

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.

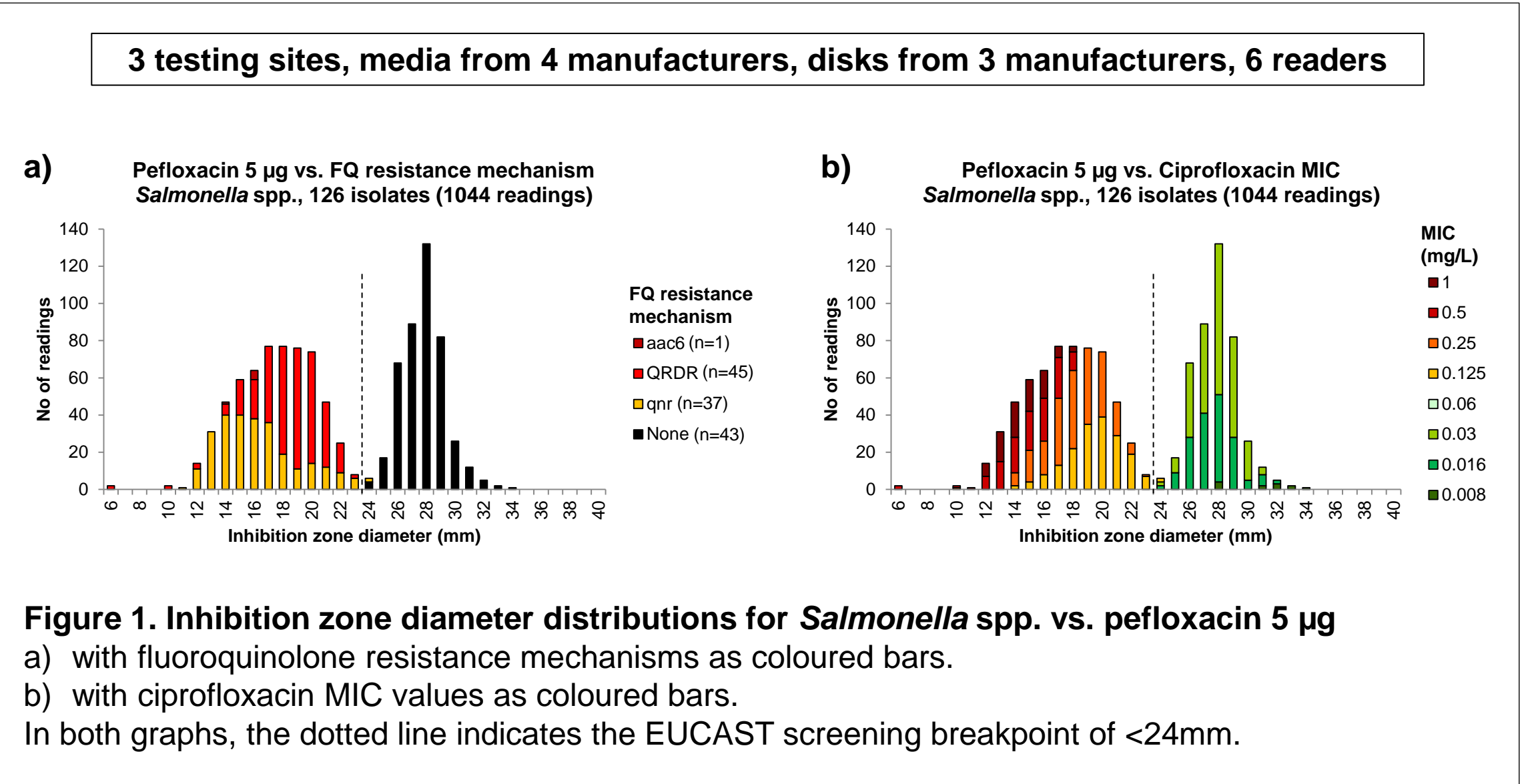


Table 1. EUCAST recommendations (EUCAST Breakpoint Table v 4.0, 2014) for testing and reporting of ciprofloxacin resistance in *Salmonella* spp.

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, <i>Salmonella</i> 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 <i>Salmonella</i> spp. with low-level ciprofloxacin resistance (MIC>0.06 mg/L). The available data relate mainly to <i>S. typhi</i> but there are also case reports of poor response with other <i>Salmonella</i> species. A. Tests with a ciprofloxacin 5 µg disk will not reliably detect low-level resistance in <i>Salmonella</i> spp. To screen for ciprofloxacin resistance in <i>Salmonella</i> spp., use the pefloxacin 5 µg disk. See Note B.
Pefloxacin (screen), <i>Salmonella</i> spp. ¹	NA	NA	5	24 ^B	24 ^B	B. Susceptibility of <i>Salmonella</i> spp. to ciprofloxacin can be inferred from the pefloxacin disk diffusion susceptibility test result.

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).

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Introduction

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has recently established clinical MIC breakpoints for *Corynebacterium* spp. (available in the EUCAST Breakpoint Table v. 4.0) and a disk diffusion method based on the EUCAST fastidious medium (MH-F).

Objectives

The objective of this study was to produce MIC and zone diameter correlations using EUCAST methods and to establish zone diameter breakpoints for *Corynebacterium* spp.

Methods

Antimicrobial susceptibility testing was performed on a collection of 258 clinical isolates of *Corynebacterium* spp. of different geographical origin: Kronoberg county, Sweden; Santander, Spain and isolates from the worldwide SENTRY Antimicrobial Surveillance Program (provided by JMI Laboratories, USA). Species identification was performed with MALDI-TOF MS. Disk diffusion was performed according to EUCAST methodology for fastidious organisms on Mueller-Hinton (MH) agar with 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F) using in-house prepared plates with agar from two manufacturers (BBL/BD and Oxoid/Thermo Fischer Scientific). MIC determination was performed with broth microdilution (BMD) according to ISO standard 20776-1 using EUCAST media for fastidious organisms (cation-adjusted MH broth with 5% lysed horse blood and 20 mg/L β-NAD). Sealed BMD plates were incubated in ambient air at 35°C. If standard incubation for 16-20 h did not result in sufficient growth, incubation was prolonged to a total time of 40-44 h, both for disk diffusion and BMD. Antimicrobial agents tested are listed in **Table 1**. Very major, major and minor errors (VME, ME and mE) for disk diffusion with the proposed zone diameter breakpoints were calculated according to ISO standard 20776-2 with BMD as reference.

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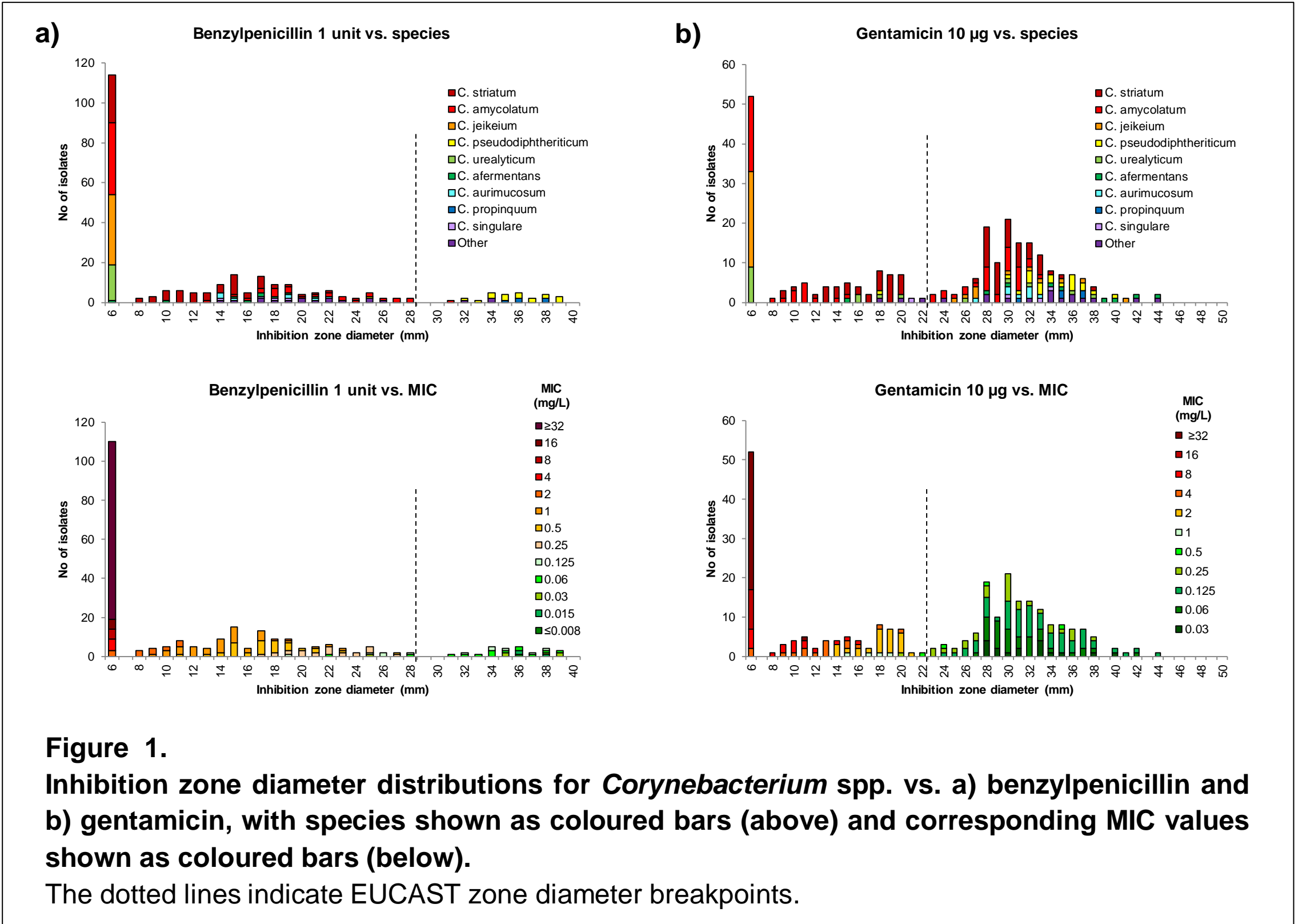


Figure 1. Inhibition zone diameter distributions for *Corynebacterium* spp. vs. a) benzylpenicillin and b) gentamicin, with species shown as coloured bars (above) and corresponding MIC values shown as coloured bars (below). The dotted lines indicate EUCAST zone diameter breakpoints.

Table 1. EUCAST breakpoints for *Corynebacterium* spp. with categorical errors for disk diffusion.

Antimicrobial agent	MIC breakpoints (mg/L)		Zone diameter breakpoints (mm)			Categorical errors					
	S≤	R>	Disk content (µg)	S≥	R<	VME	VME (%)	ME	ME (%)	mE	mE (%)
Benzylpenicillin	0.12	0.12	1 unit	29	29	0	0	8	3.1	-	-
Ciprofloxacin	1	1	5	25	25	0	0	2	0.8	-	-
Moxifloxacin	0.5	0.5	5	25	25	0	0	5	1.9	-	-
Gentamicin	1	1	10	23	23	0	0	6	2.3	-	-
Vancomycin	2	2	5	17	17	0*	0*	0*	0*	-	-
Clindamycin	0.5	0.5	2	20	20	0	0	2	0.8	-	-
Tetracycline	2	2	30	24	24	3	1.2	0	0	-	-
Linezolid	2	2	10	25	25	0*	0*	0*	0*	-	-
Rifampicin	0.06	0.5	5	30	25	0	0	0	0	1	0.4

* For vancomycin and linezolid, only susceptible isolates were included.
S = Susceptible, R = Resistant, VME = Very Major Error, ME = Major Error, mE = minor Error

Results

The collection of *Corynebacterium* spp. (n=258) comprised 20 different species, with *C. striatum* (n=78), *C. amycolatum* (n=63), *C. jeikeium* (n=35), *C. pseudodiphtheriticum* (n=20) and *C. urealyticum* (n=18) dominating. Prolonged incubation (totally 40-44 h) was needed to achieve sufficient growth for 11% (disk diffusion) and 16% (BMD) of the isolates. This was mainly for *C. jeikeium*, *C. urealyticum* and *C. pseudodiphtheriticum*.

The correlation between MIC and zone diameters for *Corynebacterium* spp. was excellent, despite the high number of different species represented. It was apparent that MICs varied between species, but it did not affect the MIC-zone diameter correlation per se (**Figure 1**). Zone diameter breakpoints were established to minimize the occurrence of VME, resulting in overall error rates as follows: VME 0.13, ME 0.99, and mE 0.04 %, see **Table 1**. The corresponding values after excluding vancomycin and linezolid because of the lack of resistant isolates are: VME 0.17, ME 1.27 and mE 0.06 %.

MIC-zone diameter correlations for *Corynebacterium* spp. based on the results in this study are available on the EUCAST website:
http://www.eucast.org/antimicrobial_susceptibility_testing/calibration_and_validation/.

Conclusions

The results from this study show that antimicrobial susceptibility testing of *Corynebacterium* spp. can be reliably performed using EUCAST methodology. Based on these data, EUCAST has established zone diameter breakpoints for *Corynebacterium* spp. calibrated to the clinical MIC breakpoints. These are available in the EUCAST Breakpoint Table v. 4.0, January 2014.

Acknowledgements

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Evaluation of retapamulin Etest.

Validation against broth microdilution and inter-laboratory variation using EUCAST media.

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Introduction

Retapamulin (RET) has *in vitro* activity against *Staphylococcus aureus* and beta-haemolytic streptococci. There are no clinical breakpoints for RET, but the epidemiological cut-off (ECOFF) values can be used to distinguish between wild-type isolates and isolates with acquired resistance mechanisms (non-wild type isolates).

Objectives

The objectives of this study were to evaluate RET Etest by validation against reference methodology and by investigation of inter-laboratory variation.

Methods

Etest (bioMérieux) MIC determination was performed on EUCAST media, un-supplemented Mueller-Hinton (MH) agar for *S. aureus* and MH with 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F) for *Streptococcus pyogenes*. Preparation of inoculum, inoculation and incubation of plates were performed according to EUCAST disk diffusion methodology. Broth microdilution (BMD) was performed on custom Sensititre plates (TREK Diagnostics/Thermo Fisher Scientific) according to ISO standard 20776-1 using cation-adjusted MH broth for *S. aureus* and EUCAST MH-F broth for *S. pyogenes*. Evaluation of RET Etest against BMD was performed at the EUCAST AST Development Laboratory for 100 methicillin-susceptible (MSSA), 100 methicillin-resistant (MRSA) *S. aureus* and 100 *S. pyogenes*. The inter-laboratory variation for RET Etest was investigated by 5 additional European laboratories using local clinical isolates (100 MSSA, 100 MRSA and 100 *S. pyogenes* per site if available) on common lots of commercial MH and MH-F plates (Oxoid/Thermo Fisher Scientific). Quality control was performed with *S. aureus* ATCC 29213 and *S. pneumoniae* ATCC 49619.

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Results

For *S. aureus*, the correlation between RET Etest and BMD was excellent, with 99 and 100% of Etest MICs within ± 1 dilution of BMD MICs for MSSA and MRSA, respectively. For *S. pyogenes*, Etest MICs tended to be higher than BMD MICs, with 39% at +1 dilution and 11% at +2 dilutions. However, 89% of the Etest MICs were still within ± 1 dilution of BMD MICs.

RET Etest distributions from 5 sites (Lab A-E) showed little variation for MSSA and MRSA with medians ranging from 0.06 to 0.12 mg/L (**Table 1**). Thirteen MRSA from one site had MICs of 64 mg/L and 12 of these belonged to the same clonal complex, CC398. The aggregated MIC distribution (MSSA and MRSA from all test sites) correlated well with the EUCAST reference distribution (**Figure 1a**). For *S. pyogenes*, the MIC distributions were wider and the variation between sites was larger than for *S. aureus*, but the aggregated distribution correlated well with the EUCAST reference distribution (**Figure 1b**). Medians for *S. pyogenes* varied from 0.015-0.06 mg/L.

Table 1.
Retapamulin MIC distributions (no of isolates) per method and test site.

MSSA (ECOFF: WT ≤ 0.5 mg/L)																	
Test and site	No of isolates	MIC (mg/L)															
		≤0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥512
BMD EUCAST Lab	100					1	67	31								1	
Etest EUCAST Lab	100					4	60	35								1	
Etest Lab A	100					3	34	63									
Etest Lab B	100					4	48	48									
Etest Lab C	100						47	52	1								
Etest Lab D	99						2	91	6								
Etest Lab E	100				1	14	62	22	1								

MRSA (ECOFF: WT ≤ 0.5 mg/L)																	
Test and site	No of isolates	MIC (mg/L)															
		≤0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥512
BMD EUCAST Lab	100					1	79	20									
Etest EUCAST Lab	100					9	81	10									
Etest Lab A	102					3	27	70	2								
Etest Lab B	100					9	70	21									
Etest Lab C	100						7	93									
Etest Lab D	100						1	61	24			1				13	
Etest Lab E	100				1	20	69	8		2							

<i>S. pyogenes</i> (ECOFF: WT ≤ 0.12 mg/L)																	
Test and site	No of isolates	MIC (mg/L)															
		≤0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥512
BMD EUCAST Lab	100			6	78	16											
Etest EUCAST Lab	100				37	55	8										
Etest Lab A	98			7	19	43	26	2	1								
Etest Lab B	38				7	19	12										
Etest Lab C	90			1	10	66	8	5									
Etest Lab D	99					16	81	1	1								
Etest Lab E	99			12	37	41	9										

Conclusions

The MIC distributions for MSSA, MRSA and *S. pyogenes* produced with retapamulin Etest in this study correlated well with EUCAST reference MIC distributions. Also, Etest MICs performed at the EUCAST AST Development Laboratory correlated well with reference BMD. We therefore conclude that retapamulin Etest can be used on EUCAST media to categorise clinical isolates of *S. aureus* and *S. pyogenes* as wild-type and non-wild type, respectively.

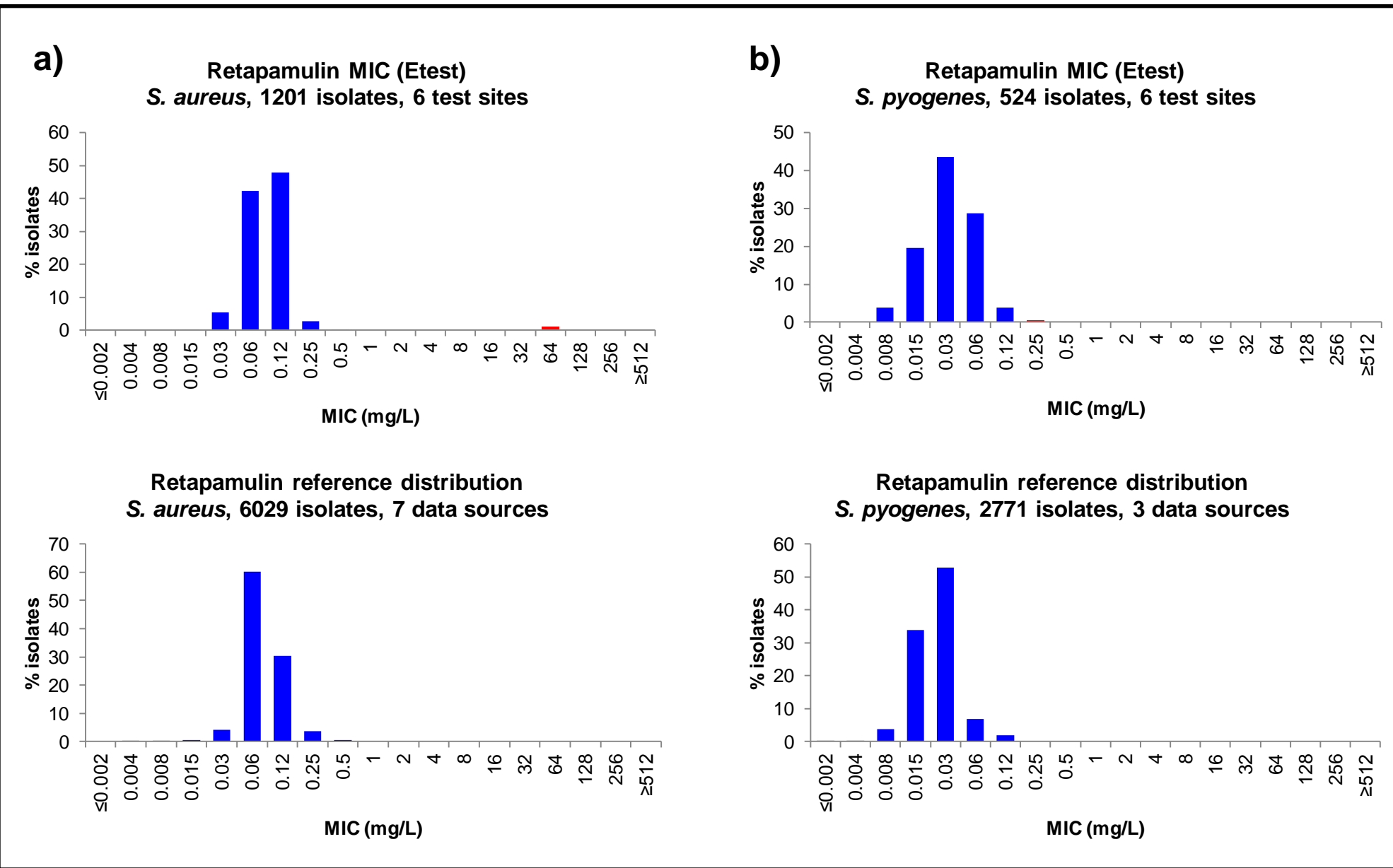


Figure 1.
Retapamulin MIC distributions for a) *S. aureus* (MSSA + MRSA) and b) *S. pyogenes*.
Aggregated Etest distributions above and reference distributions (www.eucast.org) below.

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P0282

EUCAST fluoroquinolone breakpoints in enterococci in urinary tract infections

– determination of disk diffusion criteria for susceptibility testing.

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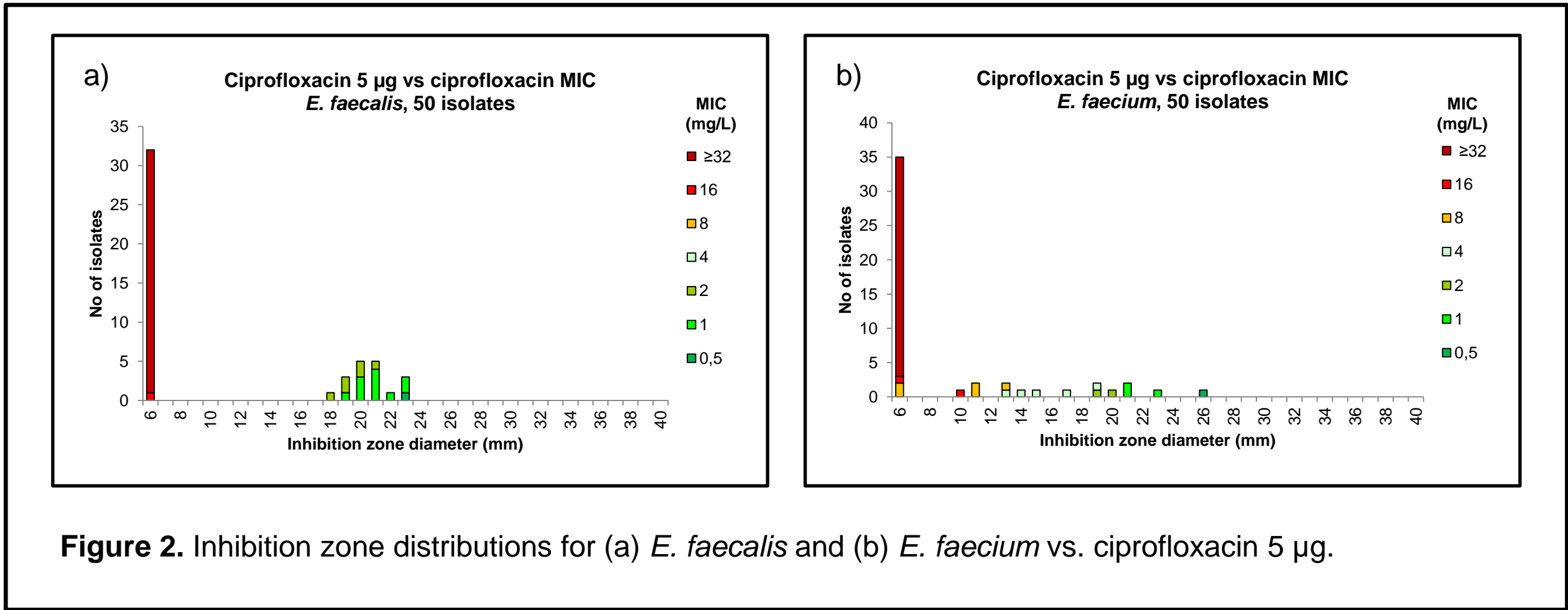
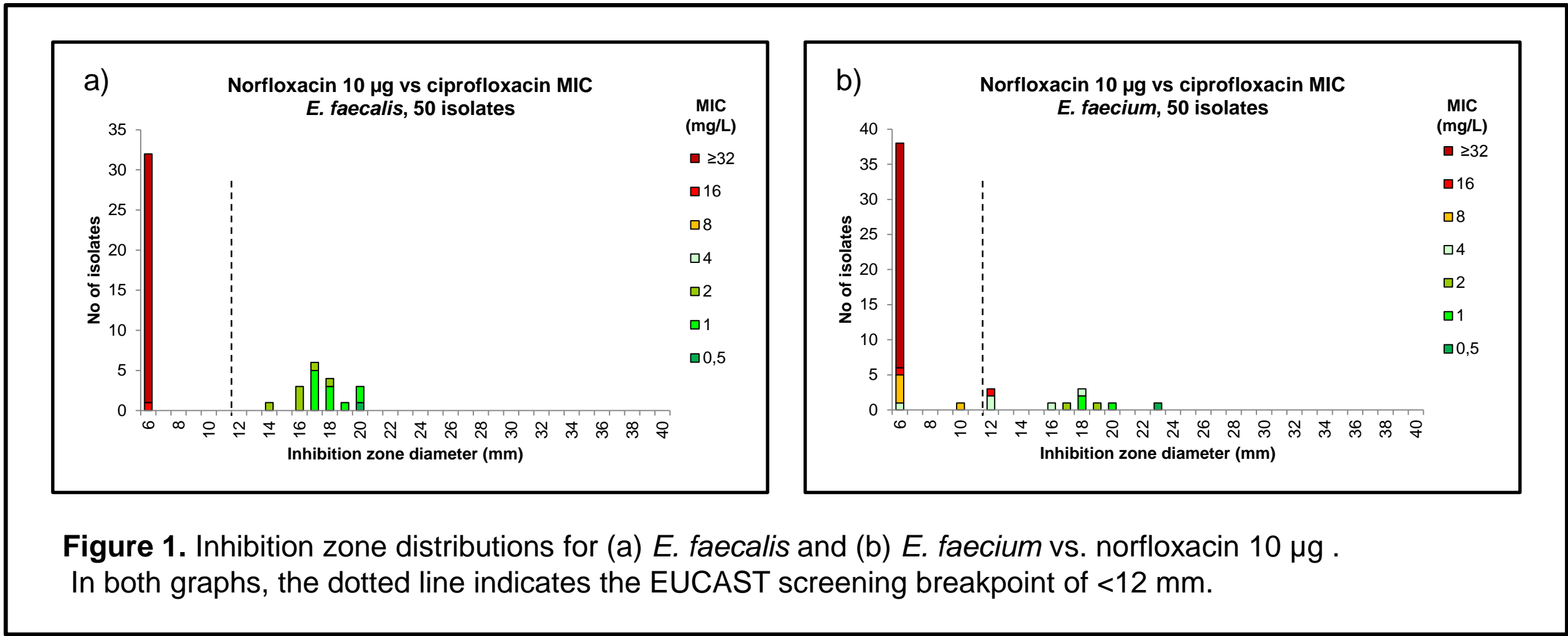
Introduction and objective

Treatment of systemic enterococcal infections with fluoroquinolones has been shown to carry a significant risk of treatment failure. However, both *in vitro* and clinical data support that enterococci should be considered possible targets for fluoroquinolone therapy in urinary tract infection (UTI).¹⁻² EUCAST has therefore recently published clinical breakpoints for ciprofloxacin and levofloxacin vs. enterococci valid for **uncomplicated UTI only** (now available in the EUCAST Breakpoint Table v. 4.0).³ The clinical breakpoints equal the epidemiological cut-off values for respective agent and species (4 mg/L for both species) .

The objective of this study was to evaluate the EUCAST recommendation to screen for fluoroquinolone resistance in enterococci using the norfloxacin 10 µg disk and a screening breakpoint of < 12 mm. This procedure is recommended for several other grampositive bacteria.

Methods

Antimicrobial susceptibility testing was performed on clinical isolates of *Enterococcus faecalis* (n=50) and *E. faecium* (n=50). Disk diffusion against norfloxacin 10 µg and ciprofloxacin 5 µg was performed according to EUCAST methodology on Mueller-Hinton agar from three manufacturers (BBL/BD, Bio-Rad and Oxoid/Thermo Fisher Scientific). Ciprofloxacin MIC values were determined by broth microdilution (BMD) on custom Sensititre plates (TREK Diagnostics/Thermo Fisher Scientific), in accordance with ISO standard 20776-1.



Results

Screening with the norfloxacin 10 µg disk clearly separated ciprofloxacin wild-type from non-wild type isolates of *E. faecalis* (Figure 1a). All ciprofloxacin-resistant isolates had a norfloxacin 10 µg zone diameter of 6 mm. For *E. faecium*, a similar distribution was seen, with 48/50 isolates classified correctly according to ciprofloxacin susceptibility (Figure 1b). However two isolates of *E. faecium* were incorrectly classified, one as ciprofloxacin susceptible and one as resistant. This was independent of the Mueller-Hinton agar used. The ciprofloxacin 5 µg disk also reliably separated ciprofloxacin wild-type from non-wild type isolates of *E. faecalis*, while in *E. faecium* an overlap in zone diameter was seen for isolates with MIC values close to the breakpoint (Figure 2).

Conclusions

Disk diffusion using norfloxacin 10 µg and a screening breakpoint of < 12 mm is a reliable method for detecting ciprofloxacin resistance in *E. faecalis* and *E. faecium*. Our data suggest that the ciprofloxacin 5 µg disk would work well in *E. faecalis* but not in *E. faecium*.

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2. Peterson J, Kaul S, Khashab M et al. *Urology* 2008; 71: 17-22.
3. EUCAST Breakpoint table 4.0 (2014); www.eucast.org.

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Evaluation of EUCAST zone diameter breakpoints for *Pseudomonas* spp.

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Introduction

Pseudomonas aeruginosa is an opportunistic nosocomial pathogen causing bacteraemia and infections in the pulmonary tract, urinary tract, burns and wounds. In addition to intrinsic resistance to a wide range of antibiotics, *P. aeruginosa* easily develops acquired resistance either by mutation in chromosomally encoded genes or by horizontal gene transfer. *P. non-aeruginosa* is less frequently recovered from clinical specimens but may be of relevance in compromised patients and has also been described to express multi-drug resistance. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has published clinical MIC and zone diameter breakpoints for *Pseudomonas* spp. These breakpoints were mainly based on data for *P. aeruginosa* since little data have been available for *P. non-aeruginosa*.

Objective

The objective of this study was to evaluate whether zone diameter breakpoints developed primarily on data from *P. aeruginosa*, would be valid for other major *Pseudomonas* species, using broth microdilution (BMD) as reference.

Methods

A total of 123 clinical isolates of *P. aeruginosa* (PA) and 192 *P. non-aeruginosa* (PNA) were selected from the worldwide SENTRY Antimicrobial Surveillance Program (JMI Laboratories, USA). Species identification was confirmed with MALDI-TOF MS. The collection of PNA contained 22 different species, which mainly belonged to the putida (n=113), stutzeri (n=35) and fluorescens (n=20) groups. Disk diffusion was performed at the EUCAST Laboratory according to EUCAST methodology on Mueller-Hinton agar from three manufacturers (BBL/BD, Bio-Rad and Oxoid/Thermo Fisher Scientific). MIC values were determined by BMD, according to ISO standard 20776-1, at JMI Laboratories. Data were analysed by EUCAST for all antibiotic agents with zone diameter breakpoints in EUCAST tables, except for netilmicin and ticarcillin (PA and PNA) and piperacillin and ticarcillin-clavulanate (PA), where no BMD MICs were available. Very major, major and minor errors (VME, ME and mE) for categorical agreement between disk diffusion and BMD were calculated according to ISO standard 20776-2.

Results

The correlation between MIC and zone diameters was excellent both for PA and PNA, although PNA more often showed broader zone distributions, sometimes bimodal and with different median values compared with PA. This is probably explained by the large number of different species represented in the PNA collection. Overall error rates (%) vs. EUCAST Breakpoint Table v. 3.1 were as follows: VME 0.2, ME 0.8 and mE 5.3 (no of tests = 3983). Error rates were similar for PA and PNA, see **Table 1**. Minor errors were most often seen with carbapenems and aztreonam, due to poor separation between susceptible, intermediate and resistant isolates. Correlation between MIC values and inhibition zones resulted in minor adjustments (± 1 mm) in zone diameter breakpoints for piperacillin, piperacillin-tazobactam, ticarcillin-clavulanate and cefepime. The doripenem MIC R breakpoint was adjusted by EUCAST and therefore also the corresponding zone diameter breakpoint. Updated breakpoints (**Table 2**), resulted in overall error rates (%) as follows: VME 0.2, ME 0.5 and mE 4.9.

MIC-zone diameter correlations for *Pseudomonas* spp. based on the results in this study are available on the EUCAST website:
http://www.eucast.org/antimicrobial_susceptibility_testing/calibration_and_validation/.

Table 1.
Error rates (%) for *Pseudomonas* spp. vs. EUCAST Breakpoint Table v. 3.1.

Antimicrobial agent	<i>P. aeruginosa</i>				<i>P. non-aeruginosa</i>			
	No of tests	VME	ME	mE	No of tests	VME	ME	mE
Piperacillin	0	-	-	-	171	0	5.8	-
Piperacillin-tazobactam	123	0	2.4	-	191	0	3.1	-
Ticarcillin-clavulanate	0	-	-	-	150	2.7	0	-
Cefepime	123	1.6	0	-	191	0	0	-
Ceftazidime	117	0	0	-	188	1.1	0	-
Doripenem	115	0	0	9.6	176	0	0	13.6
Imipenem	123	0	1.6	8.1	187	0	0	2.7
Meropenem	121	0	0.8	17.4	190	0	2.6	21.6
Aztreonam	123	0	0	8.9	191	0	0	34.0
Ciprofloxacin	120	0	0	1.7	173	0	0	2.3
Levofloxacin	119	0	0	4.2	191	0	0	0.5
Amikacin	120	0	0	8.3	158	0	0	0.6
Gentamicin	117	0.9	1.7	-	191	0	0.5	-
Tobramycin	122	0	0	-	192	0	1	-
Total	1443	0.2	0.6	4.9	2540	0.2	0.9	5.6

Conclusions

The analysis of MIC and zone diameter correlates for *Pseudomonas* spp. indicates that zone diameter breakpoints which were based mainly on data for *P. aeruginosa* could reliably be used also for other species of *Pseudomonas*. Our analysis suggested a need for a few minor adjustments in zone diameter breakpoints. These were implemented in the EUCAST Breakpoint Table v. 4.0, valid from January 2014.

Table 2. EUCAST clinical breakpoints for *Pseudomonas* spp.
EUCAST Clinical Breakpoint Table v. 4.0, January 2014.
New breakpoints highlighted in yellow, with old breakpoints in parenthesis.

Antimicrobial agent	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Piperacillin	16	16	30	18 (19)	18 (19)
Piperacillin-tazobactam	16	16	30-6	18 (19)	18 (19)
Ticarcillin	16	16	75	18 (17)	18 (17)
Ticarcillin-clavulanate	16	16	75-10	18 (17)	18 (17)
Cefepime	8	8	30	19 (18)	19 (18)
Ceftazidime	8	8	10	16	16
Doripenem	1	2 (4)	10	25	22 (19)
Imipenem	4	8	10	20	17
Meropenem	2	8	10	24	18
Aztreonam	1	16	30	50	16
Ciprofloxacin	0.5	1	5	25	22
Levofloxacin	1	2	5	20	17
Amikacin	8	16	30	18	15
Gentamicin	4	4	10	15	15
Netilmicin	4	4	10	12	12
Tobramycin	4	4	10	16	16
Colistin	4	4		Note ^A	Note ^A

Notes
A. Use an MIC method.



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P0285 Evaluation of different fluoroquinolone (FQ) antibiotics for detection of FQ resistance in *Salmonella* species by disk diffusion (DD)



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Introduction

EUCAST breakpoints for *Salmonella* species and ciprofloxacin are S ≤ 0.06 and R > 0.06 mg/L. The Ciprofloxacin 5 μ g disc which is the standard for FQ testing of Gram-negatives does not reliably distinguish between isolates with and without low-level resistance (regardless of resistance mechanism). At present, no single DD method reliably detects all resistance mechanism to FQs.

Objectives

In this study we investigated 11 different quinolone and FQ antibiotic agents, against 88 *Salmonella* isolates with diverse resistance mechanisms.

Materials and methods

88 *Salmonellae* isolates, non-Typhi belonging to 14 different *spp.* (37 wild type (WT), 31 with *qnr*, 19 with mutations in the QRDR (quinolone resistance determining region) and 1 with unknown resistance mechanism)

MIC - ciprofloxacin:

- Broth Micro dilution (BMD) according to ISO standard 20776-1.

Disk diffusion (EUCAST methodology).

- 16 different quinolone and FQ containing disks (see Table 1)
- Mueller-Hinton agar from two manufacturers; Becton Dickinson and Oxoid.
- All DD tests were performed in duplicate (read by 2 different persons) giving 4 readings per isolates i.e. a total of 352 readings per disk

Table 1. Zone diameters for different disks according to MIC for ciprofloxacin

Antimicrobial agent	Disk (μ g)	Zone diameters for Ciprofloxacin (MIC)		Readings (%) in overlapping interval
		≤ 0.06	> 0.06	
Ciprofloxacin	1	26-36	15-28	6
Ciprofloxacin	5	31-40	21-34	24
Enoxacin	10	26-34	14-25	0
Enrofloxacin	5	28-37	14-29	3
Gatifloxacin	2	25-37	13-27	14
Gatifloxacin	5	27-38	16-29	19
Levofloxacin	1	23-33	9-24	4
Levofloxacin	5	28-39	18-29	12
Lomefloxacin	10	27-37	17-29	9
Nalidixic acid	5	6-21	6-14	16
Nalidixic acid	10	9-25	6-17	38
Nalidixic acid	30	19-30	12-23	20
Norfloxacin	2	26-36	14-24	0
Ofloxacin	5	27-37	16-28	5
Pefloxacin	5	26-35	6-26	1
Sparfloxacin	5	27-38	14-31	22

Conclusions

- Enoxacin, 10 μ g, norfloxacin 2 μ g and pefloxacin 5 μ g were able to discriminate wild-type *Salmonella* spp. from isolates with resistance mechanisms / increased MICs (i.e. MIC > 0.06 mg/L).
- Further evaluation showed that pefloxacin is the better candidate. A EUCAST breakpoint of R < 24 mm and S ≥ 24 mm has now been developed for screening for FQ resistance in *Salmonella* spp. (Poster 279, ECCMID 2014).
- The study confirmed that traditional FQs i.e. ciprofloxacin 5 μ g, levofloxacin 5 μ g, or ofloxacin 5 μ g did not discriminate WT from resistant isolates.

Results

Ciprofloxacin MIC:

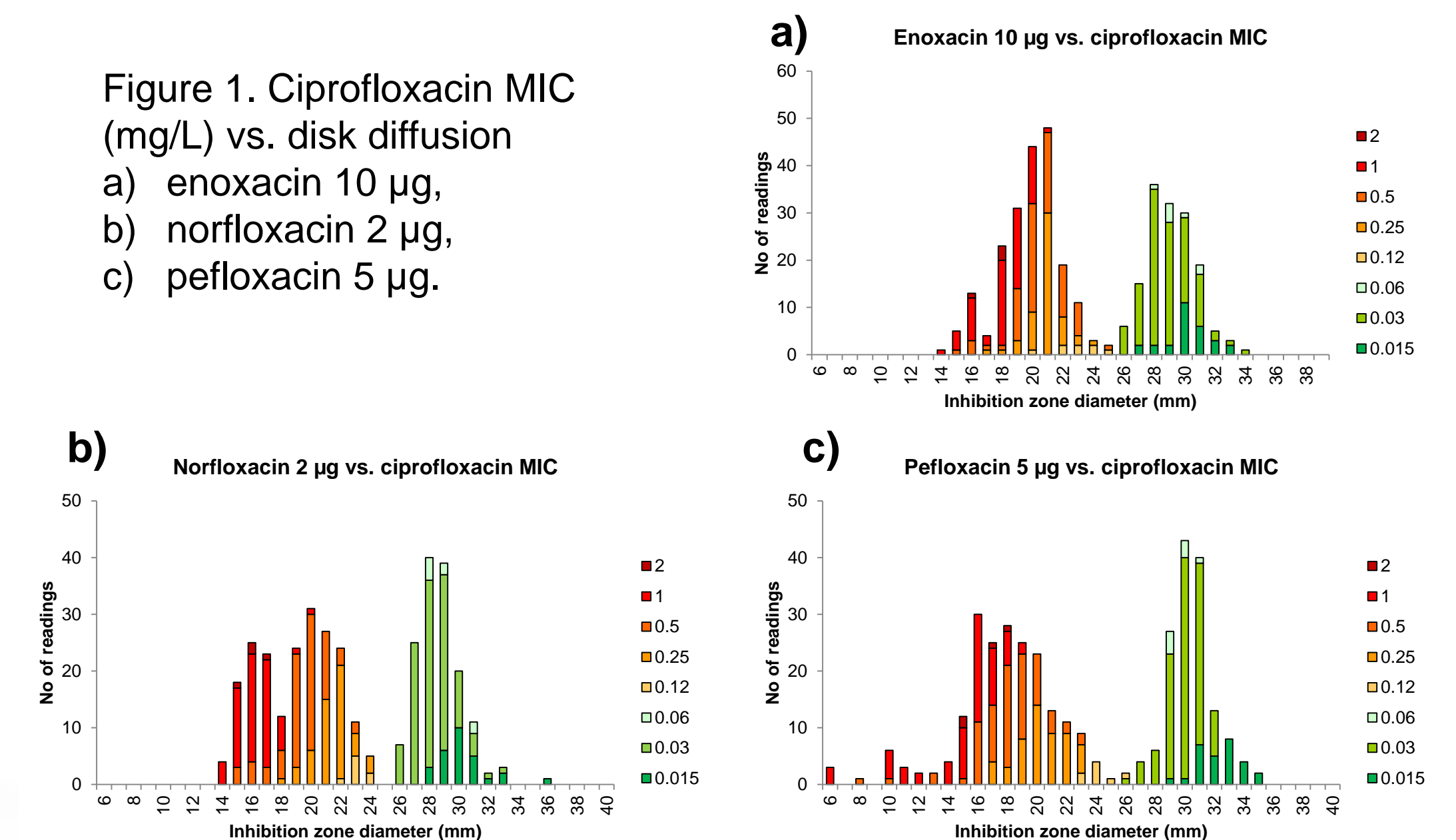
- All WT isolates had MICs ≤ 0.06 mg/L. MICs of resistant isolates were: 0.125 (n=2); 0.25 (13); 0.5 (19), 1 (16) and 2 (1) mg/L.

Disk diffusion:

- Both enoxacin 10 μ g and norfloxacin 2 μ g fully discriminated WT isolates from resistant isolates (Figure 1a-b).
- For pefloxacin 5 μ g, 1 resistant isolate had a zone of 26 mm in 1 of the 4 readings; thus overlapping the zone obtained for WT isolates (Figure 1c).
- None of the standard disks (ciprofloxacin 5 μ g, levofloxacin 5 μ g, or ofloxacin 5 μ g) could discriminate between WT and resistant isolates.

Figure 1. Ciprofloxacin MIC (mg/L) vs. disk diffusion

- a) enoxacin 10 μ g,
b) norfloxacin 2 μ g,
c) pefloxacin 5 μ g.



EUCAST objectives

EUCAST was set up to standardize susceptibility testing in Europe so that comparable results and interpretations are produced.

EUCAST structure

EUCAST is a standing ESCMID committee, funded through a contract with ECDC, with additional input, particularly related to the disk diffusion method, from ESCMID. It was formed in 1997 and restructured in 2002. A General Committee with representatives from all European Union and some non-European Union countries is led by an ESCMID-appointed Steering Committee, which includes a Chairman, Scientific Secretary, Clinical Data Coordinator, five National Breakpoint Committee representatives and three representatives of the EUCAST General Committee. Decisions are made by the Steering Committee after consultation with the General Committee and expert groups.

EUCAST General Committee

Australia Prof John Turnidge
Austria Dr Petra Apfalter
Belgium Prof Jan Verhaegen
Bosnia Dr Selma Uzunovic-Kamberovic
Bulgaria Prof Krassimir Metodiev
Croatia Dr Arjana Tambic-Andrasevic
Czech Republic Dr Helena Zemlickova
Denmark Dr Robert Skov
Estonia Dr Marina Ivanova
Finland Dr Antti Hakanen
France Prof Luc Dubreuil
Germany Prof Sören Gatermann
Greece Prof Alkiviadis Vatopoulos
Hungary Dr Ákos Tóth
Iceland Dr Karl Gustaf Kristinsson
Ireland Dr Michael F. Mulhern
Israel Dr Yoram Keness
Italy Prof Pietro Emanuele Varaldo
Latvia Dr Arta Balode
Lithuania Dr Jolanta Miciuleviciene,
Luxembourg Dr Monique Perrin
Netherlands Dr Greetje Kampinga
Norway Dr P. Christoffer Lindemann
Poland Prof Waleria Hryniewicz
Portugal Prof Jose Melo Cristino
Romania Dr Irina Codita
Russia Dr Marina Sukhorukova
Serbia Dr Lazar Ranin
Slovak Republic Prof Milan Niks
Slovenia Dr Iztok Strumbelj
Spain Prof Luis Martinez-Martinez
Sweden Dr Barbro Olsson-Liljequist
Switzerland Prof Reinhard Zbinden
Turkey Dr Deniz Gür
UK Prof Alasdair MacGowan
USA Dr Paul Ambrose
ISC – Prof Paul Tulkens
FESCI – Prof David Livermore

Pharmaceutical Industry and Susceptibility Testing Device Manufacturers

These groups are informed of EUCAST activity and consulted via an e-mail network open to all companies with an interest in antimicrobial susceptibility testing.

EUCAST Steering Committee (SC)

Chairholder Rafael Canton (to 2014)
Scientific secretary, Derek Brown (to 2014)
Clinical Data Coordinator Gunnar Kahlmeter (to 2014)
BSAC (The UK), Alasdair MacGowan (to 2014)
CA-SFM (France), Luc Dubreuil (to 2014)
CRG (The Netherlands) Johan Mouton (to 2014)
NWGA (Norway), Martin Steinbakk (to 2014)
SRGA (Sweden), Christian Giske (to 2014)
EUCAST GC representatives
Luis Martinez Martinez, Spain (to 2014)
Robert Skov, Denmark (to 2014)
Sören Gatermann, Germany (to 2015)
Iztok Strumbelj, Slovenia (2014 - 2016)
Jan Verhaegen, Belgium (2014 - 2016)

”Visiting” members from General Committees

Any national representative can attend up to two SC meeting per year. A maximum of two visiting representatives may attend each meeting. ESCMID provides up to five travel grants per year to support visiting GC members.

Appointments by ESCMID

- Chairholder, Scientific Secretary and Clinical Data Coordinator (three years)
- Three representatives from the EUCAST general committee from countries other than those with breakpoint committee representatives (two years).

Appointments by European countries

- Each country with an active national breakpoint committee appoints one representative to serve for three years on the Steering Committee .
- Each country appoints one representative to serve on the General Committee (no set term of office).

EUCAST subcommittees

Antifungal Susceptibility Testing

The Antifungal Susceptibility Testing (AFST) subcommittee is a standing EUCAST subcommittee dealing with all issues related to breakpoints and susceptibility testing for fungi. The AFST Steering Committee currently consists of Maiken C. Arendrup, (chairholder), Susan Howard (secretary), Joseph Melitiades (Clinical Data Coordinator), Johan Mouton (SC representative) and two AFST General Committee representatives, Manuel Cuenca Estrella (Spain) and Connie Lass-Flörl (Austria). Other Subcommittee members are listed on the EUCAST website.

Micafungin breakpoints for *Candida* spp. were agreed and fluconazole and anidulafungin breakpoints for *Candida* spp. were revised in February 2013. Version 6.1 of EUCAST antifungal breakpoint tables was released in March 2013.

Antimicrobial resistance mechanisms

The subcommittee on antimicrobial resistance mechanisms of clinical and/or epidemiological importance (chairholder Christian Giske) has published practical guidelines for detection of resistance mechanisms. The document is available from the EUCAST website.

EUCAST websites

The EUCAST websites continue to be developed and updated, and all EUCAST breakpoints and documents are freely available from the website at **www.eucast.org**

In the “**Antimicrobial Susceptibility Testing**” section the EUCAST methodology documents have been updated.

Under “**Clinical breakpoints**” the breakpoints tables version 4.0 have been released.

Extensive data have been added to the section on “**Calibration and Validation**” of the EUCAST disk diffusion method.

The section on “**Frequently asked questions**” provides answers to a range of frequently asked questions regarding EUCAST breakpoints and methods. It has recently been extensively revised and updated

The “**News**” section on the EUCAST website highlights new information and announces consultations on EUCAST breakpoints and methods.

The EUCAST **MIC and zone diameter website** presents MIC and zone diameter distributions of bacteria and fungi based on a continually increasing number of distributions.

EUCAST publications

All EUCAST publications are available from the EUCAST website.

Other EUCAST activities 2013-2014

Breakpoints

Breakpoint tables v 4.0 were published on the EUCAST website in December 2013. The tables included additions and revisions to several breakpoints including the following:

- MIC breakpoints for ciprofloxacin and levofloxacin in the treatment of uncomplicated urinary tract infections caused by *Enterococcus* spp. were agreed.
- Breakpoints for amoxicillin-clavulanic acid for uncomplicated urinary tract infection were agreed.
- Breakpoints for *Corynebacterium* spp. were added and breakpoints for *Pseudomonas aeruginosa* validated for other *Pseudomonas* spp.
- Guidance notes on susceptibility testing for *Burkholderia cepacia* and for topical agents were published on the EUCAST website.

Breakpoints for new agents

Breakpoints for new agents are set by EUCAST as part of the marketing authorisation process through EMA. Breakpoints were set for ceftobiprole and delamanid (anti-mycobacterial agent) and several other agents are currently at various stages of the licensing procedure.

Revision of breakpoints

Doripenem breakpoints were revised and breakpoints for other carbapenems are currently under review. Notes relating to several agents have been updated in version 4.0 of the breakpoint tables.

EUCAST disk diffusion method

Version 4.0 of breakpoints for the EUCAST disk diffusion method calibrated to EUCAST clinical MIC breakpoints is now available on the EUCAST website. Breakpoints for *Corynebacterium* spp. were added to version 4.0, together with adjustments to some other breakpoints and notes.

Graphs showing MIC-zone diameter correlations also continue to be expanded on the EUCAST website. The distributions highlight wild type populations and give epidemiological cut-off values (ECOFFs).

Implementation of EUCAST breakpoints

In Europe, the trend from using other breakpoint guidelines to using EUCAST breakpoints and methods continues. In the EARS-Net resistance surveillance external quality assessment exercise in November 2013, EUCAST breakpoints were used by 64% of participating laboratories. In the UK NEQAS international external quality assurance scheme in February 2014, over 80% of participating laboratories followed EUCAST breakpoints.

Automated systems

The manufacturers of automated susceptibility testing devices continue to implement EUCAST breakpoints in their systems. Up-to-date information on the current EUCAST status of manufacturers of automated systems and of materials for disk diffusion and gradient MIC methods is given on the EUCAST website.

EUCAST documents

A Standard Operating Procedures (SOP) for preparation and distribution of EUCAST minutes, SOP 7.0, has been published on the EUCAST website.

Translations of several EUCAST documents are available on the EUCAST website under the sections for National AST Committees (NACs) for individual countries.



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