

# Detection of reduced susceptibility to fluoroquinolones for *salmonellae spp* using alternative fluoroquinolone disks

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# Background

- Salmonella break point for ciprofloxacin is  $S \leq 0.064$  and  $R > 0.064$
- Nalidixic acid MIC and disk testing do not detect all isolates with plasmid mediated resistance mechanisms
  - *qnr* genes
- Ciprofloxacin 5  $\mu\text{g}$  disks can not separate isolates with resistance mechanisms from WT isolates

# NordicAST tabel 2.0

Fluorokinoloner	MIC-brytpunkt (mg/L)		Lappstyrka (µg)	Zonbrytpunkt (mm)		Kommentarer
	S ≤	R >		S ≥	R <	
						Nalidixinsyra har använts för resistensövervakning. Användandet av ECOFF (WT MIC ≤ 16 mg/L, zon 30 µg lapp ≥ 16 mm) har hög känslighet för detektion av kromosomal resistens men inte för plasmidmedierad resistens.
<a href="#">Ciprofloxacin</a>	<a href="#">0.5</a>	<a href="#">1</a>	<b>5</b>	<a href="#">22</a>	<a href="#">19</a>	
<a href="#">Ciprofloxacin, Salmonella spp.</a>	<a href="#">0.06</a>	<a href="#">0.06</a>	<b>5</b>	<b>31</b>	<b>31</b>	.

# Cipro 5 $\mu$ g

Zone diam	CIP MIC								Total
	0,02	0,03	0,06	0,12	0,25	0,5	1	2	
21							2		2
22						5	6		11
23						9	13	2	24
24						11	17	1	29
25						19	17	1	37
26					1	12	6		19
27					4	3	2		9
28					14	6	1		21
29				2	16	10			28
30				3	10	1			14
31		3		2	3				8
32		8		1	2				11
33		21	2						23
34	1	35	4		2				42
35	6	25	2						33
36	4	10							14
37	7	6							13
38	6	4							10
39	1								1
40	3								3
Total	28	112	8	8	52	76	64	4	352

# NordicAST tabel 3.0

Fluorokinoloner	MIC-brytpunkt (mg/L)		Lappstyrka (µg)	Zonbrytpunkt (mm)		Kommentarer
	S ≤	R >		S ≥	R <	
						Nalidixinsyra har använts för resistensövervakning. Användandet av ECOFF (WT MIC ≤ 16 mg/L, zon 30 µg lapp ≥ 16 mm) har hög känslighet för detektion av kromosomal resistens men inte för plasmidmedierad resistens.
<a href="#">Ciprofloxacin</a>	<a href="#">0.5</a>	<a href="#">1</a>	<b>5</b>	<a href="#">22</a>	<a href="#">19</a>	
<a href="#">Ciprofloxacin, Salmonella spp.</a>	<a href="#">0.06</a>	<a href="#">0.06</a>	<b>5</b>	<a href="#">33</a>	<b>Note</b>	Vid zon < 30 mm rapportera R. Vid zon 30-32 mm utför MIC-bestämning.

# Cipro 5 $\mu$ g

Zone diam	CIP MIC								Total
	0,02	0,03	0,06	0,12	0,25	0,5	1	2	
21							2		2
22						5	6		11
23						9	13	2	24
24						11	17	1	29
25						19	17	1	37
26					1	12	6		19
27					4	3	2		9
28					14	6	1		21
29				2	16	10			28
30				3	10	1			14
31		3		2	3				8
32		8		1	2				11
33		21	2						23
34	1	35	4		2				42
35	6	25	2						33
36	4	10							14
37	7	6							13
38	6	4							10
39	1								1
40	3								3
Total	28	112	8	8	52	76	64	4	352

# Material and methods

- 3 laboratories
  - EUCAST laboratory for AST, Växjö, Sweden
    - 2 persons reading
  - SSI National Reference Laboratory, Copenhagen, Denmark
    - 2 persons reading
  - CDC National Antimicrobial Resistance Surveillance Team, Atlanta, US
    - 2 persons reading

# Materials and methods

- 126 isolates
  - Examined by PCR for qnr, QRDR and aac6
    - 43 isolates with no identified resistance mechanisms
    - 37 isolates with qnr genes
    - 45 isolates with QRDR mutations
    - 1 isolate with an aac6-Ib-cr gene
      - Possible also an additional resistance mechanism – not identified
- DD
  - Media MH- 3 different manufacturers
    - VX BD, commercial  
Oxoid powder, in-house prepared
    - SSI BD, commercial  
BD powder, in-house prepared
    - CDC BD, commercial  
Remel, commercial

# Materials and Methods

- Disks ( $\mu\text{g}$ )
  - Ciprofloxacin 5
  - Ofloxacin 5
  - Levofloxacin 5
  - Nalidixic acid 30
  
- Ciprofloxacin 1
- Enoxacin 10
- Norfloxacin 2
- Pefloxacin 5

# Isolates

Serotype	No resistance	Qnr	QRDR	aac6	Total
Anatum		1			1
Aqua		1			1
Berta		1			1
Braenderup			1		1
Bredeney (grp B, lv:1,7)			1		1
Corvallis	4	6			10
Dublin (may be Rostock, no u on lum)			1		1
Duesseldorf			1		1
Emek		1			1
Enteritidis	14	3	14		31
Haardt or Blockley (need z58)			1		1
Hadar			1		1
Irumu			1		1
Mbandaka		1			1
Montevideo		2			2
Newport	1		3		4
S. 4 12; i-			1		1
Saintpaul		3	1		4
Schwarzengrund			1		1
Stanley	1	4			5
Telekebir		1			1
Thompson				1	1
Typhimurium	23	13	10		46
Virchow			5		5
Virchow (C1, r:1,2; Ent would be g,m)			2		2
Virchow (O:7 (C1), r:1,2; Hei would be B)			1		1
<b>Total</b>	<b>43</b>	<b>37</b>	<b>45</b>	<b>1</b>	<b>126</b>

# Disk Diffusion

# Cipro 5

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			

NordicAST brp



# Oflox 5

Zone diameter	Resistance mechanism			
	none	qnr	QRDR	aac6
15			1	
16		1	2	
17		12	3	
18		47		
19		58	1	1
20		54	7	3
21		14	28	2
22		17	40	
23		11	64	
24	1	7	73	
25	1	1	42	
26	4		5	
27	20		4	
28	43			
29	64			
30	49			
31	47			
32	19			
33	7			
34	2			
35	1			

# Levo 5

Zone diameter	Resistance mechanism			
	none	qnr	QRDR	aac6
18			1	
19		2	1	
20		18		
21		50	4	
22		61	5	1
23		38	15	3
24		24	47	2
25		18	60	
26		10	67	
27	5	1	45	
28	14		20	
29	29		5	
30	45			
31	56			
32	47			
33	29			
34	16			
35	13			
36	4			

# Nalidixic Acid 30

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			

# Cipro 1

Zonediam	Resistance mechanism				
	eter	none	qnr	QRDR aac6	
13				1	
14				3	
15			4	1	2
16			21	2	
17			47	1	
18			50	4	
19			37	8	
20			21	25	
21			19	35	
22			11	59	
23			11	60	
24			1	47	
25				22	
26		6		6	
27		20			
28		50			
29		60			
30		50			
31		39			
32		13			
33		11			
34		7			
35		1			
36		1			

# Nor 2

Zonediam	Resistance mechanism				
	eter	none	qnr	QRDR aac6	
11				1	
12				1	
13			1	2	2
14			13	2	2
15			42	3	
16			56	3	
17			30	8	
18			17	40	
19			30	54	
20			18	71	
21		1	9	61	
22			5	17	
23				8	
24		6	1	1	
25		19			
26		61			
27		55			
28		60			
29		25			
30		17			
31		12			
32		2			

# Enox 10

Zonediam	Resistance mechanism				
	eter	none	qnr	QRDR aac6	
12			1		
13				1	
14				1	
15			2	1	
16			11	5	1
17			45	18	3
18			58	59	2
19			41	80	
20			21	68	
21			22	27	
22			14	9	
23			3		
24		4	3	1	
25		16	1		
26		44			
27		61			
28		67			
29		29			
30		19			
31		12			
32		5			
33		1			

# Peflox 5

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			

# Peflox 5 - Gap on each agar!

## (shown for 5 of the 6 agars)

Zo	BD, SSI			
	Resistance mechanism	none	qnr	QRDR
10				
11				
12			1	
13		3		
14		8		
15		8	1	
16		6	1	1
17		5	2	
18		2	6	
19			9	
20		1	11	
21		2	10	
22		1	4	
23		1		
24				
25				
26				
27	2			
28	7			
29	17			
30	11			
31	3			
32	3			

Zo	BD, Vx				
	Resistance mechanism	none	qnr	QRDR	aac6
10				1	
11					
12			5		
13			9		
14			7	4	1
15			2	10	
16			5	8	
17			4	8	
18			1	7	
19			2	4	
20			1	2	
21					
22					
23			1		
24					
25					
26		15			
27		15			
28		10			
29		2			
30					
31					
33		1			

Zo	BD, CDC				
	Resistance mechanism	none	qnr	QRDR	aac6
10					
11					
12				1	
13			3		
14			8		
15			8		
16			4	1	1
17			6	4	
18			3	6	
19			1	9	
20			1	13	
21				7	
22			2	3	
23				1	
24			1		
25					
26					
27		7			
28		14			
29		16			
30		4			
31		1			
33		1			

Zo	Remel, CDC				
	Resistance mechanism	none	qnr	QRDR	aac6
6				1	
11					
12					
13			3		
14			2		
15			13	4	
16			7	3	1
17			3	11	
18			4	10	
19			1	10	
20			2	4	
21			2	2	
22					
23					
24			1		
25					
26		9			
27		13			
28		15			
29		1			
30		2			
31		1			
34		1			

Zo	Oxoid, Vx				
	Resistance mechanism	none	qnr	QRDR	aac6
10				1	
11			1		
12			6		
13			10		
14			5		
15			2	3	
16			6	3	1
17			3	6	
18				8	
19			1	13	
20			1	8	
21			1	2	
22			1	1	
23					
24					
25		2			
26		3			
27		11			
28		15			
29		9			
30					
31		3			
32					

 Gap zone

 Highest zone diameter for isolates with a resistance mechanism

# Summary /conclusion I

- By MIC all three FQ distinguished between isolates with and without resistance mechanisms.
  - With the present breakpoints - 8 isolates with resistance mechanisms had readings in the susceptible range for ciprofloxacin and ofloxacin
    - Probably related to the lot of the MIC panel used
- By DD neither cipro 5 $\mu$ g, levo 5, oflox 5 or nali 30 were able to reliably distinguish isolates with resistance mechanisms from WT.

# Summary /conclusion

- DD

- Alternative disks were identified
- **Pefloxacin 5** were able to reliably distinguish between isolates with and without resistance mechanisms on all tested batches of MH agar
- Pooling all results (readers, media etc) a breakpoint of

$S \geq 25$  mm

$R < 25$  mm

yielded a

sensitivity of 100%

specificity of 99,6%

# Develop QC data for pefloxacin

- $\geq 7$  laboratories each doing
  - Testing on *E. coli* 25922 - 10 times on
  - Media from 3 manufactures
    - BD
    - Thermofisher (Oxoid)
    - Remel
  - Disks from 2 different manufacturers
    - Thermofisher (Oxoid)
    - BD
    - Bio-Rad
    - Mast provides on special request

# Pefloxacin different disk manufacturers

Oxoid disks				Oxoid disks 2nd lot				BD disks				Mast disks				Bio-Rad disks			
None	qnr	QRDR		None	qnr	QRDR		None	qnr	QRDR		None	qnr	QRDR		None	qnr	QRDR	
6				6				6				6				6			
7				7				7				7				7			
8				8				8				8				8			
9				9				9				9				9			
10				10				10				10				10			
11				11				11				11				11			
12				12				12				12				12			
13				13				13				13				13			
14				14				14	3	2		14				14			
15				15				15	1	1		15	1			15			
16	1			16	1			16	1	3		16	2	2		16			
17	1			17	1			17	2	3		17	2	4		17			
18	2	2		18	1			18	1	4		18	2	6		18			
19	1	4		19	3	6		19	1	2		19	1	1		19	1	1	
20	1	3		20	1	6		20	1			20	3	2		20	1		
21	2	2		21	2	2		21	1			21				21			
22	2	3		22	1	1		22	1			22	1			22	3	3	
23	1	1		23	2			23				23				23	1	2	
24	1			24				24	2			24	1			24	1	4	
25				25				25	13			25	2			25	4	4	
26	3			26	4			26	17			26	16			26	1	1	
27	8			27	8			27	9			27	13			27			
28	24			28	19			28	3			28	13			28	4		
29	6			29	12			29	1			29				29	10		
30	3			30	2			30				30				30	21		
31	1			31				31				31				31	5		
32				32				32				32				32	5		

# Disk potency

Disc Manufacturer	Expected result (90-125%)	Ec20		Ps16	
		Bioassay Result ( $\mu\text{g}$ )	Correlation coefficient (r)	Bioassay Result ( $\mu\text{g}$ )	Correlation coefficient (r)
Mast	5 $\mu\text{g}$ (4.5-6.25 $\mu\text{g}$ )	4.69	0.9989	5.17	0.9996
Oxoid	5 $\mu\text{g}$ (4.5-6.25 $\mu\text{g}$ )	7.40	0.9990	7.37	0.9996
BD BBL	5 $\mu\text{g}$ (4.5-6.25 $\mu\text{g}$ )	3.26	0.9973	4.08	0.9996
BioRad	5 $\mu\text{g}$ (4.5-6.25 $\mu\text{g}$ )	10.66	0.9994	12.18	0.9999
Blind Control A	12.75 $\mu\text{g}$ (11.48-15.94 $\mu\text{g}$ )	12.52	0.9989	12.57	0.9996
Blind Control B	6.375 $\mu\text{g}$ (5.74-7.97 $\mu\text{g}$ )	6.51	0.9989	6.69	0.9999