

Evaluation of the Reproducibility of MIC Results of Selected Newer Fluoroquinolones in Commercial Dry-Form Panels for Clinical Use

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AMENDED ABSTRACT

Background: Newer fluoroquinolones (FQs) with higher potency and broader spectrum against Gram-positive pathogens have been developed, requiring standardization and evaluation of commercial susceptibility test panels. We evaluated the reproducibility of MIC results in commercial broth microdilution (BMD) trays for four FQs: gemifloxacin (GEM), moxifloxacin (MFX), sitafloxacin (SIT), and sparfloxacin (SPX).

Methods: Commercially prepared dry-form 96-well trays (Sensititre, TREK Diagnostics, Cleveland, OH) were inoculated using NCCLS M7-A6 methods to determine the susceptibility of 20 strains, including eight ATCC/NCCLS quality control (QC) isolates. MIC testing for each strain was performed three times daily for three days with the goal of ≥95% of MIC results ± one log₂ dilution of the expected median and/or mode. BMD MIC results were analyzed for each FQ only for those strains where all MIC results demonstrated on-scale values. Concurrent QC results were acceptable.

Results: All four FQs demonstrated 100% of MIC results within ± one log₂ dilution for same day of testing, as well as between days. SPX demonstrated the highest MIC reproducibility with 91.2% and 87.1% identical values for same day and between day results, respectively. GEM and SIT had identical reproducibility results at 89.5% for same day testing. MFX showed the lowest reproducibility at 88.9 and 82.1% for identical results on same day and between day tests. No trends of the MIC results (higher or lower) were observed for these FQ among the non-identical MIC values.

Conclusions: Reproducibility of MIC results was very high for all four FQs evaluated with ≥88.9% of test results being the same and 100% of within day and between day results being acceptable (NCCLS, M23-A2). These commercial dry-form BMD panels having extended shelf-life and room-temperature storage conditions demonstrated excellent accuracy, and are appropriate for routine use in clinical laboratories for testing of patient isolates.

BACKGROUND

Recent publications have reported the emergence of fluoroquinolone resistance in species not previously observed, as well as increasing fluoroquinolone resistance in Enterobacteriaceae, staphylococcal and streptococcal isolates collected in longitudinal surveillance programs. Resistance to fluoroquinolones has been mainly caused by target site mutations in the quinolone-resistance determining region.

This study assesses the reproducibility of commercially produced MIC panels with a challenge set of 20 Gram-positive and Gram-negative bacterial isolates for four newer fluoroquinolone antimicrobial agents. Laboratory support for clinical development for these newer fluoroquinolones requires availability of validated susceptibility testing products with extended shelf lives to be used by study site investigators as well as clinicians for testing. This report summarizes results of reagent qualifying reproducibility tests utilizing NCCLS M23-A2 guidelines.

MATERIALS AND METHODS

A total of 20 bacterial isolates including eight American Type Culture Collection (ATCC) Quality Control strains were selected for testing. Strains represented the streptococci (four), enterococci (two), staphylococci (four), Enterobacteriaceae (seven) and non-fermentative Gram-negative bacilli (three) groups. Clinical isolate identifications were confirmed by using colony morphology on standard media, routine biochemical tests and Vitek (bioMerieux, Hazelwood, MO) identification when necessary.

The isolates were tested for susceptibility using National Committee for Clinical Laboratory Standards (NCCLS) methods with commercially prepared, validated, dry-form broth microdilution panels (TREK Diagnostics Inc., Cleveland, OH) filled with cation-adjusted Mueller-Hinton broth. Streptococci were tested in cation-adjusted Mueller-Hinton broth supplemented with 2-5% lysed horse blood. The four newer fluoroquinolone antimicrobial agents were tested in triplicate for three days for a total of nine MIC values for each strain. Analysis was performed only for those strains demonstrating on-scale MIC values for each antimicrobial agent. Acceptable target reproducibility rate was considered to be ≥95% MIC results within ± one log₂ dilution.

RESULTS

- Table 1 summarizes the gemifloxacin reproducibility results for 18 strains with 89.5 and 87.0% identical results for same day tests and between day tests respectively.

- For moxifloxacin, (Table 2) only 13 strains produced on-scale MIC values with 88.9 and 82.1% identical results for same day and between day tests respectively.

- The MIC reproducibility results for the newer quinolones sitafloxacin and sparfloxacin are summarized in Tables 3 and 4. Both compounds demonstrated on-scale MIC values for 19 of the 20 selected strains. Identical MIC results were observed at a higher rate for sparfloxacin (91.2 and 87.1% for same day and between day tests, respectively) compared to sitafloxacin (89.5 and 82.5% for same day and between day tests, respectively).

- Table 5 summarizes the within day and between day percentage of MIC results which were identical and showing a ± one log₂ dilution variation. All four newer fluoroquinolones demonstrated excellent reproducibility rates with 100% of on scale results within ± one log₂ dilution.

Table 1. Results of a broth microdilution MIC reproducibility experiment with gemifloxacin tested against 18 organisms (includes quality control strains) three replicates daily for three consecutive days.											
Organism (strain no.)	Variations in log ₂ dilutions for:										
	Within daily replicates ^a					Between days ^b					
	-2	-1	Same	+1	+2	-2	-1	Same	+1	+2	
<i>S. mitis</i> (15-6797A)	0	2	6	1	0	0	4	5	0	0	
<i>S. pyogenes</i> (7-6560A)	0	0	9	0	0	0	0	9	0	0	
<i>S. pneumoniae</i> (13-3602C)	0	0	9	0	0	0	0	9	0	0	
<i>S. pneumoniae</i> (ATCC 49619)	0	0	9	0	0	0	0	9	0	0	
<i>E. faecalis</i> (19-6848A)	0	0	9	0	0	0	0	9	0	0	
<i>E. faecalis</i> (ATCC 29212)	0	0	9	0	0	0	0	9	0	0	
<i>S. epidermidis</i> (1-6777A)	0	0	9	0	0	0	0	9	0	0	
<i>S. aureus</i> (ATCC 29213)	0	1	8	0	0	0	1	8	0	0	
<i>S. aureus</i> (ATCC 25923)	0	0	8	1	0	0	0	8	1	0	
<i>E. coli</i> (ATCC 25922)	0	0	9	0	0	0	0	9	0	0	
<i>E. coli</i> (ATCC 35218)	0	0	9	0	0	0	0	9	0	0	
<i>P. aeruginosa</i> (ATCC 27853)	0	0	9	0	0	0	0	9	0	0	
<i>P. aeruginosa</i> (30-166A)	0	1	6	2	0	0	1	6	2	0	
<i>K. pneumoniae</i> (ATCC 700603)	0	2	7	0	0	0	2	7	0	0	
<i>K. pneumoniae</i> (6-25A)	0	2	7	0	0	0	2	7	0	0	
<i>A. baumannii</i> (24-60A)	0	0	7	2	0	0	0	7	2	0	
<i>S. marcescens</i> (4-95A)	0	0	7	2	0	0	4	5	0	0	
<i>E. cloacae</i> (33-71A)	0	0	8	1	0	0	2	7	0	0	
Totals	0	8 ^c	145 ^c	9 ^c	0	0	16 ^c	141 ^c	5 ^c	0	
a. Results compared to the within day consensus MIC (mode or median if three different results were encountered). b. Results compared to the all replicate consensus value for each organism (nine results). c. Reproducibility was at 100.0% ± one log ₂ dilution step, and 87.0 - 89.5% of results were identical (162 total tests).											
Table 2. Results of a broth microdilution MIC reproducibility experiment with moxifloxacin tested against 13 organisms (includes quality control strains) three replicates daily for three consecutive days.											
Organism (strain no.)	Variations in log ₂ dilutions for:										
	Within daily replicates ^a					Between days ^b					
	-2	-1	Same	+1	+2	-2	-1	Same	+1	+2	
<i>S. mitis</i> (15-6797A)	0	0	9	0	0	0	0	9	0	0	
<i>S. pyogenes</i> (7-6560A)	0	2	7	0	0	0	0	5	4	0	
<i>S. pneumoniae</i> (13-3602C)	0	0	9	0	0	0	0	8	1	0	
<i>S. pneumoniae</i> (ATCC 49619)	0	0	9	0	0	0	0	9	0	0	
<i>E. faecalis</i> (19-6848A)	0	0	9	0	0	0	0	9	0	0	
<i>E. faecalis</i> (ATCC 29212)	0	0	8	1	0	0	0	5	4	0	
<i>S. epidermidis</i> (1-6777A)	0	0	9	0	0	0	0	9	0	0	
<i>P. aeruginosa</i> (ATCC 27853)	0	0	8	1	0	0	0	8	1	0	
<i>P. aeruginosa</i> (30-166A)	0	0	7	2	0	0	0	7	2	0	
<i>K. pneumoniae</i> (ATCC 700603)	0	1	8	0	0	0	1	8	0	0	
<i>K. pneumoniae</i> (6-25A)	0	2	6	1	0	0	4	5	0	0	
<i>S. marcescens</i> (4-95A)	0	0	9	0	0	0	0	9	0	0	
<i>E. cloacae</i> (33-71A)	0	0	7	2	0	0	4	5	0	0	
Totals	0	5 ^c	104 ^c	8 ^c	0	0	9 ^c	96 ^c	12 ^c	0	
a. Results compared to the within day consensus MIC (mode or median if three different results were encountered). b. Results compared to the all replicate consensus value for each organism (nine results). c. Reproducibility was at 100.0% ± one log ₂ dilution step, and 82.1 - 88.9% of results were identical (117 total tests).											

Table 3. Results of a broth microdilution MIC reproducibility experiment with sitafloxacin tested against 19 organisms (includes quality control strains) three replicates daily for three consecutive days.											
Organism (strain no.)	Variations in log ₂ dilutions for:										
	Within daily replicates ^a					Between days ^b					
	-2	-1	Same	+1	+2	-2	-1	Same	+1	+2	
<i>S. mitis</i> (15-6797A)	0	1	6	2	0	0	0	5	4	0	
<i>S. pyogenes</i> (7-6560A)	0	0	9	0	0	0	0	9	0	0	
<i>S. pneumoniae</i> (13-3602C)	0	0	8	1	0	0	0	8	1	0	
<i>S. pneumoniae</i> (ATCC 49619)	0	0	7	2	0	0	0	7	2	0	
<i>E. faecalis</i> (19-6848A)	0	0	9	0	0	0	0	9	0	0	
<i>E. faecalis</i> (ATCC 29212)	0	0	9	0	0	0	0	9	0	0	
<i>S. epidermidis</i> (1-6777A)	0	1	8	0	0	0	1	8	0	0	
<i>S. aureus</i> (ATCC 29213)	0	1	8	0	0	0	1	8	0	0	
<i>S. aureus</i> (ATCC 25923)	0	0	9	0	0	0	0	9	0	0	
<i>E. coli</i> (ATCC 25922)	0	0	9	0	0	0	0	9	0	0	
<i>E. coli</i> (ATCC 35218)	0	0	9	0	0	0	0	9	0	0	
<i>P. aeruginosa</i> (ATCC 27853)	0	1	8	0	0	0	4	5	0	0	
<i>P. aeruginosa</i> (30-166A)	0	2	6	1	0	0	4	5	0	0	
<i>K. pneumoniae</i> (ATCC 700603)	0	0	7	2	0	0	0	7	2	0	
<i>K. pneumoniae</i> (6-25A)	0	0	9	0	0	0	0	9	0	0	
<i>P. mirabilis</i> (63-3A)	0	1	7	1	0	0	0	6	3	0	
<i>A. baumannii</i> (24-60A)	0	0	8	1	0	0	0	8	1	0	
<i>S. marcescens</i> (4-95A)	0	0	9	0	0	0	0	6	3	0	
<i>E. cloacae</i> (33-71A)	0	1	8	0	0	0	4	5	0	0	
Totals	0	8 ^c	153 ^c	10 ^c	0	0	14 ^c	141 ^c	16 ^c	0	
a. Results compared to the within day consensus MIC (mode or median if three different results were encountered). b. Results compared to the all replicate consensus value for each organism (nine results). c. Reproducibility was at 100.0% ± one log ₂ dilution step, and 82.5 - 89.5% of results were identical (171 total tests).											
Table 4. Results of a broth microdilution MIC reproducibility experiment with sparfloxacin tested against 19 organisms (includes quality control strains) three replicates daily for three consecutive days.											
Organism (strain no.)	Variations in log ₂ dilutions for:										
	Within daily replicates ^a					Between days ^b					
	-2	-1	Same	+1	+2	-2	-1	Same	+1	+2	
<i>S. mitis</i> (15-6797A)	0	0	8	1	0	0	0	8	1	0	
<i>S. pyogenes</i> (7-6560A)	0	0	9	0	0	0	0	9	0	0	
<i>S. pneumoniae</i> (13-3602C)	0	0	9	0	0	0	0	9	0	0	
<i>S. pneumoniae</i> (ATCC 49619)	0	1	8	0	0	0	0	7	2	0	
<i>E. faecalis</i> (19-6848A)	0	0	9	0	0	0	0	9	0	0	
<i>E. faecalis</i> (ATCC 29212)	0	0	7	2	0	0	0	7	2	0	
<i>S. epidermidis</i> (1-6777A)	0	0	9	0	0	0	0	9	0	0	
<i>S. aureus</i> (43-507A)	0	0	9	0	0	0	0	9	0	0	
<i>S. aureus</i> (ATCC 29213)	0	2	7	0	0	0	2	7	0	0	
<i>S. aureus</i> (ATCC 25923)	0	0	9	0	0	0	0	9	0	0	
<i>E. coli</i> (ATCC 25922)	0	0	9	0	0	0	0	9	0	0	
<i>E. coli</i> (ATCC 35218)	0	1	8	0	0	0	1	8	0	0	
<i>P. aeruginosa</i> (ATCC 27853)	0	0	9	0	0	0	0	9	0	0	
<i>P. aeruginosa</i> (30-166A)	0	0	8	1	0	0	0	8	1	0	
<i>K. pneumoniae</i> (ATCC 700603)	0	1	7	1	0	0	0	6	3	0	
<i>K. pneumoniae</i> (6-25A)	0	1	7	1	0	0	0	5	4	0	
<i>A. baumannii</i> (24-60A)	0	0	7	2	0	0	4	5	0	0	
<i>S. marcescens</i> (4-95A)	0	0	9	0	0	0	0	9	0	0	
<i>E. cloacae</i> (33-71A)	0	1	8	0	0	0	0	7	2	0	
Totals	0	7 ^c	156 ^c	8 ^c	0	0	7 ^c	149 ^c	15 ^c	0	
a. Results compared to the within day consensus MIC (mode or median if three different results were encountered). b. Results compared to the all replicate consensus value for each organism (nine results). c. Reproducibility was at 100.0% ± one log ₂ dilution step, and 87.1 - 91.2% of results were identical (171 total tests).											

Table 5. Summary of the reproducibility results for Sensititre commercial broth microdilution panels when testing four newer or investigational fluoroquinolones (13 - 19 organisms with on-scale MIC values; 621 total tests).											
Fluoroquinolone (no. tests)	Variations in log ₂ dilutions for:										
	Within daily replicates ^a					Between days ^b					
	-2	-1	Same ^a	+1	+2	-2	-1	Same ^a	+1	+2	
Gemifloxacin (162)	0.0	4.9	89.5	5.6	0.0	0.0	9.9	87.0	3.1	0.0	
Moxifloxacin (117)	0.0	4.3	88.9	6.8	0.0	0.0	7.7	82.1	10.2	0.0	
Sitafloxacin (171)	0.0	4.7	89.5	5.8	0.0	0.0	8.2	82.5	9.3	0.0	
Sparfloxacin (171)	0.0	4.1	91.2	4.7	0.0	0.0	4.1	87.1	8.8	0.0	
a. 82.1 - 91.2% of results were identical within and between daily replicates; an acceptable level of reproducibility [NCCLS M23-A2, 2001].											