseria gonorrhoeae* MtrC MtrD-MtrE efflux pump system confer different levels of antimicrobial resistance and in vivo fitness. *Mol – Microbiol*, Vol. 70, Nº 2, (October, 2008), pp. 462-478, ISSN: 0305-7453.

World Health Organization (WHO). (2001). Global prevalence and incidence of selected curable sexually transmitted infections: overview and estimates. (WHO/HIV_AIDS/2001.02, WHO/CDC/CSR/EDC/2001.10), pp. 1-43.

World Health Organization (WHO) (2003) Guidelines for the management of sexually transmitted infections. Geneva, Switzerland. Available at: http://www.who.int/hiv/pub/sti/en/STIGuidelines2003.pdf.

Zarantonelli L, Borthagaray G, Lee EH & Shafer WM. (1999). Decreased azithromycin susceptibility of *Neisseria gonorhoeae* due to mtrR mutatins. *Antimicrob Agents Chemother,* Vol. 43, Nº 10, (October, 1999), pp. 2468-2472, ISSN: 0305-7453.

Zarantonelli L, Borthagaray G, Lee E H, Veal W & Shafer WM. (2001). Decreased susceptibility to azithromycin and erythromycin mediated by a novel *mtr*(R) promoter mutation in *Neisseria gonorrhoeae. J Antimicrob Chemother*,Vol. 47, pp. 651-654, ISSN: 0305-7453.



Sexually Transmitted Infections

Edited by Prof. Nancy Malla

ISBN 978-953-51-0258-8
Hard cover, 244 pages
Publisher InTech
Published online 21, March, 2012
Published in print edition March, 2012

Sexually transmitted infections (STIs) are infections that are spread primarily through person to person sexual contact. There are more than 30 different sexually transmissible bacteria, viruses and parasites. STIs lead to high morbidity and complications. This book entitled as Sexually Transmitted Infections is not a text book but provides useful information for general reference work for physicians, researchers and students interested in the subject. Each chapter is abundant in tips useful to general readers as well. It also includes the Introductory chapter providing an overview with special emphasis on syndromic approach to the management of STIs in clinical setting.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Ángela María Rosa Famiglietti and Susana Diana García (2012). Gonorrhea in Men Sex Men and Heterosexual Men, Sexually Transmitted Infections, Prof. Nancy Malla (Ed.), ISBN: 978-953-51-0258-8, InTech, Available from: http://www.intechopen.com/books/sexually-transmitted-infections/gonorrhea-in-men-who-have-sex-with-men-and-heterosexual-men

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821