



Developments in Antimicrobial Susceptibility Testing of gonococci

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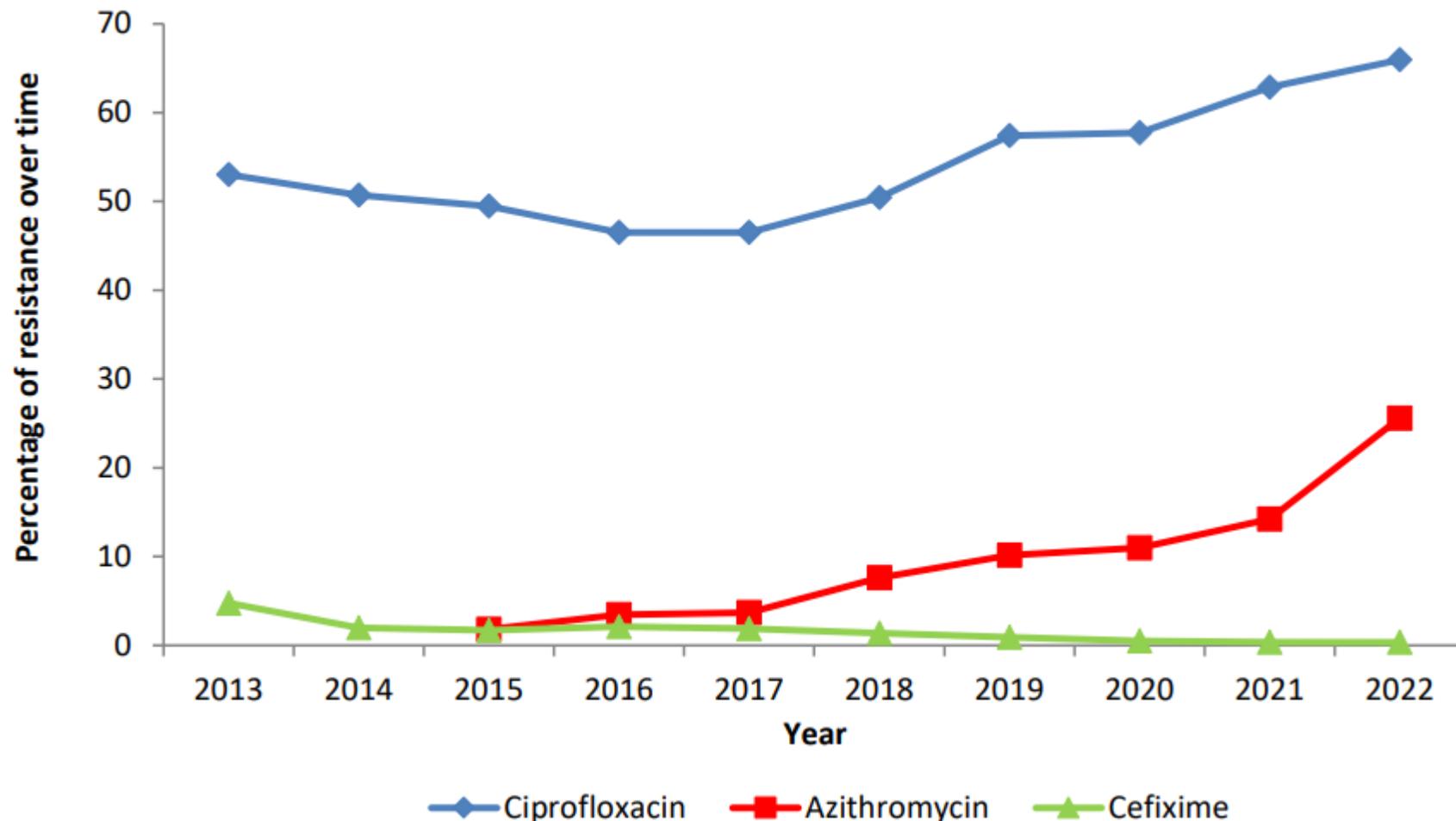
EUCAST Development Laboratory, Växjö, Sweden

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Neisseria gonorrhoeae

- The sexually transmitted disease gonorrhoea, caused by *Neisseria gonorrhoeae*, is a global health threat and is prioritized among threats against public health listed by WHO.
- Antimicrobial resistance to commonly used antimicrobial agents, including third-generation cephalosporins, is increasing worldwide.
- Most common treatment options
 - Ceftriaxone monotherapy
 - Ceftriaxone + azithromycin
 - Azithromycin monotherapy
 - Azithromycin + cefixime

Figure 1. Percentage of resistant *Neisseria gonorrhoeae* by antimicrobial and year, Euro-GASP, 2013–2022



Recent data from Asia

Ceftriaxone resistance:

- Japan: 7.9%
- China: 8.1%
- Cambodia: 18%
- Vietnam: 27%

(M. Unemo)

New treatment options?

- Zoliflodacin, a topoisomerase II inhibitor (similar to fluoroquinolones)
- Gepotidacin, a triazaacenaphthylene antibacterial that inhibits bacterial DNA gyrase and topoisomerase IV
- Good *in vitro* activity

New treatment options?

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

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

^aMICs were determined using the agar dilution technique for zoliflodacin and Etest for the other antimicrobials.

^bS, 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 $\mu\text{g/ml}$) was used to distinguish isolates with azithromycin resistance determinants (31).

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Antimicrobial susceptibility testing (AST)

- *N. gonorrhoeae* is a fastidious organism that needs rich media for cultivation and AST
- **CLSI:** Agar dilution and disk diffusion on GC agar with 1% defined growth supplement
- **EUCAST:** Disk diffusion criteria for antimicrobial susceptibility testing of *Neisseria gonorrhoeae* have not yet been defined and an MIC method should be used. If a commercial MIC method is used, follow the manufacturer's instructions. Laboratories with few isolates are encouraged to refer these to a reference laboratory for testing.
- **WHO** accepts MICs with gradient tests (various media)

Disk diffusion of *N. gonorrhoeae*?

Requirements for a reproducible and reliable disk diffusion method

- Standardised medium, available from several manufacturers, that supports good growth of *N. gonorrhoeae*
 - Poor standardisation between manufacturers for the CLSI-recommended medium
 - MH-F does not support sufficient growth of *N. gonorrhoeae*
- Reproducible results with repeated testing
- Good correlation between zone diameters and reference MICs
- Separation between susceptible and resistant isolates

Evaluation of *N. gonorrhoeae* disk diffusion

- Isolates (n=112) were provided by
 - Robert Koch Institute, Berlin, Germany,
 - Hôpital Saint-Louis, Paris, France
 - NRL Neisseria, Örebro Sweden (Magnus Unemo)
- Disk diffusion was performed on an experimental medium prepared from agar from two manufacturers (A and B)
- Growth was assessed visually for all tests
- Reproducibility was investigated by repeated testing of *N. gonorrhoeae* ATCC 49226
- Zone diameters for clinical isolates were correlated to MICs provided by the original investigators (mainly gradient test MICs)

Growth

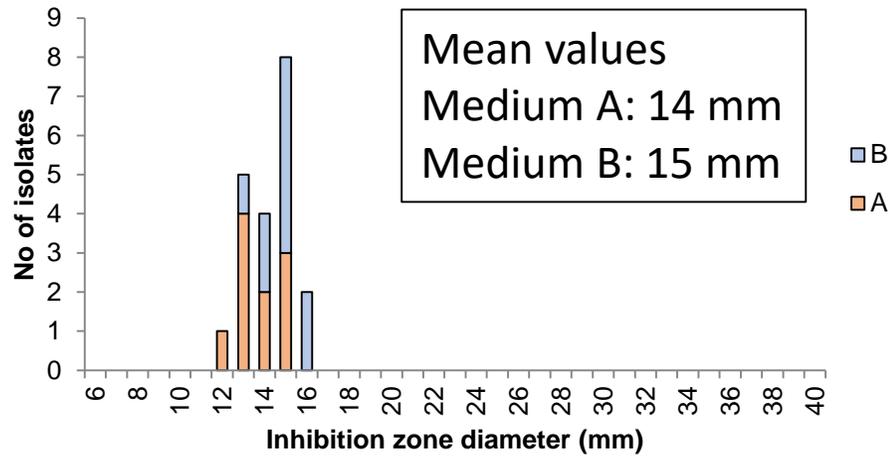
Growth	Manufact. A	Manufact. B	TOTAL (no)	TOTAL (%)
Lighter than confluent	3	8	11	4.9
Almost confluent	4	13	17	7.6
Confluent	28	52	80	35.7
Rich confluent	77	39	116	51.8

Confluent (or almost confluent) growth for 95% of the tests

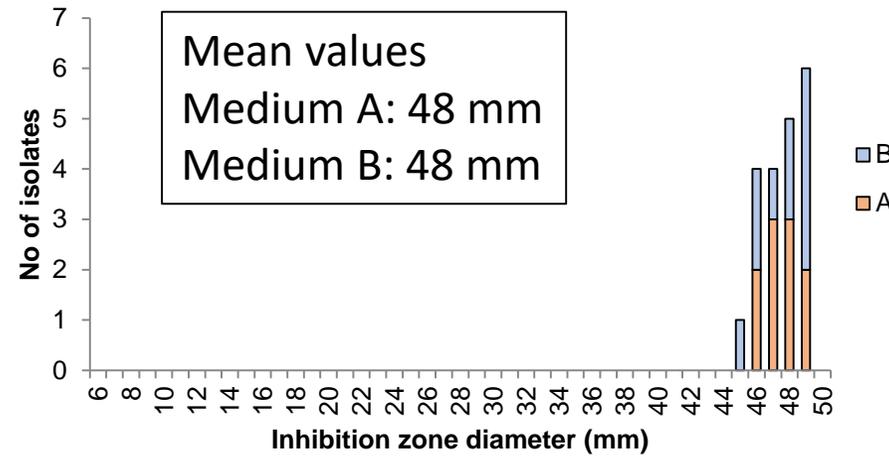
Reproducibility *N. gonorrhoeae* ATCC 49226 per manufacturer

Data from tests performed in parallel with clinical isolates

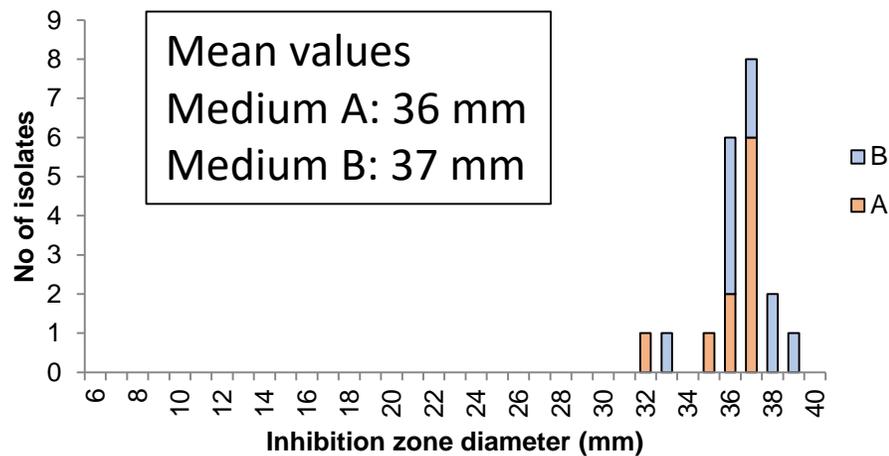
Benzylpenicillin 1 unit



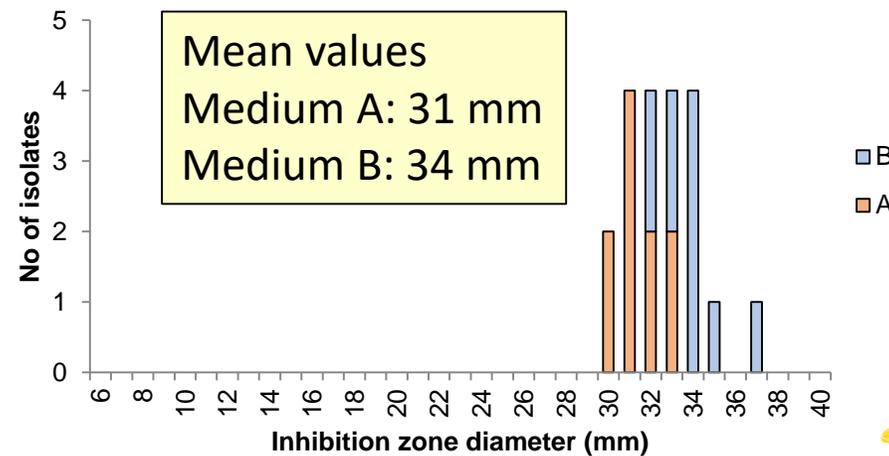
Ciprofloxacin 5 µg



Cefixime 5 µg

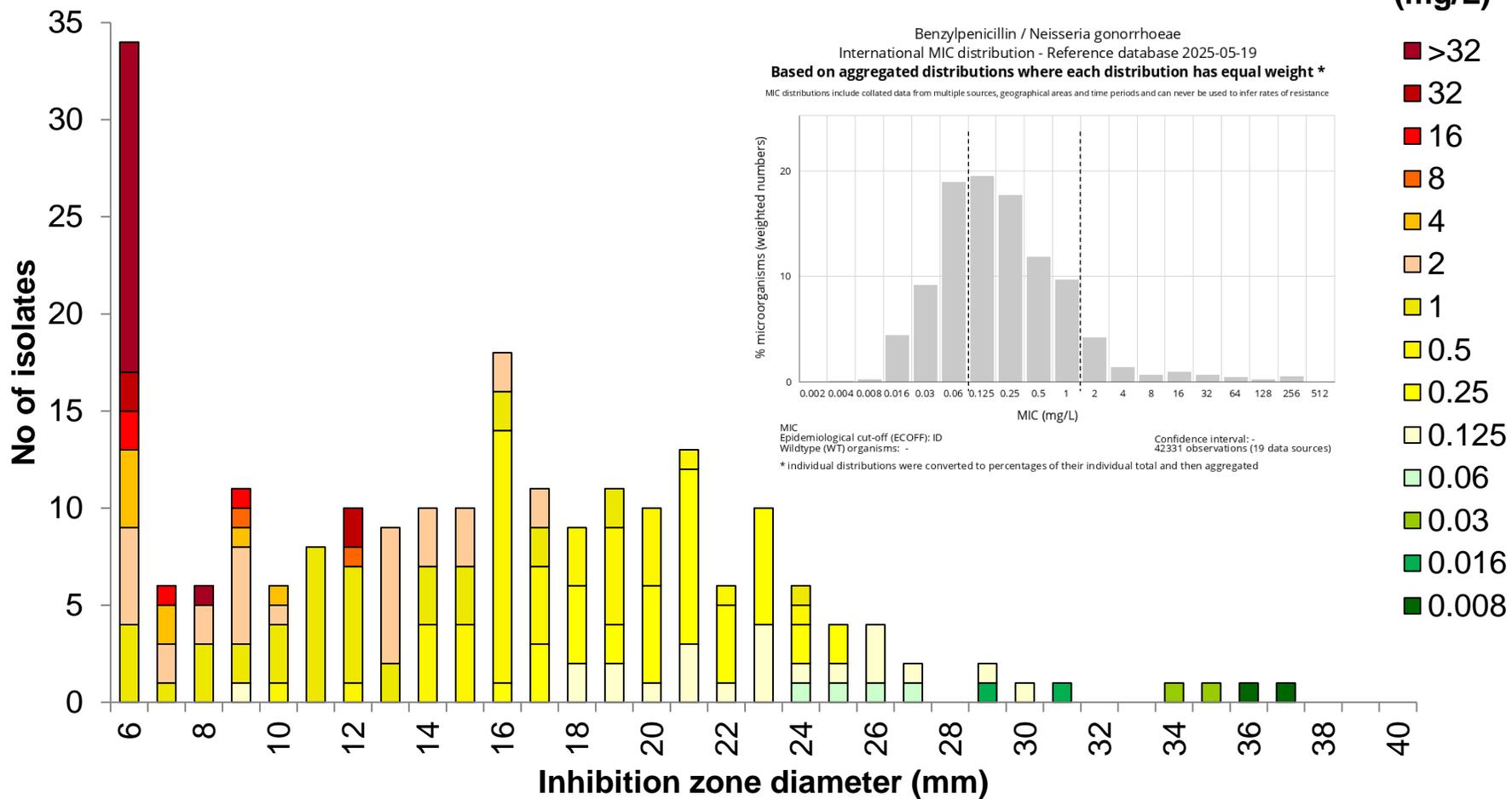


Azithromycin 15 µg

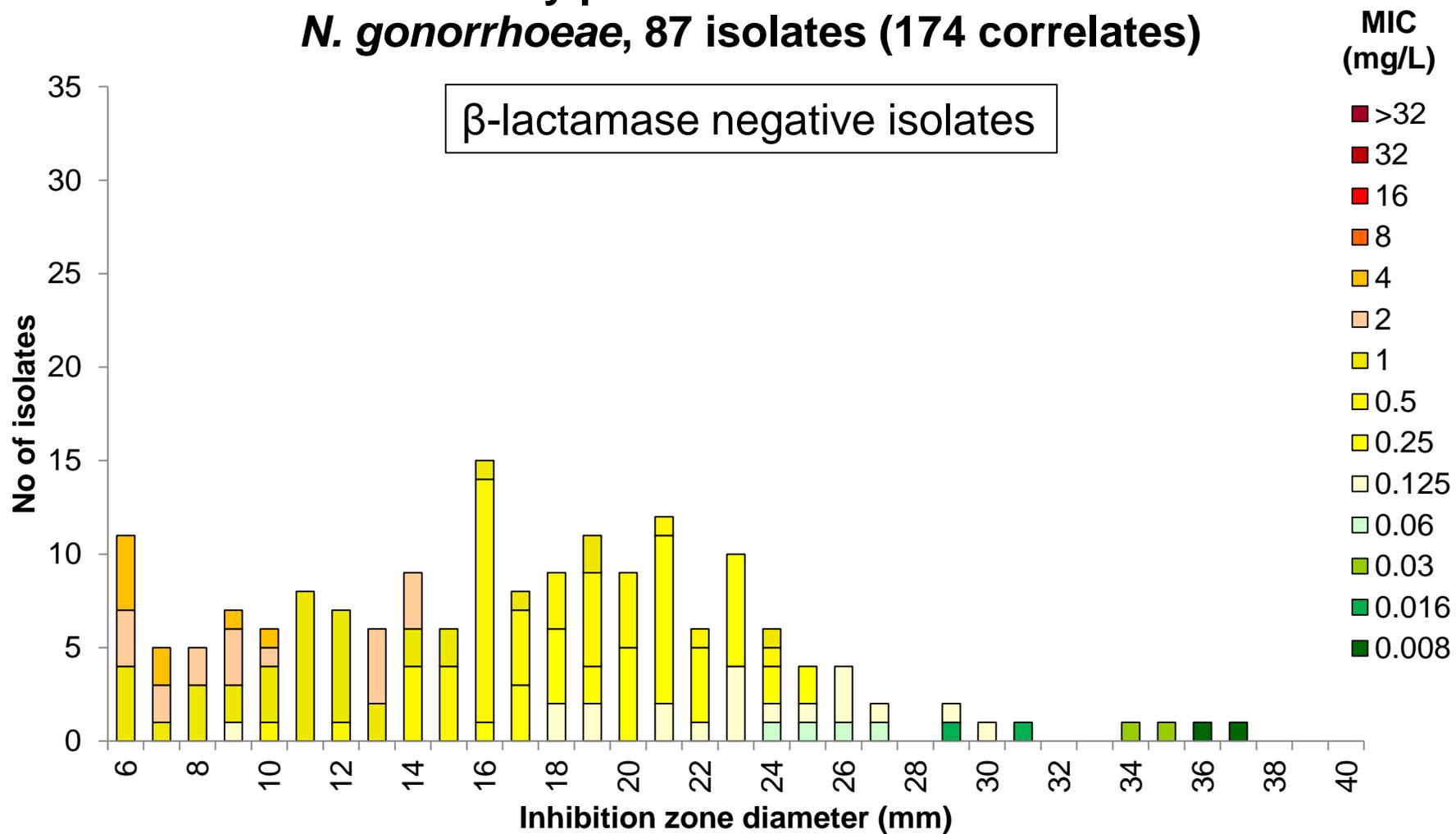


Benzylpenicillin 1 unit vs. MIC

N. gonorrhoeae, 111 isolates (222 correlates)

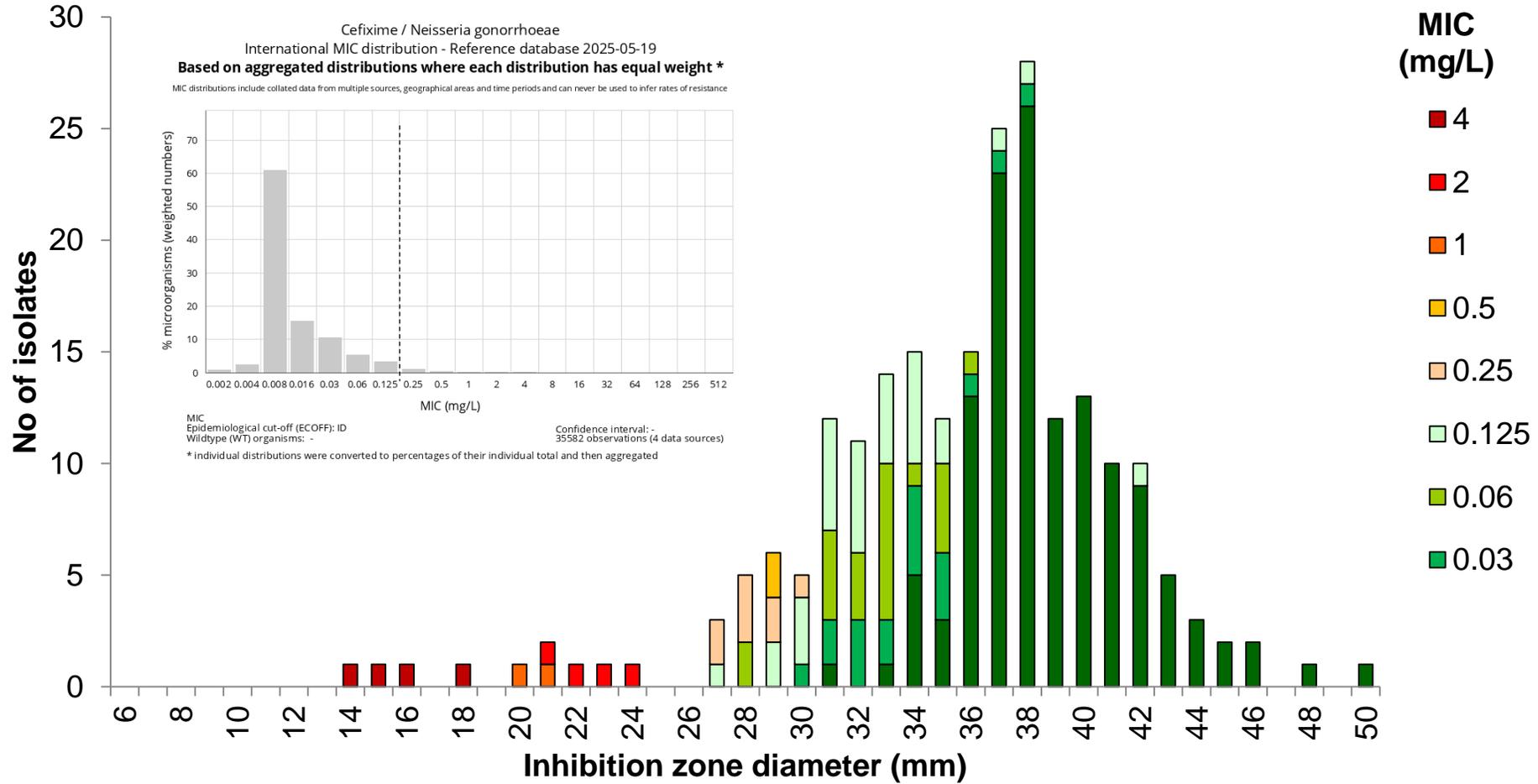


Benzylpenicillin 1 unit vs. MIC *N. gonorrhoeae*, 87 isolates (174 correlates)



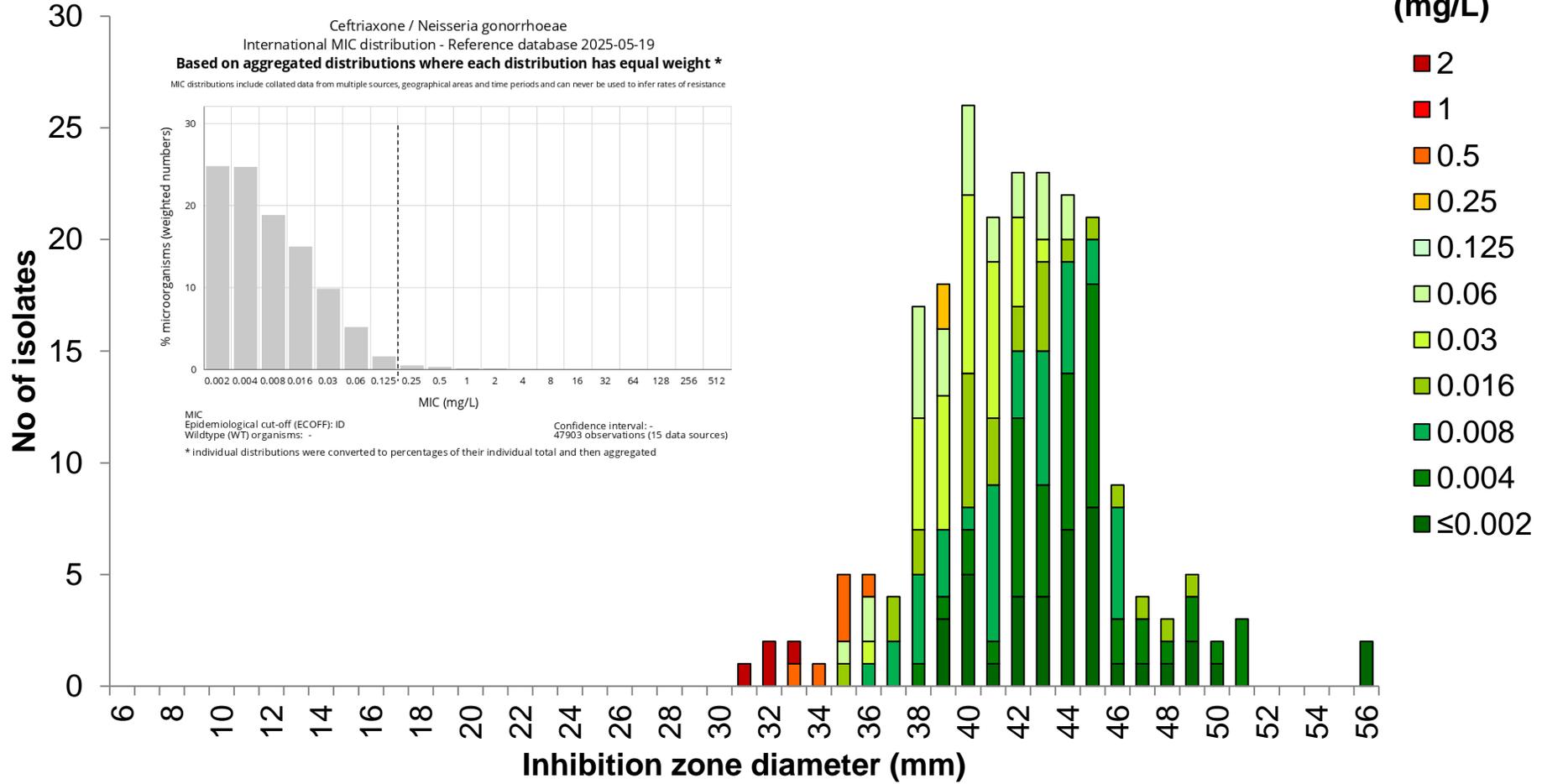
Cefixime 5 µg vs. MIC

N. gonorrhoeae, 110 isolates (220 correlates)

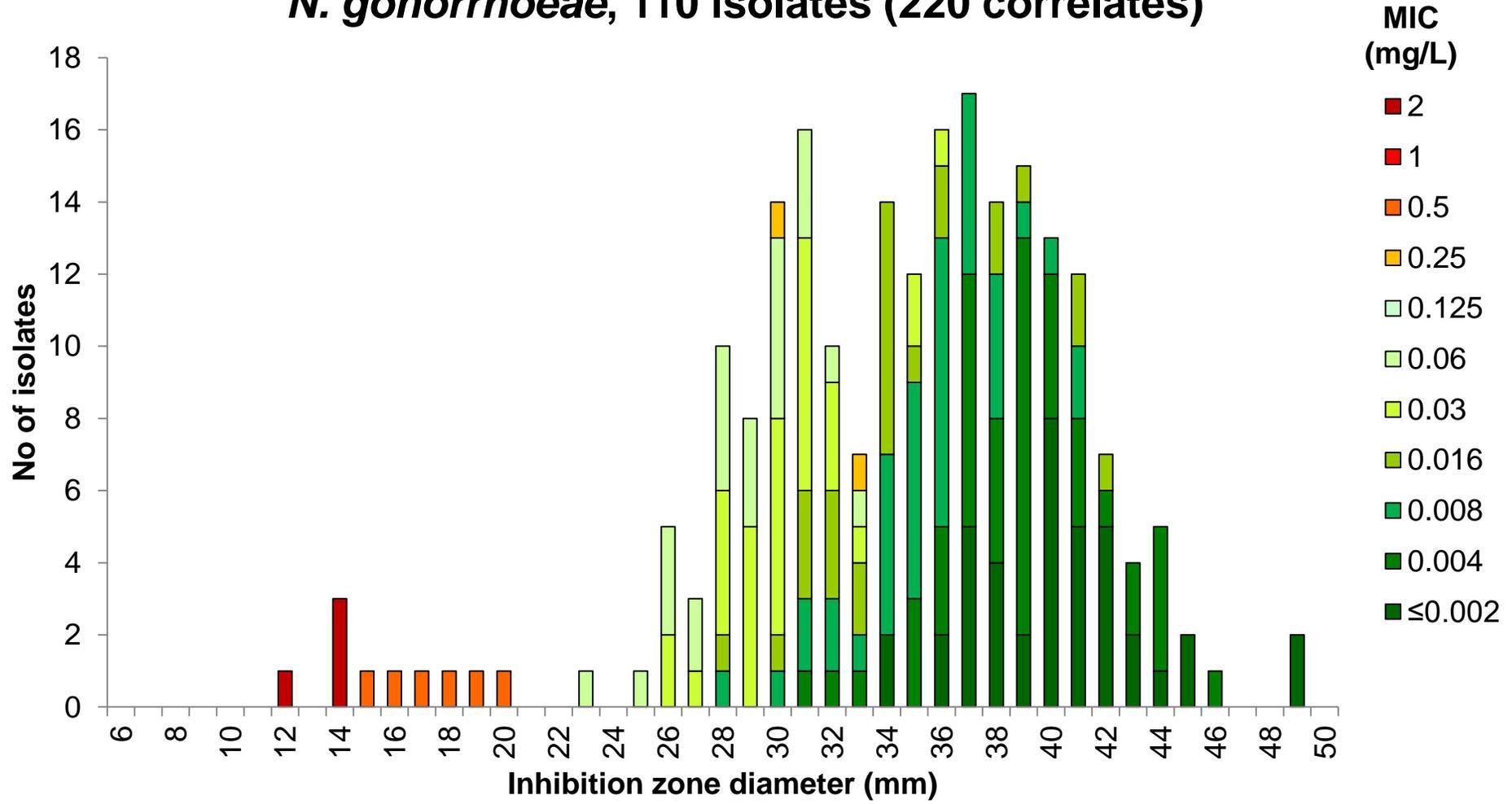


Ceftriaxone 30 µg vs. MIC

N. gonorrhoeae, 110 isolates (220 correlates)

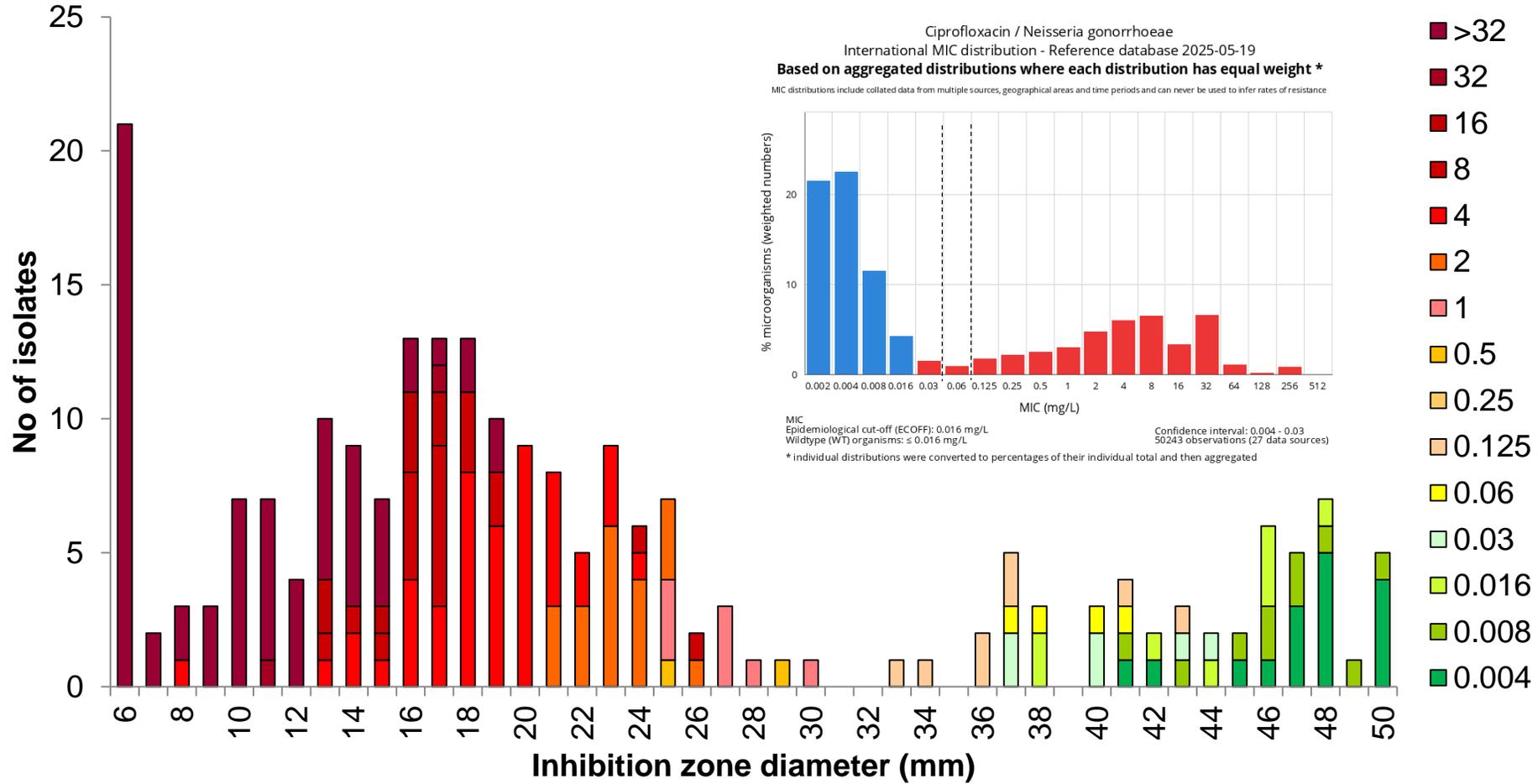


Cefotaxime 5 µg vs. ceftriaxone MIC
N. gonorrhoeae, 110 isolates (220 correlates)

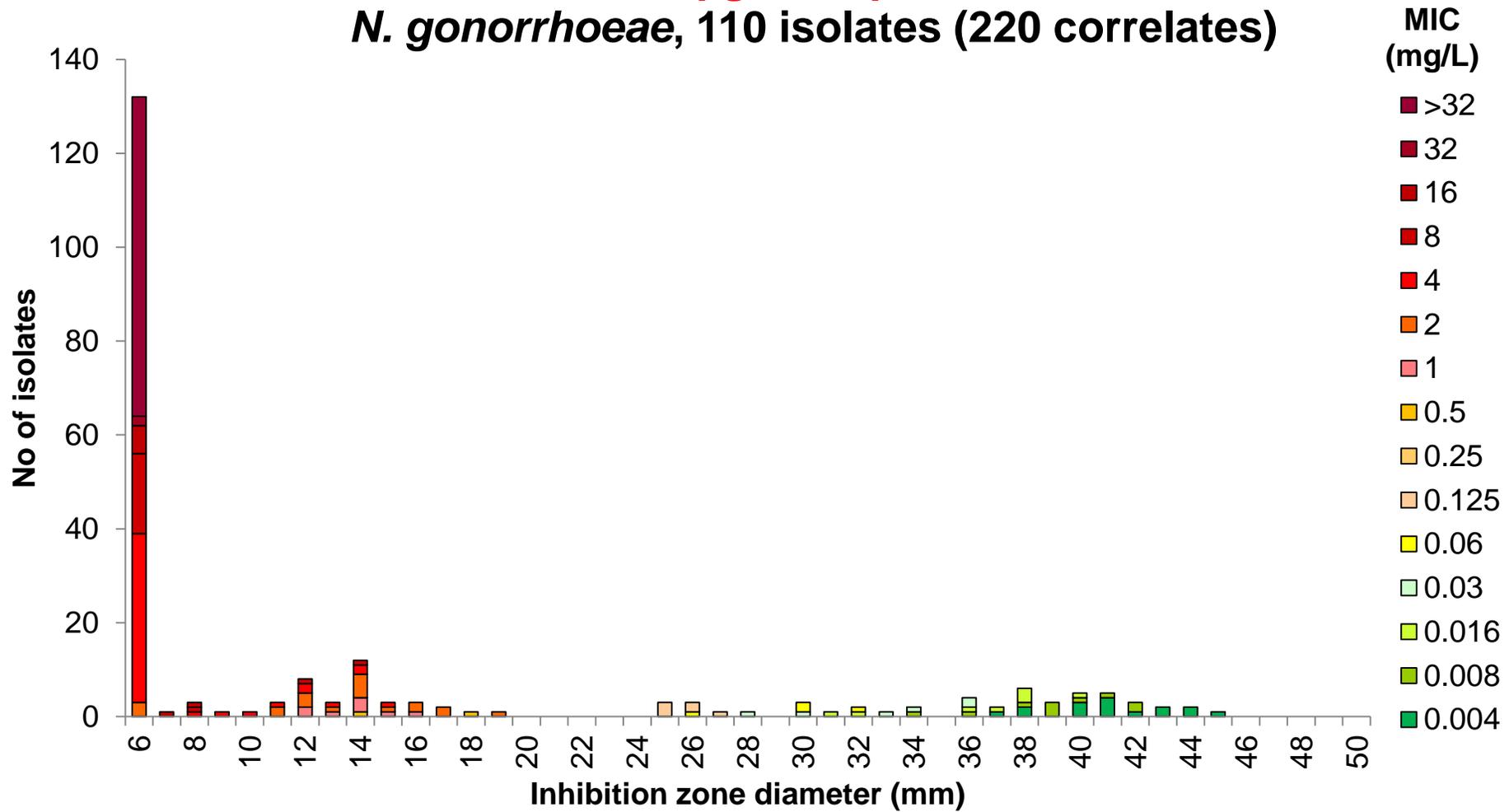


Ciprofloxacin 5 µg vs. MIC

N. gonorrhoeae, 110 isolates (220 correlates)

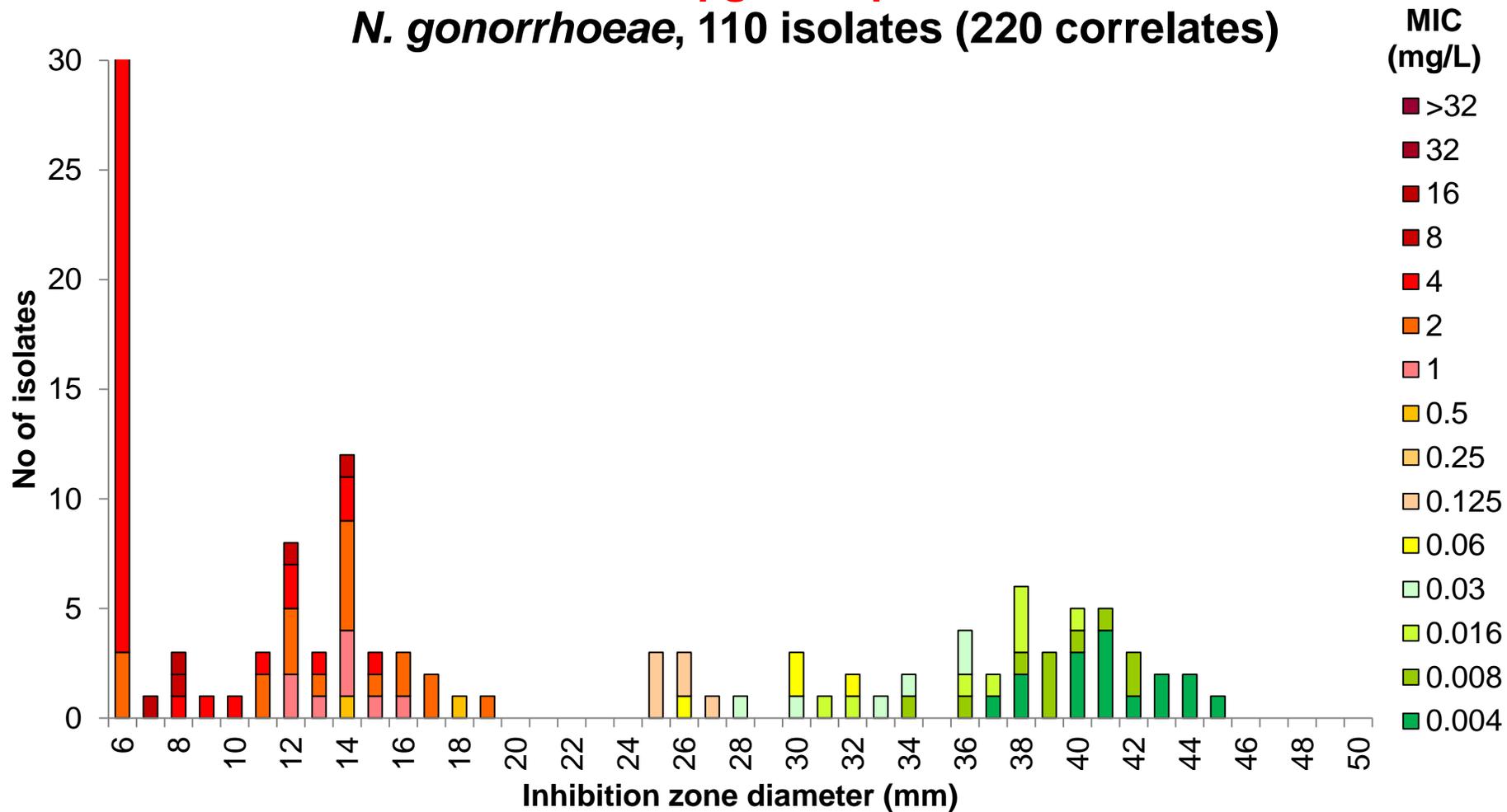


Pefloxacin 5 µg vs. ciprofloxacin MIC
N. gonorrhoeae, 110 isolates (220 correlates)



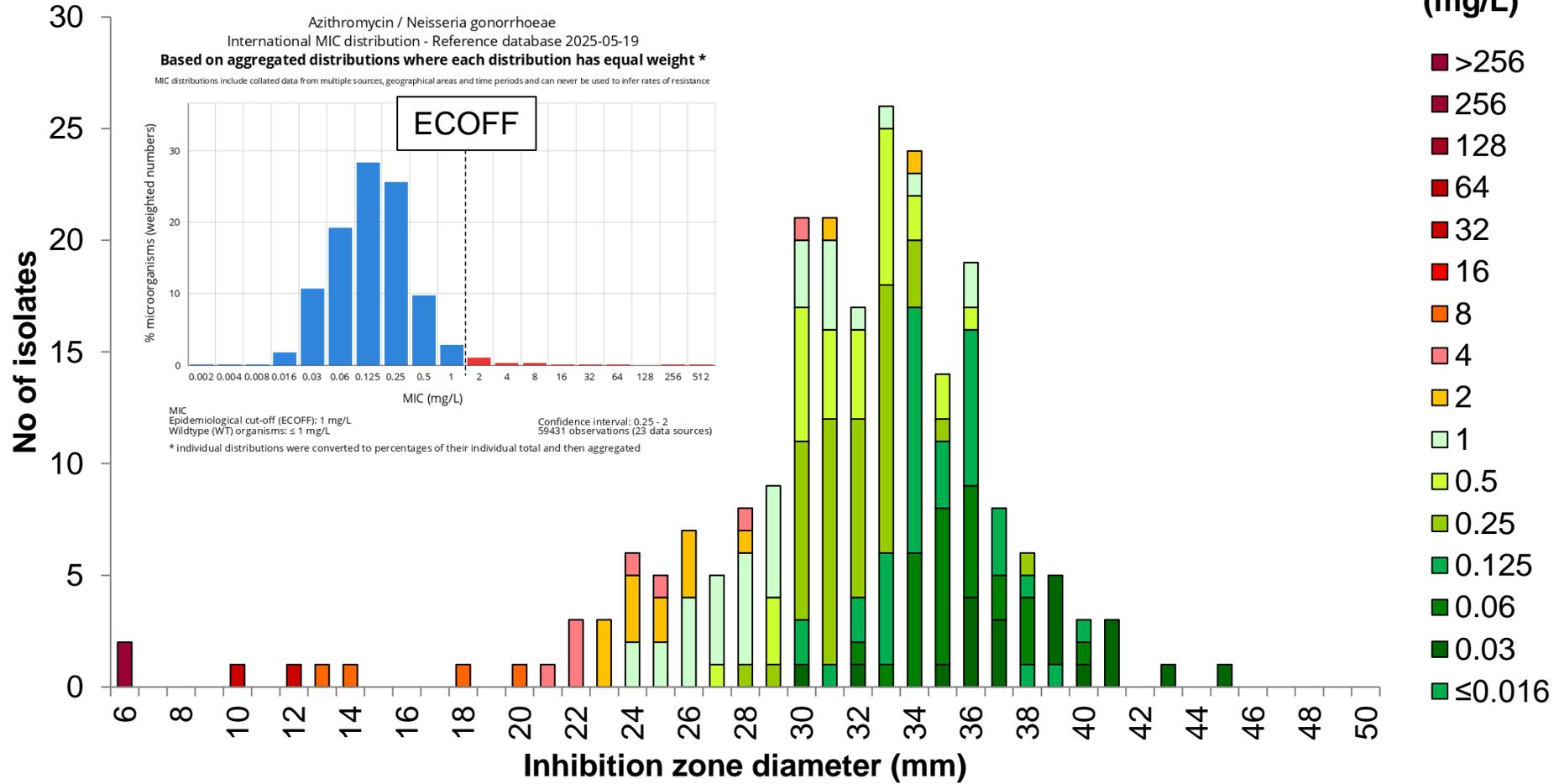
Reduced y axis

Pefloxacin 5 µg vs. ciprofloxacin MIC
***N. gonorrhoeae*, 110 isolates (220 correlates)**



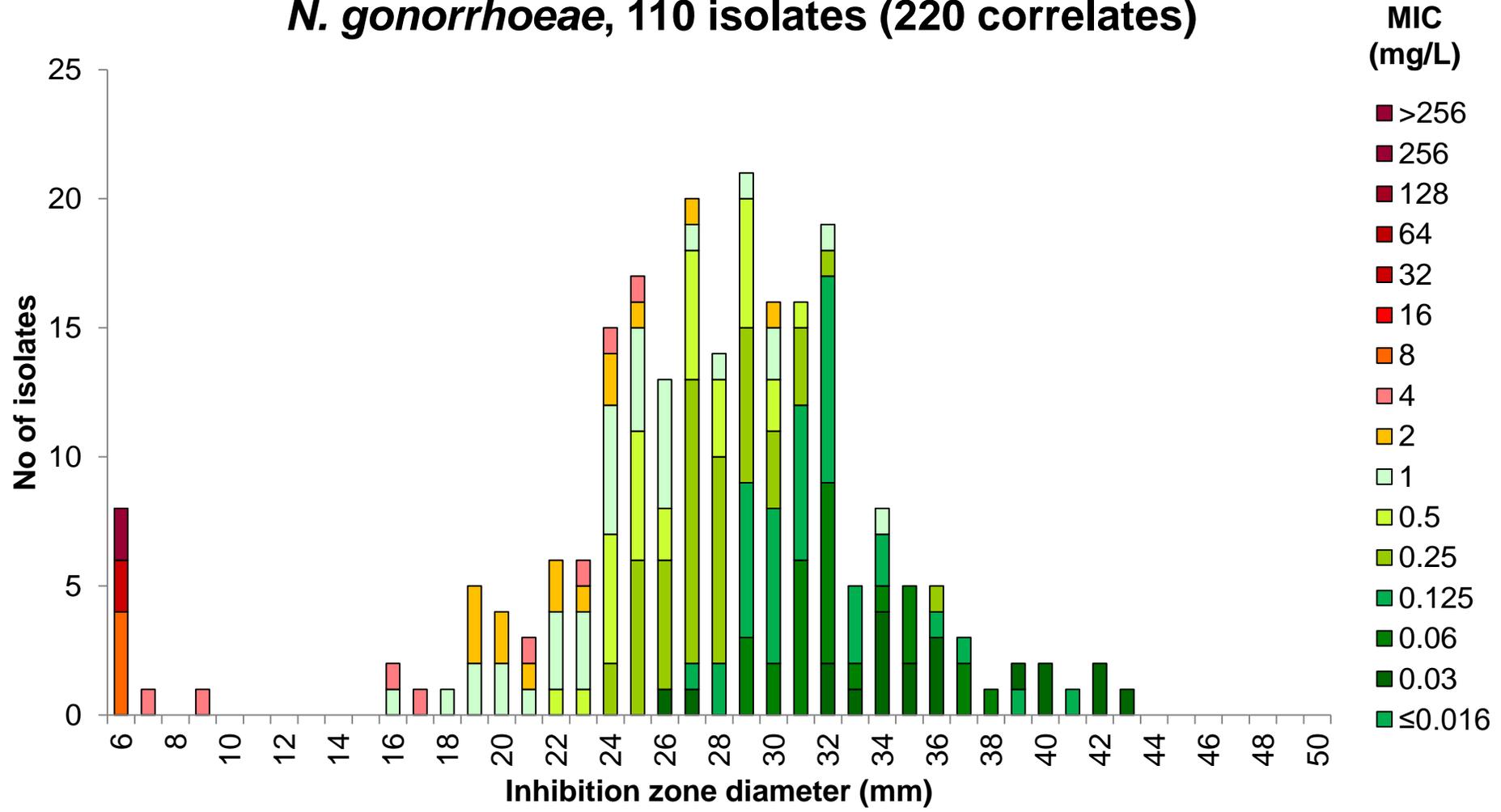
Azithromycin 15 µg vs. MIC

N. gonorrhoeae, 110 isolates (220 correlates)

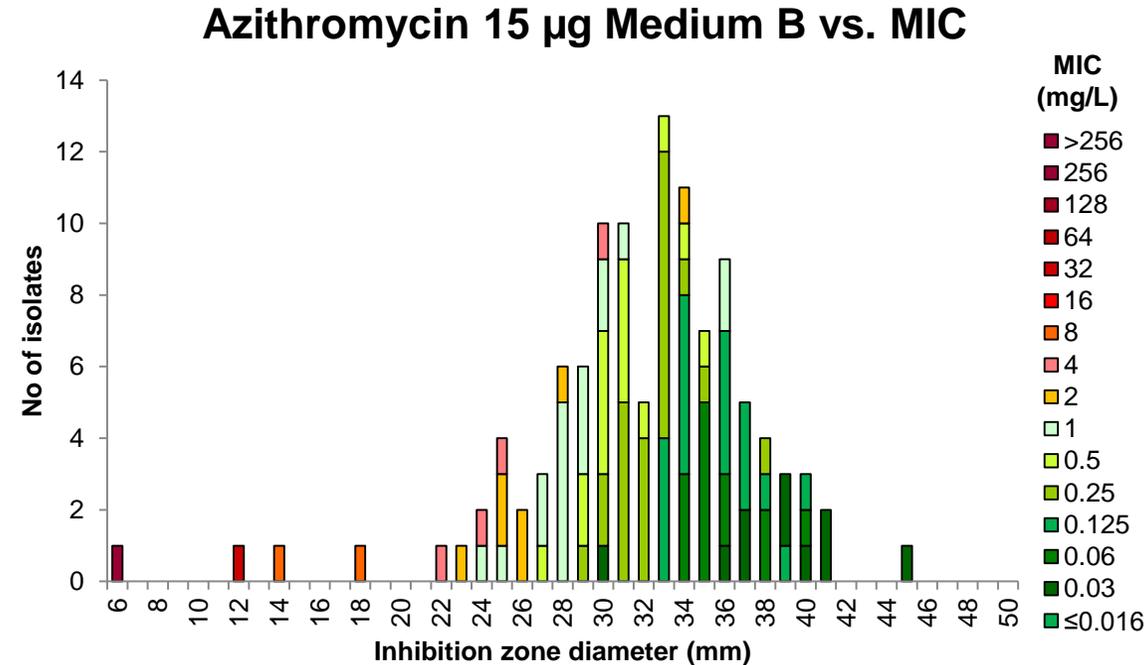
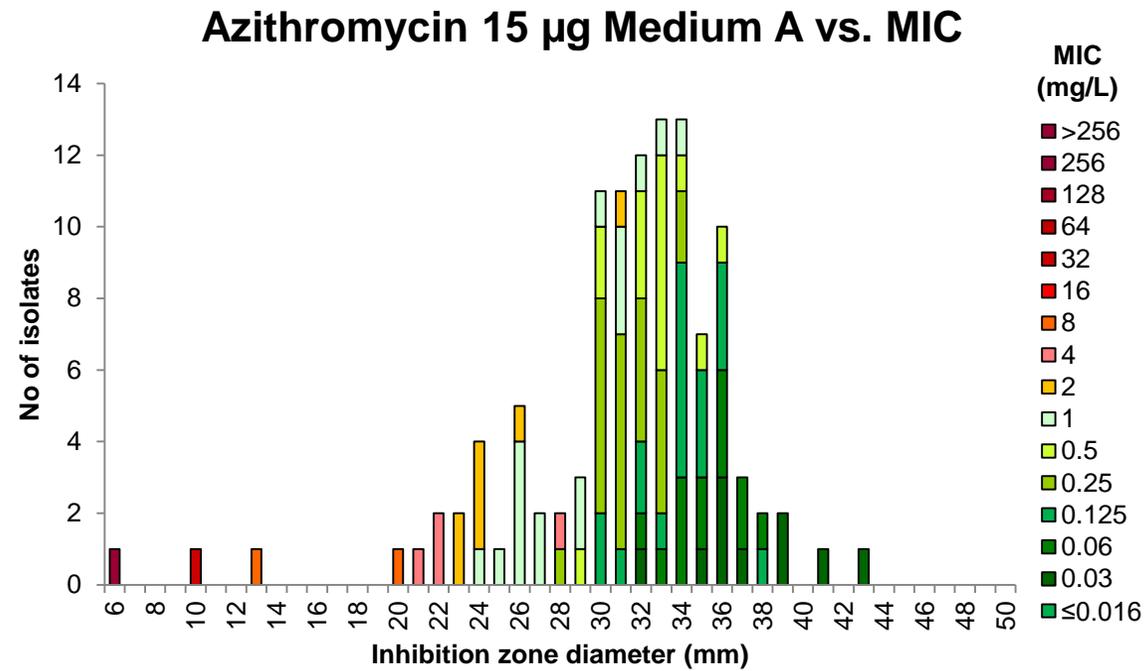


Erythromycin 15 µg vs. azithromycin MIC

N. gonorrhoeae, 110 isolates (220 correlates)

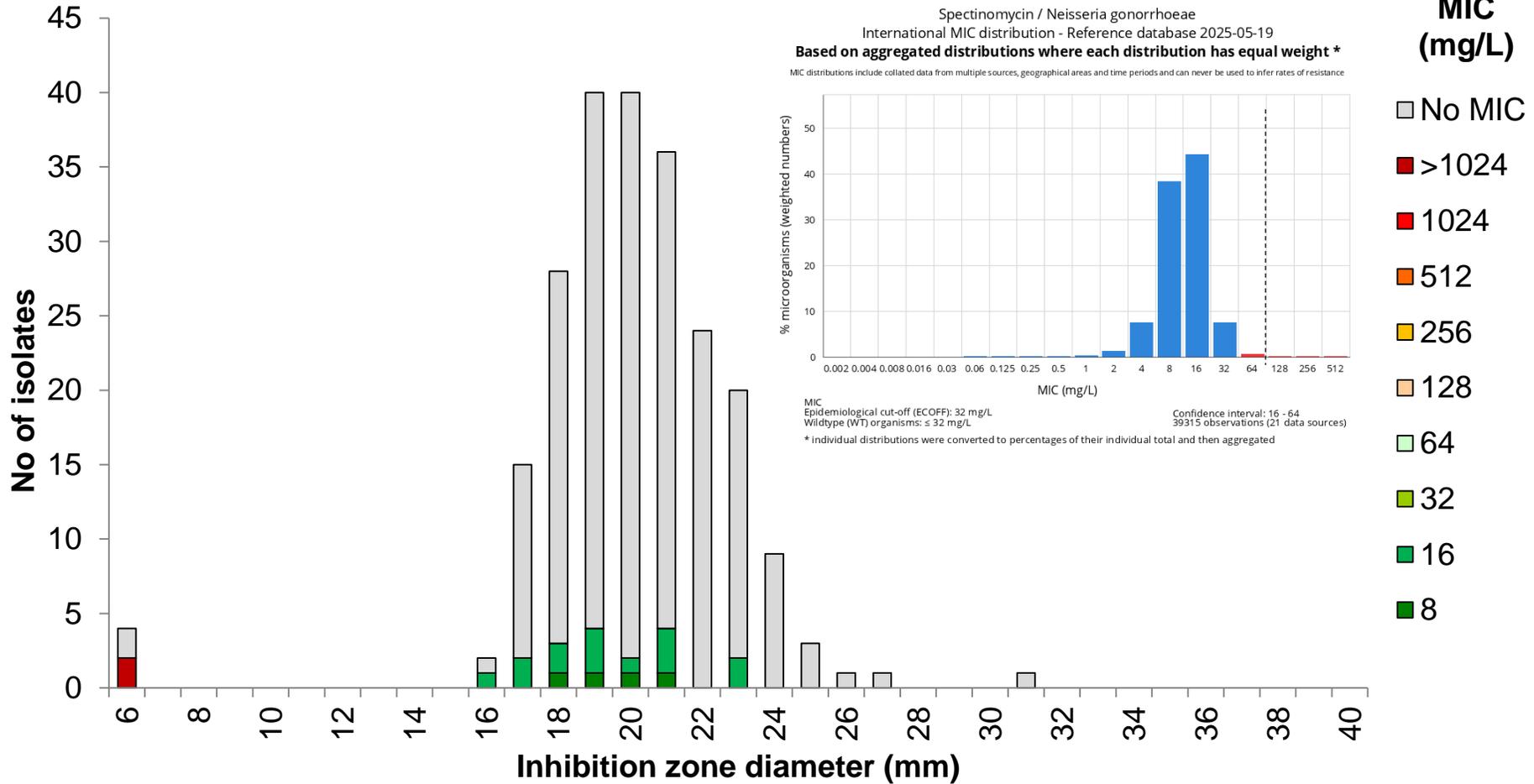


Azithromycin vs. medium manufacturer



Spectinomycin 25 µg vs. MIC

N. gonorrhoeae, 112 isolates (224 correlates)



Future work

- MIC determination
 - Compare gradient tests with agar dilution
 - Compare agar dilution on CLSI medium with EUCAST experimental medium
- Disk diffusion
 - Evaluate lower disk potencies
 - Additional testing to evaluate disk diffusion reproducibility
 - Data to establish quality control criteria
 - Test additional isolates
 - Wild-type to beta-lactam agents
 - Additional resistant isolates for cefixime, cefotaxime, ceftriaxone, azithromycin and spectinomycin

Thanks!

Isolates

- Robert Koch Institute, Berlin, Germany,
- Hôpital Saint-Louis, Paris, France
- NRL Neisseria, Örebro Sweden (Magnus Unemo)

Testing and method development

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- The EDL staff

