

Future prospects for *Neisseria gonorrhoeae* treatment

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Abstract

Gonorrhea is a sexually transmitted disease with a high morbidity burden. Incidence of this disease is rising due to the increasing number of antibiotic-resistant strains. *Neisseria gonorrhoeae* has shown an extraordinary ability to develop resistance to all antimicrobials introduced for its treatment. In fact, it was recently classified as a “Priority 2” microorganism in the WHO *Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery and Development of New Antibiotics*. Seeing as there is no gonococcal vaccine, control of the disease relies entirely on prevention, diagnosis and, especially, antibiotic treatment. Different health organizations worldwide have established treatment guidelines against gonorrhea, mostly consisting in dual therapy with a single oral or intramuscular dose. However, gonococci continue to develop resistances to all antibiotics introduced for treatment. In fact, the first strain of super-resistant *N. gonorrhoeae* was recently detected in the United Kingdom, which was resistant to ceftriaxone and azithromycin. This increasing detection of resistant gonococcal strains may lead to a situation where gonorrhea becomes untreatable. Seeing as drug resistance appears to be unstoppable, new treatment options are necessary in order to control the disease. Three approaches are currently being followed for the development of new therapies against drug-resistant gonococci: (1) novel combinations of already existing antibiotics, (2) development of new antibiotics and (3) development of alternative therapies which might slow down the appearance of resistances. *N. gonorrhoeae* is a public health threat due to the increasing number of antibiotic-resistant strains. Current treatment guidelines are already being challenged by this Superbug. This has lead the scientific community to develop new antibiotics and alternative therapies in order to control this disease.

Keywords: *Neisseria gonorrhoeae*; antibiotic resistance; gonorrhea; treatment

1. Introduction

Gonorrhea is a sexually transmitted disease (STD) caused by the obligate human pathogen *Neisseria gonorrhoeae*. This disease has a high morbidity burden, with more than 106 million new cases being diagnosed every year worldwide [1]. In fact, this morbidity is increasing exponentially due to the fact that gonococci have an extraordinary ability to develop resistances to all antimicrobials introduced for its treatment (Figure 1) [2, 3].

The issue with drug-resistant *N. gonorrhoeae* has become such that the Centers for Disease Control (CDC) classified it as a “Superbug” in 2012, already alerting about a near future in which gonorrhea would become untreatable [4]. Furthermore, the World Health Organization (WHO) classified it as a “Priority 2” microorganism in the recently published WHO *Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New antibiotics* [5]. This document highlights the importance of developing new antibiotics to treat this disease, seeing as existence of *N. gonorrhoeae* strains resistant to 3rd generation cephalosporins and fluoroquinolones have already been reported. In fact, the first “super-resistant” strain was recently reported in the United Kingdom, showing resistance against the current first line treatment which consists in dual therapy with azithromycin and ceftriaxone [6].

Seeing as there is no gonococcal vaccine, control of the disease relies entirely on prevention, diagnosis and, especially antibiotic treatment [7]. It is for this reason that the present review focuses on current treatment options and the future perspectives for the treatment of this disease.

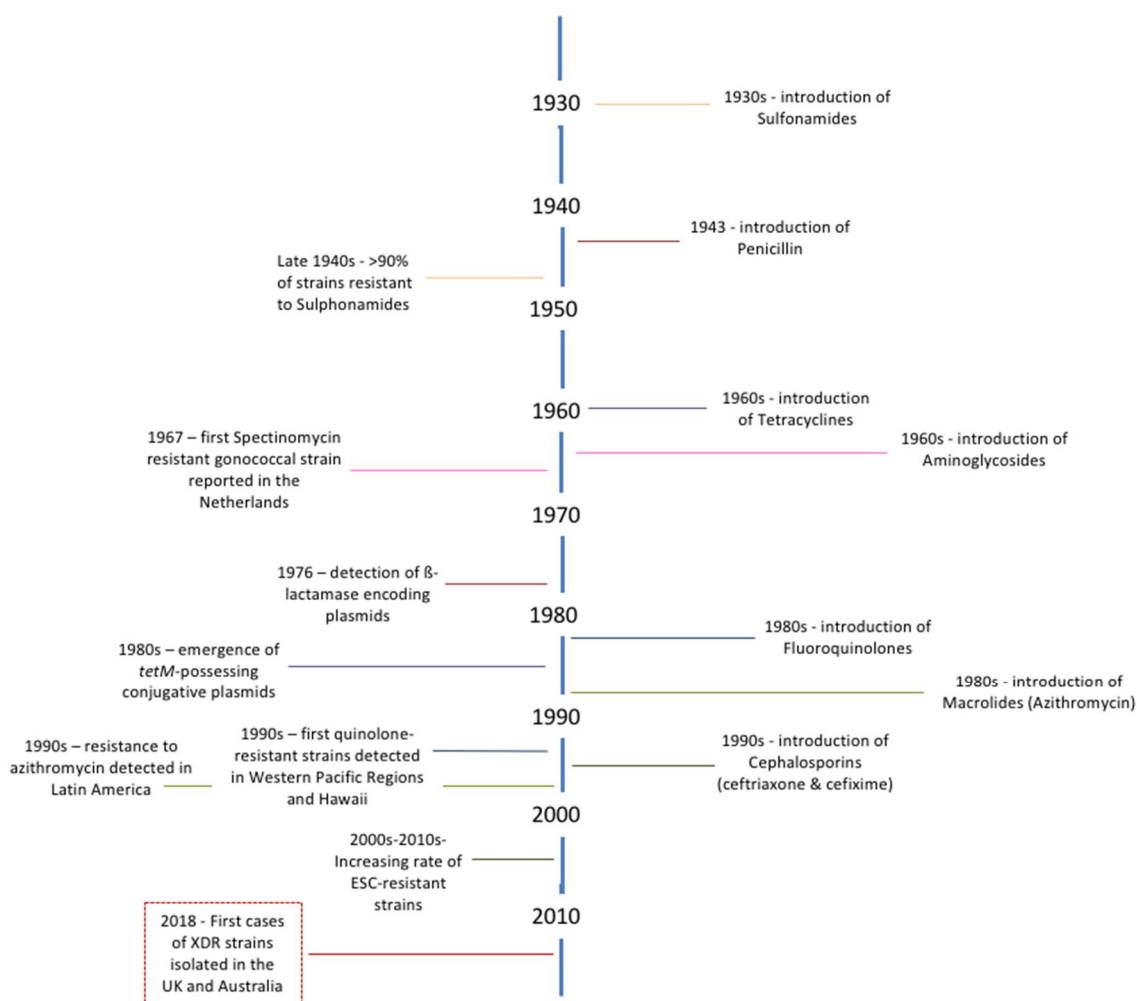


Figure 1. Timeline representing introduction and first reports of resistance for all treatments used against gonorrhoea.

2. Current treatment

Generally, treatment for gonococcal infection is given at first clinical visit, which implies that antimicrobial susceptibility is rarely performed prior to prescription. According to WHO guidelines [8], first-line antimicrobial therapy must be highly effective, widely available and affordable, lack toxicity, single dose and rapidly cure at least >95% of infected patients.

Different health organizations worldwide have established treatment guidelines against gonorrhoea, mostly consisting in dual therapy with a single oral or intramuscular dose of a third-generation cephalosporin (250-500mg IM ceftriaxone or 400mg PO cefixime) in combination with a single oral dose of 1-2g of azithromycin [9-15] (Table 1).

However, as it was mentioned earlier, these treatment options will not be useful in the near future, as they have already been reported ineffective in treating an infected patient [6]. With this in mind, it is evident that the future control of this disease relies completely on the development of new antibiotics and alternative treatments.

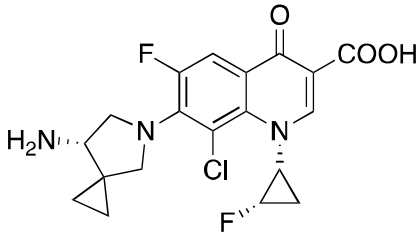
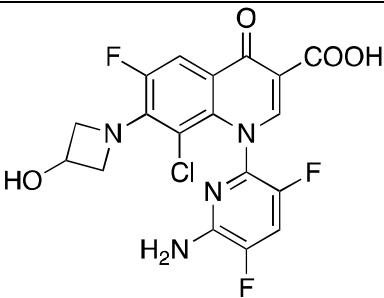
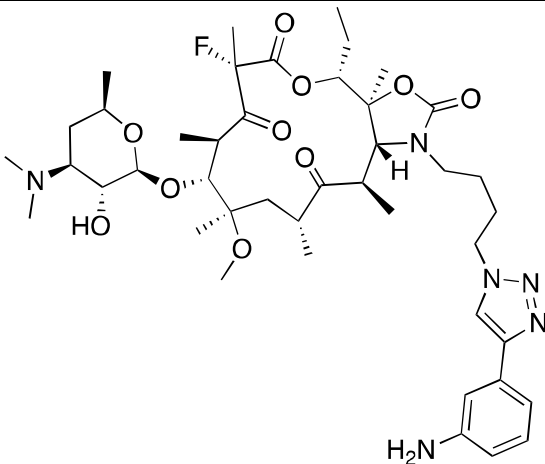
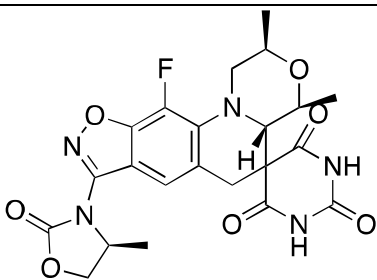
Table 1. Different treatment guidelines for gonorrhoea worldwide (all single dose).

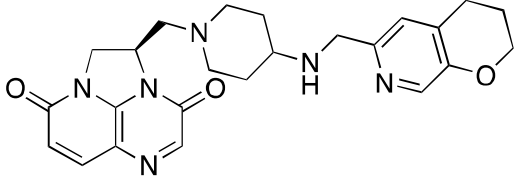
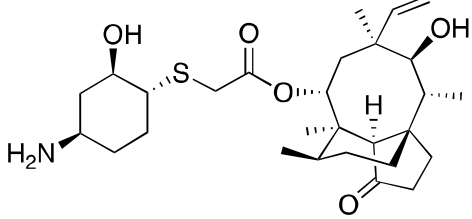
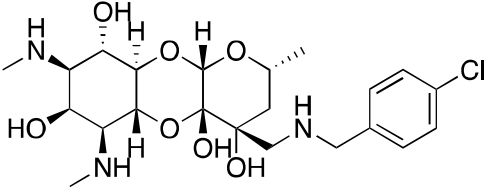
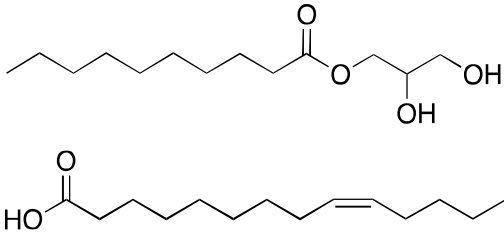
WHO [9]	Australasia [10]	Canada [11]	EEUU [12]	UK [13]	EU [14]	New Zealand [15]
Ceftriaxone 250mg IM + Azithromycin 1g PO Or Cefixime 400mg PO + Azithromycin 1g PO	Ceftriaxone 500mg IM + Azithromycin 1g PO	Ceftriaxone 250mg IM + Azithromycin 1g PO	Ceftriaxone 250mg IM + Azithromycin 1g PO	Ceftriaxone 500mg IM + Azithromycin 1g PO	Ceftriaxone 500mg IM + Azithromycin 1g PO	Ceftriaxone 250mg IM + Azithromycin 1g PO
Ceftriaxone 500mg IM + Azithromycin 2g PO Or Cefixime 800mg PO + Azithromycin 2g PO Or Gentamicin 240mg IM + Azithromycin 2g PO Or Spectinomycin 2g IM + Azithromycin 2g PO		Cefixime 800mg PO + Azithromycin 1g PO Or Spectinomycin 2g IM + Azithromycin 1g PO	Cefixime 400mg PO + Azithromycin 1g PO	Cefixime 400mg PO + Azithromycin 1g PO Or Spectinomycin 2g IM + Azithromycin 1g PO Or Cefotaxime 500mg IM + Azithromycin 1g PO	Cefixime 400mg PO + Azithromycin 2g PO Or Spectinomycin 2g IM + Azithromycin 2g PO	Spectinomycin 2g IM + Azithromycin 1g PO Or Gentamicin 240mg IM + Azithromycin 2g PO

3. Future perspectives

Seeing as drug resistance appears to be unstoppable, new treatment options are necessary in order to control the disease [16]. Three approaches are currently being followed for the development of new therapies against drug-resistant gonococci: (1) novel combinations of already existing antibiotics, (2) development of new antibiotics and (3) development of alternative therapies, which might slow down the appearance of resistances (Table 2).

Table 2. Antigonococcal agents currently under development.

FUTURE OPTIONS	NAME	ACTION MECHANISM	STRUCTURE	REFERENCE
Drug repurposing	Sitafloxacin	DNA gyrase and topoisomerase IV inhibitor		17, 18
	Delafloxacin	DNA gyrase and topoisomerase IV inhibitor		19
	Novel dual therapies	-	-	20, 21
New antibacterial agents	Solithromycin	Protein synthesis inhibitor		22-24
	Zoliflodacin	Spiropyrimidine trione topoisomerase inhibitor		25, 26

	Gepotidacin	DNA gyrase and topoisomerase IV inhibitor		22, 27, 28
	Lefamulin	Protein synthesis inhibitor		29, 30
	Aminoethyl spectinomycins	Protein synthesis inhibitor		31
Alternative therapies	IL-12	Induction of immune response	-	32
	<i>Lactobacillus crispatus</i>	Biosurfactant and acidic environment	-	33
	Monocaprin Myristoleic acid	Cell membrane disruption		34, 35

3.1. Repurposing of already existing antibiotics

Considering the fact that untreatable gonorrhea has indeed become a reality, the need for new treatment options has become a pressing issue. For this reason, the scientific community has turned to trying new combinations of already existing antibiotics as the fastest way to fight multi-resistant superbugs. Along these lines, Jönsson *et al.* studied the viability of introducing sitafloxacin, a newer-generation broad spectrum fluoroquinolone mostly used for respiratory infections, as part of a dual therapy against gonococci [17]. In the study, sitafloxacin was tested against a global gonococcal panel of 250 isolates, showing a rapid bactericidal effect with a Minimum Inhibitory Concentration (MIC) range of ≤ 0.001 -1mg/L. These results prove that sitafloxacin is a good candidate to be included in the dual antimicrobial therapy for gonorrhea in cases with cephalosporin resistance or allergy.

Along these lines, another study focuses on the evaluation of sitafloxacin and 5 additional fluoroquinolones against ciprofloxacin-resistant *N. gonorrhoeae* isolates [18]. The *in vitro* potency of sitafloxacin was substantially higher compared with the other 5 fluoroquinolones, with a MIC range of 0.03-0.5mg/L against the ciprofloxacin-resistant strains. These results further confirm the utility of sitafloxacin in a dual antimicrobial therapy.

Another fluoroquinolone currently being studied for the treatment of gonorrhea is delafloxacin [19]. Soge *et al.* evaluated the activity of delafloxacin against 117 strains of *N. gonorrhoeae*. Results showed a MIC range of ≤ 0.001 -0.25 μ g/mL, which is higher than that of ciprofloxacin, penicillin, tetracycline, azithromycin and spectinomycin. Further studies are required to correlate these promising *in vitro* results with clinical treatment outcomes.

On a similar note, Singh *et al.* assayed the potent utility of *in vitro* interactions of 21 dual therapy combinations against 95 *N. gonorrhoeae* strains [20]. Of these 21 combinations, 5 were novel introductions that are not included in any existing guidelines: gentamicin + ertapenem, moxifloxacin + ertapenem, spectinomycin + ertapenem, azithromycin + moxifloxacin and cefixime + gentamicin. All five novel combinations produced high synergistic effects against the studied strains, which suggests that further *in vivo* evaluation in clinical trials should be performed in order to include these combinations for future treatment of gonorrhea.

Gentamicin is already included in several guidelines in combination with azithromycin as an alternative treatment option when main treatment options fail. A recent study studies the synergistic effect of this combination along with gentamicin combined with 5 other antimicrobials (cefixime, ceftriaxone, spectinomycin, azithromycin, moxifloxacin and ertapenem) [21]. The study concludes that gentamicin in combination with ertapenem or cefixime could be introduced as new antimicrobial dual therapy seeing as these combinations showed maximum efficacy and synergism against 75 gonococcal strains.

3.2. New Antibiotics

However, seeing as gonococci have proven to be able to develop resistances to all antibiotics introduced for its treatment, the long-term solution includes the development of new antibiotics. Ideally, these new antibiotics should belong to antibacterial families different to the ones already included in treatment guidelines in order to delay as much as possible the appearance of resistances.

Along these lines, WHO launched the Global Antibiotic Research and Development Partnership (GARDP) in order to work with experts to draw a plan to meet the urgent need for new drugs to treat gonorrhoea [22]. Within this partnership, experts analyze current drugs in clinical development for the treatment of this disease. Currently, only 3 molecules have reached clinical trials: Solithromycin, Zoliflodacin and Gepotidacin.

Solithromycin is a broad-spectrum oral fluoroketolide which targets 3 prokaryotic ribosomal sites [23]. *In vitro* studies against 246 clinical isolates and international reference strains of *N. gonorrhoeae* showed promising results, with a MIC range of 0.001-32µg/mL, showing more activity than the antimicrobials currently recommended for its treatment. Phase II clinical trials concluded with 100% efficacy for infection in men and women for all studied sites (genital, oral and rectal) [24]. This drug is currently in Phase III trials.

As for Zoliflodacin, it has a novel action mechanism by which it inhibits the spiropyrimidinetrione topoisomerase [25]. Early *in vitro* studies showed promising results, with the compound being highly effective against clinical isolates from 21 European countries [26]. Zoliflodacin showed a MIC range of ≤0.002-0.25µg/mL,

considerably lower to that of most drugs currently being used for treatment but, most importantly, it did not present any cross-resistance to these antimicrobials.

Similarly, Farrell *et al.* studied the antigonococcal activity of Gepotidacin, a novel triazaacenaphthylene antibacterial which inhibits bacterial DNA gyrase and topoisomerase IV via a unique mechanism [27, 36]. The compound had a MIC₅₀ and MIC₉₀ of 0.12 and 0.25mg/L respectively against 25 *N. gonorrhoeae* strains, including 5 ciprofloxacin non-susceptible strains. Moreover, synergism studies showed that no antagonism occurred when gepotidacin was combined with levofloxacin, azithromycin, tetracycline and ceftriaxone; while the combination of gepotidacin with moxifloxacin had a synergistic effect. This drug candidate underwent a Phase II evaluation, showing that oral doses of gepotidacin were $\geq 95\%$ effective in treating uncomplicated urogenital gonorrhoea [28].

Along with these 3 drugs in clinical trials, other compounds being developed to treat gonorrhoea are still in early experimental phases. This is the case of Lefamulin, a novel semi-synthetic pleuromutilin, recently evaluated against 251 gonococcal clinical isolates, including multidrug-resistant and extensively-drug resistant samples [29]. The compound showed potent activity, MIC range of 0.004-2mg/L, against gonococcal isolates and no significant cross-resistance to other antimicrobials. Furthermore, this compound has also been proven to be active against the other most relevant bacterial pathogens causing STIs, *Chlamydia trachomatis* and *Mycoplasma genitalium*, proving to be a good candidate first-line antibiotic for the treatment of STIs [30]. However promising, further studies are required in order to consider the introduction of Lefamulin as a first-line treatment option.

For that matter, Butler *et al.* studied aminoethyl spectinomycins, a new class of semisynthetic analogs of the antibiotic spectinomycin, for the treatment of drug-resistant gonococci [31]. The studied compounds presented increased potency against *N. gonorrhoeae* compared to spectinomycin. Furthermore, these compounds also demonstrated activity against *C. trachomatis*, which is not observed with spectinomycin. The study concludes that aminoethyl spectinomycins are a promising alternative for spectinomycin and antibiotics such as ceftriaxone against drug-resistant gonorrhoea, with the added benefit of treating chlamydial co-infections.

3.3. *Alternative therapies*

In addition to new antibiotics, alternative therapies to combat increasingly resistant *N. gonorrhoeae* are being developed. These alternatives are mainly focused on the prevention of recurring infections rather than on the treatment of the disease. In this regard, early *in vivo* studies have been performed regarding the intravaginal administration of interleukin-12 (IL-12) in mice [32]. The study concludes that intravaginally administered IL-12 promotes Th1-driven adaptive immune response, including the production of specific anti-gonococcal antibodies which would prevent recurring infection.

On a similar note, Foschi *et al.* studied the efficacy of vaginal lactobacilli in reducing *N. gonorrhoeae* viability [33]. The study assessed the anti-gonococcal activity of 14 vaginal *Lactobacillus* strains belonging to *L. crispatus*, *L. gasseri* and *L. vaginalis*. It was found that the acidic environment associated to lactobacilli metabolism is extremely effective in counteracting gonococcal growth, with complete abolishment of gonococci viability being observed at pH<4.0. Furthermore, results showed that lactobacilli cells are able to reduce viability and co-aggregate with gonococci. This is achieved by released-surface components with biosurfactant properties produced by lactobacilli. The study concludes that specific *Lactobacillus* strains, mainly belonging to *L. crispatus*, are able to counteract gonococcal viability through multiple mechanisms, representing a new potential probiotic strategy for the prevention of infection in women.

Prophylaxis is especially important during pregnancy, seeing as neonatal conjunctivitis is commonly caused by *N. gonorrhoeae* [34]. The most common approach is ophthalmic prophylaxis with antibiotic ointments. However, due to the increasing appearance of resistances, these are becoming less effective. Churchward *et al.* studied 37 fatty acids or fatty acid derivatives for fast antigonococcal activity [35]. Two lead candidates, monocaprin and myristoleic acid, were bactericidal at 1mM and remained active in artificial tear fluid, becoming promising alternatives to conventional antibacterial ointments.

4. Conclusions

Neisseria gonorrhoeae is a public health threat worldwide due to the increasing number of antibiotic-resistant strains. Current treatment guidelines include first-line treatments as well as alternative treatments which should only be prescribed in case of allergy or presence of resistance. However, most of these guidelines are already being challenged by this “Superbug”. This has led the scientific community to develop new antibiotics and alternative therapies in order to control this disease. These new treatment options require not only high antigenococcal potency, but also no cross-resistance with current antibiotics in order to assure its applicability in the long-run. Alternative therapies, on the other hand, have focused on preventing infections rather than treating them and, therefore, controlling the disease before it has a chance of developing further resistances.

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