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High susceptibility to zoliflodacin and conserved target (GyrB) for zoliflodacin among 1209 consecutive clinical *Neisseria gonorrhoeae* isolates from 25 European countries, 2018

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Objectives: Novel antimicrobials for treatment of gonorrhoea are imperative. The first-in-class spiropyrimidinetrione zoliflodacin is promising and currently in an international Phase 3 randomized controlled clinical trial (RCT) for treatment of uncomplicated gonorrhoea. We evaluated the *in vitro* activity of and the genetic conservation of the target (GyrB) and other potential zoliflodacin resistance determinants among 1209 consecutive clinical *Neisseria gonorrhoeae* isolates obtained from 25 EU/European Economic Area (EEA) countries in 2018 and compared the activity of zoliflodacin with that of therapeutic antimicrobials currently used.

Methods: MICs of zoliflodacin, ceftriaxone, cefixime, azithromycin and ciprofloxacin were determined using an agar dilution technique for zoliflodacin or using MIC gradient strip tests or an agar dilution technique for the other antimicrobials. Genome sequences were available for 96.1% of isolates.

Results: Zoliflodacin modal MIC, MIC_{50}, MIC_{90} and MIC range were 0.125, 0.125, 0.125 and ≤0.004–0.5 mg/L, respectively. The resistance was 49.9%, 6.7%, 1.6% and 0.2% to ciprofloxacin, azithromycin, cefixime and ceftriaxone, respectively. Zoliflodacin did not show any cross-resistance to other tested antimicrobials. GyrB was highly conserved and no zoliflodacin *gyrB* resistance mutations were found. No fluoroquinolone target GyrA or ParC resistance mutations or mutations causing overexpression of the MtrCDE efflux pump substantially affected the MICs of zoliflodacin.

Conclusions: The *in vitro* susceptibility to zoliflodacin was high and the zoliflodacin target GyrB was conserved among EU/EEA gonococcal isolates in 2018. This study supports further clinical development of zoliflodacin. However, additional zoliflodacin data regarding particularly the treatment of pharyngeal gonorrhoea, pharmacokinetics/pharmacodynamics and resistance selection, including suppression, would be valuable.

Introduction

Gonorrhoea is a major health concern internationally, particularly due to the high infection prevalence and increasing resistance of *Neisseria gonorrhoeae* to all therapeutic antimicrobials. If gonorrhoea is not detected and cured, it can result in serious complications and sequelae, such as infertility and ectopic pregnancy.[1–5]

Antimicrobial resistance (AMR) in *N. gonorrhoeae* has evolved to all earlier empirical first-line or second-line treatments, i.e. sulphonamides, penicillins, tetracyclines, fluoroquinolones, early-generation macrolides and cephalosporins.[6] In many

countries, dual therapy with ceftriaxone intramuscularly plus azithromycin orally is currently the recommended empirical first-line treatment of uncomplicated gonorrhoea.[4,5,7–10] However, during the latest decade, *in vitro* and clinical resistance to ceftriaxone and particularly azithromycin has started to spread.[2,4–6,11–14] Furthermore, the first failure to cure gonorrhoea with ceftriaxone and azithromycin dual therapy was reported in 2016[15] and in 2018 the first gonococcal strain with ceftriaxone resistance combined with high-level azithromycin resistance was identified in both England and Australia.[16,17]

Improved global surveillance of the spread and evolution of AMR, enhanced understanding of the pharmacokinetics and pharmacodynamics and optimizations of current treatments and resistance/susceptibility-guided treatment using molecular assays are crucial.[12,18] However, for future management and control of gonorrhoea, novel therapeutic antimicrobials and ideally a gonococcal vaccine are essential, which have also been strongly emphasized by WHO, ECDC and CDC.[12–14,19] Only two new antimicrobials, zoliflodacin[20–30] and gepotidacin,[31–33] are currently in the later stages of clinical development for treatment of uncomplicated gonorrhoea.

Zoliflodacin is the first-in-class spiropyrimidinetrione. It is an orally bioavailable topoisomerase II inhibitor, but with the novel target GyrB and a new bactericidal mechanism of action compared with previous topoisomerase II inhibitors, such as fluoroquinolones.[20,30] According to early studies, *N. gonorrhoeae* appears to have a high *in vitro* susceptibility to zoliflodacin.[21,24–27] No clinical gonococcal isolates with zoliflodacin resistance have yet been identified; however, resistant first-step mutants, with GyrB D429A/N or K450N/T mutations generally resulting in zoliflodacin MICs of 1–2 mg/L, have been selected *in vitro*.[22,28,29] In a Phase 2 randomized controlled clinical trial (RCT), a single 3 g dose of zoliflodacin resulted in 100% microbiological cure of uncomplicated urogenital (47/47) and rectal (6/6) gonorrhoea and the cure rate for pharyngeal gonorrhoea was 78% (7/9). Zoliflodacin was well tolerated, with mostly limited transient gastrointestinal side effects.[23] In partnership with the Global Antibiotic Research and Development Partnership (GARDP), an international Phase 3 RCT to evaluate the efficacy and safety of zoliflodacin for treatment of uncomplicated gonorrhoea was initiated in 2019. However, for future licensing of zoliflodacin for treatment of gonorrhoea, more recent and comprehensive *in vitro* zoliflodacin susceptibility data for contemporary *N. gonorrhoeae* isolates internationally are also required.

We evaluated the *in vitro* activity of the first-in-class spiropyrimidinetrione zoliflodacin and the genetic conservation of the target (GyrB) and other potential AMR determinants in *N. gonorrhoeae* isolates ($n = 1209$) collected mainly during September–November 2018 from 25 EU/European Economic Area (EEA) countries and compared the activity of zoliflodacin with that of antimicrobials that are currently recommended and used for treatment of gonorrhoea internationally.

Materials and methods

Gonorrhoea patients and N. gonorrhoeae isolates

Clinical *N. gonorrhoeae* isolates ($n = 1209$; one per gonorrhoea case) from 25 EU/EEA countries, mainly cultured during September–November 2018, in the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP)[34,35] were examined. The isolates were cultured in the following countries (where available, the first ≥50 consecutive Euro-GASP 2018 isolates in each country): Austria ($n = 50$), Belgium ($n = 50$), Croatia ($n = 9$), Cyprus ($n = 4$), the Czech Republic ($n = 50$), Denmark ($n = 49$), Estonia ($n = 6$), Finland ($n = 44$), France ($n = 58$), Germany ($n = 100$), Greece ($n = 50$), Hungary ($n = 50$), Iceland ($n = 34$), Italy ($n = 50$), Latvia ($n = 5$), Malta ($n = 7$), the Netherlands ($n = 94$), Norway ($n = 49$), Poland ($n = 50$), Portugal ($n = 50$), Slovakia ($n = 50$), Slovenia ($n = 50$), Spain ($n = 100$), Sweden ($n = 50$) and the UK ($n = 100$). The isolates were obtained from mainly consecutive males ($n = 1025$), females ($n = 173$) and 11 patients not reporting gender. The median age of the males was 31 years (mean = 33 years; range = 16–86 years) and the median age of the females was 25 years (mean = 28 years; range = 1–65 years). For 24 isolates, the ages of the corresponding patients were not reported. The isolates were cultured from the following sites: urogenital ($n = 875$), anorectal ($n = 152$), pharyngeal ($n = 77$) and other ($n = 31$); the site of infection was not reported for 74 isolates. All included *N. gonorrhoeae* isolates were cultured and stored as part of the routine diagnostics (standard care) in the different countries and no patient identification information was available in the present study. Accordingly, no ethical approval was required.

N. gonorrhoeae culture and antimicrobial susceptibility testing

All isolates, previously species verified in Euro-GASP, were cultured from frozen stocks (−70°C) on GCAGP agar medium [3.6% Difco GC Medium Base agar (BD Diagnostics, Sparks, MD, USA) supplemented with 1% haemoglobin (BD Diagnostics), 1% IsoVitalex (BD Diagnostics) and 10% horse serum] for 20–24 h in a humid CO_2-enriched atmosphere at 36 ± 1°C. If there were any dubious colony morphology or MIC results, isolates were species re-verified as *N. gonorrhoeae* using MALDI-TOF MS (Bruker Daltonics, Bremen, Germany).

The MIC (mg/L) of zoliflodacin (Entasis Therapeutics, Waltham, MA, USA) for each isolate was determined by an agar dilution technique, according to CLSI guidelines (M07-A9 and M100-S24; www.clsi.org) on GCVIT agar plates [3.6% Difco GC Medium Base agar (BD Diagnostics) supplemented with 1% IsoVitalex (BD Diagnostics)]. The examined zoliflodacin concentrations ranged from 0.004 to 2 mg/L and two plates without any zoliflodacin were included for quality control. WHO reference strains A, F and P,[36,37] which have been used in several previous zoliflodacin studies,[21,26–29] were used for quality control of each testing batch. When reading the results, oxidase testing was used to resolve uncertainty regarding growth. Antimicrobial susceptibility testing of ceftriaxone, cefixime, azithromycin and ciprofloxacin was performed in Euro-GASP using MIC gradient strip tests or an agar dilution technique, as previously described.[34,35] Clinical breakpoints from EUCAST (http://www.eucast.org/clinical_breakpoints/) were applied for ceftriaxone (susceptible ≤0.125, resistant >0.125 mg/L), cefixime (susceptible ≤0.125, resistant >0.125 mg/L) and ciprofloxacin (susceptible ≤0.03, resistant >0.06 mg/L). For azithromycin, no clinical breakpoints are stated by EUCAST, so the epidemiological cut-off of azithromycin (MIC >1 mg/L) was used to distinguish isolates with azithromycin resistance determinants (considered as resistant below).

Determination of zoliflodacin resistance determinants

Whole-genome sequences were available for nearly all isolates ($n = 1162$, 96.1%) through Euro-GASP, sequenced mainly as previously described,[35] and full details will be presented elsewhere. In the present study, the zoliflodacin resistance-determining region of the target GyrB,[20–22,28–30] MtrRCDE efflux pump resistance mutations and fluoroquinolone target GyrA (S91 and D95) and ParC (D86, S87, S88 and E91) resistance determinants[6,11,35] were determined using Pathogenwatch (https://pathogen.

watch/). Potential novel resistance mutations in the *gyrB* gene were screened for using ARIBA v2.14.4.[38]

Results

Susceptibility to zoliflodacin and other examined antimicrobials

The results of the zoliflodacin susceptibility testing of the 1209 consecutive clinical *N. gonorrhoeae* isolates obtained from 25 EU/EEA countries in 2018 are summarized in Table 1.

Briefly, the MICs of zoliflodacin for all isolates ranged from ≤0.004 mg/L (2.6% of isolates) to 0.5 mg/L (0.17%, two isolates from Norway). The modal MIC, MIC_{50} and MIC_{90} were all 0.125 mg/L. The MIC_{50} was 0.032 mg/L in 1 (4%) country, 0.064 mg/L in 10 (40%) countries and 0.125 mg/L in 14 (56%) countries. The MIC_{90} was 0.125 mg/L in 17 (68%) countries and 0.25 mg/L in 8 (32%) countries (Table 1). No obvious differences between zoliflodacin MIC values were observed for isolates obtained from females compared with males or for those from patients of different ages or for those from different anatomical sites of infection (data not shown).

In Figure 1, the zoliflodacin MIC distribution for EU/EEA isolates from 2018 ($n = 1209$) is compared with the zoliflodacin MIC distribution for EU/EEA isolates from 2012–14 ($n = 873$).[26]

Briefly, the zoliflodacin MIC distributions for EU/EEA gonococcal isolates from 2018 and 2012–14[26] appeared to both mainly represent a zoliflodacin WT MIC distribution and the two distributions were nearly identical (Figure 1).

In Table 2, the susceptibility categories for ciprofloxacin, azithromycin, cefixime and ceftriaxone for the 1209 EU/EEA gonococcal isolates are shown.

Briefly, the total resistance levels to the conventional gonorrhoea therapeutic antimicrobials ciprofloxacin, azithromycin, cefixime and ceftriaxone were 49.9%, 6.7%, 1.6% and 0.2%, respectively. For the previously recommended fluoroquinolone ciprofloxacin, the resistance levels ranged from 32.0% (in Portugal) to 88.9% (in Croatia, $n = 9$). Azithromycin resistance ranged from 0% (in six countries) to 66.7% (in Croatia). Cefixime resistance was identified in 8 (32%) of the countries and ranged from 0% (in 17 countries) to 25% (in Croatia). Finally, only two (0.2%) ceftriaxone-resistant isolates were identified (one in Germany and one in Spain) (Table 2).

Table 1. Susceptibility to zoliflodacin of clinical consecutive *N. gonorrhoeae* isolates ($n = 1209$), mainly obtained during September–November 2018 from 25 EU/EEA countries

Country (number of isolates)	Modal MIC[a,b]	MIC_{50}[c]	MIC_{90}[d]	MIC range
Austria (50)	0.064/0.125	0.064	0.125	0.032–0.25
Belgium (50)	0.125	0.125	0.125	0.016–0.25
Croatia (9)	0.125	0.125	0.25	0.064–0.25
Cyprus (4)	0.125	0.032	0.125	0.032–0.125
Czech Republic (50)	0.125	0.125	0.125	≤0.004–0.25
Denmark (49)	0.064	0.064	0.125	≤0.004–0.125
Estonia (6)	0.125	0.125	0.125	0.032–0.125
Finland (44)	0.125	0.125	0.25	≤0.004–0.25
France (58)	0.125	0.125	0.125	≤0.004–0.25
Germany (100)	0.064	0.064	0.125	0.008–0.25
Greece (50)	0.125	0.125	0.125	0.032–0.25
Hungary (50)	0.064	0.064	0.125	≤0.004–0.25
Iceland (34)	0.125	0.125	0.25	0.064–0.25
Italy (50)	0.064	0.064	0.125	0.032–0.25
Latvia (5)	0.064	0.064	0.125	0.008–0.125
Malta (7)	0.064/0.125	0.125	0.25	0.064–0.25
Netherlands (94)	0.064	0.064	0.125	≤0.004–0.25
Norway (49)	0.125	0.125	0.25	≤0.004–0.5
Poland (50)	0.125	0.125	0.125	0.008–0.125
Portugal (50)	0.125	0.064	0.125	≤0.004–0.125
Slovakia (50)	0.064	0.064	0.125	0.016–0.25
Slovenia (50)	0.125	0.125	0.25	≤0.004–0.25
Spain (100)	0.064	0.064	0.125	≤0004–0.25
Sweden (50)	0.125	0.125	0.25	0.008–0.25
UK (100)	0.125	0.125	0.25	0.008–0.25
All isolates (1209)	0.125	0.125	0.125	≤0.004–0.5

[a]MIC (mg/L) determined using an agar dilution technique (www.clsi.org).
[b]Modal MIC, the most frequently occurring MIC value.
[c]MIC_{50}, MIC where 50% of the isolates are inhibited.
[d]MIC_{90}, MIC where 90% of the isolates are inhibited.

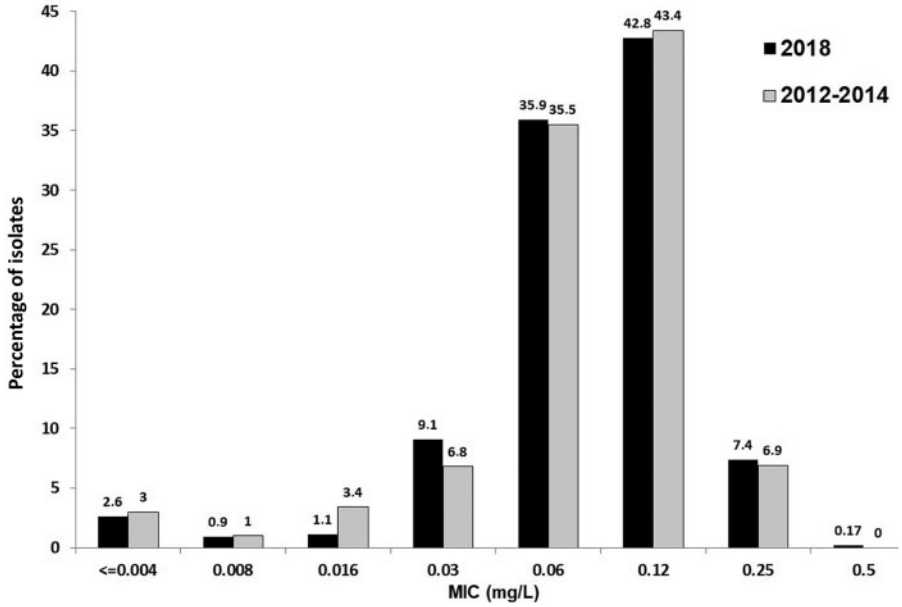

Figure 1. Zoliflodacin MIC distribution for isolates from EU/EEA countries in 2018 ($n = 1209$; black bars) compared with the MIC distribution for EU/EEA isolates from 2012–14 ($n = 873$; grey bars).[26]

No MIC correlations were identified between zoliflodacin and the other topoisomerase II inhibitor ciprofloxacin (Spearman's rank correlation coefficient = −0.14) or the other tested antimicrobials.

Zoliflodacin resistance determinants

No non-synonymous or synonymous mutations in the GyrB D429, K450 and S467N amino acid codons, i.e. where zoliflodacin first- or second-step resistance mutations have been previously selected *in vitro*,[22,28,29] were found in the 1162 whole-genome sequenced isolates. Only two non-synonymous substitutions resulting in the amino acid alterations V470L (one isolate from Spain; zoliflodacin MIC of 0.064 mg/L) and M521I (one isolate from Austria; zoliflodacin MIC of 0.125 mg/L) were found in the examined 480 bp resistance-determining region of *gyrB* that encodes the region of GyrB that surrounds the amino acid residues shown to confer resistance to zoliflodacin.[21,22] The two Norwegian isolates with the highest MIC (0.5 mg/L) were clonally related, were also susceptible to ciprofloxacin (MIC = 0.016 mg/L) and had no fluoroquinolone resistance mutations in GyrA or ParC. Both of these isolates had a mosaic *mtrR* promoter and a mosaic *mtrD* (but no 23S rRNA azithromycin resistance mutations), which probably caused the resistance to azithromycin (MICs of 2 and 4 mg/L, respectively). However, a total of 112 isolates in the dataset had a mosaic *mtrR* promoter, which also included a mosaic *mtrD* in at least 108 of them. Genetically, the mosaics presented by the two strains with a zoliflodacin MIC of 0.5 mg/L were identical to each other, but also to that of other isolates in the dataset, so we cannot confirm the contribution of this mosaicism alone to the increased zoliflodacin MIC for these two strains. In general, no fluoroquinolone target GyrA or ParC resistance mutations or mutations resulting in overexpression of the MtrCDE efflux pump appeared to substantially and/or consistently affect the MICs of zoliflodacin. Notably, WT isolates with regard to GyrA, ParC and MtrCDE AMR mutations had MICs from 0.004 to 0.25 mg/L (Figure 2).

Discussion

In this study, the novel and first-in-class spiropyrimidinetrione zoliflodacin showed high *in vitro* activity against contemporary *N. gonorrhoeae* isolates ($n = 1209$) from 25 EU/EEA countries. This study, in combination with previous studies, confirms that *N. gonorrhoeae* has a high *in vitro* susceptibility to zoliflodacin with no cross-resistance to previously used gonorrhoea therapeutic antimicrobials.[21,24–27]

The highest zoliflodacin MIC of 0.5 mg/L (confirmed in repeated testing) was recorded in two clonally related isolates (0.17% of all isolates) from Norway. In previous mostly small zoliflodacin studies, the highest zoliflodacin MIC for any clinical isolate has been 0.25 mg/L.[21,24–27] Accordingly, 0.5 mg/L is the highest zoliflodacin MIC reported for *N. gonorrhoeae*,[21,22,24–27] but no zoliflodacin target resistance mutations in GyrB or fluoroquinolone target resistance mutations in GyrA or ParC were found in these two isolates. Both isolates had genetically identical mosaics spanning *mtrR* and *mtrD*, which caused azithromycin resistance, but were also found with 100% identity in other isolates, ruling out that these mosaics alone caused the slightly higher zoliflodacin MIC. These isolates may represent the highest MIC in the zoliflodacin WT MIC distribution or they may contain some unknown determinant that slightly increases the zoliflodacin MICs.

No mutations in the GyrB D429 or K450 amino acid codons, where first-step zoliflodacin resistance mutations have been selected *in vitro*,[22,28,29] or other GyrB mutations associated with increased zoliflodacin MICs were found among clinical gonococcal isolates from 25 EU/EEA countries. In addition, no fluoroquinolone target GyrA or ParC resistance mutations appeared to affect the MICs of zoliflodacin. It has been previously shown that also general

Table 2. Susceptibility to ciprofloxacin, azithromycin, cefixime and ceftriaxone of 1209 consecutive clinical *N. gonorrhoeae* isolates obtained from 25 EU/EEA countries in 2018

Country (number of isolates)	Ciprofloxacin S/I/R (%)[a]	Azithromycin R (%)[a]	Cefixime R (%)[a]	Ceftriaxone R (%)[a]
Austria (50)	66.0/0/34.0	0	0	0
Belgium (50)	54.0/0/46.0	6.0	6.0	0
Croatia (9)	11.1/0/88.9	66.7	0	0
Cyprus (4)	25.0/0/75.0	25.0	25.0	0
Czech Republic (50)	38.0/0/62.0	18.0	0	0
Denmark (49)	59.2/0/40.8	0	0	0
Estonia (6)	66.7/0/33.3	0	0	0
Finland (44)	45.5/0/54.5	9.1	0	0
France (58)	38.0/1.7/60.3	3.4	0	0
Germany (100)	34.0/1.0/65.0	8.0	1.0	1.0
Greece (50)	44.0/0/56.0	0	6.0	0
Hungary (50)	64.0/0/36.0	0	0	0
Iceland (34)	52.9/0/47.1	17.6	0	0
Italy (50)	48.0/0/52.0	8.0	2.0	0
Latvia (5)	40.0/0/60.0	0	0	0
Malta (7)	42.9/0/57.1	28.6	0	0
Netherlands (94)	62.8/0/37.2	6.4	0	0
Norway (49)	34.7/0/65.3	18.4	0	0
Poland (50)	48.0/0/52.0	2.0	0	0
Portugal (50)	68.0/0/32.0	8.0	2.0	0
Slovakia (50)	60.0/0/40.0	2.0	0	0
Slovenia (50)	48.0/0/52.0	2.0	0	0
Spain (100)	45.0/0/55.0	10.0	8.0	1.0
Sweden (50)	44.0/0/56.0	2.0	0	0
UK (100)	58.0[b]/42.0	7.0	1.0	0
All isolates (1209)	50.1[b]/49.9	6.7	1.6	0.2

[a]S, susceptibility; I, susceptibility, increased exposure (only available for ciprofloxacin); R, resistance. According to EUCAST (http://www.eucast.org).
[b]Agar dilution breakpoint technique only distinguishing resistant (R) (MIC >0.06 mg/L) and susceptible, increased exposure (I) plus susceptible (S) (MIC ≤0.06 mg/L) isolates (used in the UK).

AMR determinants, such as overexpression of efflux pumps, particularly MtrCDE,[28] can result in increased MICs of zoliflodacin[28] and other antimicrobials;[39] however, these MIC changes are probably relatively minor in the absence of zoliflodacin GyrB target resistance mutation. Nevertheless, further studies regarding different types of mosaics in MtrRCDE and their effects on the MICs of zoliflodacin and other antimicrobials are warranted. AMR is unusual before an antimicrobial is clinically used when cross-resistance to the antimicrobials currently used is lacking, i.e. as for zoliflodacin.[21,25–27] When zoliflodacin starts to be used clinically, potential emergence of resistance should be monitored phenotypically and ideally also genetically. Nevertheless, the frequency of *in vitro* selected zoliflodacin resistance mutations has been shown to be low when evaluated as a single antimicrobial and further reduced when using antimicrobial combinations.[29]

Conclusions

The *in vitro* susceptibility to the first-in-class spiropyrimidinetrione zoliflodacin was high in contemporary, clinical isolates ($n = 1209$) collected from 25 EU/EEA countries and no cross-resistance with any of the tested conventional gonorrhoea therapeutic antimicrobials was found. An international Phase 3 RCT to evaluate the efficacy and safety of zoliflodacin for treatment of uncomplicated gonorrhoea has been ongoing since 2019. This work is performed in parallel with gonorrhoea antimicrobial stewardship initiatives by GARDP, WHO and the Foundation for Innovative New Diagnostics (FIND), including, for example, surveillance of AMR[2,3] and antimicrobial consumption, enhanced aetiological diagnostics,[40] improved AMR mutation surveillance[35,41] and rapid point-of-care tests for detection of *N. gonorrhoeae* and ideally also antimicrobial resistance/susceptibility to inform individualized treatment of gonorrhoea.[42–44] Nevertheless, it is crucial to further study also zoliflodacin pharmacokinetics/pharmacodynamics, including ideal dosing regimen, and resistance selection, including its mechanisms, fitness and suppression. To address these questions, zoliflodacin is currently being evaluated in an *N. gonorrhoeae* hollow fibre infection model for zoliflodacin.[29,45]

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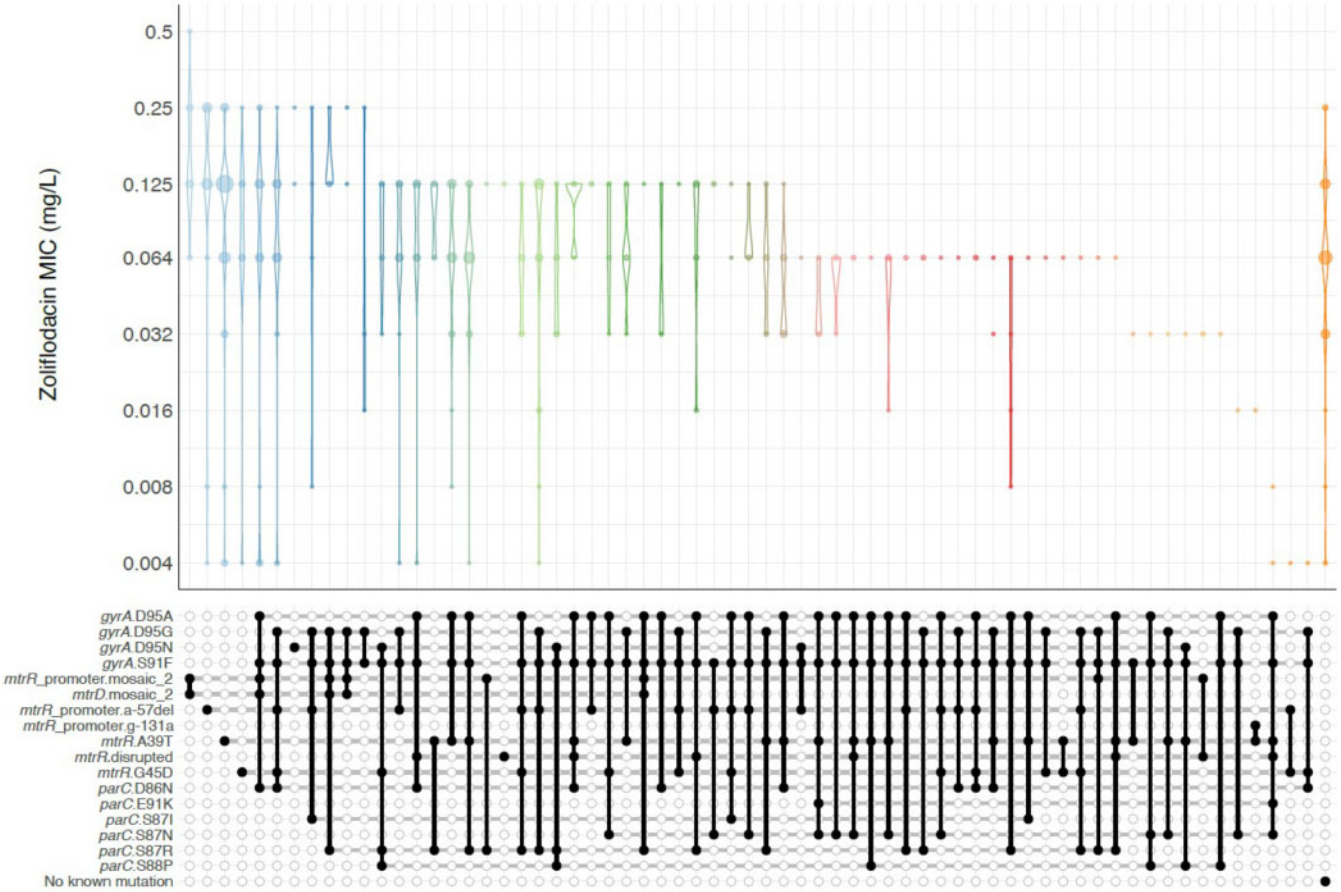

Figure 2. Distribution of MICs (mg/L) of zoliflodacin for 1162 whole-genome sequenced isolates from EU/EEA countries in 2018 with different combinations of fluoroquinolone resistance mutations in *gyrA* or *parC* and mutations resulting in overexpression of the MtrCDE efflux pump (Pathogenwatch, https://pathogen.watch/). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

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Transparency declarations

None to declare.

References

1 Rowley J, Vander Hoorn S, Korenromp E *et al.* Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. *Bull World Health Organ* 2019; **97**: 548–62P.

2 Unemo M, Lahra MM, Cole M *et al.* World Health Organization Global Gonococcal Antimicrobial Surveillance Program (WHO GASP): review of new data and evidence to inform international collaborative actions and research efforts. *Sex Health* 2019; **16**: 412–25.

3 Wi T, Lahra MM, Ndowa F *et al.* Antimicrobial resistance in *Neisseria gonorrhoeae*: global surveillance and a call for international collaborative action. *PLoS Med* 2017; **14**: e1002344.

4 WHO. WHO Guidelines for the Treatment of *Neisseria gonorrhoeae*. 2016. http://www.ncbi.nlm.nih.gov/books/NBK379221/.

5 Unemo M, Ross JDC, Serwin AB *et al*. European guideline for the diagnosis and treatment of gonorrhoea in adults. *Int J STD AIDS* 2020; doi: 10.1177/0956462420949126.

6 Unemo M, Shafer WM. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. *Clin Microbiol Rev* 2014; **27**: 587–613.

7 Workowski KA, Bolan GA. Sexually transmitted diseases treatment guidelines, 2015. *MMWR Recomm Rep* 2015; **64**: 1–137.

8 Australasian Sexual Health Alliance (ASHA). Gonorrhoea. Australian STI Management Guidelines for Use in Primary Care. 2018. http://www.sti.guidelines.org.au/sexually-transmissible-infections/gonorrhoea#management.

9 Romanowski B, Robinson J, Wong T. Gonococcal infections chapter. In: Wong T, Latham-Carmanico C, eds. *Canadian Guidelines on Sexually Transmitted Infections*. Public Health Agency of Canada, 2013. http://www.phac-aspc.gc.ca/std-mts/sti-its/cgsti-ldcits/assets/pdf/section-5-6-eng.pdf.

10 Bazzo ML, Golfetto L, Gaspar PC *et al*. First nationwide antimicrobial susceptibility surveillance for *Neisseria gonorrhoeae* in Brazil, 2015-16. *J Antimicrob Chemother* 2018; **73**: 1854–61.

11 Unemo M, Seifert HS, Hook EW 3rd *et al*. Gonorrhoea. *Nat Rev Dis Primers* 2019; **5**: 79.

12 WHO. Global Action Plan to Control the Spread and Impact of Antimicrobial Resistance in *Neisseria gonorrhoeae*. 2012. https://apps.who.int/iris/handle/10665/44863.

13 ECDC. Response Plan to Control and Manage the Threat of Multi- and Extensively Drug-Resistant Gonorrhoea in Europe. 2019 Update. https://www.ecdc.europa.eu/sites/default/files/documents/multi-and-extensively-drug-resistant-gonorrhoea-response-plan-Europe-2019.pdf.

14 CDC. Cephalosporin-Resistant *Neisseria gonorrhoeae* Public Health Response Plan. 2012. http://www.cdc.gov/std/treatment/ceph-r-responseplanjuly30-2012.pdf.

15 Fifer H, Natarajan U, Jones L *et al*. Failure of dual antimicrobial therapy in treatment of gonorrhea. *N Engl J Med* 2016; **374**: 2504–6.

16 Jennison AV, Whiley D, Lahra MM *et al*. Genetic relatedness of ceftriaxone-resistant and high-level azithromycin resistant *Neisseria gonorrhoeae* cases, United Kingdom and Australia, February to April 2018. *Euro Surveill* 2019; **24**: pii=1900118.

17 Eyre DW, Sanderson ND, Lord E *et al*. Gonorrhoea treatment failure caused by a *Neisseria gonorrhoeae* strain with combined ceftriaxone and high-level azithromycin resistance, England, February 2018. *Euro Surveill* 2018; **23**: pii=1800323.

18 Seña AC, Bachmann L, Johnston C *et al*. Optimising treatments for sexually transmitted infections: surveillance, pharmacokinetics and pharmacodynamics, therapeutic strategies, and molecular resistance prediction. *Lancet Infect Dis* 2020; **20**: e181–91.

19 Gottlieb SL, Ndowa F, Hook EW 3rd *et al*. Gonococcal vaccines: public health value and preferred product characteristics; report of a WHO global stakeholder consultation, January 2019. *Vaccine* 2020; **38**: 4362–73.

20 Basarab GS, Kern GH, McNulty J *et al*. Responding to the challenge of untreatable gonorrhea: ETX0914, a first-in-class agent with a distinct mechanism-of-action against bacterial type II topoisomerases. *Sci Rep* 2015; **5**: 11827.

21 Jacobsson S, Golparian D, Alm RA *et al*. High in vitro activity of the novel spiropyrimidinetrione AZD0914, a DNA gyrase inhibitor, against multidrug-resistant *Neisseria gonorrhoeae* isolates suggests a new effective option for oral treatment of gonorrhea. *Antimicrob Agents Chemother* 2014; **58**: 5585–8.

22 Alm RA, Lahiri SD, Kutschke A *et al*. Characterization of the novel DNA gyrase inhibitor AZD0914: low resistance potential and lack of cross-resistance in *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 2015; **59**: 1478–86.

23 Taylor SN, Marrazzo J, Batteiger BE *et al*. Single-dose zoliflodacin (ETX0914) for treatment of urogenital gonorrhea. *N Engl J Med* 2018; **379**: 1835–45.

24 Papp JR, Lawrence K, Sharpe S *et al*. In vitro growth of multidrug-resistant *Neisseria gonorrhoeae* isolates is inhibited by ETX0914, a novel spiropyrimidinetrione. *Int J Antimicrob Agents* 2016; **48**: 328–30.

25 Su X-H, Wang B-X, Le W-J *et al*. Multidrug-resistant *Neisseria gonorrhoeae* isolates from Nanjing, China, are sensitive to killing by a novel DNA gyrase inhibitor, ETX0914 (AZD0914). *Antimicrob Agents Chemother* 2015; **60**: 621–3.

26 Unemo M, Ringlander J, Wiggins C *et al*. High in vitro susceptibility to the novel spiropyrimidinetrione ETX0914 (AZD0914) among 873 contemporary clinical *Neisseria gonorrhoeae* isolates from 21 European countries from 2012 to 2014. *Antimicrob Agents Chemother* 2015; **59**: 5220–5.

27 Jacobsson S, Kularatne R, Kittiyaowamarn R *et al*. High in vitro susceptibility to the first-in-class spiropyrimidinetrione zoliflodacin among consecutive clinical *Neisseria gonorrhoeae* isolates from Thailand and South Africa. *Antimicrob Agents Chemother* 2019; **63**: e01479-19.

28 Foerster S, Golparian D, Jacobsson S *et al*. Genetic resistance determinants, in vitro time-kill curve analysis and pharmacodynamic functions for the novel topoisomerase II inhibitor ETX0914 (AZD0914) in *Neisseria gonorrhoeae*. *Front Microbiol* 2015; **6**: 1377.

29 Foerster S, Drusano G, Golparian D *et al*. In vitro antimicrobial combination testing of and evolution of resistance to the first-in-class spiropyrimidinetrione zoliflodacin combined with six therapeutically relevant antimicrobials for *Neisseria gonorrhoeae*. *J Antimicrob Chemother* 2019; **74**: 3521–9.

30 Bradford PA, Miller AA, O'Donnell J *et al*. Zoliflodacin: an oral spiropyrimidinetrione antibiotic for the treatment of *Neisseria gonorrheae*, including multi-drug-resistant isolates. *ACS Infect Dis* 2020; **6**: 1332–45.

31 Jacobsson S, Golparian D, Scangarella-Oman N *et al*. In vitro activity of the novel triazaacenaphthylene gepotidacin (GSK2140944) against MDR *Neisseria gonorrhoeae*. *J Antimicrob Chemother* 2018; **73**: 2072–7.

32 Scangarella-Oman NE, Hossain M, Dixon PB *et al*. Microbiological analysis from a phase 2 randomized study in adults evaluating single oral doses of gepotidacin in the treatment of uncomplicated urogenital gonorrhea caused by *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 2018; **62**: e01221-18.

33 Taylor SN, Morris DH, Avery AK *et al*. Gepotidacin for the treatment of uncomplicated urogenital gonorrhea: a phase 2, randomized, dose-ranging, single-oral dose evaluation. *Clin Infect Dis* 2018; **67**: 504–12.

34 Cole MJ, Spiteri G, Jacobsson S *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.

35 Harris SR, Cole MJ, Spiteri G *et al*. Public health surveillance of multidrug-resistant clones of *Neisseria gonorrhoeae* in Europe: a genomic survey. *Lancet Infect Dis* 2018; **18**: 758–68.

36 Unemo M, Golparian D, Sánchez-Busó L *et al*. The novel 2016 WHO *Neisseria gonorrhoeae* reference strains for global quality assurance of laboratory investigations: phenotypic, genetic and reference genome characterization. *J Antimicrob Chemother* 2016; **71**: 3096–108.

37 Unemo M, Fasth O, Fredlund H *et al*. Phenotypic and genetic characterization of the 2008 WHO *Neisseria gonorrhoeae* reference strain panel intended for global quality assurance and quality control of gonococcal antimicrobial resistance surveillance for public health purposes. *J Antimicrob Chemother* 2009; **63**: 1142–51.

38 Hunt M, Mather AE, Sánchez-Busó L *et al*. ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. *Microb Genom* 2017; **3**: e000131.

39 Golparian D, Shafer WM, Ohnishi M *et al*. Importance of multidrug efflux pumps in the antimicrobial resistance property of clinical multidrug-resistant isolates of *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 2014; **58**: 3556–9.

40 Wi TE, Ndowa FJ, Ferreyra C *et al*. Diagnosing sexually transmitted infections in resource-constrained settings: challenges and ways forward. *J Int AIDS Soc* 2019; **22** Suppl 6: e25343.

41 Golparian D, Bazzo ML, Golfetto L *et al*. Genomic epidemiology of *Neisseria gonorrhoeae* elucidating the gonococcal antimicrobial resistance and lineages/sublineages across Brazil, 2015–16. *J Antimicrob Chemother* 2020; **75**: 3163–72.

42 Toskin I, Govender V, Blondeel K *et al*. Call to action for health systems integration of point-of-care testing to mitigate the transmission and burden of sexually transmitted infections. *Sex Transm Infect* 2020; **96**: 342–7.

43 Ferreyra C, Osborn J, Moussy F *et al*. Developing target product profiles for *Neisseria gonorrhoeae* diagnostics in the context of antimicrobial resistance: an expert consensus. *PLoS One* 2020; **15**: e0237424.

44 Donà V, Low N, Golparian D *et al*. Recent advances in the development and use of molecular tests to predict antimicrobial resistance in *Neisseria gonorrhoeae*. *Expert Rev Mol Diagn* 2017; **17**: 845–59.

45 Drusano GL, Shields RK, Mtchedlidze N *et al*. Pharmacodynamics of ceftazidime plus avibactam against KPC-2-bearing isolates of *Klebsiella pneumoniae* in a hollow fiber infection model. *Antimicrob Agents Chemother* 2019; **63**: e00462-19.