Moxifloxacin MIC results for *Neisseria gonorrhoeae* (ATCC 49226) for an eight lab study by CLSI agar dilution methodology

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Revised abstract

Objectives: This study was performed to establish a minimum inhibitory concentration (MIC) quality control range for *Neisseria gonorrhoeae* (ATCC 49226) according to Clinical Laboratory Standards Institute (CLSI) M23-A2 for agar dilution methodology.

Methods: Moxifloxacin was tested at concentrations of 0.002–0.25 µg/mL using 3 different manufacturers of GC agar base media (Remel, Accumedia and PML) and 1% defined growth supplement (Remel). Ciprofloxacin was also tested as the control drug at concentrations of 0.0025 to 0.03 µg/mL using 1 lot of media. 10 replicates of the quality control strain were tested for each of the media lots by 7 US and 1 Canadian laboratory on each of 2 days.

Results: Of the total 434 moxifloxacin MICs evaluated, 100% were within a four-well range of 0.004 to 0.03 µg/mL and 97% were within a three-well range of 0.008 to 0.03 µg/mL. All ciprofloxacin MICs were within the CLSI established range of 0.001 to 0.008 µg/mL (90.6% of MICs at 0.004 µg/mL). The modal moxifloxacin MIC for 7 of the 8 labs and 2 of the 3 media lots was 0.016 µg/mL. The moxifloxacin MICs for 1 lab and PML media were slightly lower (mode of 0.008 µg/mL). There was also a higher incidence of poor or no growth with the PML media. The average inoculum concentration was 4.3 x 10^4 CFU/spot. MIC distributions for all labs are shown in the table.

Antimicrobial agent	MIC (µg/mL) number of occurrences						Total n
	0.001	0.002	0.004	0.008	0.016	0.03	
Moxifloxacin			13	58	334	29	434
Ciprofloxacin	1	12	145	2			160

Conclusions: When performing agar dilution MIC testing according to CLSI methodology, a quality control range for *N. gonorrhoeae* (ATCC 49226) versus moxifloxacin of 0.008 to 0.03 µg/mL is recommended.

Introduction

Neisseria gonorrhoeae is one of the primary causative organisms in pelvic inflammatory disease. Moxifloxacin has been shown to have good activity against *N. gonorrhoeae* in the context of a recent clinical trial.[1] To establish quality control ranges for moxifloxacin, an eight laboratory collaborative study was performed against *N. gonorrhoeae* strain (ATCC 49226) according to M7-A6, agar dilution methodology (CLSI).[2]

Methods

1 – Microorganism

Neisseria gonorrhoeae (ATCC 49226) (Microbiologics, lyophilized)

2 – Antimicrobial agents

Each testing laboratory was provided with frozen vials of the following antimicrobial solutions:

- Ciprofloxacin (Bayer, New Haven, CT, USA) 0.00025 to 0.03 µg/mL
- Moxifloxacin (Bayer, New Haven, CT, USA) 0.002 to 0.25 µg/mL

3 – Media

GC agar: Three lots of GC agar tubes were prepared by PML, containing 17.8 mL/tube in 20 x 135 mm glass tubes:

- Lot 1: Remel, Lot No. 220215-1
- Lot 2: Acumedia, Lot No. 220234-1
- Lot 3: PML, Lot No. 220214-1

GCHI Enrichment (Remel, Lot No. 616077)

Each testing laboratory prepared the agar dilution plates on the same day of testing. The agar was melted and sufficiently cooled and 0.2 mL of GCHI enrichment and 2 mL of antibiotic dilution was aseptically added to each tube and mixed. The entire content of each tube was poured into a sterile 100 x 15-mm Petri dish and allowed to solidify.

4 – Participating laboratories

Investigator	Institution, City, State
Saralee Bajaksouzian, Ann Windau	University Hospital, Cleveland, OH, USA
Diane Citron	R.M. Alden Research Lab., Santa Monica, CA, USA
David Hecht	Loyola University Medical Center, Maywood, IL, USA
Janet Hindler	UCLA Medical Center, Los Angeles, CA, USA
Stephen Jenkins	Mount Sinai Medical Center, New York, NH, USA
Laura Koeth	Laboratory Specialists, Inc., Westlake, OH, USA
Elizabeth Palavecino	Wake Forest University, Winston Salem, NC, USA
Robert Rennie	University of Alberta Hospital, Edmonton, Canada

5 – Agar dilution procedure

- Using a fresh culture of *N. gonorrhoeae* incubated for 48 hours, an organism suspension equivalent to a density of a 0.5 McFarland standard was prepared for 10 separate replicates. 2 µL of each of the replicates was transferred to three sets of moxifloxacin and one set of ciprofloxacin agar dilution plates.
- Plates were incubated at 36±1ºC in 5% CO_2 for 20–24 hours.
- The MIC was read and recorded as the lowest concentration of antimicrobial agent that completely inhibited growth.
- The same procedure was repeated on a second day for a total of 480 moxifloxacin results (20 replicates x 3 lots x 8 laboratories) and 160 ciprofloxacin results (20 replicates x 1 lot x 8 laboratories).
- Colony counts were performed for each replicate tested.

Results

- 97.0% of moxifloxacin MICs were within a three-well range of 0.008 to 0.03 µg/mL. All moxifloxacin MICs (n=434) were within a four-well range of 0.004 to 0.03 µg/mL. 46 results were eliminated as a result of poor or no growth on growth control plates (Tables 1 and 2 and Figures 1 and 2).
- All ciprofloxacin MICs (n=160) were within the established CLSI four-well range of 0.002-0.008 µg/mL (Table 3 and Figure 3).
- The average inoculum concentration was 4.3 x 10^4 CFU/spot.

Table 1: Moxifloxacin vs *N. gonorrhoeae* (ATCC 49226): Distribution of MICs by laboratory

MIC µg/mL	Laboratory Number (number of occurrences):								All Labs[b]
	1	2	3	4	5	6	7	8	
≤ 0.002									
0.004			13						13
0.008	3		14	11	3		10	17	58
0.016	47	40	13	49	45	60	37	43	334
0.03	10	17			2				29
0.06									
0.12									
0.25									
>0.25									
NA[a]		3	20		10		13		
Total n	60	57	40	60	50	60	47	60	434
Geo. Mean	0.017	0.019	0.008	0.014	0.015	0.016	0.013	0.013	0.014
Mode	0.016	0.016	0.008	0.016	0.016	0.016	0.016	0.016	0.016
Min	0.008	0.016	0.004	0.008	0.008	0.016	0.008	0.008	0.004
Max	0.03	0.03	0.016	0.016	0.03	0.016	0.016	0.016	0.03
Range	3	2	3	2	3	1	2	2	4
Average CFU/Spot (x10^4)	7.7	9.3	1.4	3.4	3.8	6	1.7	1.0	4.3

[a] No growth or poor growth on growth control plates (all PML media, with exception of 4 Accumedia results from lab 5)

[b] 97% of MICs were within the proposed three-well range of 0.088–0.03 µg/mL (indicated by the dashed lines)

Figure 1: Moxifloxacin vs *N. gonorrhoeae* (ATCC 49226): MIC distribution, all laboratory totals (97% within proposed range; n=434)

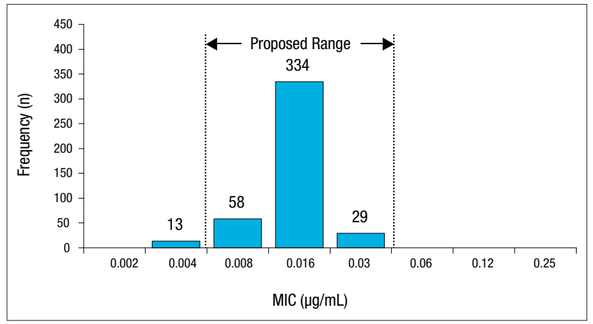


Table 2: Moxifloxacin vs *N. gonorrhoeae* (ATCC 49226): MIC distribution by lots of GC agar

MIC µg/mL	Occurrences by lot			All Lots
	Lot 1	Lot 2	Lot 3	
≤0.002				
0.004	8	5		13
0.008	11	8	38	57
0.016	141	114	80	335
0.03		29		29
0.06				
0.12				
0.25				
>0.25				
NA		4	42	
N[a]	160	156	118	434
Geo. Mean	0.014	0.016	0.012	0.014
Mode	0.016	0.016	0.016	0.016
Min	0.004	0.004	0.008	0.004
Max	0.016	0.03	0.016	0.03
Range	3	4	2	4

[a] No growth or poor growth on growth control plates

Figure 2: Moxifloxacin vs *N. gonorrhoeae* (ATCC 49226): MIC distribution by three lots of GC agar (n=434)

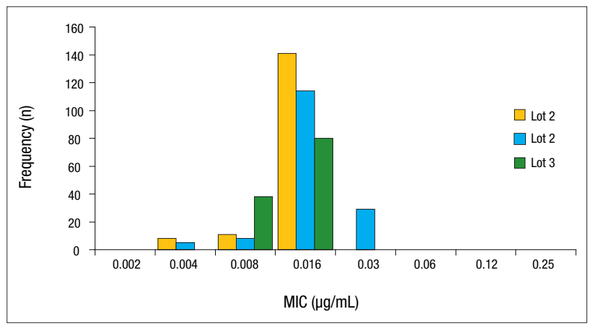
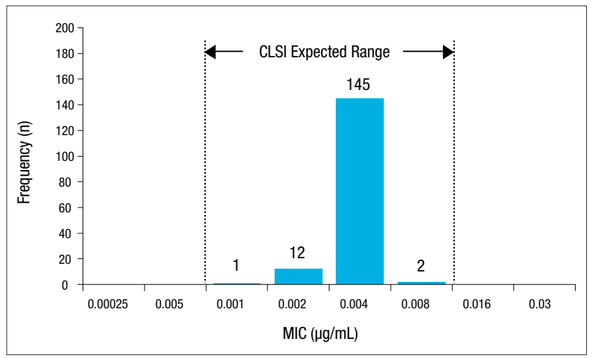


Table 3: Ciprofloxacin vs *N. gonorrhoeae* (ATCC 49226): MIC distribution by laboratory

MIC µg/mL	Laboratory Number (number of occurrences):								All Labs[a]
	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	
≤0.00025									
0.0005									
0.001			1						1
0.002		2	7				3		12
0.004	20	18	11	19	20	20	17	20	145
0.008			1	1					2
0.016									
0.03									
N	20	20	20	20	20	20	20	20	160
Geo. Mean	0.004	0.004	0.003	0.004	0.004	0.004	0.004	0.004	0.004
Mode	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
Min	0.004	0.002	0.001	0.004	0.004	0.004	0.002	0.004	0.001
Max	0.004	0.004	0.008	0.008	0.004	0.004	0.004	0.004	0.008
Range	1	2	4	2	1	1	2	1	4

[a] 100% of results were within CLSI expected range of 0.001–0.008 µg/mL (indicated by the dashed lines)

Figure 3: Ciprofloxacin vs *N. gonorrhoeae* (ATCC 49226): MIC distribution, all laboratory totals (n=160)



Conclusion

- When performing agar dilution MIC testing against *N. gonorrhoeae* according to CLSI methodology, the following quality control range is recommended

***N. gonorrhoeae* (ATCC 49226) vs moxifloxacin:**

0.008 – 0.03 µg/mL

References

1. Ross JDC, Cronje HS, Paszkowski T *et al.* Moxifloxacin versus ofloxacin plus metronidazole in uncomplicated pelvic inflammatory disease: results of a multicentre, double-blind, randomised trial. *Sex Transm Inf* 2006; 82: 446–51.
2. CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 2006 M7-A7, Vol. 26, No. 2, CLSI Wayne PA, USA.

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