

In Vitro Activity of Zoliflodacin against Contemporary *Neisseria gonorrhoeae* Isolates from the United States

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Disclosures

- Sarah McLeod and Samir Moussa are current employees of Innoviva Specialty Therapeutics, Inc., an affiliate of Entasis Therapeutics Inc.
- John Mueller is a former employee of Innoviva Specialty Therapeutics, Inc.
- Carmen Au and Suba Srinivasan are current or former employees of the Global Antibiotic Research & Development Partnership (GARDP), a non-profit Swiss-based foundation, which receives government and private-funded institutional grants/research support
- Nachum Kaplan is a consultant for GARDP
- Meredith Hackel is an employee of IHMA, Inc. and has nothing to disclose

Gonorrhea is a Growing Global Public Health Concern

Gonorrhea is the second most prevalent bacterial sexually transmitted infection¹

CDC and WHO have raised alerts regarding ceftriaxone-resistant *Neisseria gonorrhoeae*^{2,3}



U.S. Department of
Health and Human Services
Centers for Disease
Control and Prevention



RAPID COMMUNICATION

Extensively drug-resistant (XDR) *Neisseria gonorrhoeae* causing possible gonorrhoea treatment failure with ceftriaxone plus azithromycin in Austria, April 2022

1. Workowski KA, et al. MMWR Recomm Rep. 2021 Jul 23;70(4):1-187.

2. World Health Organization. Gonorrhoea (*Neisseria gonorrhoeae* infection) Fact Sheet. [https://www.who.int/news-room/fact-sheets/detail/gonorrhoea-\(neisseria-gonorrhoeae-infection\)](https://www.who.int/news-room/fact-sheets/detail/gonorrhoea-(neisseria-gonorrhoeae-infection))

3. U.S. Department of Health and Human Services and Centers for Disease Control and Prevention. Antibiotic Resistance Threats In The United States 2019. <https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf>

4. Pleininger S, et al. Euro Surveill. 2022;27(24):pii=2200455.

5. Mosbergen D. Wall Street Journal, January 29, 2023. <https://www.wsj.com/articles/new-superbug-strain-of-gonorrhea-is-outsmarting-most-antibiotics-11674947446>

6. Bergot B, et al. Euro Surveill. 2022 Dec;27(50):2200899

Resistance has expanded to include penicillin, tetracyclines, macrolides (including azithromycin), sulfonamides, and trimethoprim combinations, quinolones and, more recently, cephalosporins within a few isolated strains.

-World Health Organization 2018

HEALTH

New 'Superbug' Strain of Gonorrhea Is Outsmarting Most Antibiotics

Two cases in Massachusetts involve a novel strain more impervious to existing antibiotics than other strains in the U.S.

2023

RAPID COMMUNICATION

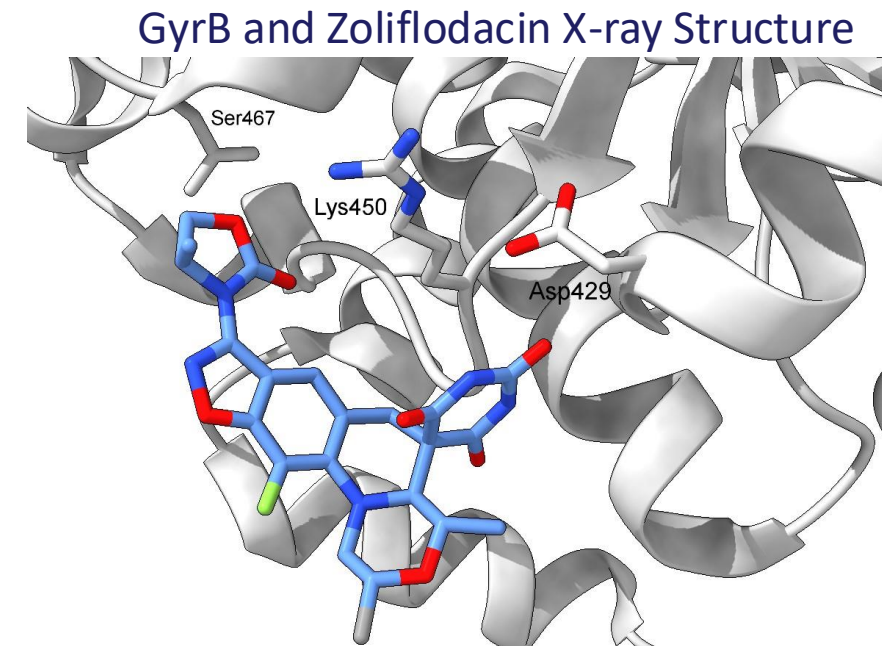
Ceftriaxone-resistant, multidrug-resistant *Neisseria gonorrhoeae* with a novel mosaic *penA-237.001* gene, France, June 2022

Zoliflodacin: For Treatment of Uncomplicated Gonorrhea

Development through a novel public-private partnership

Zoliflodacin is a first-in-class, single-dose, oral spiropyrimidinetrione antibiotic

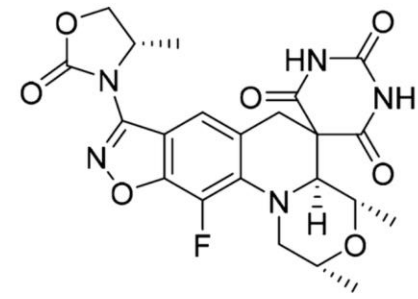
- Inhibits DNA replication with bactericidal activity:
 - Unique mode of inhibition resulting in no cross-resistance to antibiotics used to treat gonorrhea
 - Inhibits type II topoisomerases resulting in accumulation of double-stranded chromosomal breaks
 - Primary target is GyrB subunit of DNA gyrase in a distinct site from fluoroquinolones which target the GyrA subunit
- Potent in vitro activity against *Neisseria gonorrhoeae* with low propensity to develop resistance in vitro
- Achieved primary efficacy endpoint in a recent, global Phase 3 non-inferiority trial for the treatment of uncomplicated gonorrhea



PDB ID 8BP2: Morgan H. *et al.* Int J Mol Sci. 2023, 24(2), 1634

Study Design: Assessing Zoliflodacin In Vitro Activity

Recent clinical isolates of *Neisseria gonorrhoeae*



200 *N. gonorrhoeae* clinical isolates collected in USA from 2020-2021

- Isolates were provided by the CDC as part of the CDC Gonococcal Isolate Surveillance Project (GISP)
 - 100 *N. gonorrhoeae* collected in 2020
 - 100 *N. gonorrhoeae* collected in 2021
- Most isolates were sourced from male patients from urogenital body sites
- Susceptibility was determined by agar dilution by IHMA using CLSI methodology
 - Tested the following antibacterial agents:

○ Zoliflodacin	○ Ceftriaxone	○ Azithromycin
○ Ciprofloxacin	○ Cefixime	○ Penicillin
○ Gentamicin	○ Tetracycline	○ Spectinomycin

Zoliflodacin Displays Potent In Vitro Activity

200 clinical isolates of *N. gonorrhoeae* collected in 2020-2021

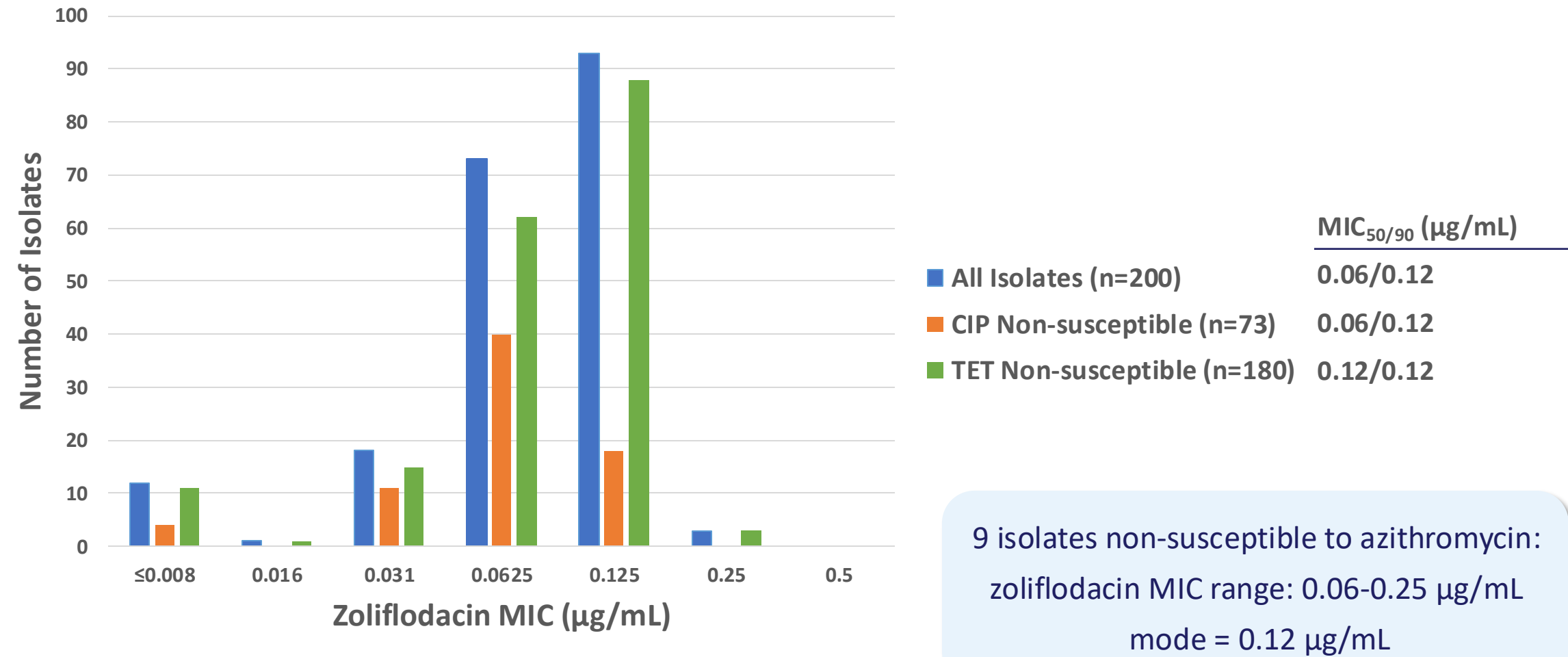
Antibiotic	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)	Range (µg/mL)	% Susceptible*
Zoliflodacin	0.06	0.12	≤0.008 - 0.25	NA
Ceftriaxone	0.008	0.015	≤0.002 – 0.06	100
Azithromycin	0.25	1	≤0.06 - >8	96.5
Ciprofloxacin	0.004	>2	0.002 - >2	63.5
Cefixime	0.015	0.03	≤0.002 – 0.12	100
Penicillin	0.5	>2	≤0.12 – >2	0
Gentamicin	8	8	2 - 16	NA
Tetracycline	0.5	2	≤0.12 – >4	10
Spectinomycin	16	32	≤4 - 32	100

- Zoliflodacin activity consistent with previous studies
- 2020 and 2021 results very similar
- All isolates susceptible to ceftriaxone
- High rate of susceptibility to azithromycin
- High rates of resistance to tetracycline and ciprofloxacin

*Based on CLSI interpretive criteria; M100 Ed. 34

Zoliflodacin Retains Activity against Antibiotic-Resistant Isolates

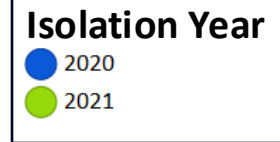
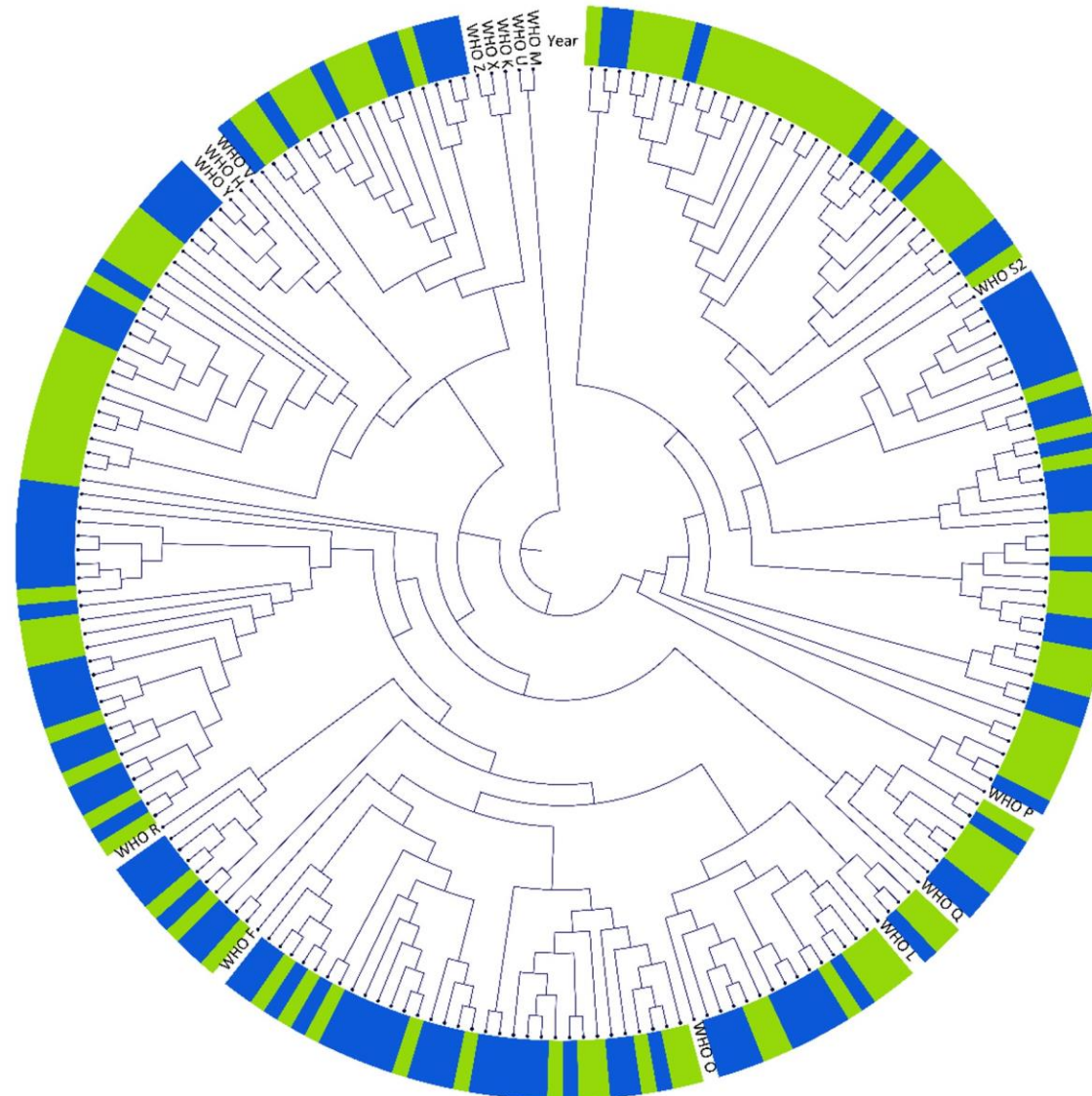
Ciprofloxacin-, tetracycline-, and azithromycin-non-susceptible *N. gonorrhoeae* isolates



Genetic Diversity of *N. gonorrhoeae* Isolates

Comparing isolates to the WHO reference strains

- High degree of genetic diversity among isolates

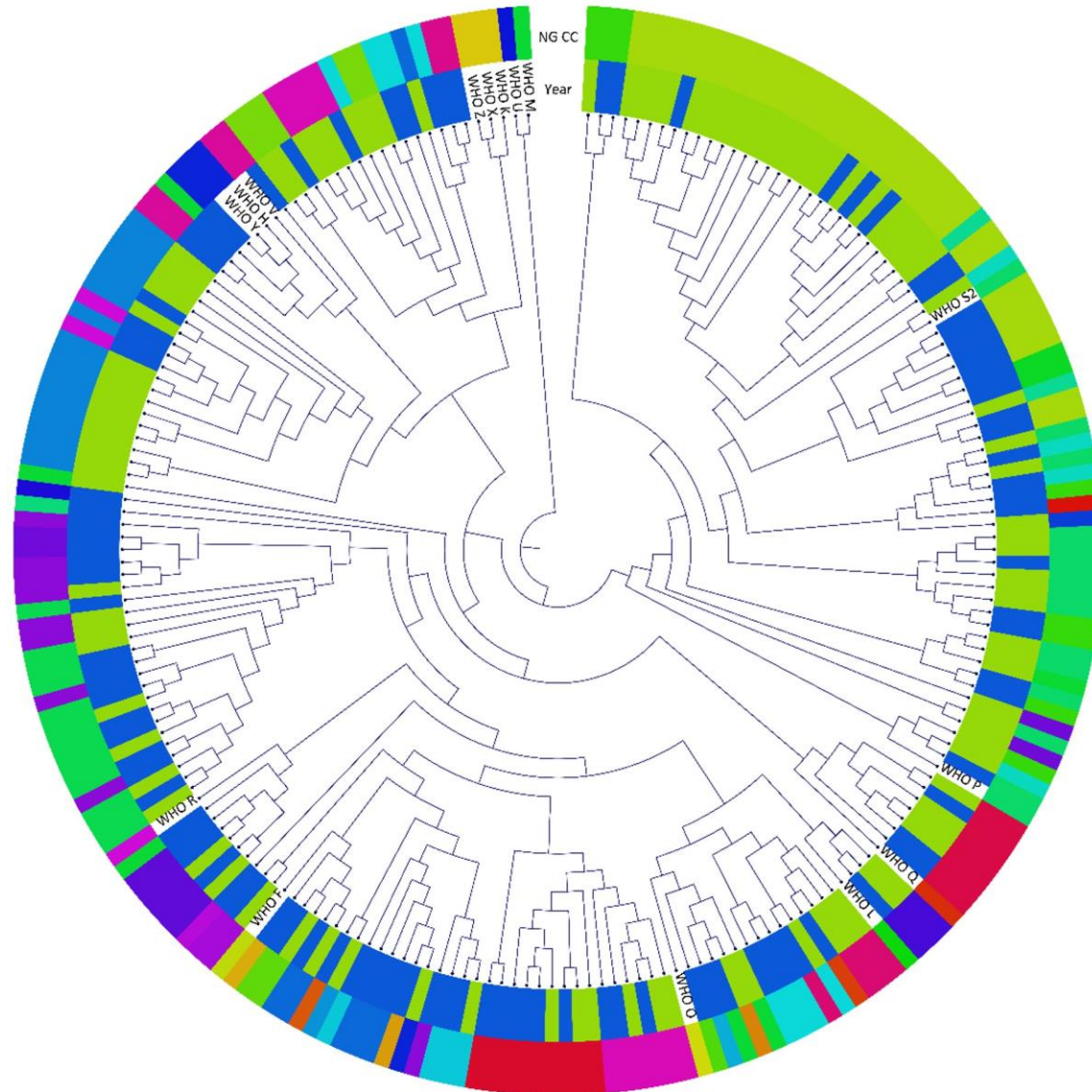


Genetic Diversity of *N. gonorrhoeae* Isolates

Comparing isolates to the WHO reference strains

- High degree of genetic diversity among isolates

- Large number of clonal complexes represented



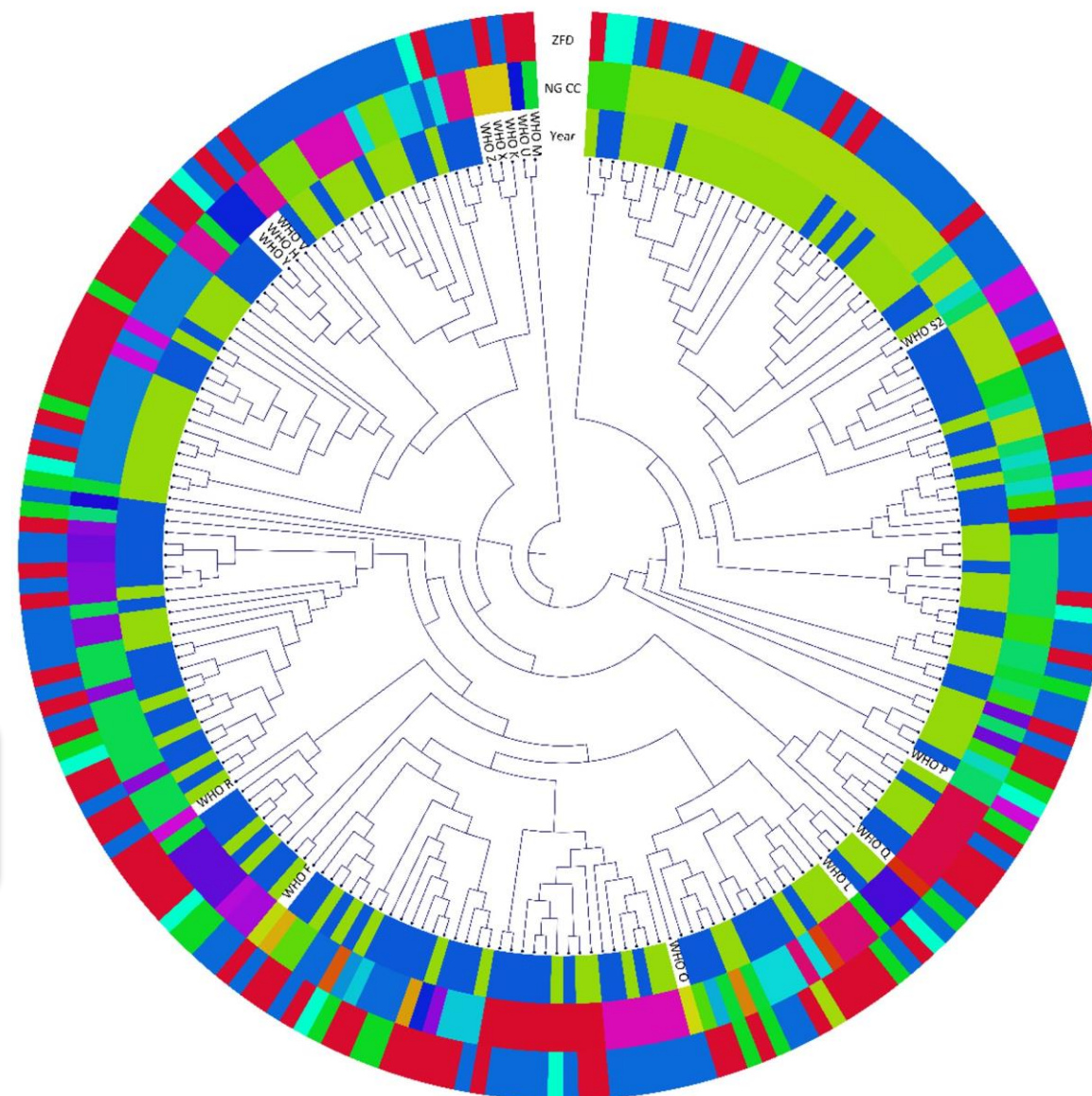
NG-STAR Clonal Complex

20	26
55	63
73	90
116	127
139	153
158	168
178	190
193	199
213	231
239	301
307	309
348	426
427	429
439	442
520	845
1096	1115
1229	1340
1387	1401
1771	1902
1958	2047
2351	3035
3087	3111
3420	novel

Genetic Diversity of *N. gonorrhoeae* Isolates

Comparing isolates to the WHO reference strains

- High degree of genetic diversity among isolates
- Large number of clonal complexes represented
- No evidence of bias in zoliflodacin MICs relative to year or clonal complex

Zoliflodacin MIC
($\mu\text{g/mL}$)

● <0.008

● 0.015

● 0.03

● 0.06

● 0.12

0.25

Zoliflodacin is Active against Two Ceftriaxone-Resistant Isolates

Neisseria gonorrhoeae clinical isolates encoding *penA* allele 60.001

AR Bank Isolate*	MLST	Year	Source	MIC (µg/mL)					
				ZFD	CTR	AZI	CIP	TET	FIX
AR-1280	1901	2019	Urethral Swab	0.12	1	0.5	>1	4	>1
AR-1281	8123	2022	Urine	0.25	1	4	>1	>8	>1

*CDC & FDA Antibiotic Resistance Isolate Bank. Atlanta, GA

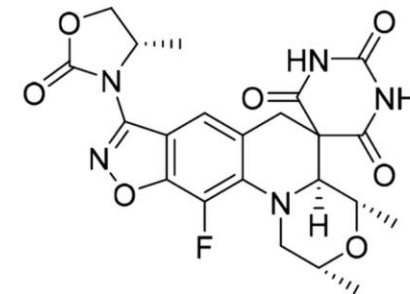
Surveillance Isolates Similar to Isolates from Phase 3 Trial

200 *N. gonorrhoeae* from US surveillance compared to 936 baseline isolates from global phase 3 trial

Antibiotic	US Surveillance		Phase 3 Trial	
	N	MIC ₅₀ /MIC ₉₀ (µg/mL)	N	MIC ₅₀ /MIC ₉₀ (µg/mL)
Zoliflodacin	200	0.06/0.12	936	0.06/0.12
Ceftriaxone	200	0.008/0.015	936	0.004/0.015
Azithromycin	200	0.25/1	936	0.12/1
Ciprofloxacin	200	0.004/>2	936	2/>2
Cefixime	200	0.015/0.03	936	0.008/0.03
Penicillin	200	0.5/>2	NA	NA
Gentamicin	200	8/8	936	8/8
Tetracycline	200	0.5/2	936	>4/>4
Spectinomycin	200	16/32	936	32/32

- Antibiotic susceptibility of *N. gonorrhoeae* from Phase 3 trial are similar to isolates from US surveillance
- In Phase 3 trial, zoliflodacin met the primary efficacy endpoint of non-inferiority compared to CTR-AZI for uncomplicated urogenital gonorrhea
- High rates of microbiological cure were observed for zoliflodacin vs urogenital, pharyngeal and rectal gonorrhea

Summary



- Zoliflodacin is an oral, single-dose antibiotic being developed for patients with uncomplicated gonorrhea, including those infected with MDR strains
- Zoliflodacin is a first-in-class spiropyrimidinetrione that inhibits DNA synthesis by targeting type II topoisomerases
- Against 200 clinical isolates of *Neisseria gonorrhoeae* collected in 2020 and 2021, all zoliflodacin MICs were within the wild-type distribution ($\text{MIC}_{50/90} = 0.06/0.12 \mu\text{g/mL}$)
- No evidence of cross resistance to other antibacterials used to treat gonorrhea, including ceftriaxone, azithromycin, ciprofloxacin and tetracycline
- These data complement the recent Phase 3 results and support the continued development of zoliflodacin for patients with uncomplicated gonorrhea

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- IHMA, Inc.
- CDC: Gonorrhea Team / GISP Project, and AR Isolate Bank
- NIAID Team