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SUSCEPTIBILITY

High *In Vitro* Susceptibility to the First-in-Class Spiropyrimidinetrione Zoliflodacin among Consecutive Clinical *Neisseria gonorrhoeae* Isolates from Thailand and South Africa

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ABSTRACT We evaluated the *in vitro* susceptibility to the first-in-class spiropyrimidinetrione zoliflodacin among recent consecutive clinical *Neisseria gonorrhoeae* isolates cultured in Thailand ($n = 99$) (in 2018) and South Africa ($n = 100$) (in 2015 to 2017). Zoliflodacin was highly active *in vitro* against all tested isolates (MIC range, 0.004 to 0.25 μg/ml; MIC_{50}, 0.064 μg/ml; MIC_{90}, 0.125 μg/ml), with no cross-resistance to any of the seven comparator antimicrobials. Our data support the initiation of the global phase 3 randomized controlled clinical trial of zoliflodacin for uncomplicated gonorrhea.

KEYWORDS South Africa, Thailand, gonorrhea, resistance, spiropyrimidinetrione, susceptibility, treatment, zoliflodacin

The sexually transmitted infection (STI) gonorrhea, including its severe complications and sequelae, is a significant public health concern globally. The World Health Organization (WHO) estimated 87 million new cases of gonorrhea globally among adults in 2016 (1). Antimicrobial resistance in *Neisseria gonorrhoeae* has been a major concern for many decades, which has severely limited current treatment options internationally. The emergence of resistance to the extended-spectrum cephalosporins threatens the effectiveness of ceftriaxone, the last remaining option for empirical first-line monotherapy of gonorrhea globally (2–17). Novel effective antimicrobials for the treatment of urogenital and extragenital gonorrhea are essential (2, 3, 18, 19).

Zoliflodacin is the first-in-class spiropyrimidinetrione and the drug in most advanced clinical development for the treatment of uncomplicated gonorrhea (19, 20). Zoliflodacin is a topoisomerase II inhibitor, like the fluoroquinolones, but with a novel target in GyrB and a distinct mechanism of action (20). A phase 2 randomized controlled clinical trial (RCT) showed that zoliflodacin (3 g, orally), in the per-protocol analyses, achieved high cure rates among patients with uncomplicated urogenital gonorrhea (100% [47/47 patients]) and rectal gonorrhea (100% [6/6 patients]) but a reduced cure rate for the small number of pharyngeal gonorrhea cases (78% [7/9 patients]). Zoliflodacin was well tolerated, with transient gastrointestinal upset being the most frequently reported adverse event (21). A multicontinent phase 3 RCT to evaluate the clinical efficacy, tolerability, and safety in patients with uncomplicated urogenital and extragenital gonorrhea is planned in 2019 (22). This phase 3 RCT will include gonorrhea patients in the United States, the Netherlands, Thailand, and South Africa. *In vitro*

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TABLE 1 MIC values for zoliflodacin and therapeutic antimicrobials currently or previously recommended for gonorrhea treatment in *Neisseria gonorrhoeae* isolates obtained in Thailand (in 2018) and South Africa (in 2015 to 2017)

Antimicrobial and isolate group	MIC (μg/ml)[a]				Susceptibility status (%)[b]		
	Range	Modal MIC	MIC_{50}	MIC_{90}	S	I	R
Zoliflodacin							
All isolates (n = 199)	0.004 to 0.25	0.064	0.064	0.125	ND		
South African isolates (n = 100)	0.004 to 0.25	0.064	0.064	0.125	ND		
Thai isolates (n = 99)	0.004 to 0.125	0.032	0.032	0.064	ND		
Ciprofloxacin-resistant isolates[b] (n = 177)	0.004 to 0.125	0.064	0.064	0.125	ND		
Ciprofloxacin-susceptible isolates[b] (n = 22)	0.032 to 0.25	0.064	0.064	0.125	ND		
Ceftriaxone, all isolates (n = 199)	<0.002 to 0.064	0.004	0.004	0.008	100	ND	0
Azithromycin, all isolates (n = 199)	0.032 to 4	0.125	0.125	0.25	99.0	ND	1.0
Ciprofloxacin, all isolates (n = 199)	<0.002 to >32	2	2	4	11.1	ND	88.9
Penicillin G, all isolates (n = 199)	0.125 to >32	>32	4	>32	0	31.7	68.3
Spectinomycin, all isolates (n = 199)	8 to 32	16	16	16	100	ND	0
Tetracycline, all isolates (n = 199)	0.25 to 128	32	32	32	5.5	6.5	87.9
Gentamicin, all isolates (n = 199)	2 to 8	8	8	8	ND		

[a]MICs were determined using the agar dilution technique for zoliflodacin and Etest for the other antimicrobials.

[b]S, susceptible; I, intermediate susceptible; R, resistant; ND, not determined (due to the lack of interpretative criteria). The EUCAST clinical breakpoints were applied for all antimicrobials with the exception of azithromycin, for which no resistance breakpoint exists; instead, the ECOFF value (1 μg/ml) was used to distinguish isolates with azithromycin resistance determinants (31).

susceptibility to zoliflodacin was proven to be high in the United States (23) and the Netherlands (24). However, the susceptibility to zoliflodacin among *N. gonorrhoeae* strains circulating in Thailand and South Africa remains unknown. In Thailand, gonorrhea is highly prevalent, particularly in certain high-risk groups, e.g., men who have sex with men (MSM) (25), and it is a major concern that extensively drug-resistant gonococcal isolates might be spreading in Thailand and other countries in Southeast Asia (13, 14). In South Africa, the prevalence of gonorrhea is very high in both women and men (26). Furthermore, the *N. gonorrhoeae* resistance to traditional therapeutic antimicrobials for gonorrhea is also high (27), and treatment failures with the extended-spectrum cephalosporin cefixime have been verified (28).

We assessed the *in vitro* susceptibility to zoliflodacin among consecutive clinical *N. gonorrhoeae* isolates cultured in Thailand (n = 99) (in 2018) and South Africa (n = 100) (in 2015 to 2017), to provide data that are crucial to obtain prior to initiating the multicontinent phase 3 RCT of zoliflodacin. The *N. gonorrhoeae* isolates were cultured from male patients with urethritis presenting to primary health care centers in Kwa Zulu Natal (n = 40) (in 2015) and Gauteng (n = 60) (in 2017) Provinces, South Africa, and from male patients (n = 37) and female patients (n = 62) attending the Bangrak STI center in Bangkok, Thailand, in 2018. Antimicrobial susceptibility profiles, including MICs, were obtained for all *N. gonorrhoeae* isolates by the agar dilution technique, according to CLSI guidelines (29), for zoliflodacin as described previously (30) and by Etest, according to the manufacturer's instructions (bioMérieux, Marcy-l'Étoile, France), for seven other antimicrobials (ceftriaxone, azithromycin, ciprofloxacin, penicillin G, spectinomycin, tetracycline, and gentamicin). The agar dilution technique and Etests were performed in parallel, using the same inoculum of the *N. gonorrhoeae* isolates. The EUCAST clinical breakpoints were applied for all antimicrobials, with the exception of azithromycin, for which no resistance breakpoint exists; instead, the epidemiological cutoff (ECOFF) value was used to distinguish isolates with azithromycin resistance determinants (31). For quality control, the WHO reference strains A, F, and P (32, 33) were included in each antimicrobial susceptibility testing run.

The results of all antimicrobial susceptibility tests are summarized in Table 1, in which the zoliflodacin MIC data are categorized as the zoliflodacin susceptibility in all isolates (n = 199), in South African isolates (n = 100), in Thai isolates (n = 99), in ciprofloxacin-resistant isolates (n = 177), and in ciprofloxacin-susceptible isolates (n = 22). The MIC data for all other tested antimicrobials are also summarized. Briefly, zoliflodacin was highly active *in vitro* against all *N. gonorrhoeae* isolates (n = 199), with

TABLE 2 Zoliflodacin MIC distributions

Country	No. (cumulative %) with zoliflodacin MIC of:						Zoliflodacin MIC_{50} (μg/ml)	Zoliflodacin MIC_{90} (μg/ml)
	0.004 μg/ml	0.016 μg/ml	0.032 μg/ml	0.064 μg/ml	0.125 μg/ml	0.25 μg/ml		
Thailand (n = 99)	10 (10)		49 (60)	37 (97)	3 (100)		0.032	0.064
South Africa (n = 100)	2 (2)	1 (3)	21 (24)	48 (72)	27 (99)	1 (100)	0.064	0.12

a MIC range of 0.004 to 0.25 μg/ml, a modal MIC of 0.064 μg/ml, a MIC_{50} of 0.064 μg/ml, and a MIC_{90} of 0.125 μg/ml. The modal MIC, MIC_{50}, and MIC_{90} were 1 MIC $\log_2$ dilution higher in the South African isolates than in the Thai isolates (Table 1). The majority of isolates (93% [n = 185]) displayed MICs ranging from 0.032 μg/ml to 0.125 μg/ml, 6.5% (n = 13) of the isolates displayed lower MICs (0.004 or 0.016 μg/ml), and only 0.5% (n = 1) had a zoliflodacin MIC of 0.25 μg/ml (Table 2).

The levels of resistance to conventional gonorrhea therapeutic antimicrobials in South African isolates and Thai isolates were 78% and 100%, respectively, for ciprofloxacin, 85% and 91% for tetracycline, 57% and 80% for penicillin G, 0% and 2% for azithromycin, 0% and 0% for spectinomycin, and 0% and 0% for ceftriaxone (Table 1). With the exception of ceftriaxone, the modal MIC, MIC_{50}, and MIC_{90} of all antimicrobials were substantially higher than those observed for zoliflodacin. The zoliflodacin MIC_{90} (0.125 μg/ml) was 16-fold higher than that of ceftriaxone (0.008 μg/ml), 2-fold lower than that of azithromycin (0.25 μg/ml), 32-fold lower than that of ciprofloxacin (4 μg/ml), and >32-fold lower than those of gentamicin, spectinomycin, tetracycline, and penicillin G (8 μg/ml, 16 μg/ml, 32 μg/ml, and >32 μg/ml, respectively). No cross-resistance between zoliflodacin and the tested conventional gonorrhea therapeutic antimicrobials was found. The modal MIC, MIC_{50}, and MIC_{90} values were identical for ciprofloxacin-susceptible and ciprofloxacin-resistant isolates; additionally, the Spearman's correlation coefficient for the zoliflodacin MICs versus the ciprofloxacin MICs was −0.064, suggesting a lack of correlation between the MICs of the two antimicrobials.

In conclusion, zoliflodacin was highly active *in vitro* against contemporary consecutive clinical *N. gonorrhoeae* isolates from Thailand and South Africa, and no cross-resistance with any of the tested conventional gonorrhea therapeutic antimicrobials was found. The levels of susceptibility to the internationally recommended therapeutic antimicrobials ceftriaxone, cefixime, azithromycin, and spectinomycin were also high. Zoliflodacin previously demonstrated potent *in vitro* activity against temporally and genetically diverse clinical *N. gonorrhoeae* isolates and international reference strains from 21 European Union/European Economic Area countries (MIC_{50}, 0.064 μg/ml; MIC_{90}, 0.125 μg/ml; MIC range, 0.002 to 0.25 μg/ml), the United States (MIC_{50}, 0.06 μg/ml; MIC_{90}, 0.125 μg/ml; MIC range, 0.008 to 0.25 μg/ml), and China (MIC_{50}, 0.03 μg/ml; MIC_{90}, 0.06 μg/ml; MIC range, ≤0.002 to 0.125 μg/ml) (23, 24, 30, 34). Zoliflodacin has additionally shown *in vitro* activity against other STI pathogens, i.e., *Chlamydia trachomatis* and *Mycoplasma genitalium* (35, 36). The results of our study support the idea that zoliflodacin is a promising novel oral therapeutic option for gonorrhea, and they were critical for the timely initiation of the multicontinent phase 3 RCT of zoliflodacin for uncomplicated gonorrhea.

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