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Literature Review
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Zoliflodacin, A Novel Antimirobial Agent Against *neisseria Gonorhoeae*: A Systematic Rreview of Quasi-Experimental Studies

Franklind Matthew[1], Maureen Miracle Stella[2], Kevin Tandarto[1], Regina Regina[3]
[1]*Doctor of Medicine, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia*
[2]*Doctor of Medicine Profession Student, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia*
[3] *Department of Dermatology and Venereology, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia*

ABSTRACT

Background: The gram-negative bacteria *Neisseria gonorrhoeae* is the source of the STI known as gonorrhea. *N. gonorrhoeae* can adapt well to humans as a host and become a pathogen with sophisticated mechanisms to avoid the innate immune response as well as suppress the adaptive immune response. Lately, zoliflodacin was shown to have antibacterial activity against *N. gonorrhoeae*. **Purpose:** The aim of this study is to evaluate the antibacterial activity of zoliflodacin against *N. gonorrhoeae*. **Methods:** We followed the PRISMA 2020 guidelines to systematically search and collect literature in the following databases: ProQuest, EBSCOhost, PubMed, ScienceDirect, Taylor&Francis, SAGE, JSTOR, and Wiley, without time limitation (until October 9th, 2022). Titles and abstracts were reviewed for relevance. The inclusion criteria were original article written in English that investigated the effects of zoliflodacin in patients with gonorrhea. From 177 studies, we retrieved five studies for this study, published between 2015 and 2021. **Results:** Zoliflodacin was highly active in vitro against *N. gonorrhoeae* isolates from European countries, China, Thailand, and South Africa, and also showed potent antimicrobial activity against multi-resistant *N. gonorrhoeae* isolates in South Korea. There were three different kinds of minimum inhibitory concentration (MIC) evaluated, with the following results: modal MIC (0.064-0.25 µg/mL), MIC50 (0.03-0.125 µg/mL), and MIC90 (0.06-0.25 µg/mL). There was no cross-resistance to antimicrobials currently or previously used for gonorrhea treatment. This MIC range could be used for further studies. **Conclusion:** This systematic review found that the overall growth inhibition of *N. gonorrhoeae* by zoliflodacin was satisfactory from a quasi-experimental study point of view.

Keywords: antimicrobial, gonorrhea, *Neisseria gonorrhoeae*, zoliflodacin.

Correspondence: Regina Regina, Departemen Dermatologi dan Venereologi Fakultas Kedokteran dan Ilmu Kesehatan Universitas Katolik Indonesia Atma Jaya Jalan Pluit Raya no.2, Jakarta Utara, 14440, Telephone: +62216693168, Email: regina@atmajaya.ac.id

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BACKGROUND

The gram-negative bacteria *Neisseria gonorrhoeae* is the source of the sexually transmitted infection (STI) known as gonorrhea.[1] The virulence of *N. gonorrhoeae* as a pathogen is a result of the development of a variety of complex systems for avoiding host initial effectors and dampening protective host defense mechanisms.[2] WHO anticipated there would be 82.4 million adult cases worldwide in 2020, with the Western Pacific area (23.4 million cases) and Africa region having the largest numbers (21.8 million).[3] For empirical first-line therapy of gonorrhea, dual antimicrobial therapy, namely ceftriaxone 250–500 mg intramuscularly and azithromycin 1-2 g orally, is currently advised in many situations.[4] One of the most significant dangers to public health that is producing considerable problems is antimicrobial resistance to successfully preventing and treating persistent diseases. The CDC estimates that over 2 million Americans contract antibiotic-resistant illnesses each year, leading to at least 23,000 fatalities.[4] According to statistics from the Gonococcal Isolate Surveillance Program (GISP), 51.3% of all

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gonococcal infections in 2018 were thought to have shown resilience to cefixime, ceftriaxone, penicillin, tetracycline, and ciprofloxacin. Resistance or decreased susceptibility were still on the rise.[5] Inhibiting transcription of genes and causing a buildup of double-strand cleavages in bacteria, zoliflodacin is a new antimicrobial drug with efficacy against bacteria type II topoisomerases. It helps to maintain and stop the cleavage covalent complex of gyrase with double-strand broken DNA.[6] One randomized phase 2 study was done and showed promising effectiveness in the treatment of gonococcal urogenital and rectal infections.[7] To date, no studies have systematically reviewed the potency of zoliflodacin against *N. gonorrhoeae*. Therefore, this systematic review aims to assess the potency of zoliflodacin as an antimicrobial agent against gonorrhea and its potential use in clinical settings.

METHODS

This review adheres to the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and also Meta-Analysis (PRISMA 2020). This study performed a thorough search to assess the association between zoliflodacin and *N. gonorrhoeae* in ProQuest, EBSCOhost, PubMed, ScienceDirect, Taylor & Francis, SAGE, JSTOR, and Wiley without time limitation (until October 9th, 2022). The search phrases used in PubMed were as follows: ((“Zoliflodacin”) AND (“Neisseria”)) OR ((“Zoliflodacin”) AND (“Neisseria gonorrhoea”)). The scope of the literature search was restricted to full-text publications in the English language. In order to find prospective research, we also manually searched study sources. The following criteria for inclusion were predetermined for studies: (1) reported as the original article; (2) experimental group received zoliflodacin; (4) those in the control group only obtained ineffective treatments, or none at all; and (5) the primary outcome was the *N. gonorrhoeae* growth inhibition. Criteria for exclusion were specified as follows: (1) no control group in the study; (2) review and case report; (3) repeated publication; and (4) lack of available data. Three separate reviewers independently evaluated the bias risk (FM, MMS, and KT). For quasi-experimental studies, using Risk of Bias In Non-randomized Studies of Interventions (ROBINS-I), a quality assessment was conducted. Each parameter's risk of bias was rated as "mild," "medium," or "severe" for all evaluated research. Differences of opinion amongst writers during the quality analysis were settled by conversation with the fourth author (RR).

RESULT

From 177 studies, we retrieved five studies for this study (Figure 1). All studies used in this review were published in English between 2015 and 2021.[8-12] Zoliflodacin was used as the intervention in all studies, although the source of zoliflodacin differs between each study: AstraZeneca Pharmaceuticals LP (n=1)[12], Entasis Therapeutics (n=2)[8,10], no data (n=2)[9,11]. There were three methods of susceptibility testing that were used: agar dilution (n=5)[8-12], disk diffusion (n=1)[9], and Etest (n=2),[10,12] all of which resulted in a minimum inhibitory concentration (MIC), except disk diffusion, which resulted in an inhibition zone. From the day these experiments were conducted, the results were assessed after 24 to 72 hours at 37°C. It is also worth mentioning that all studies included in this review used control groups that received either dimethyl sulfoxide (DMSO) as a placebo or no intervention. Considering the assessment of internal methodological bias, two out of five studies only have a low risk of bias, while the other three have a moderate risk of bias (Figure 2). In addition, none of the studies stated any conflict of interest. Even though some studies stated that they applied statistical analysis, few adequately specified the methodology used.[8-12]

Zoliflodacin was highly active in vitro against *N. gonorrhoeae* isolates from European countries, China, Thailand, and South Africa, and also showed potent antimicrobial activity against multi-resistant *N. gonorrhoeae* isolates in South Korea. There were three different kinds of MIC evaluated, with the following results: modal MIC (0.064-0.25 μg/mL), MIC50 (0.03-0.125 μg/mL), and MIC90 (0.06-0.25 μg/mL). There were no cross-resistance to antimicrobials currently or previously used for gonorrhea treatment, as indicated. This MIC range could be used for further studies (Table 1).

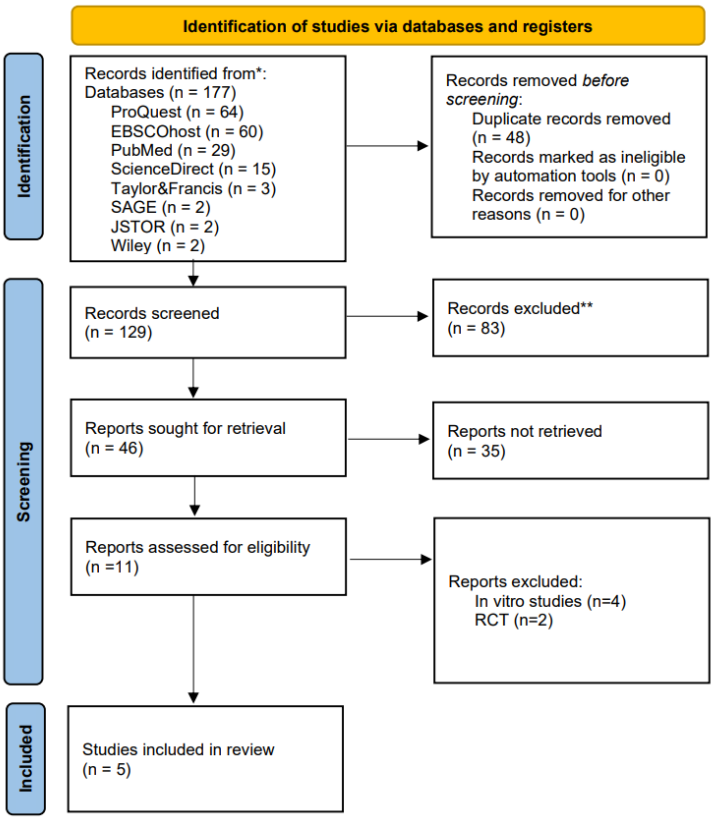

Figure 1. PRISMA 2020 flow diagram

	Risk of bias domains							
Study	D1	D2	D3	D4	D5	D6	D7	Overall
Le at al. (2021)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)
Luong et al. (2020)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(-)
Jacobsson et al. (2019)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Su et al. (2016)	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(-)
Unemo et al. (2015)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)

Domains:
D1: Bias due to confounding.
D2: Bias due to selection of participants.
D3: Bias in classification of interventions.
D4: Bias due to deviations from intended intervention.
D5: Bias due to missing data.
D6: Bias in measurement of outcomes.
D7: Bias in selection of the reported result.

Judgement:
(-): Moderate
(+): Low

Figure 2. Risk of bias for laboratory studies

Table 1. Characteristic of the included studies

Author (Year)	Zoliflodacin Source	Bacterial Isolates	Methods	Results
Le et al. (2021)[8]	Zoliflodacin (Entasis Theraeutics) (Waltham, MA, USA)	From January 2014 until December 2018, 986 gonococcal isolates were obtained from male patients suffering from symptomatic urethritis attending the STD Clinic in Nanjing, China.	Agar dilution was used to test *N. gonorrhoeae* isolates for susceptibility to zoliflodacin and 7 other antimicrobials (cefixime, tetracycline, spectinomycin, penicillin G, ciprofloxacin, azithromycin, and ceftriaxone).	The MICs of zoliflodacin ranged from <=0.002 to 0.25 mg/liter; the overall MIC50* and MIC90** were 0.06 mg/liter and 0.125 mg/liter, respectively, in 2018, increasing 2-fold from 2014. The percentage of isolates with lower zoliflodacin MICs declined sequentially in each year, while the percentage with higher MICs increased yearly (P <= 0.00001).
Luong et al. (2020)[9]	Zoliflodacin	Two hundred and fifty samples were collected, including from the pharynx, rectum, and joint fluid, in addition to the genital system. Samples were gathered from various clinics throughout South Seoul from January 2016 to December 2018.	The MICs of zoliflodacin, ceftriaxone, spectinomycin, penicillin, and ciprofloxacin were assessed using the agar dilution method. Disk diffusion was chosen as the process for quantifying antibiotic resistance.	The MIC of zoliflodacin ranges from 0.014 to 0.12 mg/L, in which MIC50* and MIC90** were 0.03, 0.06 mg/L, respectively. Additionally, the MIC modal is 0.03 mg/L, and the findings of this MIC indicate that zoliflocacin has a promising capacity for killing bacteria; penicillin (51.2%), tetracycline (78.8%), and ciprofloxacin (94.8%) were resistant to *N. gonorrhoeae*. There were no reports of ciprofloxacin and zoliflodacin cross-resistance.
Jacobsson et al. (2019)[10]	Zoliflodacin (Entasis Theraeutics) (Waltham, MA, USA)	Consecutive clinical *N. gonorrhoeae* isolates were cultured in 2015-2017 (n=100) in Africa and in 2018 (n=99) in Thailand (n=99) at a STI facility in Bangkok, Thailand, from patients with urethritis who were seen in primary care in South Africa.	By using the agar dilution method and Etest, antimicrobial susceptibility profiles, including MICs, were acquired for all *N. gonorrhoeae* isolates (gentamicin, tetracycline, spectinomycin, penicillin, ciprofloxacin, azithromycin, and	With no cross-resistance to any of the 7 comparison anti-microbials, zoliflodacin was extremely effective in vitro against all of the tested isolates (MIC90**, 0.125 g/ml, MIC50*, 0.064 g/ml; MIC range, 0.004 to 0.25 g/ml).

			ceftriaxone).	
Su et al. (2016)[11]	Zoliflodacin	In 2013, 187 clinical gonococcal strains were found in males who visited a dermatology clinic for gonococcal urethritis in Nanjing, China.	MICs of *N. gonorrhoeae* to gentamicin, tetracycline, spectinomycin, penicillin, ciprofloxacin, azithromycin, and ceftriaxone were determined by agar gel dilution.	Zoliflodacin's MIC range, MIC90**, and MIC50*, were 0.002 to 0.125 g/ml, 0.06 g/ml, and 0.03 g/ml, respectively. With a median 256-fold differential, the MICs of gonococcal isolates for ETX0914 were lesser compared to ciprofloxacin (P 0.0001). 28.8% of the isolates showed multidrug resistance, i.e., resistance to azithromycin, tetracycline, penicillin, and ciprofloxacin.
Unemo et al. (2015)[12]	Zoliflodacin (Astrazeneca Pharmaceuticals LP)	In 2014 (n = 18), 2013 (n = 846), and 2012 (n = 9), clinical N. gonorrhoeae strains cultivated from 717 male, 147 female, andl 9 patients of unknown gender in 21 European nations were analyzed. Isolates were from urogenital, pharyngeal, and anorectal specimens.	The agar dilution method was used to calculate MICs for zoliflodacin. The Etest technique was used to assess antibiotic susceptibility for the drugs ciprofloxacin, azithromycin, cefixime, and ceftriaxone.	The MIC of zoliflodacin ranges from <0.002 to 0.25 mg/L, in which the modal MIC, MIC50*, and MIC90** were 0.125, 0.064, and 0.125l mg/L, respectively. The MIC values were substantially lower than those of the other antimicrobials examined. Cefriaxone, cefixime, azithromycin, and cipofloxacin each had resistance values of 0.2%, 4,1%, 8,4%, and 55%, respectively. There was no evidence of cross-resistance with any other antibiotic.

***** A substance's MIC50 is defined as the quantity at which the survivability of microorganisms is reduced by 50%. determined in vitro.

****** A substance's MIC90 is defined as the quantity at which the survivability of microorganisms is reduced by 90%. determined in vitro.

DISCUSSION

This systematic review revealed that zoliflodacin, a novel antimicrobial agent, and, with no cross-resistance to recently used gonorrhea therapeutic antibiotics, first-in-class spiropyrimidinetrione demonstrated high in vitro efficacy against *N. gonorrhoeae* specimens from various nations. In order to stabilize the split covalent complex of DNAgyrase with dual-stranded fragmanted DNA and avoid insurance companies forming circular DNA, zoliflodacin targets the GyrB subcomponent in DNAgyrase. Despite having a comparable action mechanism to ciprofloxacin, zoliflodacin lacks the use of a Mg2+ molecule in the same way.[13] In this study, zoliflodacin was also tested on susceptible or resistant strains. The MIC of the five studies showed a range of 0.002 µg/mL, and the highest was 0.25 µg/mL, whereas it varied from 0.064 µg/mL to 0.125 µg/mL for multi-resistant *N. gonorrhoeae*. This rate was found to be lower than the MIC for the use of other antibiotics.[8-12] Three out of five studies reported that there was no cross-resistance with any of the other tested antibiotics.[9,10,12]

Initial evaluation of breakpoints for zoliflodacin, resistance (R), intermediate susceptibility (I), and susceptibility (S) was hard to determine due to the lack of interpretative criteria. Le et al. (2021)[8] showed that there was a 2-fold increase in MIC in 2018 compared to 2014, with a significant increase in the percentage of MIC every year ($p<0.0001$). An in vitro study conducted by Alm et al. (2015)[14] suggested amino acid alterations D429Nl and K450N/Tl in the *GyrB* to play significant roles in increasing MICs. A phase II clinical trial study was conducted in 2015 on 179 participants who received a single dose of 2 or 3 g of oral zoliflodacin. The microbiological cure was found in 96% of urogenital specimens, 100% of rectal specimens, and 50-82% of pharyngeal specimens.[15] Phase III of the clinical trial has also been carried out since May 2019 and is planned for completion in 2023.[13] The weakness of this study that the literature search was limited to studies in English, so it might miss studies in other languages that met the criteria. Besides that, the difference in test results is thought to be influenced by a number of technical matters. Different material specimens of origin (urogenital, pharyngeal, joint fluid, and anorectal specimens), methods (microdilution and macrodilution), and growth inhibition assays give results in the form of minimal inhibitory concentrations.

This systematic review found that the overall growth inhibition of *N. gonorrhoeae* by zoliflodacin was satisfactory from a quasi-experimental study point of view. Zoliflodacin demonstrated potent in vitro antibacterial activity against *N. gonorrhoeae*. In addition, there was no cross-resistance to antibiotics used today or in the past to cure gonorrhea. Further research is needed, especially large-scale clinical trial studies to evaluate the mechanism, side effect, and efficacy of zoliflodacin, so that it can be used as a promising antimicrobial agent can be used as a new therapy for gonorrhea for general population. Meta-analyses can also be done to analyze the significancy of zoliflodacin in gonorrhea.

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