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EUCAST disk diffusion with pefloxacin 5 µg as screen for fluoroquinolone resistance in *Salmonella* spp. Variation between media, disks and testing sites

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Introduction

There is clinical evidence that systemic infections caused by *Salmonella* spp. with low-level fluoroquinolone resistance (MIC >0.06 mg/L) may respond poorly to ciprofloxacin. Disk diffusion with ciprofloxacin 5 µg does not reliably detect such isolates. Isolates resistant due to quinolone resistance determining region (QRDR) mutations can be detected by nalidixic acid 30 µg, but the detection of isolates with *qnr*, or other plasmid-mediated mechanisms, remains uncertain. In a previous study, we showed that the pefloxacin 5 µg disk can be used to detect all currently defined fluoroquinolone (FQ) resistance mechanisms in *Salmonella* spp. (Poster 285, ECCMID 2014).

Objectives

The objectives of this study were to investigate the variation between media, disks and testing sites and also to establish a EUCAST screening breakpoint for pefloxacin 5 µg to detect FQ resistance mechanisms and ciprofloxacin resistance in *Salmonella* spp.

Methods

All tests were performed on a collection of 126 clinical isolates of *Salmonella* spp., including a large proportion of isolates with low-level FQ resistance. Disk diffusion with pefloxacin 5 µg was performed according to EUCAST methodology. Ciprofloxacin MIC values were determined with broth microdilution on custom frozen panels (TREK Diagnostics/Thermo Fisher Scientific) according to ISO standard 20776-1. The absence or presence of FQ resistance mechanisms (*qnr, aac(6')Ib-cr* and QRDR mutations) was determined by PCR and sequencing. Mueller-Hinton (MH) agar from four manufacturers (BBL/BD, Bio-Rad, Oxoid/Thermo Fisher Scientific and Remel) and pefloxacin disks from four manufacturers (BD, Bio-Rad, Mast Diagnostics and Oxoid) were investigated. Both in-house prepared and commercial plates were used. Inter-laboratory variation was evaluated by testing at three sites. Testing was also performed on *Escherichia coli* ATCC 25922 in order to establish a tentative QC target and range for pefloxacin 5 µg.

3 testing sites, media from 4 manufacturers, disks from 3 manufacturers, 6 readers

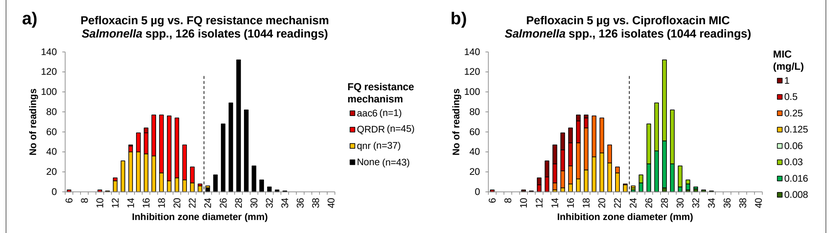

Figure 1. Inhibition zone diameter distributions for *Salmonella* spp. vs. pefloxacin 5 µg
a) with fluoroquinolone resistance mechanisms as coloured bars.
b) with ciprofloxacin MIC values as coloured bars.
In both graphs, the dotted line indicates the EUCAST screening breakpoint of <24mm.

Table 1. EUCAST recommendations (EUCAST Breakpoint Table v 4.0, 2014) for testing and reporting of ciprofloxacin resistance in *Salmonella* spp.

Enterobacteriaceae and fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbers for comments on MIC breakpoints Letters for comments on disk diffusion
	S ≤	R >		S ≥	R <	
Ciprofloxacin, *Salmonella* spp.[1]	0.06	0.06		Note[A]	Note[A]	**1**. There is clinical evidence for ciprofloxacin to indicate a poor response in systemic infections caused by *Salmonella* spp. with low-level ciprofloxacin resistance (MIC>0.06 mg/L). The available data relate mainly to *S. typhi* but there are also case reports of poor response with other *Salmonella* species. **A**. Tests with a ciprofloxacin 5 µg disk will not reliably detect low-level resistance in *Salmonella* spp. To screen for ciprofloxacin resistance in *Salmonella* spp., use the pefloxacin 5 µg disk. **See Note B.**
Pefloxacin (screen), *Salmonella* spp.[1]	NA	NA	**5**	24[B]	24[B]	**B**. Susceptibility of *Salmonella* spp. to ciprofloxacin can be inferred from the pefloxacin disk diffusion susceptibility test result.

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Results

Pefloxacin 5 µg inhibition zones were comparable for disks from BD, Mast and Oxoid. Inhibition zones for Bio-Rad disks were significantly larger and were excluded from further analysis. Inhibition zones for *E. coli* ATCC 25922 and pefloxacin 5 µg disks from BD, Mast and Oxoid ranged from 26-30 mm with a mean of 28 mm. Pefloxacin disks from additional manufacturers, as well as new batches of Bio-Rad disks, will be evaluated during 2014.

FQ resistance mechanisms were present in 65% of the isolates: *qnr* (n=37), *aac(6')Ib-cr* (n=1) and QRDR mutations (n=45). A total of 1044 inhibition zones for pefloxacin 5 µg (BD, Mast and Oxoid disks) were obtained for the 126 isolates. Although some variation between MH agars and testing sites was observed, the aggregation of all data resulted in a distribution with a minimal overlap between isolates without and with FQ resistance at 24 mm (**Figure 1a**). This was despite the large proportion of isolates with low-level FQ resistance. A screening breakpoint of <24 mm was chosen to minimise overlap between isolates without and with FQ resistance using media and disks from the majority of the investigated manufacturers. The correlation between pefloxacin 5 µg inhibition zones and ciprofloxacin MIC was also excellent (**Figure 1b**).

The EUCAST algorithm for screening and reporting of ciprofloxacin resistance in *Salmonella* spp. was published in the EUCAST Breakpoint Table v 4.0, January 2014, see **Table 1**.

Conclusions

We conclude that the pefloxacin 5 µg disk can be used to detect all currently defined FQ resistance mechanisms and ciprofloxacin resistance in *Salmonella* spp. with a screening breakpoint of <24 mm.

The test appears robust enough to allow for some variation between manufacturers and testing sites. However, a QC range for *E. coli* ATCC 25922 of 25-31 mm, with a target of 28 mm, should be used for stringent quality control of pefloxacin disks, both by manufacturers and users. The mean value of repeated tests should be within 27-29 mm (target ± 1 mm).