Emergence, prevalence and mechanisms of fluoroquinolone resistance in *Neisseria gonorrhoeae*: A literature review

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Summary

Gonorrhea is a widespread sexually transmitted infection and a major community health concern. Medication against *Neisseria gonorrhoeae* has been circulating for decades, but the bacteria have progressively become resistant to nearly all drugs administered against them. *N. gonorrhoeae* developed resistance to sulfonamides, penicillin, tetracyclines and spectinomycin, in a chronological order, throughout the 20th century. Fluoroquinolones, notably ciprofloxacin, displayed exceptional potency against the bacteria. They debuted in the 1980s, and consumption peaked during the 1990s. However, this trend was hindered by the sporadic emergence of quinolone-resistant *N. gonorrhoeae* (QRNG) that thrived in the East Asia – Western Pacific region. The ever-increasing prevalence of quinolone resistance led to the drugs' gradual exclusion from gonorrhea treatment guidelines around the globe. Fluoroquinolones directly interfere with DNA synthesis by inhibiting DNA gyrase and topoisomerase IV. The GyrA and ParC genes, which encode the homonymous structural subunits of these enzymes, are the main targets of mutations that yield quinolone resistance. Moreover, changes in the permeability of the bacterial cytoplas-

mic membrane, owing to the increase of efflux pumps, prevent the drug from reaching its target. The thorough examination of these procedures may uncover the mechanisms of antimicrobial resistance and bolster the development of new therapeutic choices against gonococcus, whereas the epidemiological study of the prevalence of QRNG is vital for public health. Azithromycin and third-generation cephalosporins are the current recommended antimicrobials issued against the disease. Nevertheless, recent findings and warnings issued by the WHO have shown that *N. gonorrhoeae* might involving into a superbug.

Key words
gonorrhea, Neisseria gonorrhoeae, quinolones, resistance, QRNG

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Introduction

The ancient malady of gonorrhea has historically been one of the most prevalent sexually transmitted diseases. An often painful and distressing ailment, gonorrhea is still widespread around the globe. *Neisseria gonorrhoeae*, commonly referred to as gonococcus, is a Gram-negative bacteria responsible for the disease. *N. gonorrhoeae* is transmitted through vaginal, anal or oral sex, and may be passed on to newborns at birth, causing ophthalmia neonatorum. It chiefly pollutes the urinal tract as well as the rectal and pharyngeal mucosal membranes.

The biomedical breakthroughs of the 20th century, that manifested in the rise of antibiotics, revolutionized the treatment of gonorrhea. However, *N. gonorrhoeae* progressively developed resistance to sulfonamides, penicillin, tetracycline and spectinomycin. In 1989, fluoroquinolones were introduced as antibiotics of choice against the disease. Fluoroquinolones are broad-spectrum drugs that would rise to prominence as the most effective antibiotic agents against gonorrhea. Sadly, gonococcal fitness in quinolone-rich environments would develop rapidly. Quinolone-resistant strains of *N. gohorrhoeae* (QRNG) emerged in East Asia during the late 1980s, and resilience quickly spread around the globe over the course of the following decades. Nowadays, the alarming prevalence of quinolone – resistant gonorrhea has rendered the drugs obsolete. The dramatic spike in quinolone resistance has been considered a major turning point in the history of gonorrhea. Reported failures in the implementation of newer drugs may have signaled the beginning of an era of untreatable gonorrhea.

A review of the appearance, spread and biochemical apparatus of QRNG, through the assessment of available literature sources, is critical for understanding the process of bacterial resilience and offers insight into the possible evolution of *N. gonorrhoeae* into a superbug. Owing to the diversity of available sources and in order to provide a well-rounded view of treatment history and antimicrobial mechanisms, the references examined are primarily reviews.

The pre-quinolone era

Before the emergence of antimicrobials, gonorrhea was treated with a variety of traditional methods and natural ingredients. Patients were generally encouraged to follow a balanced lifestyle, while most therapeutic regimens consisted of balsams and organic mixtures. During the First World War, metallic compounds -primarily employig mercury, as well as hyperthermia, were introduced to combat the disease's symptoms. Beginning in the 1930s, sulfonamides were the first antimicrobial drugs to be administered

against gonorrhea. They proved exceptionally effective, treating up to 90% of documented cases of gonorrhea. Sadly, the bacteria had become fully resistant to sulfonamides by the late 1940s.[1,2]

The advent of penicillin, a powerful β-lactam antibiotic, presented scientists with a groundbreaking treatment against gonococcal urethritis. The renowned drug is a potent inhibitor of peptidoglycan synthesis. Widely viewed as a new revolutionary antibiotic, penicillin could treat up to 95% of cases when it was first launched against *N. gonorrhoeae*, with doses as low as 45 mg. Gradually, the minimum inhibitory concentration (MIC) of penicillin was increased, and the first resistant strains emerged by 1946. Nonetheless, penicillin retained its status as an antibiotic of choice against gonorrhea. The drug's usage was widespread and further intensified during the 1960s, owing to the spread of gonorrhea stimulated by the "Sexual revolution". The recommended dosage regimens were substantially increased because of this gonorrhea epidemic.[1,2] As a result, by 1976, β-lactamase-producing strains of *N. gonorrhoeae* had developed resistance mechanisms against the antibiotic through plasmid mediation (bla_{TEM-1} and $bla_{TEM-135}$ plasmids).[2] In 1985, the discovery of non-β-lactamase-producing penicillin-resistant *N. gonorrhoeae*, possessing chromosomal mutations (penA, penB, ponA, and mtr genes) led to a severe decrease in penicillin usage against *N. gonorrhoeae*. The antibiotic was irrevocably abandoned a few years later.[3]

Following the initial applications of penicillin, tetracyclines made their commercial debut. Antibiotics of this group are inhibitors of bacterial protein synthesis and would be administered to gonorrhea patients with penicillin allergies. The MIC for tetracyclines progressively increased until the 1980s, when systematic resistance emerged in *N. gonorrhoeae* strains possessing the plasmid-mediated tetM gene.[1] Furthermore, it was discovered that certain alterations in the RpsJ gene increased the number of efflux pumps (MtrCDE pump) while simultaneously diminishing porin influx (PorB), thus hampering the drug's absorption.[2]

At this point, it is important to mention the notable case of spectinomycin, an antibiotic functionally similar to tetracyclines. The drug was made commercially available in the 1960s, right after the early emergence of plasmid-mediated penicillin resistance in *N. gonorrhoeae*. Spectinomycin was popularized in Korea among the US army ranks starting in 1981, but resistance spread quickly, with treatment failures reaching an 8.2% rate over the course of the next four years.[1,2] Certain findings demonstrate that the basic molecular fluctuations that boost gonococcal resistance to spectinomycin are mutations in certain ribosomal proteins, as well as 16s rRNA polymorphisms.[2,4] The drug eventually lost its first-line status. Despite its withdrawal, current data demonstrates that the prevalence of spectinomycin resistance in *N. gonorrhoeae* is exceptionally low.[4] According to recent data, spectinomycin constitutes an effective alternative to the current recommended antibiotic regimen, with a 98.2% cure rate against urethral and rectal gonorrhea.[5] Nevertheless, administration of the drug is recommended solely as a measure of last resort against multidrug-resistant cases of gonorrhea.

The quinolone era

The pharmaceutical concept of quinolones can be traced back in 1962, when G. Lesher discovered nalidixic acid, a naphthyridine byproduct of chloroquine synthesis.[6] The following year, several in vitro experiments conducted by McChesney *et al*., as well as a clinical trial organized by Lishman *et al*., verified the newfound agent's potency against Gram-negative pathogenic bacteria of the urinary tract. The substance was orally administered throughout its early clinical trials and proved remarkably effective against numerous bacterial species. Ward-McQuadi *et al*. evaluated the outcomes of these preliminary studies and compared them with other authors' deductions.[7] It was concluded that the novel drug displayed considerable potency against urinary tract infections (UTIs). In 1967, broad clinical usage of nalidixic acid against most UTIs was officially authorized. At that time, its utilization was slightly impeded by its elevated MIC (4-16 mg/L). The subsequent introduction of nalidixic acid analogs spawned the first generation of quinolone antibiotics. A multitude of agents were launched in the 1970s, including pipemidic acid, cinoxacin and oxolinic acid, yet most were not competent enough against UTIs. It is now widely accepted that first-generation quinolone agents display average potency against Gram-negative bacteria.[8] Following their introduction, the first resistant bacterial strains promptly emerged.[9] The subsequent emergence of second-generation fluoroquinolones constituted a paramount development in quinolone history. Deriving from the addition of fluoride to native quinolone molecules, these substances were coined fluoroquinolones, and were rapidly popularized. Second-generation quinolone antibiotics proved exceptionally effective against a much broader spectrum of bacteria compared to their predecessors.[6] Eventually they would be succeeded by a third and fourth group of quinolone antibiotics. In context, Table 1 classifies some of the most common quinolone drugs by generation (Table 1).[8,10]

Table 1 Most common quinolones classified into generations

First generation	Second Generation	Third Generation	Fourth Generation
Nalidixic Acid	Ciprofloxacin	Levofloxacin	Trovafloxacin
Pipemidic Acid	Norfloxacin	Sparfloxacin	Alatrofloxacin
Oxolinic Acid	Ofloxacin	Gatifloxacin	
Cinoxacin	Enoxacin	Moxifloxacin	
	Lomefloxacin		

The year 1989 marks the beginning of the quinolone era of gonorrhea treatment. Penicillin officially ceased to be listed as a recommended treatment for gonorrhea,[11] while fluoroquinolones premiered against the disease, as stipulated by the CDC's guidelines.[12] They were introduced as antibiotics of choice in 1993.[13] Second-generation quinolones, such as ciprofloxacin, ofloxacin and norfloxacin, displayed great potency against a variety of both Gram-negative and -to a lesser extent- Gram-positive bacteria. The great anti-gonococcal effectiveness of these drugs propelled them into becoming the most commonly used antibiotics against gonorrhea.[14] Ciprofloxacin in particular was deemed an exceptionally powerful agent against *N. gonorrhoeae*, as can be inferred from several clinical trials of the late 1980s, that compared its effectiveness against *N. gonorrhoeae* and *C. trachomatis.* (Table 2).[15-20]

Through most clinical trials, it was concluded that gonorrhea could be treated by a single oral dose of 250 mg ciprofloxacin. Yet in 1993, the CDC's guidelines opted physicians to issue single-doses of 500mg of orally administered ciprofloxacin, owing to reported treatment failures of the 250mg regimen in the UK.[12]

The trend that evolved from the growing popularity of fluoroquinolones would inevitably lead to their empirical prescription and misuse. Consequently, systematic resistance first emerged in East Asia, and spread to the United States during the late 1990s - 2000s. Soon, the prevalence of bacterial resilience had grown significantly and resistance against fluoroquinolones reached alarming levels in many regions of the world. We have summarized findings from a choice of 1990s trials demonstrating the growing trend of low susceptibility against ciprofloxacin (Table 3).[21-25] It is important to note that numerous patient cases examined in these reports claimed to have contracted gonorrhea in East Asian countries, notably the Philippines and Thailand, thus supporting a migration hypothesis for the spread of QRNG.

Most of these studies were conducted prior to the standardization of Minimum Inhibitory Concentration thresholds, which would be established to distinguish between different types of QRNG. Subsequent papers classify the susceptibility of *N. gonorrhoeae* strains to ciprofloxacin and other quinolones in accordance with Knapp's criteria,[11] which indicate a ciprofloxacin concentration of 0.125mg/L -within a 500mg dose- as a threshold for low susceptibility.[10] However, resistance thresholds are susceptible to local guidelines or distinct causative factors, and thus may vary. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has established an MIC of >0.06 mg/L as the point of ciprofloxacin resistance, whereas the Clinical Laboratory Standards Institute (CLSI) recommends a >0.25 mg/L breakpoint for resistance.[13,26] Up to the 2010s, MICs of >0.125 mg/L also applied to Grepafloxacin (400mg) and Lomefloxacin (400mg). An MIC of >0.5 mg/L indicated decreased susceptibility to Ofloxacin (400mg), Enoxacin (400mg), Norfloxacin (80mg) and Fleroxacin (400mg).[10]

Distribution of QRNG around the world

Following the introduction of fluoroquinolones, resistance emerged in *S. aureus* and *P. aeruginosa* strains.[27] The first instance of high levels of fluoroquinolone resistance in *N. gonorrhoeae* was reported in the Netherlands,[13,28] followed by sporadic occurrences across the world. During the early 90s, numerous reports on QRNG emerged from the Philippines, Hong Kong and Japan, while sporadic reports originated from Australia, Canada, the UK and the US.[12]

The South-East Asia and Western Pacific regions were the main geographical areas where the great prevalence of fluoroquinolone resistance first presented a major health issue. In Japan, after sporadic occurrences of QRNG, a major upheaval in resistance was caused in the early 1990s, as clinically detected QRNG strains accounted for 40% of the total cases within the country. By the end of the decade this rate was doubled, reaching 80%, which led to the exclusion of fluoroquinolones from the Japanese anti-gonococcal treatment guidelines in 1999.[13] In Hong Kong, the rate of reported quinolone-resistant strains of *N. gonorrhoeae* increased more than twenty-fold

Table 2 Comparison of 6 preliminary trials on the effectiveness of ciprofloxacin against *Neisseria gonorrhoeae*

Researchers	No. of patients	Dosage regimen	Success Rate
Hart *et al*. (1984)	35 In vitro strains	0.01 mg/L	100%
Loo *et al.* (1985)	10, subsequently 54	500 mg, single oral dose, subsequently reduced to 250 mg	100%
Arya *et al.* (1986)	29 (22 with *N. gonorrhoeae* and 7 with *N. gonorrhoeae* and *C. trachomatis*)	500 mg, twice daily, for one week	100%
Roddy *et al.* (1986)	49	Single oral dose, 250 mg	100% (urethral)*
Shahmanesh *et al.* (1986)	44	Single oral dose, 250 mg for 18 subjects, 100 mg for 26	100%
Avonts *et al.* (1988 correspondence)	47	Single oral dose, 250 mg for 22 subjects, 100 mg for 25	100%

**Out of 49 patients with urethral infections, 49 were cured, while out of 5 patients with rectal infections, 4 were cured, resulting in a cumulative success rate of 98%.*

Table 3 Comparison of 8 clinical trials on the early emergence of *Neisseria gonorrhoeae* strains with low susceptibility to ciprofloxacin

Researchers	No. of patients	MIC threshold for low susceptibility	Low susceptibility Rate
Gransden *et al.* (UK, 1991) (Research conducted on 1989 data)	959	>0.008 mg/L	2.4 %
Ison *et al.* (UK, 1991)	896	>0.008 mg/L	6.25%
Tanaka *et al.* (Japan, 1995)	27 (1981 – 1984)	>0.125 mg/L	3.7%
CDC (USA, 1994)	450 (JaN. 1992 – June 1993, Ohio)	0.13-0.25 mg/L	5.6%
	124 (Nov. – Dec. 1993, Ohio)	0.13-0.25 mg/L	13.7%
	37 (Penicillinase – producing strains, May 1993 – Feb 1994, Hawaii)	2 mg/L	8%
Tanaka *et al.* (Japan, 1995)	79 (1992 – 1993)	>0.125 mg/L	46.8%
Knapp *et al.* (Philippines, 1995)	92	>0.125 mg/L	54.3% to nalidixic acid, of which 84% to ciprofloxacin

within two years, from 0.5% in 1992 to 10.4% in 1994, while in 1995 the strains with low susceptibility to ciprofloxacin and ofloxacin accounted for 36% of all cases. The same year, this rate amounted to 54% in the Philippines and 22% in Thailand.[12] In India, where usage of fluoroquinolones against gonorrhea commenced in 1990, resistance to norfloxacin emerged in 1996, whereas a steep increase in the prevalence of QRNG was observed over the course of the following decade, with rates rising from 22% in 1999 to 67.3% in 2001.[10,11] 2005 saw the abandonment of quinolones for gonorrhea therapy in the country.[11] In Australia, certain low susceptibility strains were first reported in Sidney, in 1984. However, by 1991 fully resistant QRNG with ciprofloxacin MICs of up to 16mg/L were emerging across the country. The prevalence of QRNG in Australia increased from 3.1% in 1995 to 20% in 2000.[10] Meanwhile, the available yearly reports issued by Australian Gonococcal Surveillance Programme indicate a ~16% rise in ciprofloxacin resistance, from 14% in 1997 to 30% during the 2nd quarter of 2016.[29,30] As reported by the World Health Organization (WHO), the national rates of QRNG in 2008, based on 8731 strains of *N. gonorrhoeae* across the Western Pacific and South East Asia Regions were: Australia 54.2%, Bhutan 95%, Brunei 73.7%, China 99.9%, Hong Kong 98.6%, India 100%, Japan 77.4%, South Korea 95.7%, Laos 11%, Malaysia 81.4%, Mongolia 75.8%, Myanmar 83.3%, New Caledonia 3.3%, Papua New Guinea 0%, Philippines 85.7%, Sri Lanka 76.5%, Singapore 80.6%, Thailand 97.1% and Vietnam 99.3%.[31] Nowadays, nearly all countries in the East Asian epicenter of QRNG report great resistance rates, with 9 countries documenting ciprofloxacin resistance rates of >90%.[32]

In North America, resistance emerged sporadically, but was also spread from East Asia to the United States through the sex industry. During the 1990s, fluoroquinolone usage in the United States increased by 40%.[33] In 1994, several patients in Hawaii were reportedly non-sensitive to fluoroquinolone therapy, with numerous strains of *N. gonorrhoeae* displaying resistance to high doses of ciprofloxacin (0.13 – 0.25 mg/L, whereas the MICs had been less than 0.06 mg/L).[23] In 1995 the CDC published a worrying report, conducted in 9 gonorrhea patients from Colorado and Washington. The results raised suspicions about the emergence of high-level quinolone resistance, as the MIC for ciprofloxacin and ofloxacin was documented at 8mg/L.[34] Despite the rapid spread of resilient gonorrhea, the overall prevalence of QRNG across the United States remained lower than 1% during the 1990s. By 2000 QRNG rates were on the rise, particularly in Hawaii and California. It should be noted that several patients examined in these States had contracted the disease while overseas.[2,13] During the 2001-2005 period QRNG prevalence was linearly increased, reaching 9.4% in 2005.[13] By 2002 fluoroquinolones had already been excluded from regional gonorrhea treatment guidelines in both California and Hawaii. Subsequently, the CDC removed fluoroquinolones from its gonorrhea treatment guidelines for homosexual males,[13] and eventually in 2007 the drugs were eliminated from the CDC's general gonorrhea treatment recommendations.[2]

The spread of QRNG in Europe followed a pattern similar to the one observed in North America. As in most developed countries, QRNG first emerged sporadically and then advanced into the continent from overseas. Regional resilience might also have spawned spontaneously. Around 1999, reduced susceptibility of *N. gonorrhoeae* strains had been reported in the UK, Finland, France, Spain, The Netherlands and Greece. Research demonstrated that various cases of gonorrhea were contracted outside the continent, with most strains originating from Southeast Asia.[28] In Greece, a series of comprehensive reports demonstrated that between 1990 and 1993, the prevalence of QRNG was observed in merely 3.4% of all examined strains, whereas the follow-up report of 1994 – 2004 revealed that resistant strains accounted for 16.5% of all cases in 2004.[35,36] Consumption peaked during the 2007-2008 period, as the country became the top user of fluoroquinolones within Europe. In a 2010 study, it was discovered that fluoroquinolone resistance was almost doubled from 32.7% in 2005 to 62.9% in 2008, with the vast majority of strains being classified as highly resistant.[37,38] In Spain, first traces of QRNG were detected in 2000 and 2002.[10] In a relevant study, the prevalence of ciprofloxacin – resistant *N. gonorrhoeae* rose from 8.8% in the 2003-2005 period to 23.6% during the 2006-2007 period.[39] By 2004, some reported national rates of QNRG, accounting for both reduced susceptibility and high resistance to ciprofloxacin were thus: Spain 8.7%, The Netherlands 2,23%, France 0.7%, Germany (Berlin) 6%, The UK/London 7.2% (solely high-resistance strains), Denmark 6.8%, Sweden 17.8% and Greece 8.1%.[10] In 2013, selected ciprofloxacin resistance rates were: Spain 65.6%, The Netherlands 34.5%, France 44.6%, Germany 63.4%, The UK 32.1%, Denmark 58.2%, Sweden 60%, Greece 72%, Italy 63%, Austria 71.6% and Belgium 56.4%.[26]

In Africa and South America, resistance to fluoroquinolones was almost non-prevalent during the 1990s. The reduced availability of fluoroquinolones in the African continent prevented the exponential spread of QRNG. Owing to the rarity of trials and reviews from the continent, few data could be extracted during the 1990s, and resistance was perceived as low

to non-existent.[14] Recently, a systematic review of studies published during 2013-2016 in Africa revealed a ciprofloxacin resistance rate of at least 37.5% among 658 reported isolates of *N. gonorrhoeae* across the continent.[40] In South America, few resistant strains emerged in Brazil, the Caribbean and Uruguay during the 90s.[14] Nowadays, the prevalence of ciprofloxacin resistance is elevated in most South American countries. The total rate for ciprofloxacin resistance in South America and the Caribbean was increased from 1.6% in 1997 to 42.1% in 2011.[41] Meanwhile, reports on QRNG remain scarce in the Middle East. In Israel, however, resistance was first documented in the early 1990s, whereas a 2000 study demonstrated a 64% rate of ciprofloxacin resistance in Tel Aviv.[10]

Mechanisms of fluoroquinolone resistance

In order to obtain a solid grasp on the mechanisms of quinolone resistance, one must examine the ways Gram-negative bacteria, including *N. gonorrhoeae*, utilize special enzymes to process DNA. Bacteria employ type II topoisomerases, enzymes responsible for maintaining the integrity of the bacterial DNA chromosome. These enzymes are also charged with managing the torsional tension of the DNA molecule and promoting crucial biochemical operations. Though various cellular functions, the DNA helix is susceptible to supercoiling. For example, as DNA helicases move forward during the expansion of replications forks, positive super-helical twists in the DNA begin to accumulate ahead of them. Once the process is completed, the two daughter DNA molecules are interlinked. Type II Topoisomerases are responsible for reducing positive DNA supercoiling and separating the two distinct DNA chromosomes. Positive supercoiling may create inhibitory effects, but negative supercoiling can substantially accelerate the vital processes of DNA replication and transcription, as well as recombination in certain bacterial strains, owing to the fact that it promotes the rapid unwinding of the double helix. Thus the proper functioning of the protein complexes in charge of these operations is pivotal for the proliferation of *N. gonorrhoeae*. The enzyme responsible for the creation of subtractive twisting that results in negative supercoiling is DNA Gyrase, a type II bacterial topoisomerase composed of two GyrA and two GyrB subunits. DNA gyrase binds to DNA and performs supercoiling reactions, boosted by the hydrolyzation of ATP. It also removes unwanted chromosomal knots, assists in the folding of the DNA helix and prevents positive supercoiling. Topoisomerase IV is another type II topoisomerase that separates conjoined DNA molecules, balances the density of superhelices and relaxes positive supercoils. Compared to its counterpart, it is more potent in unlinking DNA strands during replication but cannot induce negative supercoiling in the molecule like gyrase. The molecule of topoisomerase II is a heterotetramer comprised of two ParC and two ParE subunits.[1,6,27,33,38,42,43]

Both enzymes function by slicing the DNA in points that are 4bp apart, thus forming units with free 5′ overhanging ends. The 5′ termini are then bonded with the enzymes' active site, which contains tyrosine.[6] This pair of complementary single-stranded breaks engulf the active site, thus creating a gap though which another DNA chain may move to relieve DNA supercoiling or separate itself from its daughter molecule.[43] The subunits GyrA and GyrB are encoded by homonymous genes in the chromosome of *N. gonorrhoeae*, as are ParC and ParE. The homologous subunits GyrA and ParC consist of the enzymes' active sites, whereas GyrB and ParE are comprised of an ATPase domain, as well as a topoisomerase-primase domain that binds with catalytic metal ion molecules.[6]

The bactericidal function of fluoroquinolones relies on the systematic inhibition of DNA synthesis through the deactivation of the above mechanisms. The drug molecules achieve this by binding onto the enzyme-DNA complexes and suppressing enzyme activity, prompting the accumulation of supercoils and resulting in catastrophic fracturing of the bacterial DNA in organisms such as *N. gonorrhoeae*. For the inhibition of each enzyme-DNA complex, two fluoroquinolone molecules are needed, although the inhibition of Topoisomerase IV requires a greater quinolone concentration than that of DNA gyrase.[1] Once a vital biological process is interrupted due to an inhibited enzyme, the bacterial DNA is rendered fragile and may break, thus triggering volatile DNA repair pathways that may prove fatal to the cell.[6] Quinolones stabilize the cleaved enzyme-DNA complex, block relegation and break the DNA helix. The drug molecules bind on specific enzyme target sites, called quinolone resistance-determining regions (QRDR), through a metal-water hydrogen bond (ion bridge). It has been demonstrated that this hydrogen bond is primarily forged between the enzyme's serine, and the drug molecule's keto acid, which anchors an active metal ion. Apart from serine, glutamic acid or asparagine have been shown to participate as well.[6]

Fluoroquinolone resistance in *N. gonorrhoeae* primarily derives from certain mutations of type II topoisomerase genes in the bacterial chromosomal DNA. Scientists had traced the resistant gyrA genes since the early days of nalidixic acid.[43] The discovery of topoisomerase in the 1990s further encouraged research

and expanded the available literature on QRNG. During this decade, the scientific community managed to uncover the precise modifications in the structural subunits of the aforementioned enzymes, which rendered the bacteria less susceptible to the new drugs. Resistant strains typically possess mutated GyrA and ParC genes.[38] Within the GyrA gene, tyrosine or phenylalanine were found to replace serine at codon 91, whereas aspartic acid was substituted by glycine, tyrosine or asparagine at codon 95. On the other hand, valine and lysine replaced glutamic acid at codon 91 of the ParC gene, while aspartic acid was substituted by asparagine at codon 86 and serine by asparagine at codon 87.[44,45] More mutations were uncovered through years of scientific research and genotypic analysis. It can be inferred that the most common mutated enzyme genotypes in QRNG are the $GyrA_{Ser91Phe\text{-}Asp95Gly}$ (codon 91: Serine to phenylalanine, codon 95: aspartic acid to glycine), and the $ParC_{Ser87Arg}$ (codon 87: serine to arginine).[46-48] As these codons produce specific amino acids capable of formulating the bonds between quinolone molecules and QRDR sections, their altered counterparts in mutant alleles are impotent and thus present a fluctuating rate of immunity towards quinolones. Strains with affected GyrA subunits are ten times more resistant to fluoroquinolones than non-mutated strains.[6,38] The reason is that GyrA is considered the primary target enzyme for mutations, while ParC is viewed as a secondary focus. The MICs for mutated strains are augmented according to the multitude and severity of genome alterations.[27] Strains with apparent mutations in both Gyra and ParC display a 10-to-100-fold increase in resilience.[6,38] The fatal cellular destruction sequences that are triggered by DNA damage and repair failures, are frequently averted through the SOS response.[6]

N. gonorrhoeae has also displayed high rates of fitness in quinolone-rich environments owing to the development of plasmid-mediated resistance (PMQR, Plasmid - Mediated Quinolone Resistance). A considerable portion of bacterial plasmids carry quinolone resistance factors and genes belonging to the broad qnr family,[33] of which more than 100 have been identified. Qnr genes encode pentapeptides that safeguard the integrity of the bacterial genome, by diminishing the number of active DNA-topoisomerase complexes through the inhibition of enzymic ATP-induced processes. Thus, quinolone molecules have less sites to bind onto, meaning less potential to repress enzyme function and to induce DNA fracturing. Although primarily encountered in plasmids, the qnr genes may also be traced within the chromosomes of numerous gram-negative and gram-positive bacteria.[6,49] An additional protein accredited with providing quinolone resistance is AAC(6′)-Ib-cr, a plasmid-mediated variant of aminoglycoside acetyltransferase. Its gene may be located within integrons, adjacent to other PQMR genes.[49] The molecular product is a mutated aminoglycoside acetyltransferase, with most common amino acid variations being Trp102Arg and Asp179Tyr). This molecule is capable of performing an acetalization reaction on fluoroquinolones containing a free nitrogen within their skeleton, such as ciprofloxacin and norfloxacin, thus reducing their antimicrobial potency.[6,49] Other plasmid-mediated proteins responsible for quinolone resistance are QepA1 and QepA2 (traced in human infections) and OqxAB (traced in animal infections).[6] These genes code surface efflux pumps that force quinolones out of the cell, thus reducing their intracellular concentration and lowering their overall bactericidal effectiveness.[49] Likewise, quinolone accumulation can be lowered by chromosome-encoded efflux pumps.[6] The generic bacterial MDR efflux pumps (multidrug resistance pumps) are a common resistance mechanism employed by numerous species of both gram-negative and gram-positive microorganisms. The structural features of most fluoroquinolones grant them passage through the outer and cytoplasmic membranes of the bacteria via porins. In contrast, MDR pumps function by propelling fluoroquinolone molecules outside the cell. Even though their genome may be transferred on a cell-to-cell basis via plasmids, their degree of expression is often susceptible to chromosomal mutations.[27] The most important MDR efflux pumps in *N. gonorrhoeae* are namely MtrD, FarB, MacB and NorM.[50]

The Post-quinolone era

Apart from second generation fluoroquinolones, newer drugs such as levofloxacin and trovafloxacin started being introduced. Third generation quinolones acted against an extensive array of microorganisms but proved lackluster in certain cases, when compared to their second-generation counterparts. Their reduced availability, coupled with perilous cardiovascular and phototoxicity side effects, renders them scarce. The latest fourth generation fluoroquinolones are also demonstrating effectiveness against gram-negative, gram-positive and anaerobic microorganisms, but may cause hepatotoxicity.[8,51] Sitafloxacin, a novel fourth-generation fluoroquinolone has demonstrated significant in vitro activity against ciprofloxacin-resisting *N. gonorrhoeae*, with MICs of 0.03 – 0.6 mg/L. However, the documented increase in the drug's MIC, in view of GyrA and ParC mutations, renders its administration solely appropriate under a dual therapy regimen.[52]

The quinolone era of gonorrhea treatment officially ended in 2007, when the CDC ceased listing quinolones as recommended drugs against the disease.[2,5,53] In regions with high QRNG prevalence, some countries had already removed fluoroquinolones from local recommended anti-gonococcal regimens. Following the abandonment of fluoroquinolones, the scientific community turned to macrolides and cephalosporins. However, resistance to those agents has already begun to manifest in *N. gonorrhoeae*.[54]

Macrolides were introduced in 1950 with the discovery of erythromycin. 1980 saw the emergence of azithromycin, a notable macrolide antibiotic with great potency against *N. gonorrhoeae*. Macrolides inhibit protein synthesis by interfering with the 50S ribosomal subunits of the target bacteria. Their development was well underway during the 1980s, and they were rapidly gaining popularity prior to and alongside quinolones. The first instances of resistance were reported in Latin America during the late 1990s, and since then have been traced all around the world.[1,2] By 2014, gonococcal resilience against azithromycin had been documented in 45 countries and centered around the European and Western Pacific regions.[32]

The latest commercially available agents against gonorrhea are third generation cephalosporins. Cephalosporin antibiotics debuted in 1945,[55] but were made widely available in 1964 with the introduction of cefalotin.[1] Extended spectrum cephalosporins, primarily cefixime and ceftriaxone, were employed against *N. gonorrhoeae* after the abandonment of fluoroquinolones. Cephalosporins are β-lactam antibiotics that function similarly to penicillin, inhibiting bacterial cell wall synthesis by halting its peptidoglycan enrichment. Unfortunately, strains of *N. gonorrhoeae* with mutations in penicillin-binding protein genes exhibited resistance to the drugs. The affected genetic code roughly comprises the same genes that are altered in penicillin-resistant strains (PenA, PonA, PenB, mtR). Resilience against cefixime emerged in East Asia, especially Japan, and was later observed across Europe, North America and South Africa.[2,56] Resistance to ceftriaxone has also been documented sporadically, thus sparking the fear of untreatable gonorrhea.[57] In 2014, instances of low susceptibility or resistance to extended-spectrum cephalosporins had been recorded in 23 of the 45 reporting WHO countries.[32]

Conclusions, measures and implications

Through a retrospective examination of available literature, it can be deduced that antimicrobial resistance (AMR) in *N. gonorrhoeae* follows a recurring pattern; it spontaneously emerges, then rapidly spreads and may gradually dwindle. Scientists frequently observe new bacterial strains with low susceptibility to obsolete antibiotics, such as spectinomycin. However, this tendency is poor and the strategy of reusing older agents proves inadequate. The case of fluoroquinolones constitutes a model for antibiotic resistance. They debuted successfully and were rapidly popularized. Resilience developed in East Asia and might have been spread through the sex industry to other parts of the world. During the mid-2000s, resistance grew to alarming rates around the world. Despite their novelty, originality and effectiveness, they were withdrawn within a 30-year period because of soaring levels of bacterial resilience. Until now, cases of reduced QRNG rates have scarcely been reported. Scientists must therefore remain vigilant and aspire to decipher the critical AMR processes within the intricate genome of gonococcus.

The current recommended treatment regimens for gonorrhea do not include fluoroquinolones, but rely on cephalosporines and azithromycin instead. In order to impede the ever-increasing risk of widespread cephalosporin resistance, a dual therapy regimen has been recommended, which relies on the combination of two antibiotic types to treat gonorrhea.[56] As of 2015, the CDC recommends combining 250 mg ceftriaxone via a single-dose intramuscular (IM) injection with a single oral dose of 1g of azithromycin. This applies to the treatment of all types of gonococcal infections, including urethral, rectal, cervical and pharyngeal. Alternatively, a single oral dose of 1g azithromycin and 400 mg cefixime is advised. Though not available in the US, spectinomycin is also recommended to patients with cephalosporin allergies.[5] The WHO recommendations correspond to the CDC's, though two more alternatives are included: 400mg single oral dose of cefixime coupled with 1g single oral dose of azithromycin. The WHO also advocates single therapy to counter antimicrobial susceptibility, specifically instructing ceftriaxone (single dose, 250 mg IM), cefixime (single dose, 400mg orally) and spectinomycin (single dose, 2g IM).[58]

Fluoroquinolones were often administered empirically and arbitrarily. In many countries, physicians would prescribe the drugs without considering patient examination data or adhering to treatment protocols. Moreover, the standardization of a high-yield single-dose regimen to combat gonorrhea rendered the bacteria exceptionally resilient over time.[54] Physicians and hospitals need to be strict when prescribing antibiotics, and are obliged to adhere to international regulations. Preemptive action must also be taken to prevent the spread of gonorrhea and, by extension,

the increase of resistance. Once an infected patient is reported, his most recent sexual partners also need to be registered and examined. Dual treatment with the recommended regimen and sexual abstinence are encouraged as preventative measures. Furthermore, health education of both sexual partners is vital.[5,59] It is worth mentioning that the prevalence of gonorrhea –and by extension QRNG– may be much greater than thought, as precise data on asymptomatic patients cannot be calculated.[59]

The introduction of novel drugs to combat gonorrhea is a never-ending struggle to which the scientific community is devoted. New promising agents are being researched, such as AZD0914, a spiropyrimidinetrione that obstructs DNA Gyrase and Topoisomerase. The propitious novel agent appears effective against standard *N. gonorrhoeae* strains with low susceptibility or resistance against ciprofloxacin, penicillin, tetracycline and ceftriaxone.[60] Ideally, a vaccine for gonorrhea would resolve the global issue of gonorrhea once and for all. Until recently, corresponding research had yielded few positive results. Nevertheless, recent findings shed light on the pathophysiology of gonococcal surface antigens and receptors, and investigate the immunological memory of T-helper-1 cells in mice. Such advancements could signal the development of a revolutionary vaccine that would exploit bacterial mechanisms and structural features, as well as immunization models, and might hopefully present a cure for gonorrhea.[56,61] Even though such novelties are still a long way before commercialization, there are substances whose research is nearing completion, such as solithromycin (phase III), zoliflodacin (phase II) and gepotidacin (phase II). The development of new antibiotics against gonococcus is bolstered by a joint effort between the WHO and the Drugs for Neglected Diseases initiative, culminating in the formation of the Glocal Antibiotic Research and Development Partnership. The discovery and delivery of efficacious anti-gonococcal drugs are current priorities of this institution.[59] Other candidate antimicrobials that exhibit therapeutic properties against gonorrhea are gentamycin, an aminoglycoside that has already been used against the bacteria, solithromycin, a fluoroketolide inhibitor of protein synthesis, as well as ertapenem and tigecycline. However, the effectiveness and reliability of these agents pale in comparison to those of fluoroquinolones or ESCs.[56]

The close inspection of resistance mechanisms and mutations may hold the key to preventing quinolone resistance. Bacteria with low susceptibility towards quinolones are incubated under specific conditions, to determine the MIC below which no mutations occur. This value, coined "Mutant Protective Concentration" (MPC), may pinpoint both quantitative and qualitative elements of fluoroquinolone resistance, such as specific mutation factors and the degree of bacterial fitness they cause.[33]

Furthermore, the regulation of certain DNA pathways, such as the SOS response, might potentiate the efficacy of existing antibiotics, especially quinolones. A 2017 study on mutated strains of *E. coli* revealed that the inhibition of the SOS response might reduce quinolone MICs and render bacteria more vulnerable to obsolete antibiotics, such as fluoroquinolones. The successful suppression of the SOS response yielded an 8-fold reduction in the MIC of ciprofloxacin, whereas the MIC of other fluoroquinolones was reduced up to 15 times.[62]

A measure of pivotal significance for understanding AMR in gonorrhea is the global monitoring of the disease, currently conducted through several surveillance programs run by the world's largest health establishments. The CDC instituted the Gonococcal Isolate Surveillance program (GISP) in 1986 to survey AMR mechanisms in *N. gonorrhoeae* within the USA. The WHO has initiated several surveillance projects which fall under the Gonococcal Antimicrobial Surveillance Program (GASP). The program was launched in the 1990s but the WHO revamped and strengthened it in 2009. Since then, it has been coined "The Enhanced GASP" or EGASP. Researchers and coordinators reporting to the EGASP follow strict protocols and regulations to scrutinize the prevalence of AMR in *N. gonorrhoeae*. However, conventional observation and registration methods are lacking in efficacy, whereas AMR surveillance in certain regions of the world remains insufficient or unregulated. Therefore, it is imperative that new monitoring systems are established to comprehensively inspect the progress of gonococcal AMR in a judicious manner. The EGASP is poised to conduct assiduous surveillance of gonococcal AMR in WHO member states by collecting laboratory results, analyzing government-issued data and handling statistics reports from private or regional healthcare providers. Moreover, several localized initiatives have been founded since the emergence of AMR in *N. gonorrhoeae*, such as the Gonococcal Resistance to Antimicrobial Surveillance Program (GRASP) in the UK, the Australian Gonococcal Surveillance Program (AGSP) and the European Gonococcal Antimicrobial Surveillance Program (Euro-GASP).[2,32,53]

As more cephalosporin-resistant *N. gonorrhoeae* strains are being isolated around the world, the fear of untreatable gonorrhea is constantly looming. In 2016 the United Nations supported the WHO's objectives against sexually transmitted infections, which aim to reduce the incidence of gonorrhea by 90% until

2021. However, on July 2017, the WHO issued a worrying news release on the emergence of untreatable gonorrhea. Resistance to ciprofloxacin was documented in 97% of WHO reporting countries, whereas azithromycin and ESC resilience were reported in 81% and 66% of countries respectively. Ominously, strains of multidrug-resistant and incurable *N. gonorrheae* have been documented.[59] Notwithstanding the planned eradication of gonorrhea, we might be running out of options to treat the disease. *N. gonorrhoeae* may be evolving into an untreatable superbug. Under those perilous circumstances, it is of paramount importance that the scientific community acts in unison to attentively monitor the menacing disease, institute novel drugs and develop a competent gonococcal vaccine.

Conflict of interests

The authors declare no conflict of interest regarding the publication of this article.

Περίληψη

Εμφάνιση, επιπολασμός και μηχανισμοί αντοχής στις φλουοροκινολόνες της *Neisseria gonorrhoeae*: ανασκόπηση της βιβλιογραφίας

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Η γονόρροια είναι ένα ευρέως διαδεδομένο σεξουαλικά μεταδιδόμενο νόσημα που αποτελεί μεγάλο κίνδυνο για τη δημόσια υγεία λόγω κυρίως της αντοχής του υπεύθυνου παθογόνου, *Neisseria gonorrhoeae*, στο σύνολο των χημειοθεραπευτικών που έχουν χρησιμοποιηθεί μέχρι σήμερα ως θεραπεία. Σταδιακά η *N. gonorrhoeae* κατά τη διάρκεια του 20ού αιώνα ανέπτυξε αντοχή, με χρονολογική σειρά, σε σουλφοναμίδες, πενικιλίνη, τετρακυκλίνες και σπεκτινομυκίνη. Οι φθοριοκινολόνες, ιδιαίτερα η σιπροφλοξασίνη, αποδείχθηκαν εξαιρετικά αποτελεσματικές έναντι του παθογόνου με αποτέλεσμα να αποτελέσουν θεραπεία εκλογής για τη γονόρροια. Η χρήση τους ξεκίνησε τη δεκαετία του 1980 και κορυφώθηκε τη δεκαετία του 1990. Ωστόσο, η εμφάνιση στελεχών *N. gonorrhoeae* με αντοχή στις κινολόνες (quinolone-resistant *N. gonorrhoeae,* QRNG), αρχικά σε περιοχές της Ανατολικής Ασίας και Δυτικού Ειρηνικού και στη συνέχεια σε όλο τον κόσμο, είχε σαν αποτέλεσμα την μείωση της χρησιμοποίησής τους ως θεραπεία εκλογής για τη γονόρροια και την σταδιακή κατάργησή της από τα προτεινόμενα εμπειρικά θεραπευτικά σχήματα. Οι φθοριοκινολόνες επιδρούν άμεσα στην σύνθεση του DNA με την παρεμπόδιση της DNA γυράσης και της τοποισομεράσης IV. Τα γονίδια GyrA και ParC, που κωδικοποιούν τις δομικά ομόλογες υπομονάδες των παραπάνω ενζύμων, είναι οι κύριοι στόχοι πολλών μεταλλάξεων που επιφέρουν αντοχή στις κινολόνες. Επιπλέον, αλλαγές στη διαπερατότητα της βακτηριακής κυτταροπλασματικής μεμβράνης, λόγω της αύξησης του αριθμού των αντλιών εκροής, εμποδίζει το φάρμακο από το να φτάσει στον στόχο του. Η αναλυτική μελέτη των μηχανισμών αυτών μπο-

ρεί να βοηθήσει στην ανάπτυξη νέων αντιμικροβιακών ουσιών. Σήμερα, η αζιθρομυκίνη και οι κεφαλοσπορίνες είναι τα αντιβιοτικά εκλογής για τη θεραπεία της γονόρροιας. Βέβαια, η επιδημιολογική έρευνα της αντοχής της *N. gonorrhoeae* είναι μεγάλης σημασίας για τη δημόσια υγεία. Δυστυχώς πρόσφατα ευρήματα και προειδοποιήσεις του Παγκοσμίου Οργανισμού Υγείας συνηγορούν στο ότι η *N. gonorrhoeae* ενδέχεται να εξελίσσεται σε παν-ανθεκτικό μικροοργανισμό.

Λέξεις κλειδιά
γονόρροια, Neisseria gonorrhoeae, κινολόνες, αντοχή, QRNG

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