Original Article

Comparative In Vitro Activity of Sitafloxacin Against *Neisseria gonorrhoeae* Isolated from Thai Patients

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Objective: To investigate the in vitro activity of sitafloxacin compared to that of ceftriaxone, cefixime, and ciprofloxacin against *Neisseria gonorrhoeae* isolated from Thai patients.

Materials and Methods: Antimicrobial susceptibility testing of ceftriaxone, cefixime, ciprofloxacin, and sitafloxacin in 52 strains of *N. gonorrhoeae* isolated from Thai patients was performed by disk diffusion method and agar dilution method to determine the inhibition zone diameters and minimum inhibitory concentrations [MICs] of ceftriaxone, cefixime, ciprofloxacin, and sitafloxacin.

Results: All *N. gonorrhoeae* isolates were susceptible to ceftriaxone (MIC ≤0.025 mg/L, zone diameter ≥35 mm) and cefixime (MIC ≤0.025 mg/l, zone diameter ≥31 mm). All *N. gonorrhoeae* study isolates were resistant to ciprofloxacin (MIC ≥1 mg/l, zone diameter ≤27 mm). The inhibition zone diameters of sitafloxacin against *N. gonorrhoeae* study isolates were 41 mm or more in 35 isolates (67%), and ranged from 28 to 40 mm in 17 isolates (33%). When the interpretative inhibition zone diameter of resistance to ciprofloxacin (27 mm or less) was applied to sitafloxacin, no *N. gonorrhoeae* study isolates were considered resistant to sitafloxacin. The MIC_{50} and MIC_{90} of sitafloxacin against *N. gonorrhoeae* study isolates was 0.06 and 0.12 mg/L, respectively. When the interpretative MIC of ciprofloxacin resistance (more than 1 mg/L) was applied to sitafloxacin, no *N. gonorrhoeae* study isolates were considered resistant to sitafloxacin.

Conclusion: Sitafloxacin is active against ciprofloxacin-resistant *N. gonorrhoeae*. A clinical study in sitafloxacin therapy to treat gonococcal urethritis caused by ciprofloxacin-resistant *N. gonorrhoeae* should be considered.

Keywords: In Vitro Activity, *Neisseria gonorrhoeae*, Sitafloxacin, Fluoroquinolone

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Neisseria gonorrhoeae is the causative bacteria of gonorrhea, which is a common sexually transmitted infection with an estimate from the World Health Organization [WHO] of 78 million new cases worldwide in people aged 15 to 49 years in 2012[(1)]. *N. gonorrhoeae* is included in the priority list of antibiotic-resistant bacteria announced by the WHO in February 2017[(2)]. Most isolates of *N. gonorrhoeae* worldwide are resistant to ciprofloxacin; as such, ciprofloxacin is no longer an effective therapy for *N. gonorrhoeae* infection[(3)]. The current recommended antibiotic therapy for *N. gonorrhoeae* infection is a combination of extended-spectrum cephalosporin (ceftriaxone) and azithromycin[(3)]. However, the emergence and spread of *N. gonorrhoeae* isolates with decreased susceptibility or resistance to combined extended-spectrum cephalosporins and azithromycin has been increasing over the past few years[(4-6)]. Therefore, research and development of new antimicrobial agents, especially oral agents that are active against resistant *N. gonorrhoeae*, are urgently needed. Sitafloxacin (DU-6859a) is an advanced generation fluoroquinolone with good activity against many gram-positive, gram-negative, and anaerobic bacteria, including strains that are resistant to other fluoroquinolones[(7)]. Sitafloxacin, an oral fluoroquinolone, was recently approved in Japan and Thailand for treatment of respiratory tract infection and genitourinary tract infection.

Given that sitafloxacin is active against many ciprofloxacin-resistant bacteria, the aim of the present study was to investigate the in vitro activity of sitafloxacin compared to that of ceftriaxone, cefixime, and ciprofloxacin against *N. gonorrhoeae* isolated from Thai patients.

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Table 1. Inhibition zone diameter and MIC interpretative criteria for ceftriaxone, cefixime, and ciprofloxacin against N. gonorrhoeae

Antimicrobial agent	Disk content	Inhibition zone diameter (mm)			MIC (mg/L)		
		S	I	R	S	I	R
Ceftriaxone	30 µg	≥35	-	-	≤0.25	-	-
Cefixime	5 µg	≥31	-	-	≤0.25	-	-
Ciprofloxacin	5 µg	≥41	28 to 40	≤27	≤0.06	0.12 to 0.5	≥1

MIC = minimum inhibitory concentration; S = susceptible; I = intermediate; R = resistant

Materials and Methods

Antibiotic susceptibility testing of 52 isolates of *N. gonorrhoeae* collected from different Thai patients in 2014 was performed at the Division of Infectious Diseases and Tropical Medicine, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

The antibiotics that were tested were ceftriaxone, cefixime, ciprofloxacin, and sitafloxacin. The inhibition zone diameters of ceftriaxone disk (30 µg), cefixime disk (5 µg), ciprofloxacin disk (5 µg), and sitafloxacin disk (5 µg) were determined by disk diffusion method according to the reference standards of Clinical and Laboratory Standards Institute [CLSI] 2013. The minimum inhibitory concentrations [MICs] of ceftriaxone, cefixime, ciprofloxacin, and sitafloxacin were determined by agar dilution method according to protocols set forth by the CLSI (M07-A10, 2015). Gonococcal [GC] Medium Base (Difco™; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) with 1% defined growth supplement (Vitox Supplement; Oxoid; Thermo Fisher Scientific, Waltham, MA, USA) was used. Inoculum preparations were made by direct colony suspension, using colonies from overnight chocolate agar plates incubated in 5% CO_2 and adjusted to 0.5 McFarland turbidity (108 CFU/mL). Bacterial suspensions were then diluted with cation-adjusted Mueller-Hinton Broth to final inocula of approximately 10^4 CFU/spot that were applied to the medium using a multipoint spot inoculator. Inoculated agars were incubated at 36°C in 5% CO_2 for 24 hours. The MIC was defined as the lowest concentration of antimicrobial agent that inhibited visible growth on agar. The control strain was *N. gonorrhoeae* ATCC 49226. The interpretative inhibition zone diameter and interpretative MIC for ceftriaxone, cefixime, and ciprofloxacin against *N. gonorrhoeae* are shown in Table 1.

Results

Inhibition zone diameter of tested antibiotics

All study isolates of *N. gonorrhoeae* collected in 2014 were susceptible to ceftriaxone with inhibition zone diameters of 35 mm or larger, and to cefixime with inhibition zone diameters of 31 mm or larger. All study isolates of *N. gonorrhoeae* were resistant to ciprofloxacin with inhibition zone diameters 27 mm or smaller. The inhibition zone diameters of sitafloxacin were 41 mm or larger in 35 isolates (67%) and ranged from 28 to 40 mm in 17 isolates (33%). When the interpretative inhibition zone diameter of resistance to ciprofloxacin (27 mm or smaller) was applied to sitafloxacin, no *N. gonorrhoeae* study isolates were considered resistant to sitafloxacin.

Table 2. MICs of ceftriaxone, cefixime, ciprofloxacin, and sitafloxacin against *N. gonorrhoeae*

Antimicrobial agent	MIC range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	% susceptible
Ceftriaxone	0.001 to 0.016	0.008	0.016	100%
Cefixime	0.002 to 0.06	0.008	0.008	100%
Ciprofloxacin	1 to >32	4	24	0%
Sitafloxacin	0.016 to 0.25	0.06	0.12	100%*

MIC = minimum inhibitory concentration

* MIC <1 mg/L is considered susceptible

MICs of tested antibiotics

The MICs of tested antibiotics are shown in Table 2. All study isolates of *N. gonorrhoeae* were susceptible to ceftriaxone with MICs less than 0.25 mg/L, and to cefixime with MICs of less than 0.25 mg/L. All study isolates of *N. gonorrhoeae* were resistant to ciprofloxacin with MICs of more than 1 mg/L. The MICs of sitafloxacin were much lower than those of ciprofloxacin. MIC_{50} and MIC_{90} of sitafloxacin against *N. gonorrhoeae* study isolates was 0.06 and 0.12 mg/L, respectively. When the interpretative MIC of ciprofloxacin resistance (of more than 1 mg/L) was applied to sitafloxacin, no *N. gonorrhoeae* study isolates were considered resistant to sitafloxacin.

Discussion

The results of the present study revealed the MIC of sitafloxacin against *N. gonorrhoeae* isolated from Thai patients to be much lower than that of ciprofloxacin, which indicated that sitafloxacin is much more active against *N. gonorrhoeae* than ciprofloxacin,

including the isolates that are resistant to ciprofloxacin. There was no official interpretative criteria for susceptibility of *N. gonorrhoeae* to sitafloxacin. As an alternative, the authors used the interpretative criteria for susceptibility of *N. gonorrhoeae* to ciprofloxacin to determine susceptibility of *N. gonorrhoeae* to sitafloxacin. The authors found that none of the study isolates of *N. gonorrhoeae* were resistant to sitafloxacin by both inhibition zone criteria and MIC criteria. Our observations of good activity of sitafloxacin against ciprofloxacin-resistant *N. gonorrhoeae* are similar to those reported from several studies conducted in Japan[8-13]. It should be noted that no ceftriaxone-resistant and/or azithromycin-resistant *N. gonorrhoeae* isolates were included in the present study. Therefore, the activity of sitafloxacin against ceftriaxone-resistant and/or azithromycin-resistant *N. gonorrhoeae* isolates from Thai patients is unknown. Sitafloxacin is an oral fluoroquinolone with favorable bioavailability and a very high concentration of active drug in urine. Sitafloxacin may be an ideal antibiotic for therapy of GC urethritis. Accordingly, clinical study in the efficacy of sitafloxacin as a therapy for GC urethritis caused by ciprofloxacin-resistant *N. gonorrhoeae* should be considered.

Conclusion

Sitafloxacin is active against ciprofloxacin-resistant *N. gonorrhoeae*. A clinical study in sitafloxacin therapy to treat GC urethritis caused by ciprofloxacin-resistant *N. gonorrhoeae* should be considered.

What is already known on this topic?

Most *N. gonorrhoeae* strains isolated from Thai patients are resistant to ciprofloxacin.

What this study adds?

Sitafloxacin is active against ciprofloxacin-resistant *N. gonorrhoeae* isolated from Thai patients.

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Potential conflicts of interest

The authors declare no conflict of interest.

References

1. World Health Organization. 2016. WHO Guidelines for the treatment of *Neisseria gonorrhoeae* [Internet]. 2016 [cited 2017 Oct 10]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK379221/.
2. World Health Organization. List of bacteria for which new antibiotics are urgently needed [Internet]. 2017 [cited 2017 Oct 10]. Available from: http://www.who.int/mediacentre/news/releases/2017/bacteria-antibiotics-needed/en/.
3. Costa-Lourenço APRD, Barros Dos Santos KT, Moreira BM, Fracalanzza SEL, Bonelli RR. Antimicrobial resistance in *Neisseria gonorrhoeae*: history, molecular mechanisms and epidemiological aspects of an emerging global threat. Braz J Microbiol 2017;48:617-28.
4. Cole MJ, Spiteri G, Jacobsson S, Woodford N, Tripodo F, Amato-Gauci AJ, et al. Overall low extended-spectrum cephalosporin resistance but high azithromycin resistance in *Neisseria gonorrhoeae* in 24 European Countries, 2015. BMC Infect Dis 2017;17:617.
5. Katz AR, Komeya AY, Kirkcaldy RD, Whelen AC, Soge OO, Papp JR, et al. Cluster of *Neisseria gonorrhoeae* isolates with high-level azithromycin resistance and decreased ceftriaxone susceptibility, Hawaii, 2016. Clin Infect Dis 2017;65:918-23.
6. Barbee LA, Soge OO, Katz DA, Dombrowski JC, Holmes KK, Golden MR. Increases in *Neisseria gonorrhoeae* with reduced susceptibility to azithromycin among men who have sex with men in Seattle, King County, Washington, 2012-2016. Clin Infect Dis 2018;66:712-8.
7. Anderson DL. Sitafloxacin hydrate for bacterial infections. Drugs Today (Barc) 2008;44:489-501.
8. Sato K, Hoshino K, Tanaka M, Hayakawa I, Osada Y. Antimicrobial activity of DU-6859, a new potent fluoroquinolone, against clinical isolates. Antimicrob Agents Chemother 1992;36:1491-8.
9. Deguchi T, Yasuda M, Nakano M, Kanematsu E, Ozeki S, Ishihara S, et al. Antimicrobial activity of a new fluoroquinolone, DU-6859a, against quinolone-resistant clinical isolates of *Neisseria gonorrhoeae* with genetic alterations in the GyrA subunit of DNA gyrase and the ParC subunit

of topoisomerase IV. J Antimicrob Chemother 1997;39:247-9.
10. Tanaka M, Nakayama H, Haraoka M, Saika T, Kobayashi I, Naito S. Susceptibilities of *Neisseria gonorrhoeae* isolates containing amino acid substitutions in GyrA, with or without substitutions in ParC, to newer fluoroquinolones and other antibiotics. Antimicrob Agents Chemother 2000; 44:192-5.
11. Hamasuna R, Yasuda M, Ishikawa K, Uehara S, Takahashi S, Hayami H, et al. Nationwide surveillance of the antimicrobial susceptibility of *Neisseria gonorrhoeae* from male urethritis in Japan. J Infect Chemother 2013;19:571-8.
12. Hamasuna R, Yasuda M, Ishikawa K, Uehara S, Hayami H, Takahashi S, et al. The second nationwide surveillance of the antimicrobial susceptibility of *Neisseria gonorrhoeae* from male urethritis in Japan, 2012-2013. J Infect Chemother 2015;21:340-5.
13. Hamasuna R, Ohnishi M, Matsumoto M, Okumura R, Unemo M, Matsumoto T. In vitro activity of sitafloxacin and additional newer generation fluoroquinolones against ciprofloxacin-resistant *Neisseria gonorrhoeae* isolates. Microb Drug Resist 2018;24:30-4.