REVIEW
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Zoliflodacin: a hope to treat antibiotic-resistant *Neisseria gonorrhoeae*

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Competing interests
The authors declare no conflicts of interest.

Abbreviations
WHO, world health organization; CDC, center of disease control; SARS COV-2, severe acute respiratory syndrome; QIDP, qualified infection disease product; FDA, food and drug administration; HFIM, hollow fiber infection model; GARDP, global antibiotic research development program; Tmax, time to reach maximum concentration; msec, millisecond; NIAID, national institute of allergy and infectious diseases; CI, confidence interval; ECG, electrocardiography; AUC, area under curve.

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Abstract
Background: *Neisseria gonorrhoeae* is a gram-negative diplococcus that leads to sexually transmitted infection. *N. gonorrhoeae* is an obligate human pathogen that causes infection to the mucus-secreting epithelial cells both in males and females. In 2017 the center for disease control and the World Health Organization published the list of global priority pathogens-12 with denting therapeutic options, including antibiotic-resistant *N. gonorrhoeae*. **Methods:** we thoroughly characterized zoliflodacin antibiotic, its clinical trials and effect on human health by using different keywords like “zoliflodacin”, “COVID-19”, “clinical trials” from different data sources like Pub-Med, Google-Scholar, and Science-Direct. **Results:** Zoliflodacin shows a therapeutic approach against *N. gonorrhoeae*. It acts by inhibiting bacterial type 2 topoisomerase with the binding sites in bacterial gyrase. It shows promising results against *N. gonorrhoeae*. Zoliflodacin is effective in treating gonococcal urogenital and rectal infection. **Conclusion:** Currently, antibiotic is the only option to treat *N. gonorrhoeae* with no vaccine available to treat it. The new drug, zoliflodacin, specifically targets antibiotic-resistant gonorrhea and it has given a hope to researchers. This review elaborates the discovery of zoliflodacin, its mechanism of action, current clinical trials, and its effectiveness.

Keywords: *Neisseria gonorrhoeae*; zoliflodacin; COVID-19; antibiotic-resistance; treatment

Introduction

Neisseria gonorrhoeae is gram-negative diplococci having coffee beans shape appearance and have flattened adjacent sides. *N. gonorrhea* is 0.6–1 mm in diameter [1]. *N. gonorrhoeae* belongs to the genus *Neisseria*. Bacterial meningitis is caused due to two pathogenic species *N. gonorrhoeae* and *Neisseria* meningitides (also known as the meningococcus) [2]. Gonorrhea is one of the common and oldest sexually transmitted infections affecting both men and women, particularly between 15 and 24 years old. In modern society, it is one of the most common and prevalent sexually transmitted diseases [3]. *N. gonorrhoeae* is an obligate human pathogen that causes infection to the mucus-secreting epithelial cells both in males and females. Gonorrhea can present as urethritis and cervicitis or urethritis in men and women, respectively. The extragenital sites include the pharynx, rectum, conjunctiva, and rarely, systemically in both sexes. The common route of transmission of *N. gonorrhoeae* includes vaginal and or rectal intercourse, fellatio, cunnilingus, and perinatal [4]. Over the centuries, there have been drastic changes in its diagnosis and treatment. *N. gonorrhoeae* shows less susceptibility to the antibiotic treatment therefore antibiotic resistant *N. gonorrhoeae* is raised concern about the public health [3]. In 2016, World Health Organization (WHO) estimated the global prevalence of urogenital gonorrhea to be 0.9% in women and 0.7% in men, corresponding to a total of 30.6 million gonorrhea cases worldwide [5]. According to WHO, in 2016, there were approximately 87 million new gonococcal infections that had occurred among the age group 15–49 years. Gonorrhea cases are rising in many countries. The prevalence has been found to be more in a certain populations, such as gay, bisexual, racial/ethnic minorities, indigenous populations, and sex workers [6]. In 2017, the center for disease control (CDC) and WHO published the list of global priority pathogens-12 with denting therapeutic options, including antibiotic-resistant *N. gonorrhoeae* [7]. The review aims to educate about a drug, Zoliflodacin, for the treatment of antibiotic-resistant *Neisseria gonorrhoeae.*

Treatment history of gonorrhea

Before antibiotics came into being, metals were tried against the infection. This included mercury, arsenic, bismuth, gold, etc. In the 16th century, mercury was used to inject into the urethra of the gonorrhea patient. In the 18th century, quantity and quality of pus was the determining factor for the choice of treatment; like those with mild symptoms received bland fluid and those with severe condition received bloodletting and urethral lavage [8]. In 1930s, the sulfa drug was introduced for the treatment of gonorrhea. But by 1944, the resistance and treatment failure due to sulphonamide was observed [9]. In 1940s, penicillin antibiotic was introduced and gonorrhea was the first disease for which it was used. In the late 1950s and early 1960s, gonorrhea was treated with penicillin. Penicillin was preferred for treating gonorrhea for over forty years. After that, in early 1970s, the era of penicillin as a first line agent for gonorrhea was ended. In 1976, due to the penicillin resistance, new drugs non-penicillin antibiotics were introduced. These include Norfloxacin and fluoroquinolones, which have shown superior efficacy and tolerability. The fluoroquinolones were widely used for gonorrhea till 2007. After the development of resistance to fluoroquinolones, the second-and third-generation cephalosporins, with cefoxitin and cefotaxime, were also effective as gonorrhea therapy. Until 2012, oral cephalosporin cefixime was recommended as first-line therapy for gonorrhea [3]. There is no vaccine available to treat gonorrhea, and hence, antibiotic therapy is the only option to treat this infection. At the same time, there is increasing resistance to the last line options for treatment of *N. gonorrhoeae*.

COVID-19 treatment and antibiotic resistance

COVID-19 is a contagious disease that severely affects the respiratory tract and lungs. The first case was identified in the Wuhan, Hubei Province, China, in December 2019 [9]. This pandemic, the profoundly infectious irresistible illness, caused serious intense respiratory condition SARS-CoV-2 and has catastrophically affected the world's socioeconomics bringing about more than 2.9 million deaths worldwide, arising as the most significant worldwide medical emergency since the time of the flu pandemic of 1918 [10]. Many drugs and antibiotics are repurposed in the treatment of COVID-19, whereas they show promising results in affected individuals [11]. Drugs like hydroxychloroquine/chloroquine, remdesivir, azithromycin, ivermectine, and oseltamivir are being used in the treatment of COVID-19 and used at high doses [12]. This excessive use of antibiotics causes resistance to different disease treatments. Antibiotics become resistant in the treatment of *N. gonorrhoeae* and it leads to superbug. Superbug has been reported in several countries, including France, Japan, Spain, the United Kingdom, and Australia [13]. The cases of *N. gonorrhoeae* have increased by 63% since 2014, which alternatively facilitates the human immunodeficiency virus [14]. Among the repurposed drugs during COVID-19, azithromycin has caught the limelight. It is administered along with chloroquine or hydroxychloroquine in patients with COVID-19 [11, 15]. Azithromycin and other macrolides have been largely used to treat infections caused due to gram-positive microorganism. Azithromycin also shows satisfactory activity against different gram-negative microorganisms, like *N. gonorrhoeae*. Resistance to antimicrobial agents is widespread and unrestricted use has been associated with the development of resistance toward azithromycin and other related macrolides [15]. Now, azithromycin shows resistance against bacteria, and there are three mechanisms that are responsible for getting resistance to azithromycin: (1) Due to alteration in the target site. (2) Due to alteration in the antibiotic transport. (3) Due to modification of the antibiotic. In *N. gonorrhoeae*, due to modification of the ribosomal attachment site, there occur changes in the permeability and antibiotic transport. Specifically, alteration of 23S rRNA ribosomal target by the genetic mutation and methylase-associated modification can describe the resistance of azithromycin. Mutation affects the peptidyl transferase loop of domain V of 23S rRNA can describe the high level of resistance. From the bacterial cell, efflux pumps help to export toxic compounds, antibacterial peptides, and several antibiotics. In *N. gonorrhoeae*, *MtR* (CDE) encoded efflux pump is responsible to export macrolides and also shows chromosomal resistance to penicillin, tetracyclines, and quinolones. This efflux pumps system is regulated by protein coded by repressor *MtR* gene and activator *MtA* gene. Different mutations in this gene show less susceptibility and low-level resistance to azithromycin. The main source to identify the resistance of azithromycin on *N. gonorrhoeae* is the in-vitro susceptibility test. Susceptibility testing for azithromycin was added in 2001 when six of 2,350 isolates (0.26%) were found to have a minimal inhibitory concentration at 1 mg/L. It was suggested that if more than 5% of strains become resistant to azithromycin, then it should not be given to that particular patient [16]. There is no vaccine available to treat gonorrhea; hence antibiotic therapy is the only option to treat this infection. Since 1980, penicillin and tetracycline have been used as first-line therapy; later, ciprofloxacin and cefixime came into the picture. Nowadays, a combination therapy of ceftriaxone and azithromycin is used [17]. The two isolates H041 and F89 show the complete resistance to ceftriaxone and this leads to ceftriaxone-resistant gonorrhea, which will be most difficult to treat and possibly untreatable [17]. The antibiotics like cephalosporins, tetracyclines, macrolides, sulfonamides, penicillins, and fluoroquinolones, which are used against gonorrhea have lost their efficacy because of resistance [18]. Sulphonamides inhibit folic acid synthesis by targeting the gonococcal dihydropteroate synthase enzyme. Over synthesis of p-aminobenzoic acid or alteration in the *folP* gene encoding the drug targets dihydropteroate synthase, which causes the sulphonamide resistance in the treatment of *N. gonorrhoeae* [19].

Zoliflodacin

A patent application titled "Compounds and Methods for Treating Bacterial Infections" was filed by Gregory S. Basarab and co-inventors at the AstraZeneca lab Waltham, MA in 2014. This invented

compounds were derivatives of spiro (isoxazolo (4, 5-*g*)) (oxazino (4, 3-*a*) quinoline pyrimidine) trione. Among the derivatives, there was a compound, AZD0914, that came to be known as zoliflodacin [20]. Zoliflodacin has been developed for the treatment of *N. gonorrhoeae*. It is a new first class antibacterial agent called the spiropyrimiditrione. Zoliflodacin is bactericidal, act by inhibiting bacterial type 2 topoisomerase with the binding sites in bacterial gyrase that are distinct from fluoroquinolones [21]. Zoliflodacin has a unique mechanism of action; it is not cross-resistant to any other classes of gyrase inhibitors such as fluoroquinolones, aminocoumarins, or novel bacterial topoisomerase inhibitors [22]. It has potent antibacterial activity against *N. gonorrhoeae*, including multi-drug-resistant strains (minimal inhibitory concentrations ranging from ≤ 0.002 to 0.25 μg/mL) [21]. EX0914 inhibits the DNA biosynthesis and replication by an accumulation of DNA double-strand cleavages and in contrast to fluoroquinolones, prevention of relegation, resulting in a bactericidal activity against *N. gonorrhoeae*. The susceptibility of ETX0914 against *N. gonorrhoeae* strain is high and this is a qualified infection disease product (QIDP) said by the U.S. food and drug administration (FDA) [23]. Zoliflodacin undergoes two phases 1 clinical trials. The first clinical trial investigated the safety, tolerability, and pharmacokinetics of zoliflodacin in a fed and fasted state while second focuses on its absorption, distribution, metabolism, and excretion (ADME). Zoliflodacin shows linear pharmacokinetic, good oral bioavailability, and no significant safety findings [24]. Zoliflodacin is a powder formulation for oral suspension used to treat uncomplicated gonorrhea. One randomized phase 2 study was done, in which oral zoliflodacin with the dose 2 g or 3 g and ceftriaxone with the dose 500 mg was administered intramuscularly. The microbiological cure rates for urogenital gonorrhea in per-protocol analysis were 97.96% (48/49) and 100% (47/47) for zoliflodacin 2 g and 3 g, respectively. The microbiological cure rates for rectal infections were 100% (4/4) and 100% (6/6) while for pharyngeal infection it is 66.67% (4/6) and 77.78% (7/9), respectively. Zoliflodacin shows promising effectiveness in treatment of gonococcal urogenital and rectal infection as compared to pharyngeal infection, which is generally more difficult to treat than urethral, cervical or rectal gonorrhea [25]. Zoliflodacin shows effective results alone or in combination antimicrobial therapy for gonococci. It is rapidly bactericidal against gonococci with low resistance emergency potential [26]. Hollow fiber infection model (HFIM) was used to examine the relationship between gepotodacin exposure and prevention of on-therapy resistance amplification in *N. gonorrhoeae* [27]. The aim of in-vitro HFIM study was to examine the pharmacodynamic of zoliflodacin against *N. gonorrhea*. The aim of performing dose-range and dose-fractionation studies in HFIM is to: 1) identify the dynamically linked pharmacodynamic indications for zoliflodacin in *N. gonorrhoeae* kill and resistance suppression; 2) determine the dynamic rate of *N. gonorrhoeae* killing with zoliflodacin; 3) examine optimal zoliflodacin dosing for gonorrhea. The results of this study give the information about the concentration-dependent killing of *N. gonorrhoeae* with a whole dose of zoliflodacin, the importance of examining multiple divergent *N. gonorrhoeae* strains, and the suppression of antimicrobial resistance emergence [25]. Global Antibiotic Research Development Program (GARDP) and Entasis Therapeutics initiated global phase 3 trial of zoliflodacin for the treatment of gonorrhea. This is a milestone for the affected people. In this study, there are 1,000 adult participants with urogenital gonorrhea enrolled randomize (2:1) from the United States, Netherland, Thailand, and South Africa. These patients are receiving either zoliflodacin 3 g or combination of ceftriaxone and azithromycin and will assessed one week later for persistence of infection [28]. Figure 1 shows the structure of zoliflodacin. Table 1 describes various treatments for *N. gonorrhoeae* during different time periods and their effects. Table 2 describes the clinical studies of zoliflodacin.

Table 1 Treatment effects of various treatments for *N. gonorrhoeae*

Century & year	Treament	Effect of treatment	Restistant year
16th century	Mercury	Terrible side effects causing neuropathies, kidney failure, sever mouth ulcer, loss of teeth, many patients died of mercury poisoning.	1910
18th century	Mild-bland fluid Sever-bloodletting Urethral lavage	It was a painful procedure involved introduction of a catheter through the urethra and flushing the urethra with water at 46–50°C. The success of the treatment was directly proportional to the discomfort experienced by the patient during the procedure. The treatment was repeated for 2–3 consecutive days.	-
1930	Sulfa drugs/Sulfonamide	It cause due to oversynthesis of p-aminobenzoic acid and alteration in the folP gene encoding the drug target DHPS.	1944
1940	Penicillin	Resistance to penicillin has developed through: 1. Inability to access/target penicillin-binding protein (PBP) enzyme 2. Inhibition of binding to PBP via modification of the enzyme 3. Hydrolysis/inactivation of the antibiotic by beta-lactamases	1976
1976	Norfloxacin fluoroquinolones	The resistance occur due to efflux pumps and mutations to the gyrA and parC gene, which encodes DNA gyrase.	2007
2007	2nd and 3rd generation cephalosporins: Cefoxitin Cefotaxime	The exact mechanism of resistance is not fully known but some study shows the similar results with fluoroquinolones.	2012
1977	Azithromycin	Resistance to macrolides may result from modification of the ribosomal target by: 1. rRNA methylase-associated modification of the 23S rRNA 2. Specific mutations in the 23S rRNA 3. From an over expressed efflux pump system	2017

Table 2 Clinical studies of zoliflodacin

Sponsors and collaborators	Study title	Design	Number of participants enrolled	Outcomes
Astra Zeneca	A study to assess the safety, tolerability, and pharmacokinetics of AZD0914	Phase 1 randomized, placebo-controlled, single-center study	100	• In the single ascending dose study, time to maximum concentration of drug in serum (T_{max}) between 1.5–2.3 h. • Urinary excretion < 5.0% of the total dose of zoliflodacin. • In the fed state, absorption was (T_{max}, 4 h) • In the ADME study (3,000 mg orally), the PK profile of zoliflodacin similar to that of the ascending dose study and a median T_{max} of 2.5 h. • The major clearance pathway was via metabolism and elimination in feces with low urinary recovery of unchanged drug is approximately 2.5%. • Metabolites accounting for 56% of the dose excreted in the feces [29].
National institute of allergy and infectious diseases (NIAID)	Through QT/QTC (TQT) clinical trials to evaluate the effect of zoliflodacin on cardiac repolarization in healthy male and female subject.	Phase 1 randomized, double-blinded, four-period crossover	72	• The primary hypothesis to be tested is that administration of zoliflodacin 2 g and 4 g, the upper bound of the one-sided 95% confidence interval (CI) of treatment effect on delta QTcF is & get;/ = 10 msec for at least one of the ECGh assessments, against the alternative hypothesis that all mean effects are & lt; 10 msec. • The primary objective is to evaluate the effect of zoliflodacin on the corrected QT interval of the ECG using Fridericia's formula (QTcF) [30].
Drugs for neglected diseases	Study to investigate effect of food and safety of a new formulation of zoliflodacin	Phase 1 parallel, open-label, randomized, cross-over, single-center study	48	Results are not reviled yet [31].
National institute of allergy and infectious diseases (NIAID)	A study to evaluate the safety, tolerability, and plasma PK of a single oral dose of zoliflodacin in healthy male and female volunteers.	Phase 1 non-randomized	8	Zoliflodacin shows linear pharmacokinetic, good oral bioavailability, and no significant safety findings [32].
National institute of allergy and infectious diseases (NIAID)	Randomized, open label phase-2 study of oral AZD0914 in the treatment of gonorrhea	Phase 2 randomized	180	• The study is designed to assess the safety and efficacy of an antimicrobial investigational product. • It administered in adults to treat uncomplicated urogenital gonorrhea compared to treatment with ceftriaxone [33].
Global antibiotics research and development partnership	Zoliflodacin in uncomplicated gonorrhea	Phase 3 randomized	1092	• Safety and efficacy of a single dose of zoliflodacin will be assessed compared to a combination of a single dose of ceftriaxone and azithromycin. • Microbiological cure rate of pharyngeal gonorrhea, rectal gonorrhea, urogenital gonorrhea, *Neisseria gonorrhoeae* will be determined after administration of a single dose of zoliflodacin compared to a combination of a single dose of ceftriaxone and azithromycin. • The plasma concentration will be evaluated (included Area under the Curve (AUC) over 36 hours) after a single dose of zoliflodacin [33].

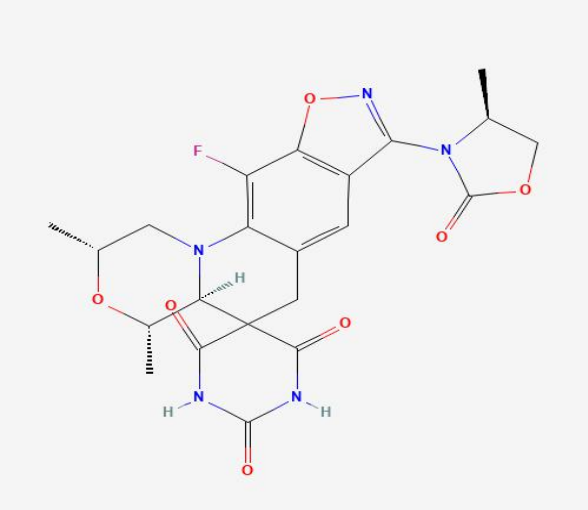

Figure 1 Structure of zoliflodacin

Conclusion

Neisseria gonorrhoeae is one of the oldest sexually transmitted diseases that has witnessed of drastic changes in the diagnosis and treatment over the centuries. *Neisseria gonorrhoeae* is showing resistance to almost all antibiotics used in the treatment. Overuse of antibiotics during the COVID-19 pandemic has led to the superbug. In 2017, the center for disease control and the World Health Organization published the list of global priority pathogens-12 with denting therapeutic options, including antibiotic-resistant *N. gonorrhoeae*. It is a need for better control of gonococcal disease to enhance global surveillance of resistance and improvement in treatment. Zoliflodacin caught the limelight to treat *N. gonorrhoeae*. It shows promising results against antibiotic-resistant *N. gonorrhoeae*. Zoliflodacin is effective in treating gonococcal urogenital and rectal infection. Zoliflodacin is an important milestone against *N. gonorrhoeae*. Zoliflodacin is a safe drug with minimum side effects. The side effects are mainly in the gastrointestinal system only.

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