Salmonella spp. Fluoroquinolone Testing (continued)

Summary of FQ WG Decisions
6-29-14

Salmonella spp. Fluoroquinolone Resistance

Genotype	Phenotype	
	Ciprofloxacin MIC (µg/ml)	Nalidixic Acid
Wild type (No resistance)	0.008-0.06	Usually susceptible
Chromosomal *gyr*A (single mutation)	0.12 - 2.0	Usually resistant
Chromosomal *gyr*B (single mutation)	0.12 – 0.5	Usually susceptible
Chromosomal *gyr*A, *gyr*B (multiple mutations)	≥4.0	Resistant
PMQR (e.g. qnr or aac(6')-lb-cr)	0.12 - 2.0	Often susceptible

PMQR, plasmid-mediated quinolone resistance - newer mechanism and less common than chromosomal gyrase mutations

Salmonella spp.

Fluoroquinolone AST and Reporting History

CLSI Standard	Fluoroquinolone Breakpoints
M100-S21 (2011)	One set of breakpoints for all Enterobacteriaceae including *Salmonella* spp. Nalidixic acid screen for reduced ciprofloxacin susceptibility in extraintestinal isolates of *Salmonella* spp.
M100-S22 (2012)	Lower ciprofloxacin breakpoints for *S. Typhi* and extraintestinal *Salmonella* spp.
M100-S23 (2013)	Lower ciprofloxacin, levofloxacin and ofloxacin breakpoints for use with all *Salmonella* spp.
M100-S24 (2014)	No changes

Unanswered Question:

What disk can best predict reduced FQ susceptibility?

Enterobacteriaceae (Table 2A)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm) S	SDD	I	R	MIC Interpretive Criteria (µg/mL) S	SDD	I	R	Comments
FLUOROQUINOLONES											
NOTE: Reevaluation of fluoroquinolones is ongoing. See comment (2).											
B	Ciprofloxacin	5 µg	≥21		16–20	≤15	≤1		2	≥4	(34) For testing and reporting of *Enterobacteriaceae* except for *Salmonella* spp.
B	Levofloxacin	5 µg	≥17		14–16	≤13	≤2		4	≥8	
B	Ciprofloxacin	5 µg	≥31		21-30	≤20	≤0.06		0.12–0.5	≥1	(35) For testing and reporting of *Salmonella* spp. (including *S.* Typhi and *S.* Paratyphi A–C). See comment (2).
B	Levofloxacin	–	–		–	–	≤0.12		0.25–1	≥2	(36) If MIC testing is not performed or if interpretive criteria cannot be implemented, see comment (39).
B	Ofloxacin	–	–		–	–	≤0.12		0.25–1	≥2	
U	Lomefloxacin or	10 µg	≥22		19–21	≤18	≤2		4	≥8	
U	ofloxacin	5 µg	≥16		13–15	≤12	≤2		4	≥8	
U	Norfloxacin	10 µg	≥17		13–16	≤12	≤4		8	≥16	
O	Enoxacin	10 µg	≥18		15–17	≤14	≤2		4	≥8	
O	Gatifloxacin	5 µg	≥18		15–17	≤14	≤2		4	≥8	
O	Gemifloxacin	5 µg	≥20		16–19	≤15	≤0.25		0.5	≥1	(37) FDA-approved for *Klebsiella pneumoniae*.
O	Grepafloxacin	5 µg	≥18		15–17	≤14	≤1		2	≥4	
Inv.	Fleroxacin	5 µg	≥19		16–18	≤15	≤2		4	≥8	

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm) S	SDD	I	R	MIC Interpretive Criteria (µg/mL) S	SDD	I	R	Comments
QUINOLONES											
O	Cinoxacin	100 µg	≥19		15–18	≤14	≤16		32	≥64	See comment (24).
O	Nalidixic acid	30 µg	≥19		14–18	≤13	≤16		–	≥32	(38) These interpretive criteria are for urinary tract isolates of *Enterobacteriaceae* and for all isolates of *Salmonella*. (39) Until laboratories can implement the current interpretive criteria for ciprofloxacin, levofloxacin, and/or ofloxacin, nalidixic acid may be used to test for reduced fluoroquinolone susceptibility in *Salmonella*. Strains of *Salmonella* that test resistant to nalidixic acid may be associated with clinical failure or delayed response in fluoroquinolone-treated patients with salmonellosis. Note that nalidixic acid may not detect all mechanisms of fluoroquinolone resistance.

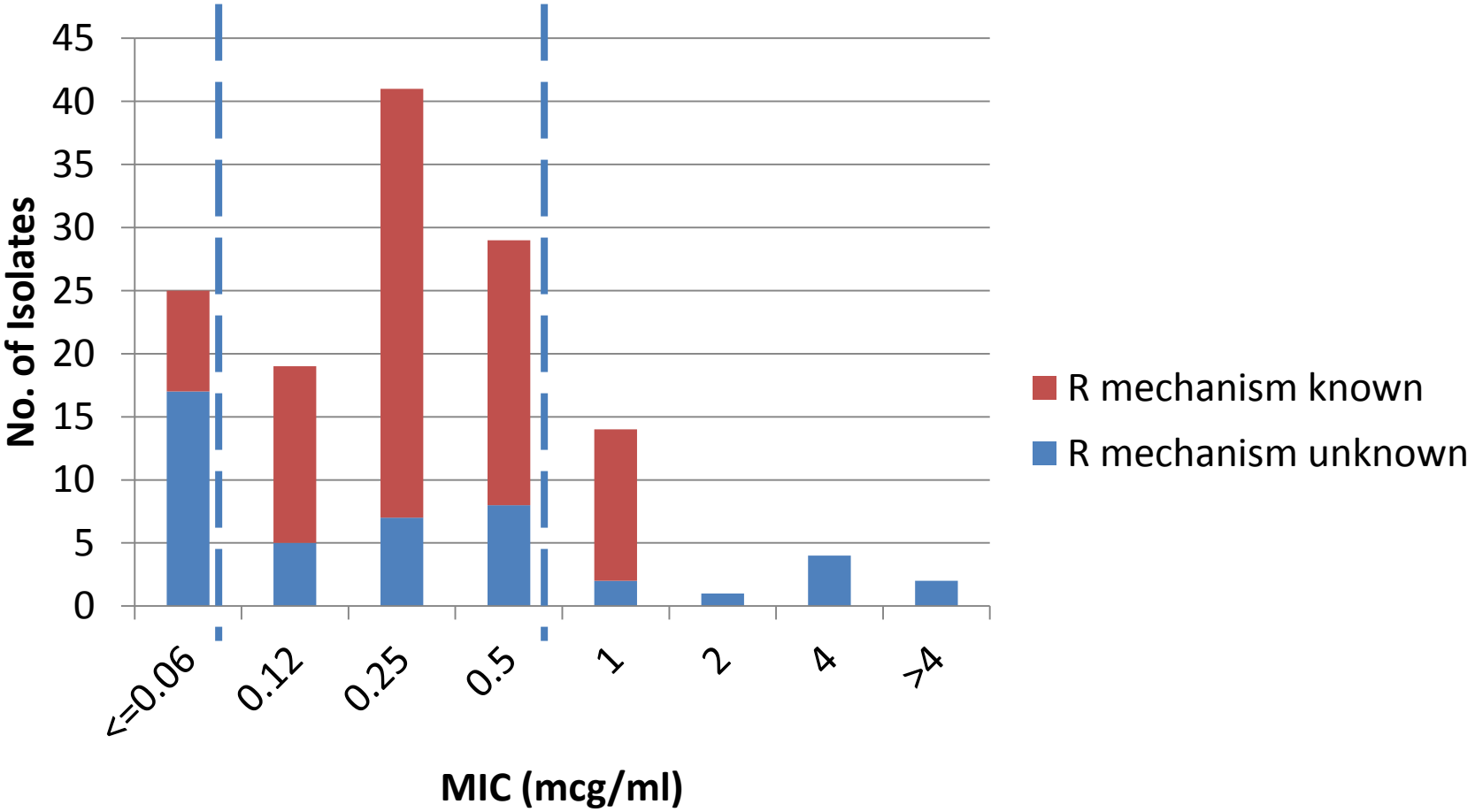
Salmonella spp.

Agent	MIC (µg/ml)			Zone (mm)		
	Susc	Int	Res	Susc	Int	Res
Ciprofloxacin						
CLSI	≤0.06	0.12-0.5	≥1	≥31	21-30	≤20
FDA (Typhi)	≤0.06	0.12-0.5	≥1	≥31	21-30	≤20
FDA (Enterobac.)	≤1	2	≥4	≥21	16-20	≤15
EUCAST (Salmonella)	≤0.06	-----	≥0.12 (>0.06)	------Use Peflox disks------		
Levofloxacin						
CLSI	≤0.12	0.25-1	≥2	-----	-----	-----
FDA (Enterobac.)	≤2	4	≥8	≥17	14-16	≤13
EUCAST (Enterobac.)	≤1	-----	≥4 (>2)	≥22	19-21	≤18
Ofloxacin						
CLSI	≤0.12	0.25-1	≥2	-----	-----	-----
FDA (Enterobac.)	≤2	4	≥8	≥16	13-15	≤12
EUCAST (Enterobac.)	≤0.5	-----	≥2(>1)	≥22	19-21	≤18
Pefloxacin						
EUCAST	-----	-----	-----	≥24	-----	<24

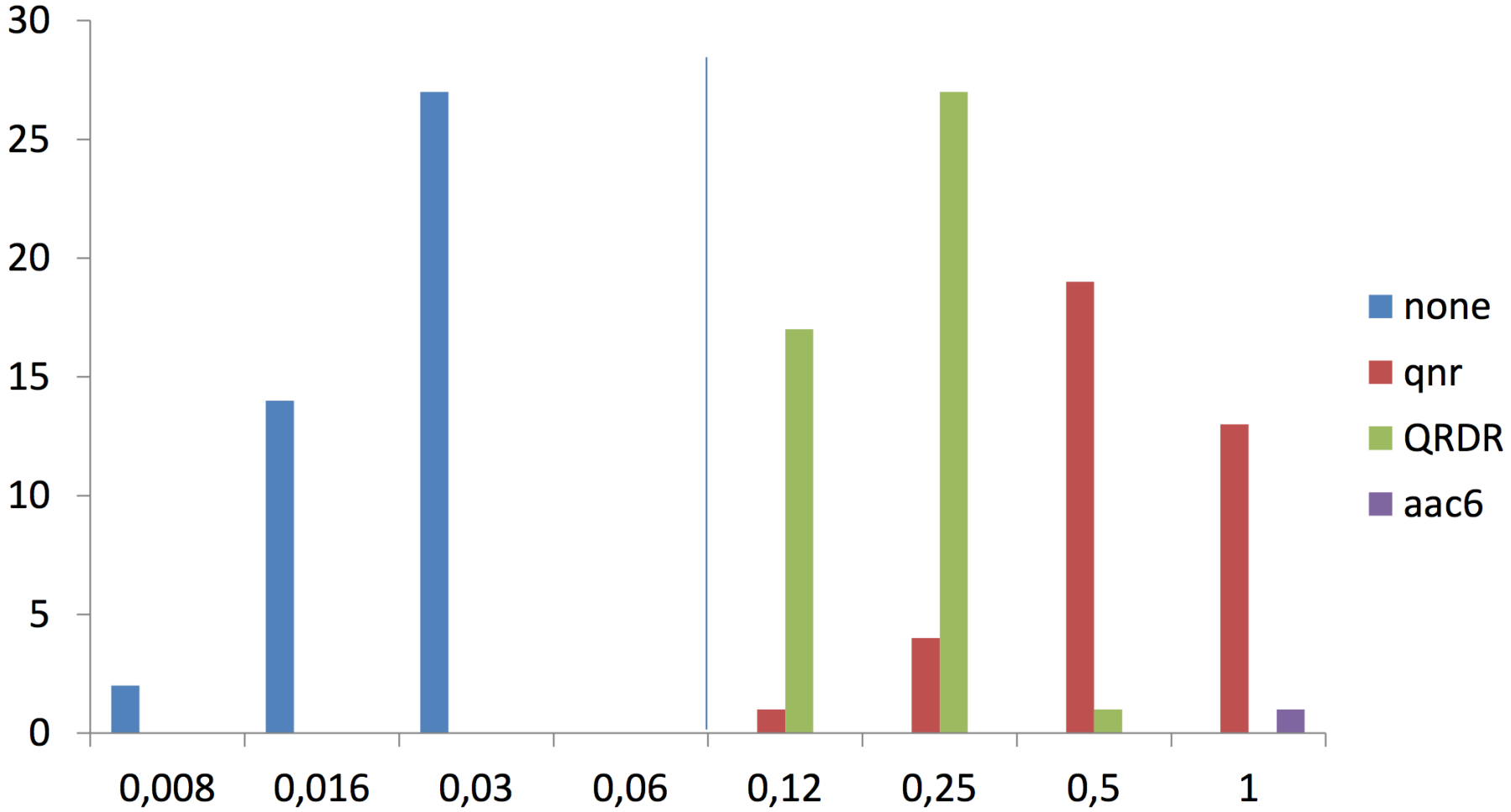
FQ WG June 2014 Presentations

- Data presented from EUCAST (Robert Skov)
- Data presented from UCLA

UCLA Studies: Distribution of Ciprofloxacin MICs (n=136)



Ciprofloxacin MIC (after retest) vs resistance mechanism



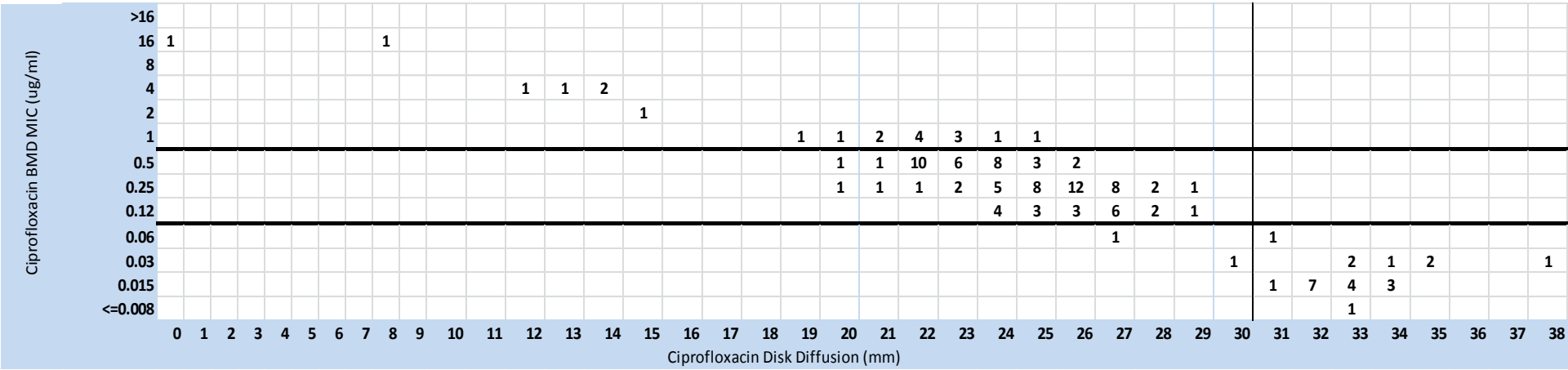
UCLA Data: MIC vs. DD for Select FQs

Agent	CA (%)	VME	ME	mE
Ciprofloxacin	89.0	0	0	11.0
Levofloxacin	94.9	0	0	5.1
Ofloxacin	80.1	0	0	19.9
Nalidixic Acid	90.4	0	0	9.6

Surrogate Disk for FQ Resistance in *Salmonella* spp.

- Our definition –
  - A disk and zone cutoff that most reliably identifies *Salmonella* isolates that are not susceptible to FQs based on current ciprofloxacin susceptible or not susceptible (I + R) MIC breakpoints
  - Assumption – ciprofloxacin MIC (using CLSI reference method) accurately differentiates FQ susceptible from FQ not susceptible isolates
- Errors calculated:
  - VME = total false S/total not susceptible
  - ME = total false not susceptible/total susceptible

Ciprofloxacin MIC vs Ciprofloxacin DD (n=136)



	Current BP (%)
VME	0
ME	8.0
CA	98.5
	≤30 mm

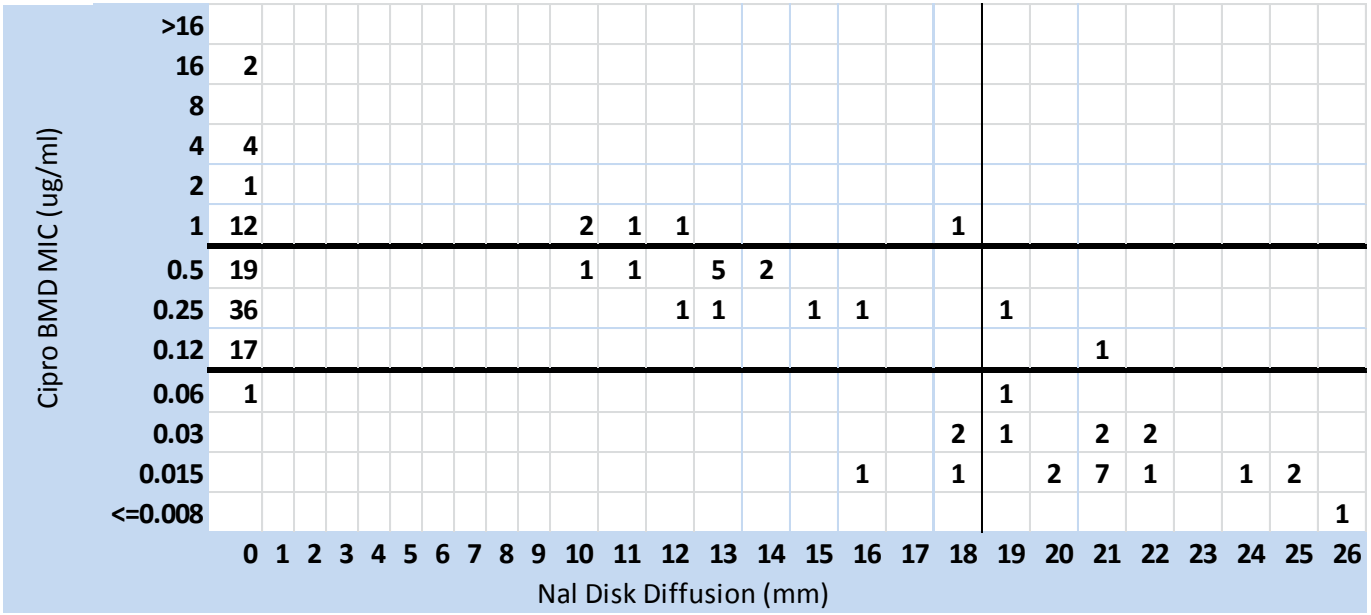
Ciprofloxacin 5 µg vs fluoroquinolone resistance mechanism

CLSI Enterobactereaceae Resistant Breakpoint

CLSI Enterobactereaceae Susceptible Breakpoint

Zone diameter	Resistance mechanism			
	none	qnr	QRDR	aac6
20		3		3
21		8		3
22		18	2	
23		50	2	
24		42	6	
25		39	12	
26		21	57	
27		19	67	
28		11	57	
29	1	9	35	
30	6	2	25	
31	23		6	
32	50		1	
33	61			
34	44			
35	29			
36	23			
37	14			
38	3			
39	4			

Ciprofloxacin MIC vs Nalidixic Acid DD (n=136)

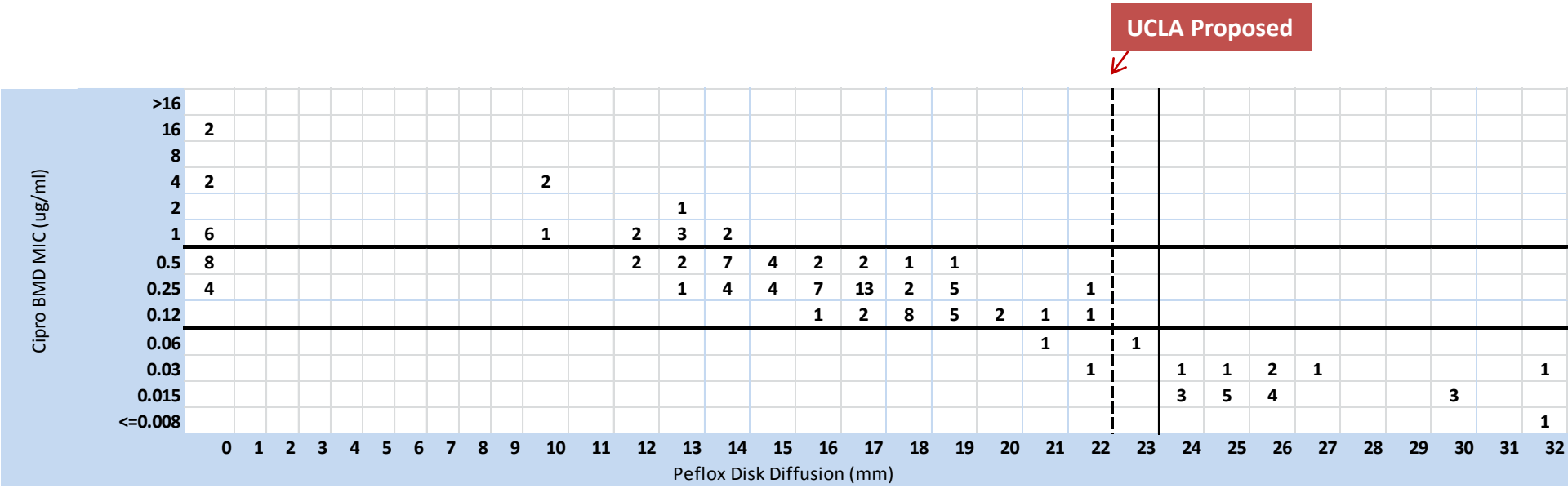


	Current BP (%)
VME	1.8
ME	20.0
CA	94.9
	≤18 mm

Nalidixic acid 30 µg vs fluoroquinolone resistance mechanism

Zone diameter	Resistance mechanism			
	none	qnr	QRDR	aac6
6		13	270	
7				
8		4		
9		2		
10		17		
11		23		
12		42		
13		40		1
14		20		1
15		23		3
16		14		1
17		9		
18	1	3		
19	4	1		
20	13			
21	30	2		
22	53	2		
23	60	3		
24	60	3		
25	21	1		
26	3			
27	6			
28	5			
29	2			

Ciprofloxacin MIC vs Pefloxacin DD (n=136)

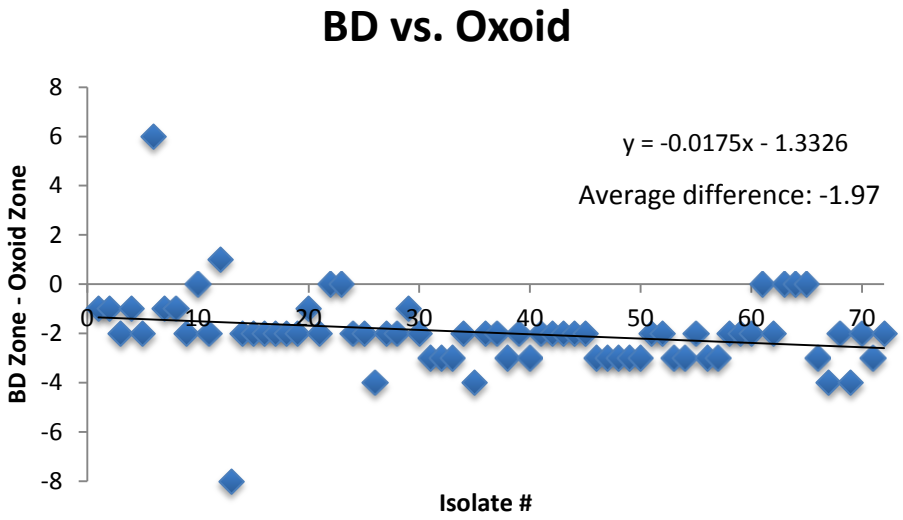


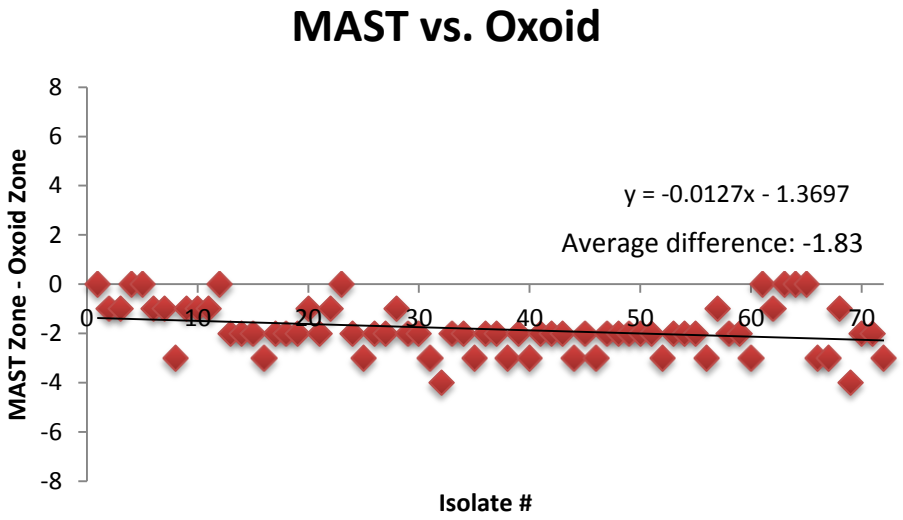
	EUCAST (%)	UCLA Proposed BP (%)
VME	0	0
ME	12.0	8.0
CA	97.8	98.5
	≤23 mm	≤22 mm

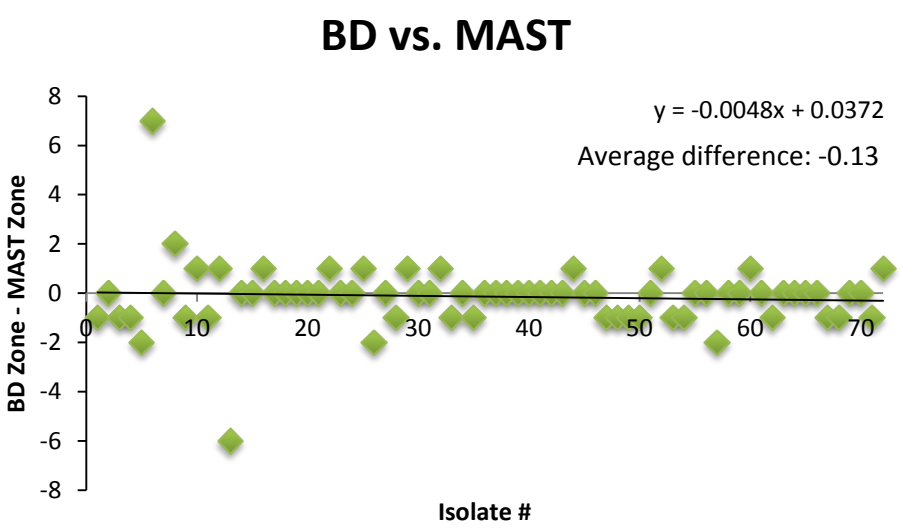
Pefloxacin 5 µg vs fluoroquinolone resistance mechanism

Zone diameter	Resistance mechanism			
	none	qnr	QRDR	aac6
6			2	
10			2	
11		1		
12		11	3	
13		31		
14		37	4	1
15		38	18	
16		33	16	5
17		30	34	
18		13	46	
19		5	52	
20		8	49	
21		7	31	
22		4	12	
23		3	1	
24	1	1		
25	2			
26	28			
27	51			
28	73			
29	63			
30	21			
31	11			
32	5			
33	2			
34	1			

Proposed screenings breakpoint



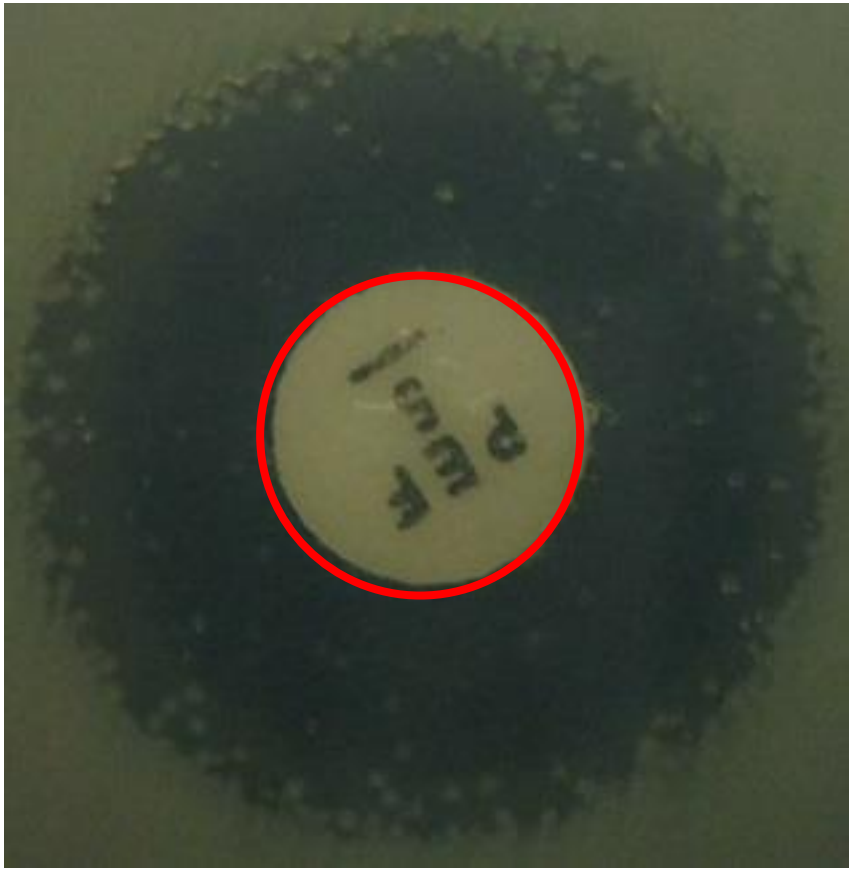


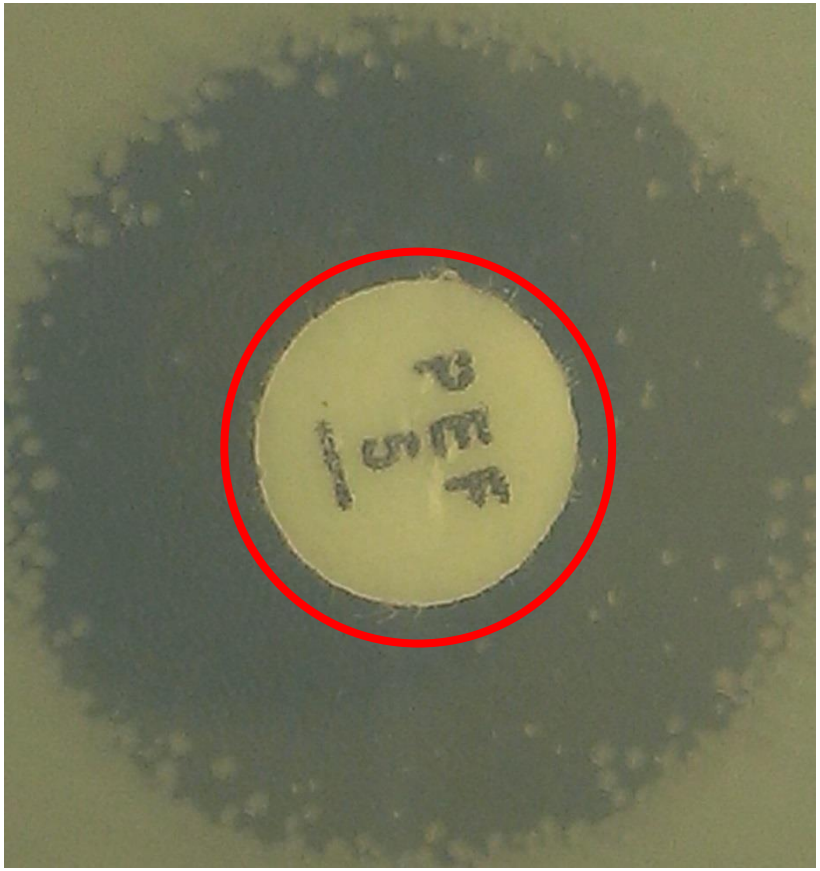


Three lots of pefloxacin disks tested

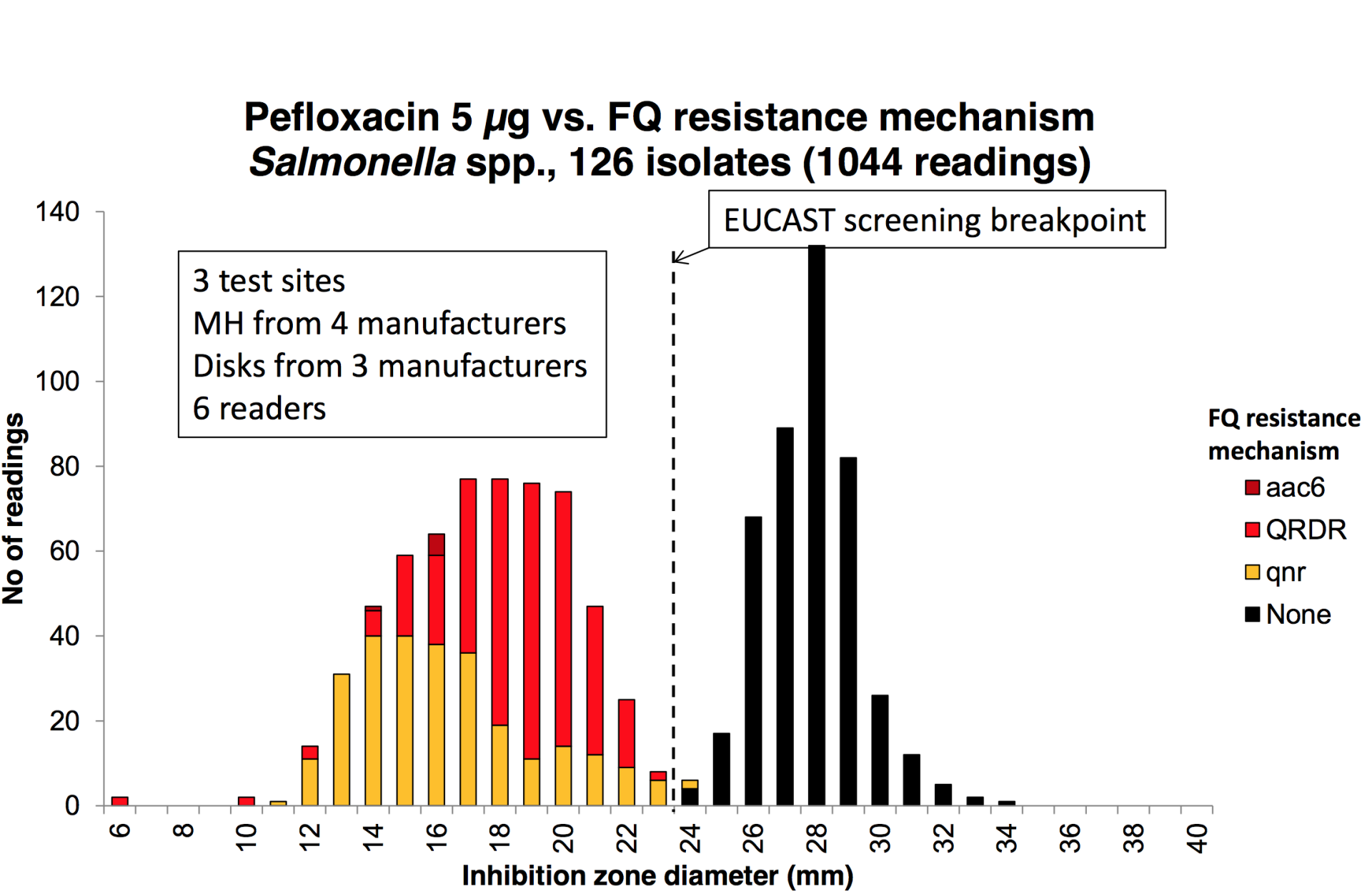
- Significantly larger zones with Oxoid disks vs. BD and MAST ($p<0.0001$)
  - ≈2 mm larger zones
- No difference in S/R interpretation for 72 isolates tested

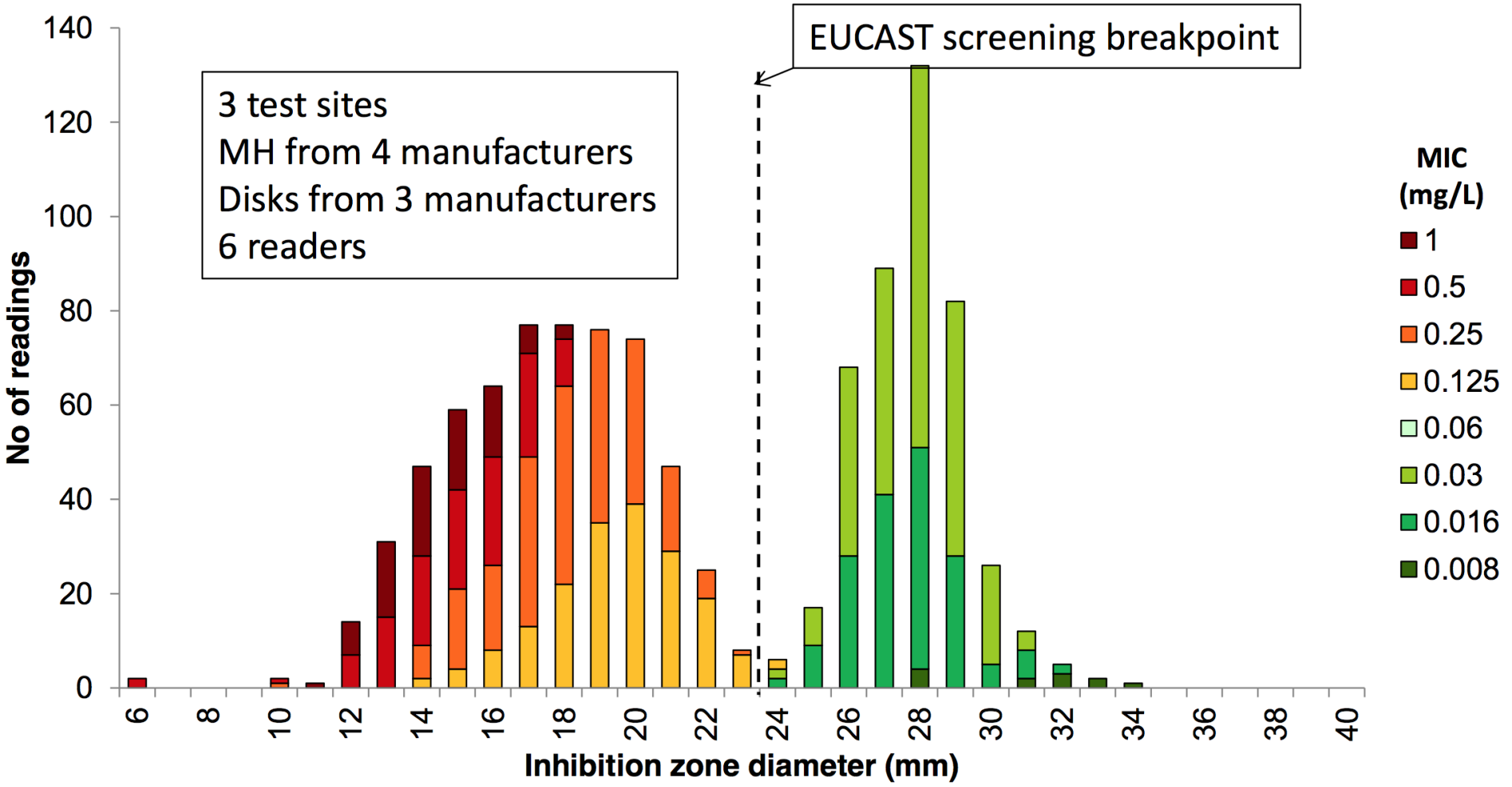
Pefloxacin 5 µg Zones[1]





[1] BBL MHA II (single lot); Oxoid disks (single lot); 18 h incubation
20/47 ciprofloxacin non-susceptible isolates appeared similar
(Phase I)


Pefloxacin 5 µg vs. FQ resistance mechanism
Salmonella spp., 126 isolates (1044 readings)
EUCAST screening breakpoint
3 test sites
MH from 4 manufacturers
Disks from 3 manufacturers
6 readers
No of readings
0
20
40
60
80
100
120
140
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
Inhibition zone diameter (mm)
FQ resistance mechanism
aac6
QRDR
qnr
None


Pefloxacin 5 µg vs. Ciprofloxacin MIC
Salmonella spp., 126 isolates (1044 readings)
EUCAST screening breakpoint
3 test sites
MH from 4 manufacturers
Disks from 3 manufacturers
6 readers
No of readings
Inhibition zone diameter (mm)
MIC (mg/L)
1
0.5
0.25
0.125
0.06
0.03
0.016
0.008

WG Vote

- Add Pefloxacin to M100 as a surrogate for ciprofloxacin resistance (7-0-0)
  - ≤23 mm R
  - ≥24 mm S
- Remove nalidixic acid (for *Salmonella*), ciprofloxacin disk diffusion breakpoints (6-1-0)
- Remove associated language for nalidixic acid from M100 (and associated documents)

- Discussion points:
  - pefloxacin not available everywhere (yet)
  - Issues with MHA brand (only one brand tested at UCLA, BBL, but more problems with ciprofloxacin noted when 3 manufacturers used)
  - nalidixic acid still very useful in South America (and ciprofloxacin DD has problems)
  - aac resistance mechanism will not necessarily be detected by pefloxacin
  - M23 QC study performed, presented at this meeting to QC WG

Enterobacteriaceae (Table 2A)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm) S	SDD	I	R	MIC Interpretive Criteria (µg/mL) S	SDD	I	R	Comments
FLUOROQUINOLONES											
NOTE: Reevaluation of fluoroquinolones is ongoing. See comment (2).											
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B	Ofloxacin	–	–		–	–	≤0.12		0.25–1	≥2	
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B	Ofloxacin	–	–		–	–	≤0.12		0.25–1	≥2	
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