



Kinolonresistens hos *Salmonella* spp. och övriga Enterobacteriaceae

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Kinoloner

- Kinoloner - nalidixansyre først introduceret i 1962
 - Fluorokinoloner – ciprofloxacin i 1987
- Virkningsmekanisme:
 - Katalyserer den negative supercoiling af DNA som er nødvendig for initiering af DNA replikation (gyrase)
 - Initierer splatningen af den dobbeltstrengede helix (topoisomerase)

Resistens mekanismer

- Mutation i bindings site
 - QRDR
- Protektion af bindings site
 - Qnr
- Acetylering
 - AAC(6)-Ib-cr
- Efflux
 - OqxAB, QepA

Mutation i bindingsite

- Hyppigst mutationer i QRDR (quinolone resistance determination region) (kromosomal)
 - Specielt *gyrA*
 - Codon 83 og 87
 - Ses også i *gyrB*, *parC* og *parE*
 - Restens niveau
 - Single mutationer
 - Cipro MIC - kun let forhøjet (0.125-1)
 - Nalidixansyre R

Protektion af bindingsite

- Dannelse af pentapeptide repeat proteiner der beskytter bindingsite
 - *qnr* – gener
 - *qnr* A,B,S,C,D
 - Plasmid medieret (1998)
 - *qnr* findes naturligt i visse vandbakterier bla. *Shewanella*
 - Resistens
 - Cipro MIC - let forhøjet (0.125-1)
 - Nalidixansyre S

Acetylering

- AAC(6)-Ib-cr forårsager acetylering af flourokinoloner, der indeholder piperzinyll secondary amines
 - Giver resistens overfor Ciprofloxacin, norfloxacin
 - AAC(6)-Ib er et aminoglycosid acetyltransferase enzym
 - - cr varianten har 2 aminosyre ændringer Trp102Arg and Asp179Tyr,
- Resistens
 - Cipro MIC - let forhøjet (0.125-1)
 - Nalidixansyre S

Efflux og impermeabilitet

- Både impermeabilitet og forøget efflux kan øge resistensen
- Hyppigst tale om kromosomale forandringer
- 2 plasmid medierede efflux mekanismer
 - OqxAB, QepA
- Resistens
 - Cipro MIC - let forhøjet (0.125-1)
 - Nalidixansyre S

Epidemiologi

- Mutationer I QRDR
 - Langt den hyppigste
- Salmonella
 - Danmark – 6500 isolater
 - 11% Nalidixan R ~ QRDR R
 - 0.8% qnr
 - 0% *aac6'-Ib-cr*
 - Internationalt 2300 isolater
 - *qnrA* 0,2%
 - *qnrB* 1,0%
 - *qnrS* 2,4%
 - *aac6'-Ib-cr* 6,4%

Epidemiologi

- Enterobacteriaceae
 - Internationalt 70 arbejder – 21000 isolater – prævalencen formentlig overestimeret som følge af inklusions kriterier
 - *qnrA* 1,5%
 - *qnrB* 4,6% } mest non E. coli
 - *qnrS* 2,4%
 - *aac6'-Ib-cr* 10,8% - mest E.coli
- QepA – få studier!! <1%

Enterobacteriaceae

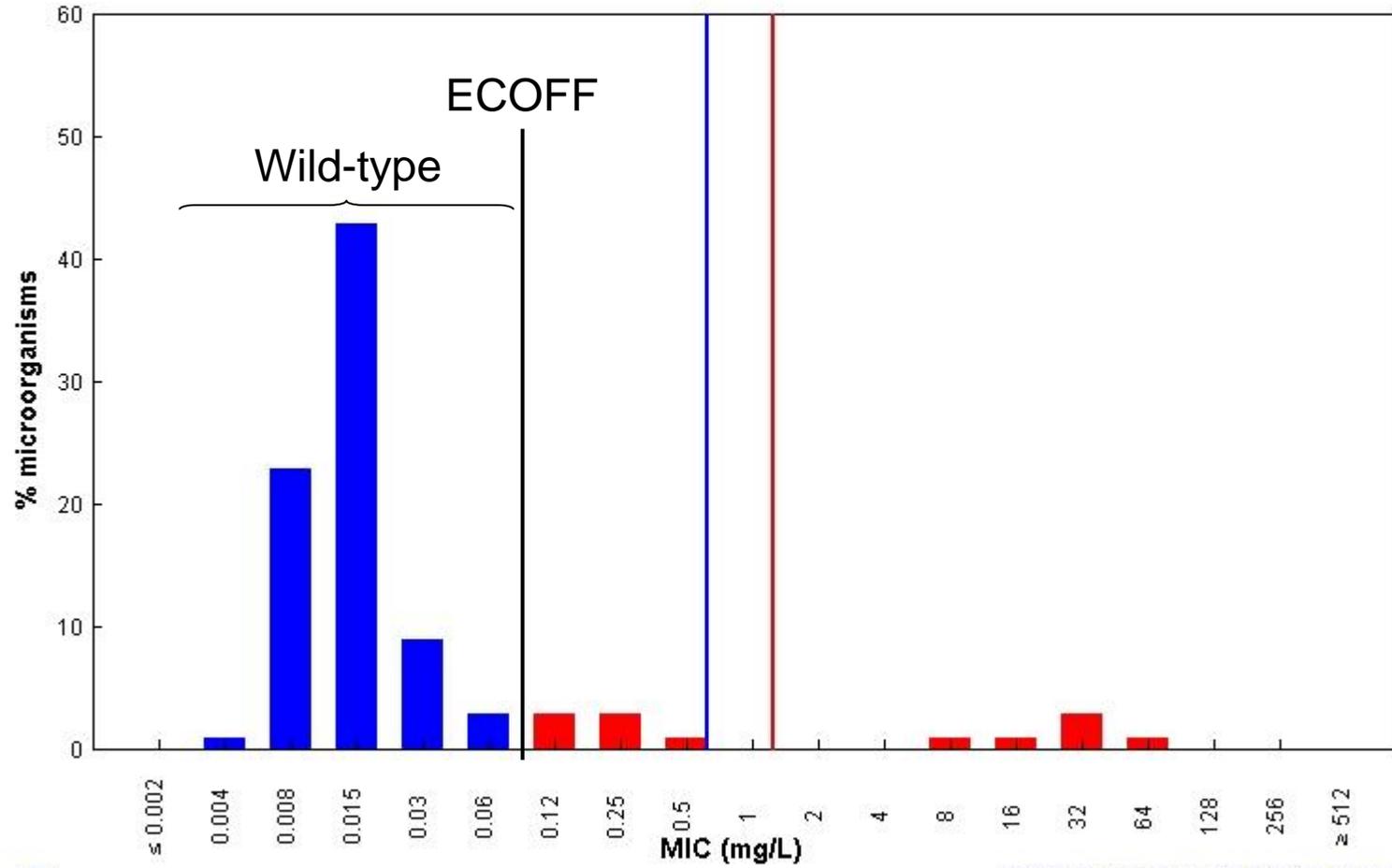
NordicAST brytpunktstabell v. 2.0

Fluorokinoloner	MIC-brytpunkt		Lappstyrka (µg)	Zonbrytpunkt	
	S ≤	R >		S ≥	R <
Ciprofloxacin	0.5	1	5	22	19
Ciprofloxacin, <i>Salmonella</i> spp.	0.06	0.06	5	31	31
Levofloxacin	1	2	5	22	19
Ofloxacin	0.5	1	5	22	19

”Nalidixinsyra har använts för resistensövervakning. Användandet av ECOFF har hög känslighet för detektion av kromosomal resistens men inte för plasmidmedierad resistens.”

Ciprofloxacin / Escherichia coli
EUCAST MIC Distribution - Reference Database 2012-05-14

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

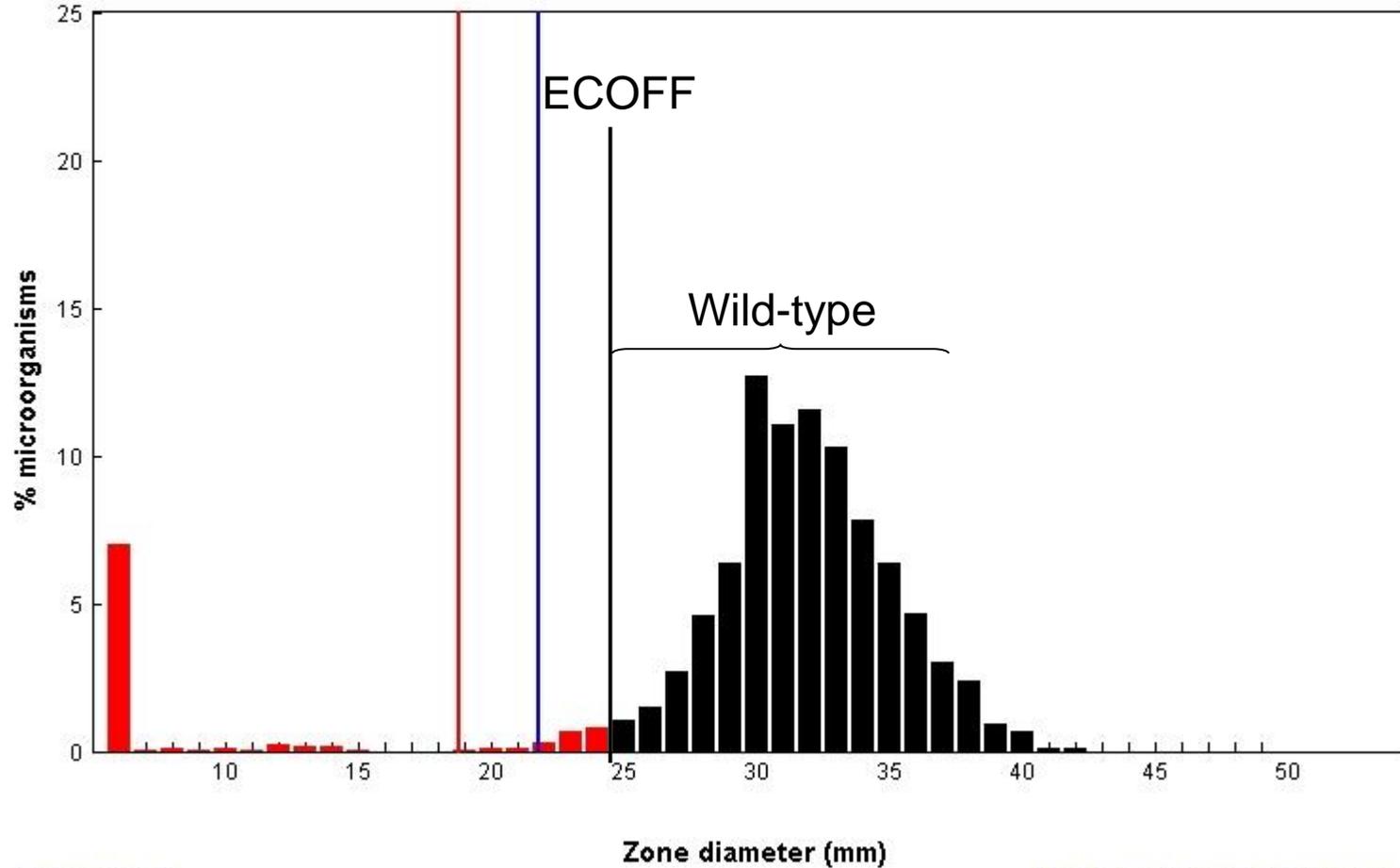


MIC
Epidemiological cut-off: WT ≤ 0.064 mg/L

16702 observations (55 data sources)
Clinical breakpoints: S ≤ 0.5 mg/L, R > 1 mg/L

Ciprofloxacin / Escherichia coli
EUCAST zone diameter distribution - Reference database 2012-05-14
EUCAST disk diffusion method

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



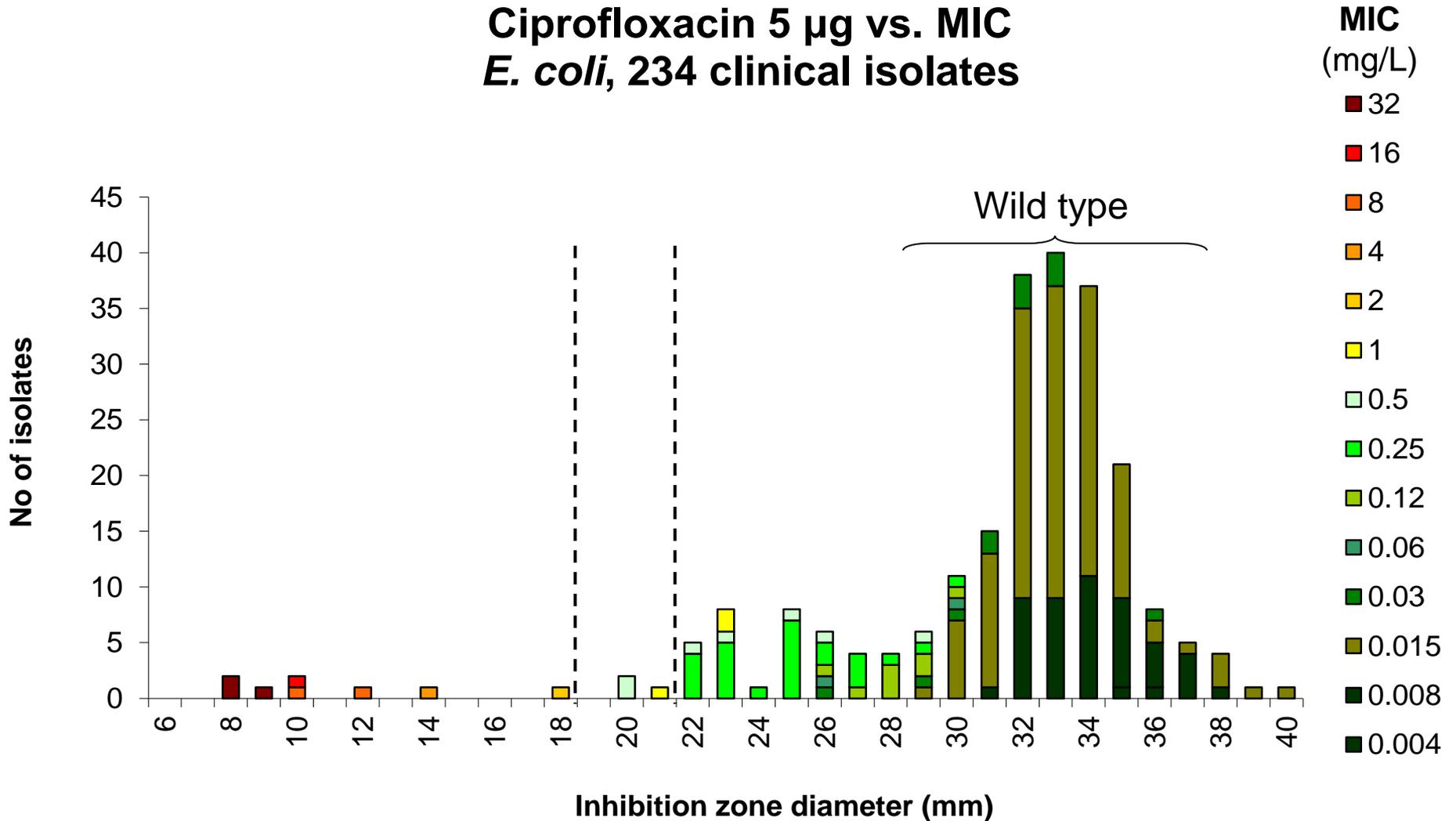
Disk content: 5

Epidemiological cut-off: WT \geq 25 mm (MIC \leq 0.064 mg/L)

6434 observations (6 data sources)

Clinical breakpoints: S \geq 22 mm, R $<$ 19 mm (S \leq 0.5 mg/L, R $>$ 1 mg/L,)

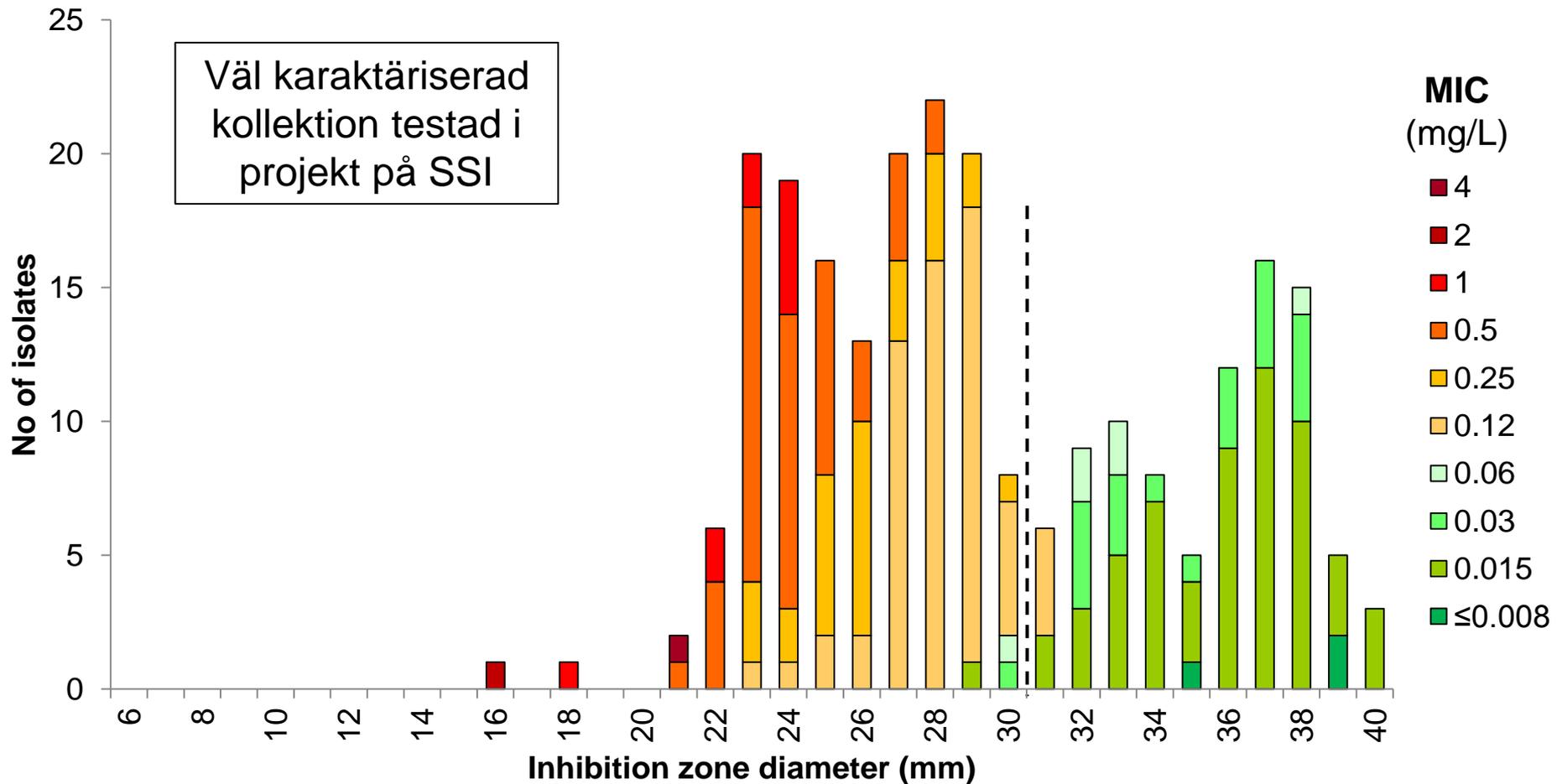
Ciprofloxacin 5 µg vs. MIC *E. coli*, 234 clinical isolates



Breakpoints		ECOFF
MIC	$S \leq 0.5$, $R > 1$ mg/L	$WT \leq 0.06$ mg/L
Zone diameter	$S \geq 22$, $R < 19$ mm	

Ciprofloxacin 5 ug vs. MIC

Salmonella spp., 237 clinical isolates



Breakpoints

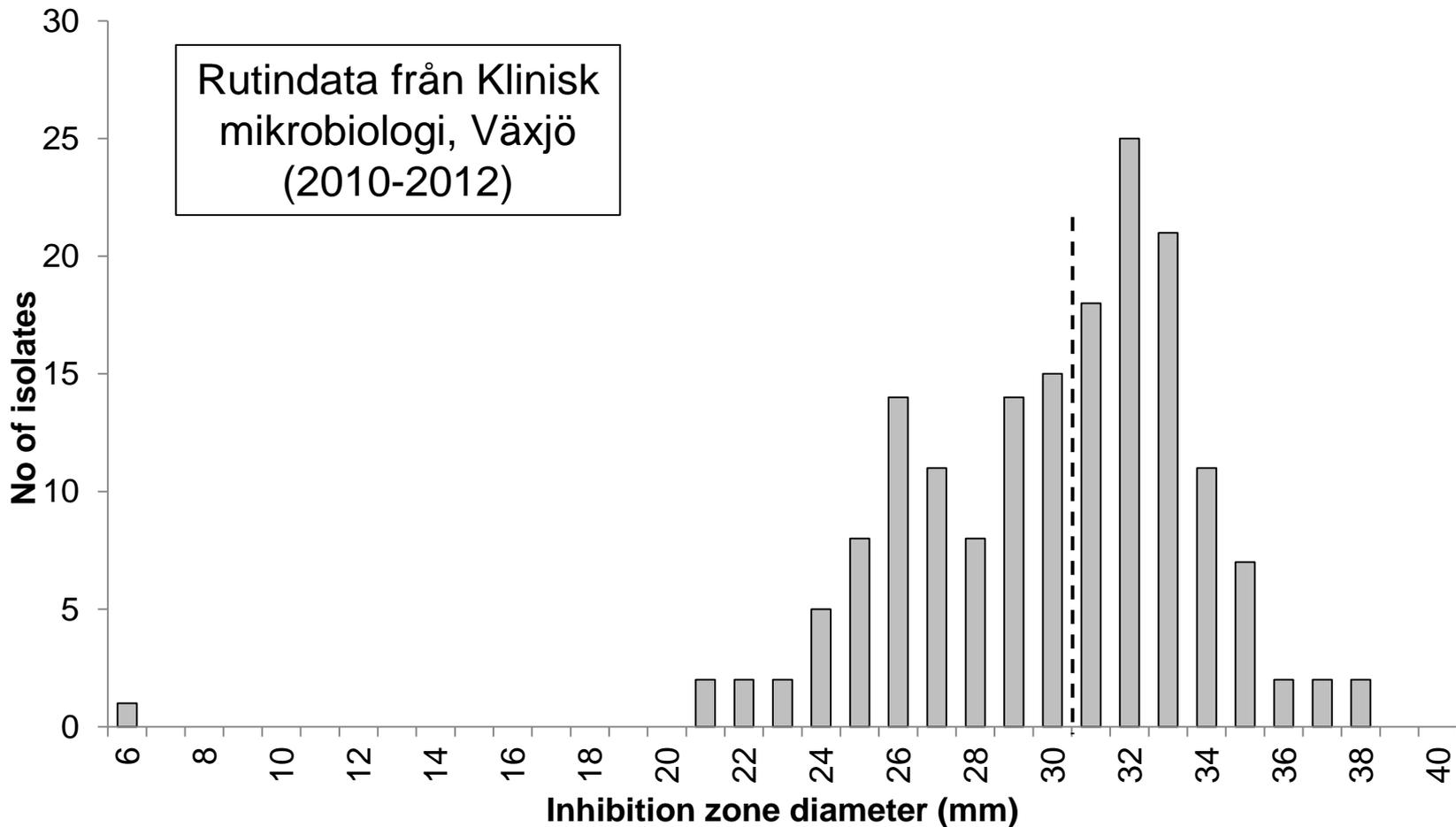
MIC S ≤ 0.06, R > 0.06 mg/L

Zone diameter S ≥ 31, R < 31 mm

ECOFF

WT ≤ 0.06 mg/L

Salmonella spp. with Ciprofloxacin 5 µg 170 clinical isolates from routine work



Breakpoints

MIC S ≤ 0.06, R > 0.06 mg/L

Zone diameter S ≥ 31, R < 31 mm

ECOFF

WT ≤ 0.06 mg/L

Screening för kinolonresistens hos *Salmonella* spp.

Pilotprojekt

Statens Serum Institut

EUCAST

Material

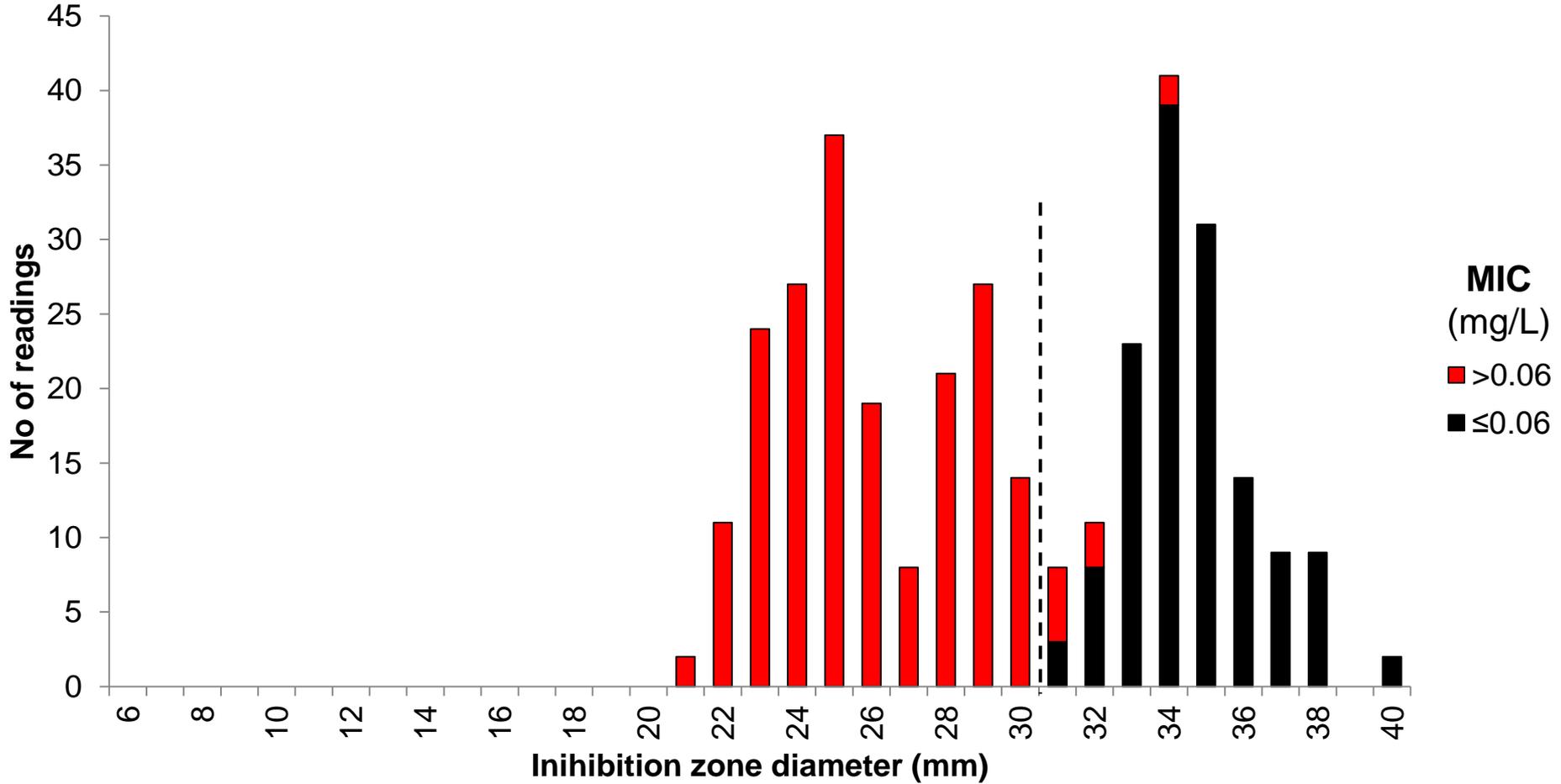
- 89 isolat
 - 37 känsliga isolat
 - Ciprofloxacin MIC <0.125 mg/L
 - Nalidixic acid MIC <8 mg/L
 - 32 med qnr
 - Ciprofloxacin MIC 0.125-2 mg/L
 - Nalidixic acid MIC 8->64 mg/L
 - 20 med mutationer i QRDR
 - Ciprofloxacin MIC 0.125-1 mg/L
 - Nalidixic acid MIC >64 mg/L

Metoder

- MIC-bestämning med buljongspädning
 - Ciprofloxacin och nalidixinsyra
- Lappdiffusion
 - 16 olika antibiotikalappar, inklusive nalidixinsyra och ciprofloxacin i låga konc.
 - Mueller Hintonagar från 2 leverantörer
 - Två oberoende avläsare
 - 4 avläsningar per zon

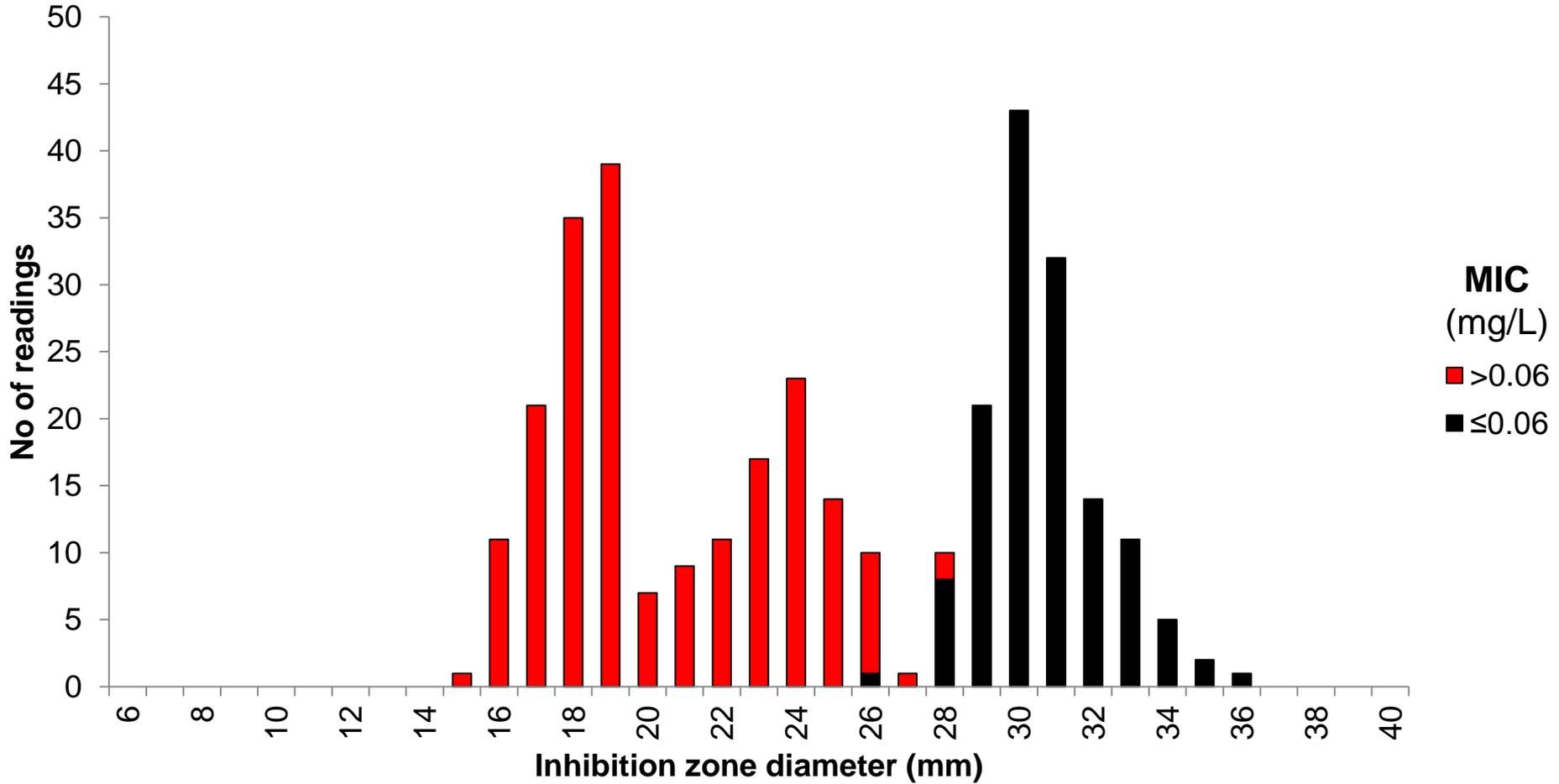
Ciprofloxacin 5 μ g vs. MIC

Salmonella spp., 89 clinical isolates x 4

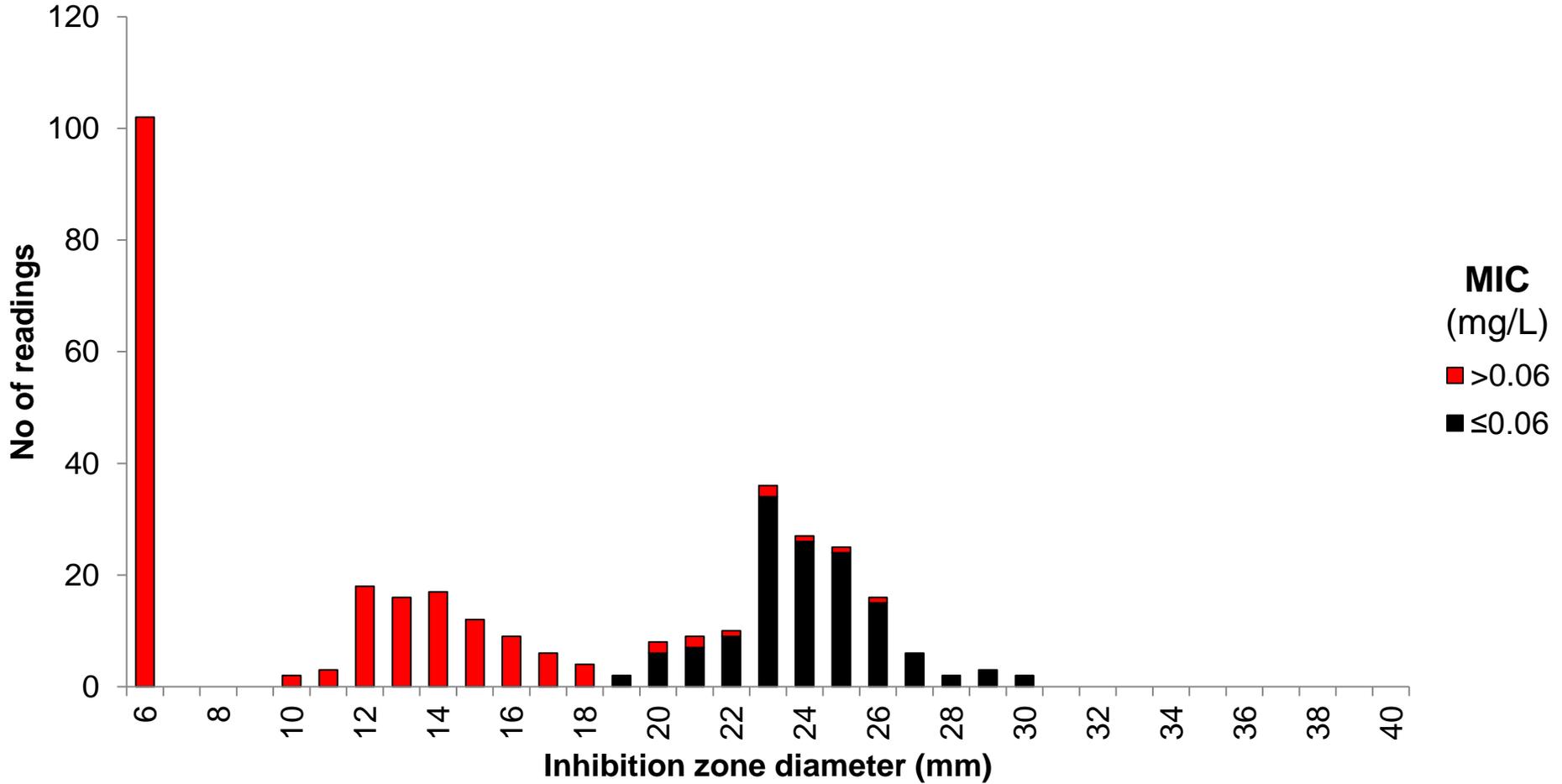


Ciprofloxacin 1 μ g vs. MIC

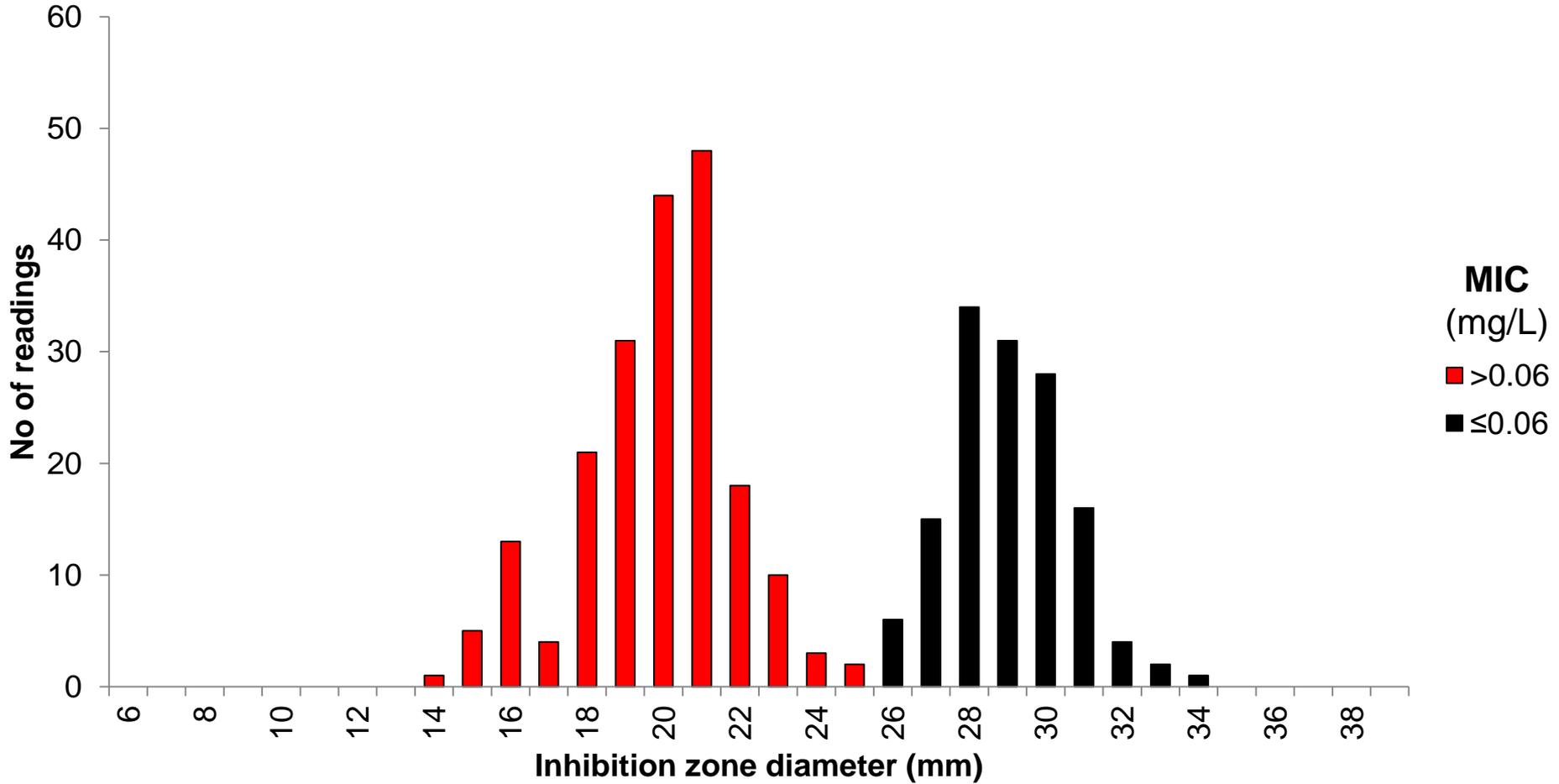
Salmonella spp., 89 clinical isolates x 4



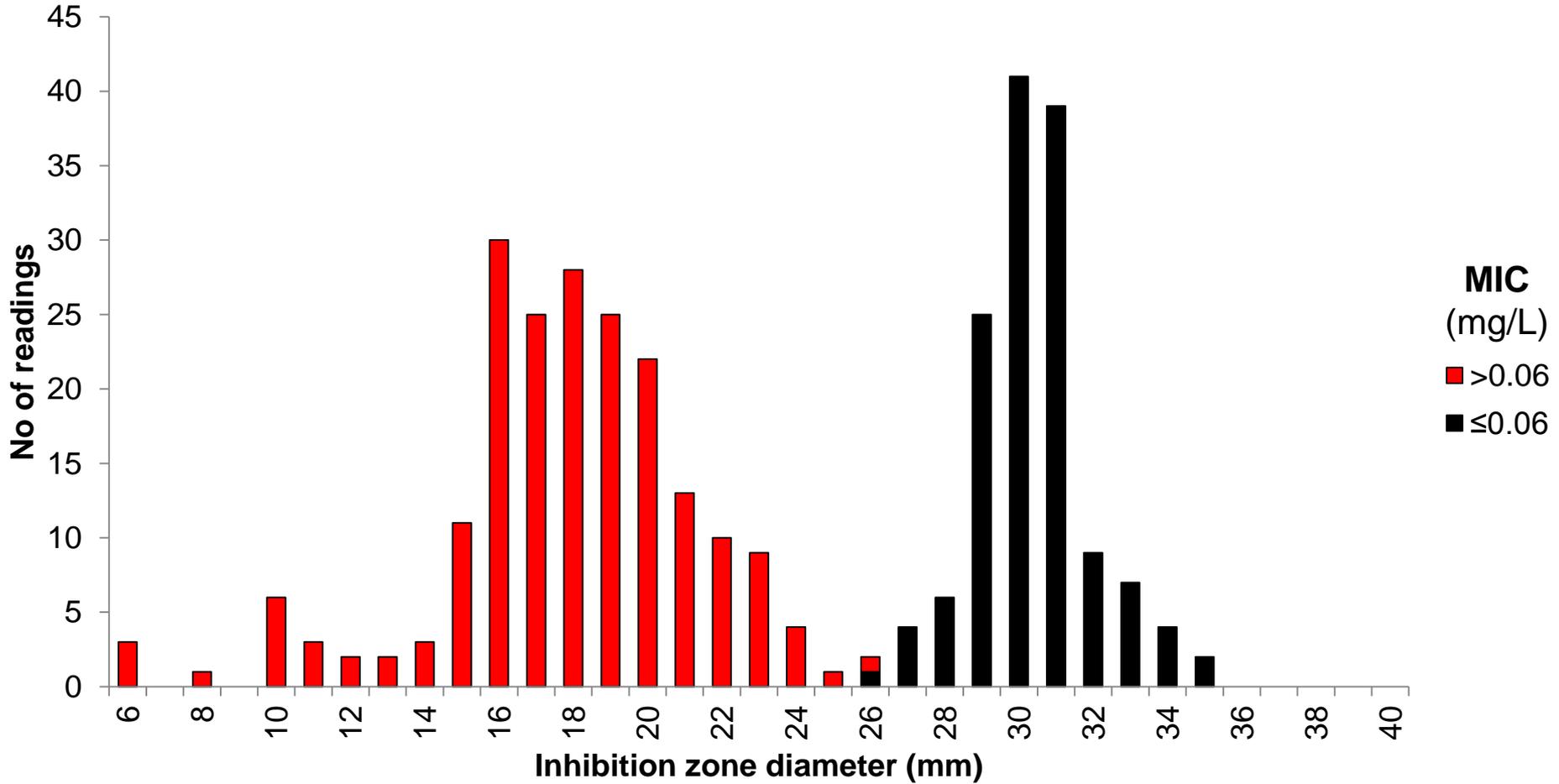
Nalidixic acid 30 μ g vs. Ciprofloxacin MIC
***Salmonella* spp., 89 clinical isolates x 4**



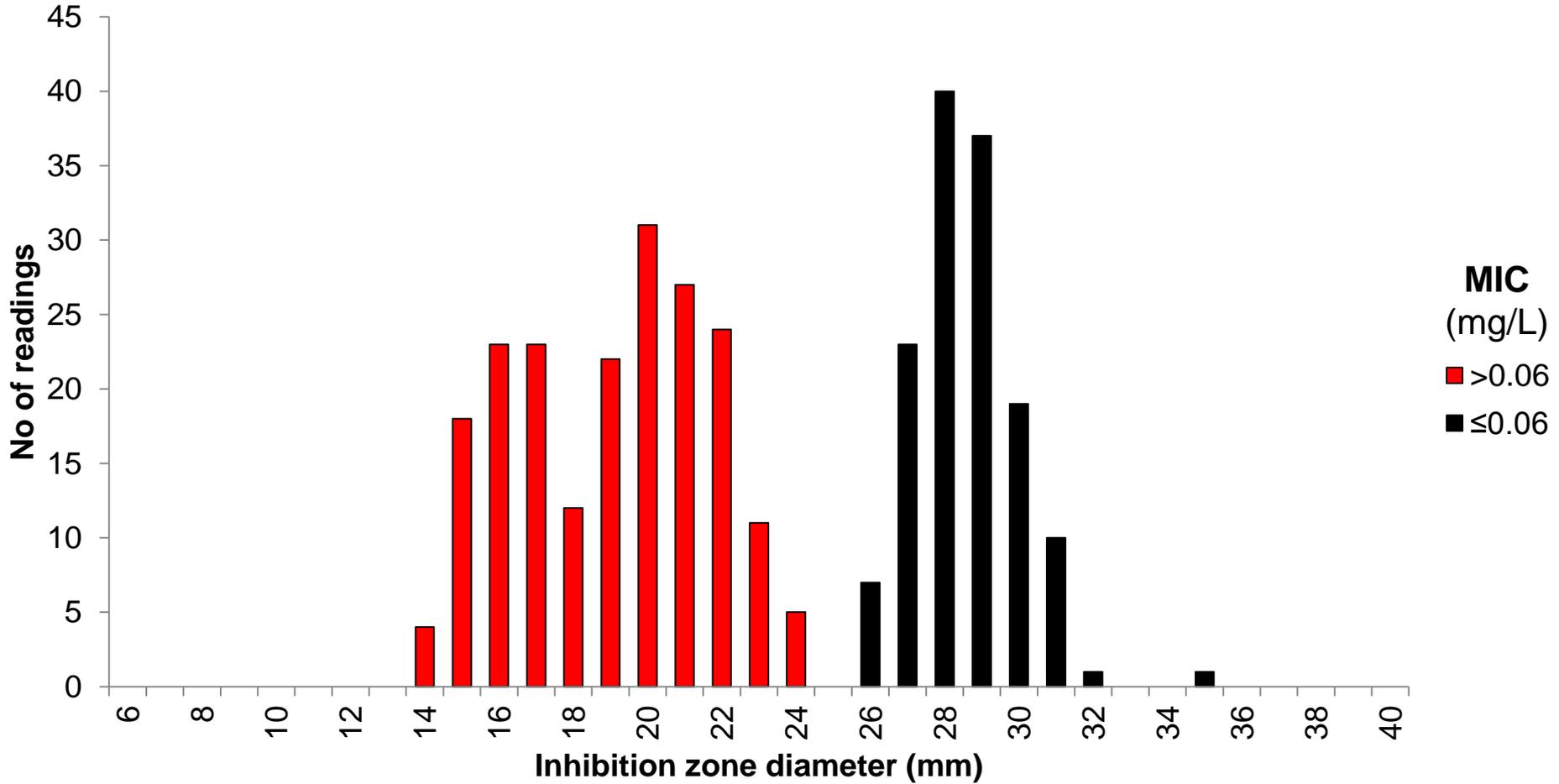
Enoxacin 10 μ g vs. Ciprofloxacin MIC
***Salmonella* spp., 89 clinical isolates x 4**



Pefloxacin 5 μ g vs. Ciprofloxacin MIC *Salmonella* spp., 89 clinical isolates x 4



Norfloxacin 2 μ g vs. Ciprofloxacin MIC *Salmonella* spp., 89 clinical isolates x 4



Validering

- Samarbete med CLSI
 - 6-7 deltagande laboratorier
 - 110-160 isolat
 - Lappar från 2 leverantörer
 - Mueller Hintonagar från 2-3 leverantörer

Konklusioner

- QRDR mutationer er langt den hyppigste resistens mekanisme!
 - De andre resistens mekanismer er væsentlig lavere
- Cipro 5 µg disks er ikke i stand til at adskille sensitive fra isolater med nedsat følsomhed
 - Lav MIC ved alvorlige salmonella isolater
 - Ciprofloxacin 1 µg men også norfloxacin 2 µg, pefloxacin 5 µg og enoxacin 10 µg udviser bedre separation
 - Multicenter studie på vej der skal afklare den metodologiske variation samt om de er brugbare