

Screening for fluoroquinolone resistance in *Staphylococcus aureus* and *Streptococcus* spp. using norfloxacin and EUCAST disk diffusion criteria

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Introduction

EUCAST recommends the use of the norfloxacin (NOR) 10-µg disk to screen for fluoroquinolone (FQ) resistance in *Staphylococcus* spp., *Streptococcus pneumoniae* and *Streptococcus* groups A, B, C and G. Norfloxacin screen negative isolates (wild type for NOR) can be considered devoid of FQ resistance mechanisms. These can be reported susceptible to all FQs with clinical breakpoints, with the exception of *S. pneumoniae* where wild-type (WT) isolates should be reported intermediate for ofloxacin (not shown) and ciprofloxacin. Norfloxacin screen positive isolates (non-wild type for NOR) should be tested for susceptibility to individual agents. The norfloxacin screen is a useful tool in areas where FQ resistance is still uncommon in *Staphylococcus* and *Streptococcus* spp.

Objectives

The objective of this study was to evaluate the norfloxacin 10-µg disk as a screen for fluoroquinolone resistance using EUCAST criteria for *Staphylococcus* spp. (<17 mm) and *Streptococcus* spp. (<12 mm).

Methods

Clinical isolates of *S. aureus* (98), *S. pneumoniae* (100), *S. pyogenes* (33) and *S. agalactiae* (32), were selected from the SENTRY collection (JMI Laboratories, USA). These included isolates susceptible and resistant to relevant FQs. MIC values were determined by broth microdilution, according to the ISO standard 20776-1, at the EUCAST Laboratory for *S. aureus* and at JMI Laboratories for streptococci. For streptococci, Mueller-Hinton broth was supplemented with 5% lysed horse blood (by repeated freezing and thawing) and 20 mg/L β-NAD (MH-F broth). All disk diffusion tests were performed at the EUCAST Laboratory according to EUCAST methodology using Mueller-Hinton agar (+/- 5% defibrinated horse blood and 20 mg/L β-NAD) from two manufacturers (BD and Oxoid/Thermo Fisher Scientific). The NOR disk screen test was evaluated by correlating NOR zone diameters to MICs for ciprofloxacin, levofloxacin and moxifloxacin, respectively.

Results

All NOR screen negative isolates were categorised by MIC as wild type for all other FQs investigated (Table 1). Of the NOR screen positive isolates, 8 of 40 *S. aureus*, 11 of 22 *S. pneumoniae* and 1 of 2 *S. pyogenes* were reported wild type according to MIC for one or more FQs. Of these isolates, three *S. aureus*, seven *S. pneumoniae* and one *S. pyogenes* were wild type for all FQs investigated. Inhibition zone diameter distributions for norfloxacin with *S. aureus* and *S. pneumoniae* are shown in Figure 1.

Table 1. Number of isolates categorised as wild type and non-wild type for norfloxacin and different fluoroquinolones.

Microorganism	Ciprofloxacin		Levofloxacin		Moxifloxacin	
	WT	non-WT	WT	non-WT	WT	non-WT
<i>S. aureus</i> (n=98)						
Norfloxacin WT (n=58)	58	0	58	0	58	0
Norfloxacin non-WT (n=40)	3	37	8	32	8	32
<i>S. pneumoniae</i> (n=100)						
Norfloxacin WT (n=78)	78	0	78	0	78	0
Norfloxacin non-WT (n=22)	8	14	10	12	10	12
<i>S. pyogenes</i> (n=33)						
Norfloxacin WT (n=31)	-	-	31	0	31	0
Norfloxacin non-WT (n=2)	-	-	1	1	1	1
<i>S. agalactiae</i> (n=32)						
Norfloxacin WT (n=27)	-	-	27	0	27	0
Norfloxacin non-WT (n=5)	-	-	0	5	0	5

WT = Wild Type, - = Not analysed due to lack of FQ breakpoints.

Conclusions

For *S. aureus* and *Streptococcus* spp., the EUCAST norfloxacin screen test reliably predicts susceptibility to all fluoroquinolones with clinical breakpoints. However, the test overcalls resistance and norfloxacin screen positive isolates must be tested for susceptibility to individual fluoroquinolones, which is also supported by this study. Due to the high sensitivity and low specificity, the norfloxacin screen test is useful only in areas with low prevalence of fluoroquinolone resistance in relevant species.

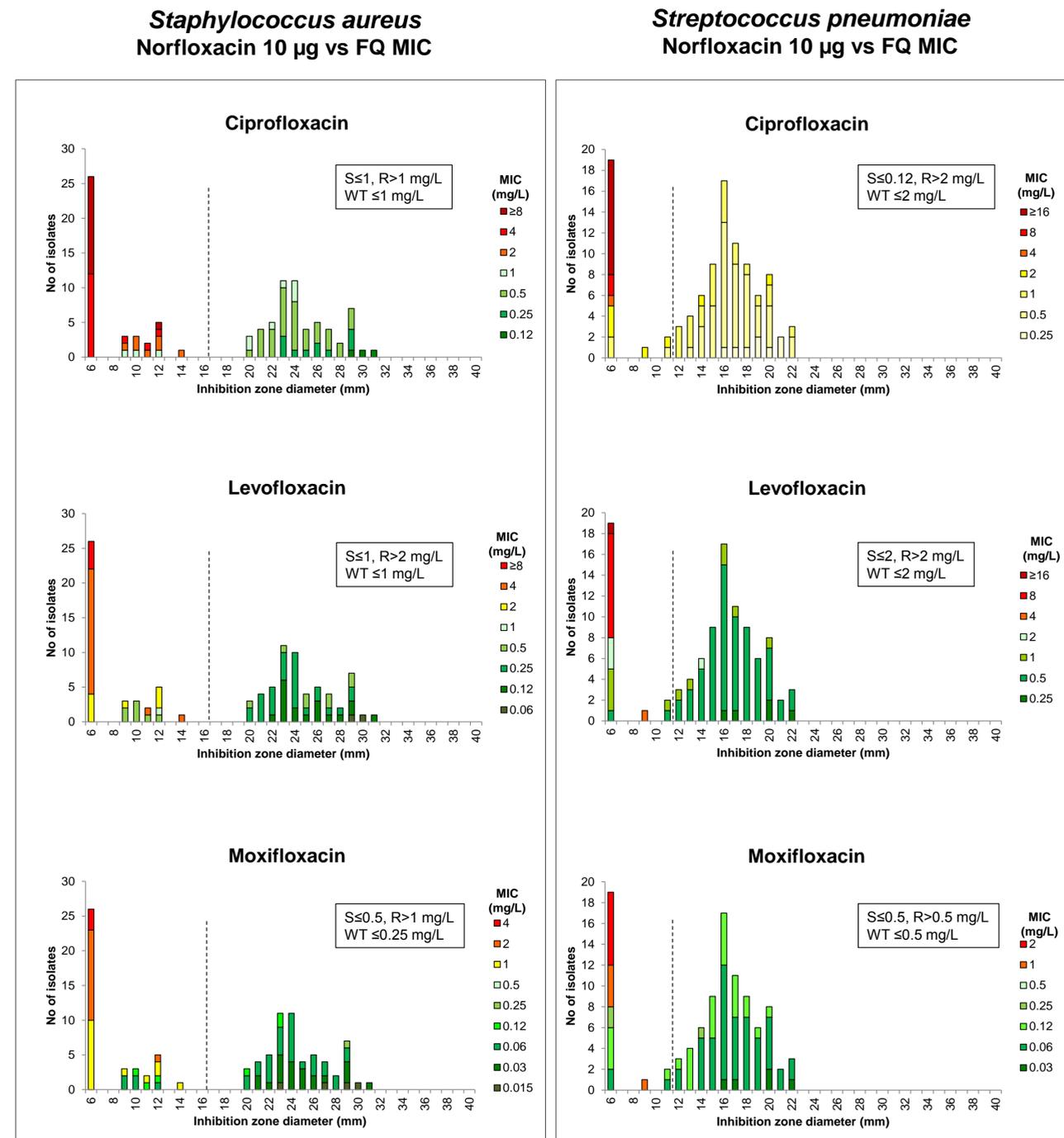


Figure 1. Inhibition zone diameter distributions for norfloxacin 10 µg with *S. aureus* (n=98) to the left and *S. pneumoniae* (n=100) to the right. MIC values for different FQs are shown as coloured bars. EUCAST screen breakpoints for norfloxacin (<17 mm for *S. aureus* and <12 mm for *S. pneumoniae*) are shown as dotted lines.