P0279
Poster Session I
EUCAST antimicrobial susceptibility testing

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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Objectives

There is clinical evidence for ciprofloxacin to indicate a poor response in systemic infections caused by *Salmonella* spp. with low-level fluoroquinolone resistance (MIC >0.06 mg/L). Disk diffusion with ciprofloxacin 5 µg does not reliably detect such isolates. Isolates resistant due to QRDR (quinolone resistance determining region) mutations can be detected by nalidixic acid 30 µg but the detection of isolates with *qnr*, or other plasmid-mediated mechanisms, remains uncertain. We have shown that the pefloxacin 5 µg disk can be used to detect all currently defined fluoroquinolone resistance (FQR) mechanisms in *Salmonella* spp. (to be published). The objectives of this study were to investigate the variation between media, disks and testing sites and to establish a screening breakpoint for pefloxacin 5 µg vs. *Salmonella* spp.

Methods

Disk diffusion for pefloxacin 5 µg was performed on a collection of 126 clinical isolates of *Salmonella* spp. according to EUCAST methodology. The absence or presence of FQR mechanisms (*qnr, aac(6')Ib-cr* and QRDR mutations) was determined by PCR and sequencing. Mueller-Hinton (MH) agar from four manufacturers (BD, Bio-Rad, Oxoid/Thermo Fisher Scientific and Remel) and pefloxacin disks from four manufacturers (BD, Bio-Rad, Mast Diagnostics and Oxoid) were investigated. 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.

Results

Pefloxacin 5 µg inhibition zones were comparable for disks from three manufacturers (BD, Mast and Oxoid). Inhibition zones for Bio-Rad disks were significantly larger and were excluded from further analysis. Although some variation between MH agars and testing sites was observed, the aggregation of all 972 readings for the 126 isolates resulted in a distribution with a neglectible overlap (0.6% of readings) between isolates without and with FQR at 24 mm (Table 1). For each MH agar, isolates without FQR were separated by 2-4 mm from isolates with FQR. 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.

Conclusions

We conclude that the pefloxacin 5 µg disk can be used to screen for all currently defined FQR mechanisms in *Salmonella* spp. and that a screening breakpoint of 24 mm can be used to separate isolates without and with FQR. The test appears sufficiently robust 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).

Table 1
Inhibition zone diameter vs. FQ resistance mechanisms for pefloxacin 5 µg and *Salmonella* spp. tested at three sites (972 readings totally for 126 clinical isolates) using disks and agar from several manufacturers.
The suggested screening breakpoint is shown as a line.

Zone diameter (mm)	FQ resistance mechanism			
	None (43 isolates)	qnr (37 isolates)	QRDR (45 isolates)	aac6 (1 isolate)
6			2	
7				
8				
9				
10			2	
11		1		
12		11	3	
13		31		
14		40	6	1
15		40	19	
16		37	21	5
17		35	41	
18		18	58	
19		8	59	
20		13	54	
21		10	33	
22		8	15	
23		4	2	
24	4	2		
25	17			
26	64			
27	81			
28	113			
29	70			
30	24			
31	12			
32	5			
33	2			
34	1			
35				
36				
37				
38				
39				
40				