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In-vitro Activities of Zoliflodacin and Solithromycin Against *Neisseria gonorrhoeae* Isolates from Korea

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Novel antimicrobial agents are continually developed to address the global threat of multidrug-resistant *Neisseria gonorrhoeae*. Promising candidates include zoliflodacin and, possibly, solithromycin. We evaluated their *in-vitro* activities against gonococcal isolates collected in Korea. In total, 250 *N. gonorrhoeae* isolates obtained across Korea between 2016 and 2018 were used to determine the minimum inhibitory concentrations (MICs) of 10 therapeutic agents using the CLSI agar dilution method. Most isolates (94.8%, 237/250) demonstrated non-susceptibility to penicillin G, tetracycline, and ciprofloxacin, and susceptibility to ceftriaxone and spectinomycin was substantially high. The half-maximal IC (MIC50) and 90% IC (MIC90) values for zoliflodacin were 0.03 and 0.06 μg/mL, respectively; 0.06 and 0.12 μg/mL, respectively, for solithromycin; and 0.03 and 0.12 μg/mL, respectively, for ceftriaxone. Notably, no cross-resistance was observed between zoliflodacin and ciprofloxacin, despite both targeting DNA topoisomerase II enzymes. Zoliflodacin and solithromycin demonstrated significant *in-vitro* activity against multidrug-resistant *N. gonorrhoeae* isolates, and zoliflodacin has shown non-inferiority to ceftriaxone/azithromycin dual therapy in a clinical phase 3 trial. Collectively, our findings highlight the potential of zoliflodacin as a novel therapeutic agent for gonococcal infections, particularly in the context of rising multidrug resistance, and highlight the need for continued surveillance and development of alternative antimicrobial strategies.

Key Words: Antimicrobial resistance, Azithromycin, Cefixime, Ceftriaxone, Gonorrhea, *Neisseria gonorrhoeae*, Sexually transmitted infections, Solithromycin, Spectinomycin, Zoliflodacin

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Neisseria gonorrhoeae infections have historically responded well to antimicrobial therapies; however, the emergence of resistant strains presents a growing challenge. The WHO estimated 82.3 million new cases of gonococcal infection among adults worldwide in 2020, with substantial prevalence in different regions. In 2012, the WHO launched a comprehensive global action plan to combat antimicrobial resistance in gonococcal infections, and the United Nations World Health Assembly approved the WHO Global Health Sector Strategy for Sexually Transmitted Infections 2022–2030, which aims for a 90% reduction in global gonorrhea incidence [1].

The lack of preventive vaccines, reliable diagnostics, and effective antimicrobial treatments exacerbates the urgency of addressing gonorrhea, especially because *N. gonorrhoeae* can develop antimicrobial resistance rapidly. Currently, ceftriaxone is the recommended treatment for uncomplicated gonorrhea in Korea, the USA, and most other countries globally, with spectinomycin and azithromycin serving as alternative options. However, emerging evidence indicates increasing resistance to ceftriaxone and azithromycin, with treatment failures confirmed in regions including the UK, other European countries, and Australia [2, 3]. These concerning trends highlight the need for exploring alternative treatments.

Recently developed antimicrobial agents, such as oral zoliflodacin, solithromycin, and gepotidacin, have shown promise in clinical phase 3 randomized controlled trials (RCTs). Zoliflodacin, a first-in-class spiropyrimidinetrione, functions as a topoisomerase II inhibitor targeting the GyrB subcomponent of DNA gyrase, whereas solithromycin, a next-generation macrolide, binds to multiple ribosomal sites to effectively target macrolide-resistant bacteria [4, 5]. Recently, zoliflodacin showed non-inferiority to dual therapy with ceftriaxone plus azithromycin for treating urogenital gonorrhea in a clinical phase 3 RCT [6]. However, solithromycin did not achieve efficacy in the treatment of urogenital gonorrhea in a clinical phase 3 RCT [7]. We further evaluated the clinical potential of zoliflodacin and solithromycin by assessing the *in-vitro* susceptibility of *N. gonorrhoeae* isolates collected in Korea between 2016 and 2018.

In total, 250 clinical *N. gonorrhoeae* isolates from private clinics and hospitals across Korea obtained as part of a national sentinel surveillance program for gonococcal resistance supported by the Korea Disease Control and Prevention Agency were used. This study was approved by the Institutional Review Board of Severance Hospital (approval No.: 4-2016-0359). The requirement for informed consent was waived as no demographic or other patient-identifying information was collected. Genital swab specimens were inoculated on Thayer–Martin agar and incubated at 35±1°C in a humidified 5% CO_2-enriched atmosphere. Species were identified using conventional microbiological methods or MALDI-TOF Biotyper (Bruker, Billerica, MA, USA) mass spectrometry. The isolates were stored in skim milk at −70°C until analysis.

Antimicrobial susceptibility was evaluated using the CLSI agar dilution method. The culture medium used was GC agar supplemented with IsoVitaleX [8]. The minimum inhibitory concentrations (MICs) and half-maximal IC (MIC50) and 90% IC (MIC90) values of zoliflodacin, solithromycin, ceftriaxone, cefixime, penicillin G, gentamicin, spectinomycin, ciprofloxacin, azithromycin, and tetracycline were determined. QC was ensured using the *N. gonorrhoeae* reference strain ATCC 49226.

The *in-vitro* activities of zoliflodacin and the nine other antimicrobial agents against *N. gonorrhoeae* are summarized in Table 1. Notably, all *N. gonorrhoeae* isolates were inhibited by zoliflodacin at concentrations ≤0.12 μg/mL, with a MIC range of ≤0.015–0.12 μg/mL and MIC50 and MIC90 values of 0.03 μg/mL and 0.06 μg/mL, respectively. Solithromycin exhibited an MIC range of ≤0.015–0.5 μg/mL, with MIC50 and MIC90 values of 0.06 μg/mL and 0.12 μg/mL, respectively.

Table 1. MICs of zoliflodacin, solithromycin, and eight antimicrobials previously or currently used to treat *Neisseria gonorrhoeae* in Korea and susceptibilities of the isolates

Antimicrobial agent	MIC (μg/mL)			N (%) isolates		
	Range	MIC50	MIC90	Susceptible	Intermediate	Resistant or non-susceptible
Zoliflodacin	≤0.015–0.12	0.03	0.06	NA	NA	NA
Solithromycin	≤0.015–0.5	0.06	0.12	NA	NA	NA
Penicillin G	0.06–>128	2	16	1 (0.4)	121 (48.4)	128 (51.2)
Ceftriaxone	≤0.008–0.5	0.03	0.12	249 (99.6)	NA	1 (0.4)
Cefixime	≤0.008–0.5	0.06	0.25	225 (90.0)	NA	25 (10.0)
Gentamicin*	0.25–32	4	8	131 (52.4)	118 (47.2)	1 (0.4)
Spectinomycin	8–64	32	32	247 (98.8)	3 (1.2)	0 (0)
Ciprofloxacin	≤0.06–64	8	16	6 (2.4)	7 (2.8)	237 (94.8)
Azithromycin	≤0.06–32	0.25	0.5	248 (99.2)	NA	2* (0.8)
Tetracycline	0.12–>128	4	64	7 (2.8)	46 (18.4)	197 (78.8)

Susceptibility was tested according to CLSI guidelines.
*Susceptibility was tested according to the calibrated dichotomous sensitivity method used in Australian laboratories [10].
Abbreviations: MIC, minimum inhibitory concentration; NA, not applicable.

Of the 250 isolates tested, 94.8% (237/250) demonstrated non-susceptibility to penicillin G, tetracycline, and ciprofloxacin. Nearly all isolates were susceptible to ceftriaxone (99.6%, 249/250) and spectinomycin (98.8%, 247/250). The MIC range for ceftriaxone was ≤0.008–0.5 μg/mL, with MIC50 and MIC90 values of 0.03 μg/mL and 0.12 μg/mL, respectively. Gentamicin had a MIC range of 0.025–32 μg/mL, with MIC50 and MIC90 values of 4 μg/mL and 8 μg/mL, respectively, and 52.4% (131/250) of isolates were susceptible. The MIC range for azithromycin was ≤0.06–32 μg/mL, with MIC50 and MIC90 values of 0.25 μg/mL and 0.5 μg/mL, respectively. The MIC distributions for zoliflodacin and solithromycin ranged from ≤0.015 to 0.12 μg/mL and from ≤0.015 to 0.5 μg/mL, respectively. The MICs of both agents were substantially lower than those of ceftriaxone, azithromycin, and ciprofloxacin (Fig. 1). Zoliflodacin had a low MIC even in strains exhibiting a high MIC for ciprofloxacin; therefore, its comparative activity (ciprofloxacin MIC/zoliflodacin MIC) increased as the ciprofloxacin MIC increased. This finding indicates the absence of cross-resistance between the two agents (Fig. 2A).

Zoliflodacin demonstrated significant *in-vitro* activity against all isolates tested, with MIC values being substantially lower than those of the currently recommended ceftriaxone and azithromycin. Zoliflodacin, a novel oral spiropyrimidinetrione, inhibits DNA topoisomerase II activity by selectively targeting GyrB [3]. This mechanism differs from that of fluoroquinolones, which target the GyrA subunit of DNA gyrase and ParC subunit of topoisomerase IV, thereby explaining the absence of cross-resistance between zoliflodacin and ciprofloxacin. These findings highlight the utility of zoliflodacin in treating fluoroquinolone-resistant gonococcal infections.

Despite the unsatisfactory results of a clinical phase 3 RCT on

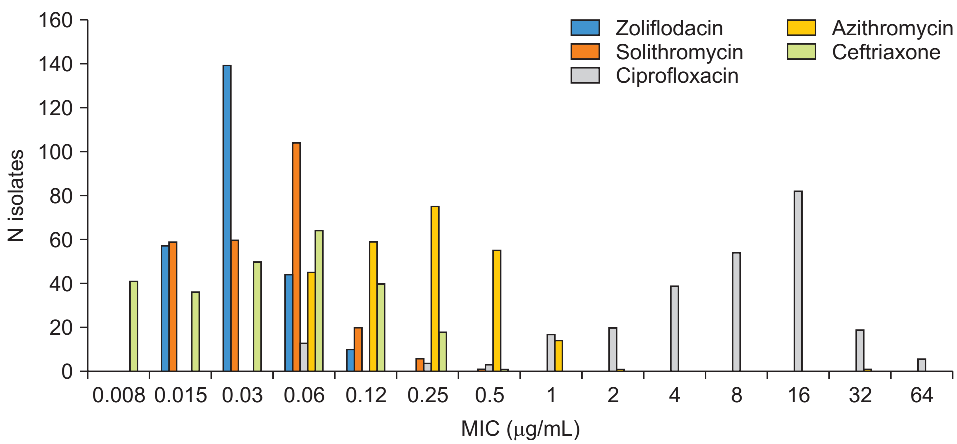

Fig. 1. MIC (μg/mL) distributions for zoliflodacin, solithromycin, ciprofloxacin, azithromycin, and ceftriaxone in 250 *Neisseria gonorrhoeae* isolates collected in Korea between 2016 and 2018.
Abbreviation: MIC, minimum inhibitory concentration.

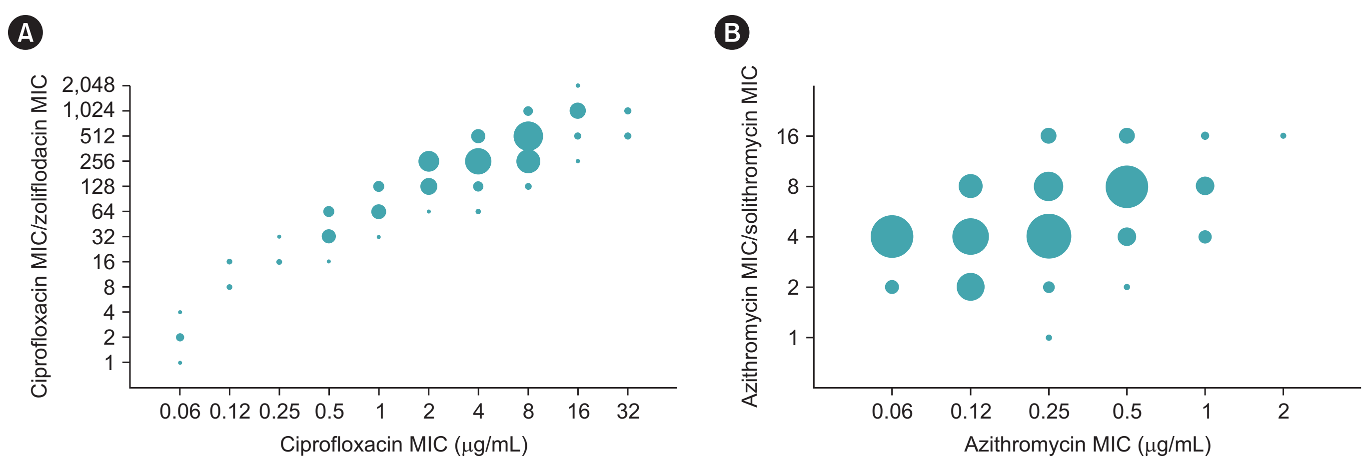

Fig. 2. Comparison of antimicrobial activities. (A) Zoliflodacin versus ciprofloxacin. (B) Solithromycin versus azithromycin. Circle size corresponds to the number of isolates.
Abbreviation: MIC, minimum inhibitory concentration.

solithromycin for treating urogenital gonorrhea [7], solithromycin demonstrated promising *in-vitro* activity against *N. gonorrhoeae* and excellent efficacy in treating resistant *Streptococcus pneumoniae* and other respiratory pathogens in concurrent trials, suggesting its potential as a replacement for fluoroquinolones [7, 9].

Our findings demonstrate the potent *in-vitro* activity of zoliflodacin and its lack of cross-resistance with existing treatment agents. Combined with the results from the recently completed clinical phase 3 RCT [6], these data support the utility of zoliflodacin as a promising novel oral antimicrobial agent for treating gonococcal infections. Further studies (such as clinical trials including more extragenital gonococcal infections) and enhanced global surveillance of antimicrobial susceptibility are warranted to elucidate its role in managing gonococcal infections amid the rise in multidrug resistance.

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AUTHOR CONTRIBUTIONS

Seo YH prepared the specimens and conducted the experiments. Lee K and Lee H designed and planned the study. Dinh LN and Roh KH analyzed the results. Dinh LN and Liu C led the manuscript writing. Lee H and Unemo M supervised the study. All authors provided critical feedback that shaped the research and final manuscript. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST

None declared.

RESEARCH FUNDING

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