

# Neisseria Gonorrhoeae infection, current state of antimicrobial susceptibility. Literature review

## Abstract

A review of the most recent and updated literature on the current global situation of *Neisseria Gonorrhoeae* infections is presented. According to the Center for Disease Control and Prevention (CDC), *Neisseria Gonorrhoeae* infection is one of the most important threats of antibiotic resistance in the United States and the world. In 1970 the first reports of resistance by isolating penicillinase-producing strains were identified and more recently, ceftriaxone-resistant *N. gonorrhoeae* strains have appeared. In Mexico, so far, there are no reports of ceftriaxone-resistant *Neisseria gonorrhoeae*; however, the first report dates from 1987 where De la Cruz and collaborators reported that up to 26.3% of gonorrhea isolates from 148 patients were penicillinase-producing strains. The emergence of *N. Gonorrhoeae* strains resistant to relatively modern and very useful antibiotics in daily medical practice raises awareness of the clinical and microbiological landscape in which we find ourselves, that is why alternative therapies such as vaccines and new drugs with antibiotic potential that address emerging resistance need to be considered.

**Keywords:** gonorrhea, resistance, antibiotics, susceptibility, ceftriaxone

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Sánchez Tinajero Ángel,<sup>1</sup> González Cueto Elizabeth,<sup>2</sup> Ruiz Santillán Danna Patricia,<sup>2</sup> Becerril Vargas Eduardo,<sup>3</sup> Martínez Orozco José Arturo,<sup>1</sup> Delgado Cueva Andrea Iraís,<sup>1</sup> Romero Aburto Tzirari Yaneli,<sup>4</sup> Alvarado Peña Nestor<sup>1</sup>

<sup>1</sup>Infectious Diseases Department, National Institute of Respiratory Diseases (INER), México

<sup>2</sup>National School of Biological Sciences (ENCB), México

<sup>3</sup>Clinical Microbiology Laboratory, National Institute of Respiratory Diseases (INER), México

<sup>4</sup>Pulmonology Department, National Institute of Respiratory Diseases (INER), México

**Correspondence:** Sánchez Tinajero Ángel, Medical Surgeon, Postgraduate in Chemical-Biological Sciences, Assigned to the infectology service for immunocompromised patients. National Institute of Respiratory Diseases (INER). Calzada de Tlalpan no. 4502, Delegación Tlalpan, Colonia Sección XVI, Mexico City. C.P. 14080, Mexico, Email [angelsan111@hotmail.com](mailto:angelsan111@hotmail.com)

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## Introduction and epidemiology

A review of the most recent and updated literature on the current global situation of *Neisseria Gonorrhoeae* infections is presented. Gonorrhea is a sexual transmission disease (STD),<sup>1</sup> caused by the so called superbug<sup>2,3</sup> *Neisseria gonorrhoeae*, which is an intracellular Gram-negative diplococcus that primarily and exclusively infects human mucosa and may secondarily, spread to other tissue types.<sup>4,5</sup> The disease is associated with high morbidity and socioeconomic consequences<sup>2,4,6</sup> and remaining to be a public health problem worldwide.<sup>3,7-11</sup> The groups most at risk of acquiring this infection are adolescents, older adults and men who have sex with men (MSM), among others. MSM have been a very important core group since the 1980s, first with the spread of TetM-expressing *N gonorrhoeae* and, second, with the dissemination of fluoroquinolone-resistant *N gonorrhoeae*.<sup>12</sup> According to Lewis DA (2013), MSM-associated sexual networks have been critical to the spread of gonococci resistant to third generation extended spectrum cephalosporins. As well, military personnel, travellers, drugs users, young adults, older man and members of street gangs have also been linked to the transmission of antimicrobial-resistant gonorrhea.<sup>12,5</sup> There was also found an association between resistant infections and heterosexual men.<sup>13</sup> In 2016, 376 million new curable sexually transmitted diseases were reported, with gonorrhea being a major one, just below *C. Trachomatis*, with an estimated 87 million new cases in 2016 in people aged 15-49<sup>14,15</sup> The estimated incidence of gonorrhea in women is 20/1000 and in men 26/1000.<sup>16</sup> Globally, there are estimated 78 million cases of

gonorrhea infection occurring per year.<sup>17</sup> Global prevalence increased from 0.8 to 0.9% in women and from 0.6 to 0.7% in men.<sup>18</sup>

The United States Centers for Disease Control and Prevention (CDC) reported about 400,000 NG cases in 2015, but estimates nearly 820,000 total infections annually due to the underreporting of asymptomatic undetected cases.<sup>19</sup> The estimated risk of transmission is higher when having unprotected sex, and the estimated probability of transmission varies based on the type of sexual practice, being about 50% per penis-to-vaginal sex act, 20% vaginal-to-penis each act, 63% urethral-to-pharynx, 9% pharyngeal-to-urethral, 84% urethral-to-rectum and only 2% rectal-to-urethral.<sup>16</sup> Risky sexual practices (sex without a condom) during international travel can encourage the spread of resistant gonorrhea strains around the world.<sup>16,13</sup> Gonorrhea has an incredible ability to adapt to the host and evade its immune system, which can lead to limited immunity, therefore individuals can become repeatedly infected. As well as states of antimicrobial resistance.<sup>10,20</sup> Signs and symptoms can range from asymptomatic infection (more common in women), rectal infections, pharyngeal infections, cervicitis,<sup>16</sup> transurethral discharge, and dysuria to disseminated forms when untreated, such as pelvic inflammatory disease in women, epididymitis in males and infertility<sup>7,5</sup> gonococcal arthritis, and even meningitis.<sup>21</sup> Chorioamnionitis, premature rupture of membranes, premature birth or first trimester abortions<sup>22</sup> and congenital blindness in offspring<sup>23</sup> also may occur. This infection also enhances the transmission of HIV.<sup>2</sup>

The diagnosis of this pathology is mainly clinical; however, IDSA and FDA guidelines recommend nucleic acid amplification (NAATS) as the standard method for confirmatory diagnosis.<sup>25,26</sup> Nevertheless nucleic acid-based amplification testing methods cannot measure antimicrobial susceptibility.<sup>19</sup> Thus enhanced molecular diagnostics that distinguish among *N. gonorrhoeae* infections with antimicrobial resistance versus reduced susceptibility, are needed to help guide antibiotic treatment.<sup>19,22</sup> The importance of timely diagnosis lays, in addition to avoiding disseminated forms of gonococcus, in the prevention of non-curable sexually transmitted infections such as HIV and HBV since gonococcal infection creates a state of vulnerability for the acquisition of these latter infections.<sup>18,26</sup> Control of gonorrhea relies on prevention, appropriate diagnostics and effective antimicrobial treatment.<sup>21,27</sup> First-line treatment of this infection is standardized worldwide; for uncomplicated gonococcal infections without reported resistance, WHO and CDC recommend dual therapy with ceftriaxone 250mg (IM) + azithromycin 1gr (orally), both single dose. As an alternative regimen to ceftriaxone, they recommend cefixime 400mg in a single dose.<sup>28–32</sup> When faced with a clinical failure scenario and with reports of resistance, they propose a regimen with higher doses of ceftriaxone (500mg) and azithromycin (2gr), even taking into consideration gentamicin and spectinomycin, both accompanied by azithromycin.<sup>18,33</sup>

In Europe, probably because of the greater number of cases of multidrug resistance presented by the gonococcus<sup>14,34</sup> a higher dose regimen (500mg ceftriaxone plus azithromycin 2g) is used as first-line treatment.<sup>35</sup> The United Kingdom, on the other hand, proposes a more aggressive treatment regimen (1g of ceftriaxone), probably because despite the few reports of resistance, there has been a gradual increase in the WCC to 0.125mg/l. In the UK, azithromycin is not considered as part of the initial treatment because, according to evidence, the synergy of both drugs (in vitro) to ensure effectiveness is inconclusive. As an alternative, once susceptibility is known, the use of quinolones is recommended despite the 36.4% resistance reported in 2017.<sup>14</sup> In Mexico, a single dose of ceftriaxone 500 mg is used as a first-line treatment for genital gonococcal infection in adults, plus 1g of azithromycin, considering as an alternative regime cefixime 400mg in a single dose plus azithromycin 1g.<sup>21</sup>

## Overview of antimicrobial susceptibility and resistance transition in the world

Sulfonamides, developed in the 1930s, were one of the first widely used classes of antibiotics, but had a relatively short life as gonococcal therapy because resistance emerged within 10 years of their introduction.<sup>23</sup> The first truly remarkably effective antigonococcal agent, penicillin G, was introduced in 1943 and was effective for about 40 years (albeit by repeated increases in the dose).<sup>2</sup> Over the course of those 4 decades the minimum inhibitory concentration (MIC) gradually rose along with the recommended dose of penicillin for gonorrhea treatment. By the late 1980s, penicillin stopped to be an adequate treatment, and almost at the same time resistance to tetracyclines (alternative therapy), also appeared. Third-generation cephalosporins and quinolones became the recommended therapies in the late 1980s, but resistance to quinolones emerged rapidly, originally in East and Southeast Asia. By the mid-1990s quinolone-resistant gonorrhea was detected in North America. By 2006, 39% of gonorrhea isolates from MSM in the United States were quinolone resistant, and quinolones were removed from the CDC gonorrhea treatment guidelines. Since 2006, the CDC has recommended only one class of antimicrobials as

a first line therapy for gonorrhea: the cephalosporins.<sup>23</sup> More than 30 years after the first report of *N. Gonorrhoeae* with multi-drug resistance and the establishment of the Gonococcal Isolate Surveillance Project (GISP), to monitor trends in antimicrobial resistance, the CDC has labeled *N. Gonorrhoeae* infections as a threat because of the incidence of resistant strains infections in the United States and the world.<sup>14,19</sup> A strain of *N. Gonorrhoeae* is defined as multidrug-resistant (MDR) when resistance or reduced susceptibility to a first-line drug (extended spectrum cephalosporines or spectomycin) is associated with at least two other families of antimicrobials. Extreme drug-resistant (XDR) strains are defined as those resistant or with decreased susceptibility to 2 first-line medicines and at least three other antimicrobials (macrolides, fluoroquinolones, penicillin's, tetracycline, aminoglycosides, and carbapenems). The first XDR strain was isolated from a sex worker in Japan but it was not associated with further transmissions.<sup>18,19</sup>

Further back in time, in 1970, a penicillinase-producing strain of gonococcus was described, plasmid-mediated mechanisms (Figure 1), which confer a high level of antimicrobial resistance.<sup>36,37</sup> Other acquired resistance mechanisms have been described for *Neisseria gonorrhoeae* antibiotic flow pumps for multiple antibiotics, which have been studied and considered as therapeutic targets.<sup>38</sup> Resistance can also be acquired by point mutations, horizontal gene transfer, and alternating drug bindin 1g.<sup>19</sup> Exposure of *Neisseria spp.* to antimicrobials can result in the selection of resistant strains, due to spontaneous gene mutations and/or acquisition of whole or parts of resistance genes. The commensal *Neisseria spp.* frequently inhabits the human body and, consequently, is exposed more frequently to antimicrobials than the transiently acquired gonococcus. This way, resistance may emerge in commensal *Neisseria spp.*, which act as a reservoir of AMR genes that can be readily transferred to gonococci through transformation.<sup>2</sup> Early in the previous decade, increased resistance to azithromycin in the UK was described in other parts of the world and by 2017 resistance was found in 9.2% (14) of all strains associated with disease to other families of antibiotics (sulphonamides and quinolones).<sup>21</sup>

In 2016, 83% of GISP member countries reported at least one isolate of azithromycin-resistant *N. Gonorrhoeae*, and 49% reported decreased susceptibility to cefixime and 22% with reduced susceptibility to ceftriaxone.<sup>18</sup> In the GISP, a prospective study was carried out in which the resistance of *N. Gonorrhoeae* was determined from September 1987 to December 1988, obtaining that up to 16.8% of the isolates presented chromosome-mediated resistance to penicillin, tetracycline or cefoxitin, however, all the isolates were sensitive to ceftriaxone.<sup>39</sup> A 30% of the member countries of the antimicrobial surveillance program that conducted gonococcal susceptibility testing for cephalosporins reported that more than 5% had a decreased susceptibility to broad-spectrum cephalosporins. Fifty-nine countries reported resistance to ciprofloxacin.<sup>24</sup> To support international surveillance of gonococcal resistance, the World Health Organization (WHO) founded Gonococcal Antimicrobial Surveillance Program (GASP) in 1990. From 2009 to 2014, the total number countries reporting to GASP increased from 56 to 77. Of the 77 countries reporting to GASP, 66% reported isolates with any resistance/decreased susceptibility of *N. gonorrhoeae* to cefixime or ceftriaxone, 81% with any resistance/decreased susceptibility of NG to AZI, and 97% with any resistance/decreased susceptibility to ciprofloxacin, for at least 1 year from 2009 to 2014. Notably, there are large gaps in data on AMR NG in Africa, Central America (extending up to Mexico), and the Middle East with adjacent countries in Asia.<sup>19</sup>

## Overview of antimicrobial susceptibility and transition of resistance in the world

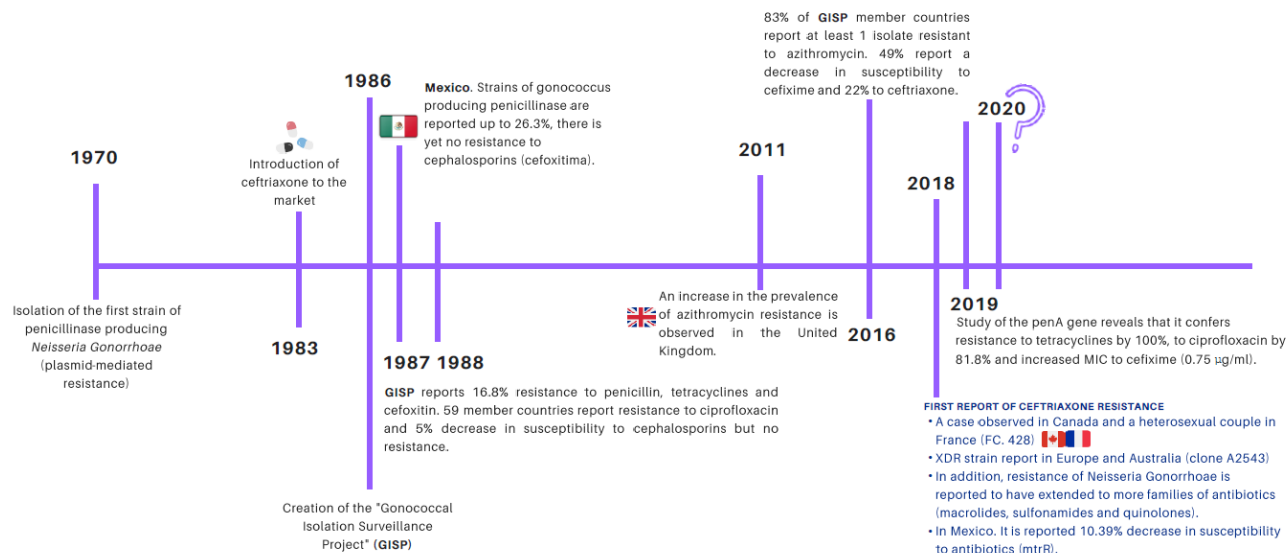


Figure 1 Plasmid-mediated mechanisms.

More recently, worldwide strains of *Neisseria gonorrhoeae* have appeared to be resistant to the first line treatment, ceftriaxone.<sup>40</sup> In 2018 in Canada there was reported a case of gonococcus resistant to this drug, as well as a heterosexual couple in southwestern France, the 3 cases molecularly identified by nucleic acid amplification test and corresponded to a genetic resistance multilocus previously identified and originated in Japan (FC428) (1903/NG-MAST 3435/NGSTAR 233).<sup>41–44</sup> This FC428 strains are susceptible to azithromycin. In March 2018, there was reported the first strain of gonorrhoea isolated from a heterosexual man in the United Kingdom, named A2543 that was resistant to both, ceftriaxone and azithromycin.<sup>31,45</sup> The probability of resistance increases when only a single gene mutation is required to change to a resistant phenotype, and less likely when multiple mutation sites are needed, which is why it has taken the gonococcus so long to develop such resistance.<sup>36</sup> In February 2020, it was reported that several alterations in the bacterial genome of gonococcus are necessary to express resistance.<sup>25</sup> In October 2019, in Ghana, the penA gene was sequenced in several gonorrhoea isolates, finding resistance to tetracyclines in 100% to ciprofloxacin in 81.8% and an isolate with a MIC at high cefixime (0.75 µg/ml) which contained a penA-34 mosaic allele. A study conducted in China and published in January 2020, analyzed 3 strains of gonorrhoea with resistance to ceftriaxone and found a new penA gene mosaic allele (penA-121,001), reflecting genetic combination, the authors concluded, that commensal strains of *Neisseria* (*N. Cinerea*, *N. Perflava*) may serve as resistance mediators in gonococcus.<sup>18</sup> Fluoroquinolones are no longer considered by the CDC as a therapeutic option for gonorrhoea infection, as they can be up to 90% resistant.<sup>18</sup> The average time between the introduction of a new antimicrobial drug and the first report of bacterial resistance to it, varies depending on the antimicrobial in question, being ceftriaxone the drug with the longest time since its introduction to the market to the first report of treatment failure (30 years).<sup>36,46</sup> In 2018 the first broad-spectrum beta-lactamase (BLEE) was discovered in a *Neisseria*

*gonorrhoeae* strain.<sup>47</sup> However, the strain described was sensitive to ceftriaxone, which may result in a possible further development of this resistance mechanism by this diplococcus.

### In México...

In Mexico, so far, there are no reports of gonococcus resistance to ceftriaxone. However, the first report on this subject dates from 1987 where De la Cruz and cols. reported that up to 26.3% of gonorrhoea isolates from 148 patients were penicillinase producers and up to 4.7% had chromosome-mediated resistance.<sup>48</sup> Similarly, Conde-Glez et al<sup>49</sup> report gonorrhoea strains resistant to spectinomycin, ampicillin, penicillin, erythromycin, chloramphenicol, and tetracyclines in frequencies from 9 to 41.3%; however, they do not identify strains resistant to cephalosporins (cefotaxime).<sup>49</sup> The most recent Mexican study was conducted by Escobedo Guerra et al. in 2018, which found that up to 10.39% of 60 samples positive for *N. Gonorrhoeae* had a mutation for reduced susceptibility to antibiotics in the mtrR gene promoting the region of the mtrCDE locus.<sup>50</sup> However, this study did not perform resistance testing with the different antibiotics to confirm or disprove resistance in the bacteria. Although existing antimicrobial resistance surveillances in some hospitals in Mexico, these studies are focused on one or few organisms and a limited set of tested antibiotics.<sup>51</sup>

### Discussion

Galeno in 130 B.C. coined the term gonorrhoea (which means in Greek "outflow or seed") by the mistaken impression of considering the discharge to be spermatorrhea. This disease was described for the first time, already in modern times (1879), by the German doctor Albert Neisser, who had the note the constant presence of a particular bacterium with coccoid morphology, in purulent discharges of infected patients.<sup>52</sup> Although the first reports of gonorrhoea resistance

to antimicrobials were found in 1970, it was not until 1986 that the Gonococcal Isolation Surveillance Project (GISP) was created, which reports alarming rates of resistance to multiple families of antibiotics considered as first-line treatment, which makes it necessary to find alternative therapies for this infection. Around the world, the emergence of these new *N. Gonorrhoeae* strains with resistance to several families of antimicrobials challenges the ease with which this infection is treated so far. Although there are no recent reports of ceftriaxone resistance in Mexico, we can find studies that show that gonococcus is not completely sensitive, it already presents genetic mutations associated with resistance to some drugs, even though they are not currently considered the first line, should be given more attention by Mexican physicians and researchers and should serve as an alert to carry out more susceptibility studies in the country. Increasing *Neisseria gonorrhoeae* resistance to extended-spectrum cephalosporins (ESCs), the last remaining option for first-line empirical monotherapy, poses an urgent public health threat. However, the genetic basis of reduced susceptibility to ceftriaxone is not completely understood: while most ceftriaxone resistance in clinical isolates is caused by target site mutations in *penA*, *porB*, and *mtrR* genes some isolates lack these mutations. So gene/protein mutations cannot entirely explain the mechanism of ESC-resistance in *N. gonorrhoeae*.<sup>11,53–56</sup>

Singh A and collaborators, in their 2020 research underpin ESC resistance is conferred by mosaic variants of penicillin-binding protein 2 (PBP2) that have diminished capacity to form acylated adducts with cephalosporins.<sup>57</sup> In the global overview and emerging resistance in which we find ourselves, several proposals arise for the saving of first-line antibiotics against gonococcal infection, such as that put forward by A.K. Zienkiewies et al<sup>58</sup> in September 2019, which proposes the carrying out of new tests to identify susceptibility to second-line antibiotics such as quinolones and in this way deal with growing resistance to ceftriaxone.<sup>58</sup> Even suggesting carbapenems (ertapenem), gepotidacin, and zoliflodacin (which are still in clinical development) as potential treatments for extremely drug-resistant gonorrhea (XDR).<sup>36,38,59</sup> In this way Public Health England reported on a man who had acquired multidrug-resistant *N. gonorrhoeae*. This isolate of *N. gonorrhoeae* showed high-level resistance to azithromycin as well as to the last remaining recommended first-line antibiotic (ceftriaxone) needing the use of a broad-spectrum carbapenem antibiotic (ertapenem). In the absence of the mass availability of a new drug for *N. gonorrhoeae* in the near future, an increase in ceftriaxone resistance might lead to consideration of routine use of carbapenems, given their high in-vitro activity and history of safety and tolerability. However, the implications of a shift to a carbapenems such as ertapenem as first-line empirical therapy for *N. gonorrhoeae* are multiple.<sup>59</sup> The Zienkiewicz AK et al<sup>58</sup> study found out that discriminatory point of care testing for drug sensitivity in both patients and their partners can result in a 70% decrease in ceftriaxone use.<sup>58</sup>

Novel, nontraditional therapeutic, and vaccine approaches to combat MDR NG infection are currently being investigated.<sup>19</sup> The possibility of a vaccine against gonococcal infection has been raised at various times, and this interest has recently increased due to the growing emergence of gonorrhea strains that are resistant to first-line drugs. Nevertheless, a wide variation of gonococcal surface antigens and other factors in the immune response against *N. gonorrhoeae* undermines the possibility of the development of this therapeutic resource. However, several studies have made increasingly plausible efforts to create a prototype vaccine with the development of infection models based on genetically modified cell lines.<sup>60</sup> A study in New

Zealand found decreased rates of gonorrhea after a mass immunization campaign against meningococcus B, but the importance of this cross-immunization is not entirely clear.<sup>18</sup> In the face of the growing wave of resistance, prevention in ethnic groups is key (transsexual population, homosexuals, indigenous populations and sex workers),<sup>16</sup> is one of the most powerful therapeutic weapons, promoting condom use, guaranteeing access to health services in marginalized communities, diagnostic and susceptibility testing will always be the most effective and safe way to address the health problem we face. In December 2019 Alhashimi et al<sup>26</sup> discovered that salicylamide, an analgesic and antipyretic drug, has antimicrobial activity against 40 different strains of *Neisseria gonorrhoeae*, in addition to having a synergistic effect with ceftriaxone, azithromycin and quinolones, this tributary to amide and hydroxyl groups that are among its components and to which antibiotic activities are attributed.<sup>26</sup>

Other therapeutic approaches include: FH/Fc fusion molecules and monoclonal antibodies. A fusion protein has been engineered that on the one hand binds to a complement regulator binding site, present on all gonococci, called factor H, and, on the other hand, possesses an Fc domain that engages complement and kills the organism. Solithromycin is a 4th-generation macrolide and the first fluoroketolide. It exhibits in vitro activity against a number of urogenital pathogens including NG, *Chlamydia trachomatis*, *Mycoplasma spp.*, and *Ureaplasma spp.*<sup>19</sup> Another immunotherapeutic molecule under development for gonorrhea treatment is the chimeric (mouse/human) 2C7 antibody. It has been tested, intravaginally and parenterally, in the mouse animal model. Because this antibody and FH/Fc target different sites on the organism, their combination may be additive. The 2C7 epitope, against which the 2C7 antibody was developed, forms the basis for a novel gonococcal vaccine.<sup>19</sup> Bradford P & Miller A O'Donnell J & Mueller J<sup>61</sup> describe Zoliflodacin, the first drug in a novel class of topoisomerase inhibitors for the treatment of uncomplicated gonorrhea and it has shown potent in vitro activity against 100 gonococcal isolates and a lack of cross-resistance to other antibiotic classes. Because its mechanism of action is distinct from fluoroquinolones, they hypothesized that zoliflodacin will be effective in treating fluoroquinolone resistant infections.<sup>19,61</sup> Recently in March-April 2020, there was reported by Pool J. and collaborators that methyl dopa and carbamazepine, prevented and cured cervical cell infection by multidrug-resistant gonococci by blocking the gonococcal-Complement Receptor 3 I-domain interaction.<sup>62</sup> Elkashif A & Seleem M<sup>55</sup> investigated auranofin as a potential novel antigonorrheal agent. Which in combination with azithromycin, ceftriaxone, cefixime or tetracycline showed an additive effect against four *N. gonorrhoeae* strains, suggesting the possibility of using auranofin in dual therapy. And was found superior to ceftriaxone in reducing the secretion of the pro-inflammatory cytokine IL-8 by endocervical cells infected with *N. Gonorrhoeae*.<sup>55</sup> Although those novel therapeutic and preventive approaches provide hope in curtailing gonococcal infections, more research and development will be required to deliver an approved, affordable treatment for *Neisseria gonorrhoeae* resistant strains.

## Conclusion

The emergence of *N. Gonorrhoeae* strains resistant to relatively modern and very useful antibiotics in daily medical practice raises awareness of the clinical and microbiological landscape in which we find ourselves, and the advent of the transformation of hitherto relatively controlled infectious problems into real public health problems. It is necessary to consider alternative therapies such

as vaccines and new drugs with antibiotic potential that address emerging resistance, including conducting susceptibility studies in a standardized manner for all patients prone to this infection or with a clinical or microbiological diagnosis of gonorrhea and thus, identify resistant cases.

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## Conflicts of interest

The authors declare that they have no conflict of interest.

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