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Re-Evaluations of the NCCLS Quality Control Guidelines for Gatifloxacin and Garenoxacin (BMS284756) When Susceptibility Testing *H. influenzae* and *S. pneumoniae*

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ABSTRACT

Background: Gatifloxacin (GATI) is a new fluoroquinolone widely used in clinical practice and garenoxacin (GARE; formerly BMS284756) is a novel, investigational des(6)F-quinolone. Preliminary QC ranges have been approved for publication by the NCCLS, but early results from laboratories using these reagent drugs indicates a possible need for adjustment. NCCLS methods (M2-A7, M7-A5) and study designs (M23-A2) were followed in this reported study.
Methods: The QC ranges needing re-evaluation were the GATI and GARE disk diffusion tests with *S. pneumoniae* (SPN) ATCC 49619 and the MIC QC range for GARE using *H. influenzae* (HI) ATCC 49247. Eight laboratories participated in the M23-A2 study using two disk lots, three agar MH lots, four HTM broth lots and replicates of 480 (disk zones) and 320 (MICs) per quinolone tested. Controls utilized were levofloxacin and moxifloxacin (disks) or GATI and clarithromycin (MICs). Average inoculum by colony counts sampled from broth microdilution trays was 3.5 x 10^5 CFU/ml.
Results: The GARE results for HI showed a MIC mode at 0.008 μg/ml, the upper limit of the current, published NCCLS range. The proposed correction could be 0.002-0.015 μg/ml or 0.004-0.015 μg/ml, each encompassing 100.0% of reported results. All control drug results were within NCCLS limits. For GARE and GATI SPN zone diameters, a minor (1 mm) modification was proposed for GARE (26-34 mm; 96.3% of results reported), and GATI results did not indicate the need for change (24-31 mm; 98.3% of results). All control drug zone diameter results were within published ranges.
Conclusions: Continuous monitoring of QC range accuracy is maintained by the NCCLS and where problems are suspected, M23-A2 style multicenter trials are again performed. Re-evaluations of GATI and GARE QC guidelines only required two very minor alterations (one $\log_2$ dilution; one mm) in current ranges to achieve ≥95% accuracy.

INTRODUCTION

The newer quinolone antimicrobial agents offer a wide spectrum of activity against many pathogens, but each individual drug can possess differences in their potency against Gram-positive and/or Gram-negative organisms, anaerobic pathogens, pseudomonads, mycobacteria and other atypical pathogens. Gatifloxacin, is a newer fluoroquinolone with a methoxy substitution at the C-8 position. This side chain produces several beneficial antimicrobial effects; including increased activity against Gram-positive bacteria and some anaerobes, improved potency and a decrease in the rate of development of resistance, and reduced quinolone phototoxicity.

Garenoxacin is a novel, investigational des-fluoro(6)quinolone which has been shown to be less toxic in mice and possess excellent activity against a wide variety of pathogens, including bacteria responsible for community-acquired respiratory tract infections and even some atypical bacteria such as *Mycobacterium, Mycoplasma, Legionella* and *Chlamydia*. Both gatifloxacin and garenoxacin have been previously studied for quality control (QC) ranges for MIC and disk diffusion test methods; however, recent indications of "out-of-control" results for gatifloxacin and garenoxacin tested against *S. pneumoniae* ATCC 49619 by the disk diffusion method and garenoxacin by using the MIC method with *H. influenzae* ATCC 49247 has led to this re-evaluation of the current QC ranges for these drugs and test methods.

Table 1. Inter- and intra-laboratory comparisons of garenoxacin MIC results versus *H. influenzae* ATCC 49247 for an eight medical center protocol meeting the study design guidelines found in NCCLS M23-A2.

	Laboratory code (occurrences):								
MIC (μg/ml)	A	B	C	D	E	F	G	H	Total
0.002									0[a,c]
0.004	13		20			12		9	54[a,b,c]
0.008	27	40	20	40	40	28	40	31	266[a,b,c]
0.015									0[a,b]

a. Proposed four log dilution QC range (100.0% of results).
b. Proposed three log dilution QC range (100.0% of results).
c. Previously published QC range (100.0% of results).

MATERIALS & METHODS

An eight laboratory study group was utilized in the re-evaluation of gatifloxacin and garenoxacin disk diffusion tests for *S. pneumoniae* ATCC 49619 and garenoxacin MIC QC ranges for *H. influenzae* ATCC 49247. The study group consisted of the following laboratories: The Cleveland Clinic Foundation (G. Hall), Cleveland, OH; The JONES Group/JMI Laboratories (A. Fuhrmeister), North Liberty, IA; Michigan State University (G. Stein), East Lansing, MI; TREK Diagnostics (C. Knapp), Cleveland, OH; University of Alberta (R. Rennie), Edmonton, AB, Canada; University of Texas (A. Wanger), Houston, TX; University of Washington (T. Fritsche), Seattle, WA; and Strong Memorial Hospital (D. Hardy), Rochester, NY. Each laboratory followed the National Committee for Clinical Laboratory Standards (NCCLS) established M23-A2 guidelines, as well as, the M2-A7 test methods for antimicrobial disk susceptibility testing and the M7-A5 [NCCLS, 2000b] test methods for broth dilution antimicrobial susceptibility testing.

The disk diffusion re-evaluation for *S. pneumoniae* ATCC 49619 consisted of three prepared Mueller-Hinton agar lots supplemented with 5% lysed horse blood (Remel, Lenexa, KS; PML, Wilsonville, OR; BBL, Sparks, MD). Two different disk lots (Remel, Lenexa, KS; BBL, Sparks, MD) were used for the investigational antimicrobials, gatifloxacin and garenoxacin, as well as for the internal control, levofloxacin. Due to limited manufacturers of moxifloxacin disks (second internal control), we were unable to use two different disk lots for the study, thus, the same lot number was tested in duplicate on each agar plate. The experiments were done daily for 10 days generating two zones on the three different media lots with a total of 480 zone diameter values for each of the investigational and internal control drugs.

The broth microdilution MIC re-evaluation for *H. influenzae* ATCC 49247 consisted of four lots of Haemophilus Test Medium (Difco, Detroit, MI; Hardy, Santa Maria, CA; BBL, Sparks, MD [two lots]) in a frozen-form, reference broth microdilution panel prepared by TREK Diagnostics (Cleveland, OH). All panels were kept frozen at -80ºC until used. The antimicrobial agents and their role in the study were as follows: garenoxacin and gatifloxacin (internal control) powders were obtained from Bristol-Myers Squibb (Princeton, NJ), and clarithromycin laboratory standard compound (a second internal control) was obtained from Abbott Laboratories (Chicago, IL). Each laboratory tested the QC strain daily for 10 days generating 320 total values for the investigational drug, garenoxacin and 160 total values for each of the internal controls. Colony counts were also performed by subculturing in a quantitative manner onto drug-free plates. The counts ranged from 3.0 x 10^4 to 1.0 x 10^6 CFU/ml with an average for all participating laboratories at 3.5 x 10^5 CFU/ml (target inoculum at 5.0 x 10^5 CFU/ml).

The proposed QC ranges for the re-evaluation were optimized using the statistical method and the method that contains ≥ 95% of all reported results as recommended by NCCLS M23-A2 guidelines. Each disk diffusion value and MIC was tabulated and compared between laboratories and media broth or agar lot that could produce variations in the results.

Table 2. Inter- and intra-laboratory comparisons of the garenoxacin zone diameter results versus *S. pneumoniae* ATCC 49619 for an eight medical center protocol meeting the study design guidelines found in NCCLS M23-A2.

	Laboratory code (occurrences):								
Zone diameter (mm)	A	B	C	D	E	F	G	H	Total
26	3			12					15[a,c]
27	8			24		6	7		45[a,b,c]
28	21		1	13		9	13		57[a,b,c]
29	18	4	18	9		15	23		87[a,b,c]
30	7	14	33	2	7	11	15	1	90[a,b,c]
31	3	24	8		13	8	2	9	67[a,b,c]
32		14			17	9		12	52[a,b,c]
33		4			20	2		14	40[a,b,c]
34					3			6	9[a]
35								10	10
36								8	8

a. Proposed QC range includes ≥ 95% of results (96.3% of results).
b. Proposed QC range using median-statistical method (91.3% of results) of the NCCLS.
c. Previously published QC range (94.4% of results).

RESULTS

- Table 1 summarizes the garenoxacin MIC results for *H. influenzae* ATCC 49247 for all eight laboratories. While 100.0% of all results were within the previously published QC range of 0.002 - 0.008 μg/ml, the mode for each laboratory and media lot was at the extreme of the dilution series (0.008 μg/ml). Since there have been reports of garenoxacin MICs at 0.015 μg/ml by other laboratories and diagnostic panel producers [personal communications; NCCLS QC Working Group], it seems that a modification of the current QC range would be prudent. A proposal for a modified QC range for garenoxacin MIC results versus *H. influenzae* ATCC 49247 would either include a three or four $\log_2$ dilution MIC range; 0.004 - 0.015 μg/ml (includes 100.0% of results) or 0.002 - 0.015 μg/ml (includes 100.0% of results). By expanding the QC range to a four $\log_2$ dilution range (proposed range), it would encompass the previous low end results plus the current higher MIC results reported in this study.

- Table 2 summarizes the garenoxacin zone diameter results for *S. pneumoniae* ATCC 49619 for the eight laboratory study. The variable modal value for each of the eight laboratories ranged from 27 to 33 mm, possibly indicating the difficulty in reading *S. pneumoniae* zone diameter results against this and other quinolones. The overall modal value for all eight laboratories was 30 mm which seems to indicate a one mm increase in the modal zone diameter compared to prior results. If the published QC range (26 - 33 mm) was applied to this study, only 94.4% of the results would be "in control"; however, if the new modal value was used and the NCCLS statistical method applied (27 - 33 mm) only 91.3% of the results would be included. Thus, the range should be modified to include ≥ 95% of the results as described by the M23-A2 guidelines, adjusting the proposed QC range to 26 - 34 mm for garenoxacin against *S. pneumoniae* ATCC 49619 (96.3% of current results).

- The gatifloxacin zone diameter results for *S. pneumoniae* ATCC 49619 exhibited similar variation with the modal values by each laboratory (data not shown). Again, this variation demonstrated the difficulty in reading zone diameter results, but did not indicate a need for changing the current disk diffusion QC range for gatifloxacin against *S. pneumoniae* ATCC 49619 to be changed. The current QC zone diameter range of 24 to 31 mm encompassed 98.3% of all laboratory gatifloxacin results.

- Table 3 provides a summary for the QC ranges for garenoxacin using MIC and disk diffusion test methods and for the gatifloxacin disk diffusion test method.

Table 3. Proposed QC ranges for MIC and disk diffusion test methods.

Quinolone/organism	MIC proposed range (μg/ml)	Disk diffusion proposed range (mm)	% in range
Garenoxacin			
H. influenzae ATCC 49247	0.002-0.015[a]	-	100.0
S. pneumoniae ATCC 49619	-	26 - 34[b]	96.3
Gatifloxacin			
S. pneumoniae ATCC 49619	-	24 - 31[c]	98.3

a. Previously published QC range = 0.002 - 0.008 μg/ml.
b. Previously published QC range = 26 - 33 mm.
c. Previously published QC range = 24 - 31 mm, no change required.

CONCLUSIONS

- The re-evaluation of the zone diameter and MIC results of garenoxacin and gatifloxacin illustrates the need for continued monitoring of the QC organisms used by clinical laboratories for internal quality assurance.

- When the occasional reports of "out-of-control" results are made to the NCCLS QC working group, susceptibility test manufacturers or laboratory inspectors, it seems in the best interest of laboratory medicine to follow up via structured re-evaluation of the problematic QC ranges.

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