

JE ROSS, RN JONES

JMI Laboratories, North Liberty, Iowa, USA

## Abstract

**Background:** The increasing prevalence of resistances in staphylococci, especially CA-MRSA, has brought renewed interest in fusidic acid (FA). A promising feature of FA is the lack of cross resistance with other antimicrobial classes, as a result of the unique mode of action that works by inhibiting bacterial protein synthesis at the translation stage. This study was performed to establish quality control (QC) ranges for broth microdilution (BMD) and disk diffusion (DD) and follows the CLSI M23-A3 (2008) guideline document. The results are presented as proposed QC ranges for two ATCC strains (*S. aureus* ATCC 29213 [SA] and *S. pneumoniae* ATCC 49619 [SPN]).

**Methods:** CLSI BMD and DD methods were utilized in an eight laboratory study design compliant with M23-A3 specifications. For BMD, four media lots (three manufacturers) of cation-adjusted Mueller-Hinton (MH) broth (with 2-5% lysed horse blood for testing SPN) were evaluated and three agar lots for the DD method. Ten replicate tests were performed for each QC organism generating 320 BMD values per strain (640 total) and 480 DD zones (two lots of FA disks were tested; 960 total zones). Levofloxacin and linezolid were used as controls.

**Results:** The table lists the recommended QC MIC and DD ranges for FA. Modal MIC values (% of total) observed were: SA at 0.12 µg/ml (77.5) and SPN at 8 µg/ml (60.6). No significant differences were noted among media lots or testing site performance for FA using BMD. Using DD, one laboratory reported zones that were significantly smaller than other participants and therefore was excluded from analysis.

QC organism (ATCC no.)	FA MIC/Disk zone diameters (% in range):	
	Proposed range for BMD (µg/ml)	Proposed range for DD (mm)
<i>S. aureus</i> ATCC 29213	0.06 – 0.25 (97.8)	24 – 32 (99.8)
<i>S. pneumoniae</i> ATCC 49619	4 – 32 (100.0)	8 – 16 (97.1)

**Conclusions:** Proposed MIC and DD QC ranges for FA will guide clinical or reference laboratories involved in the testing of clinical trial isolates and facilitate the regulatory review process.

## Introduction

The increasing prevalence of resistances in staphylococcal organisms has brought renewed interest in fusidic acid (sodium fusidate). A promising feature of fusidic acid is the lack of cross resistance with other antimicrobial classes, as a result of the unique mode of action that works by inhibiting bacterial protein synthesis at the translation stage.

These broth microdilution and disk diffusion quality control studies of fusidic acid were performed following the Clinical Laboratory Standards Institute (CLSI) M23-A3 (2008) guideline document using eight laboratories, different manufacturers of media and two antimicrobial control agents. The results are presented as proposed quality control ranges in µg/ml concentrations and zone diameters measured in mm for two American Type Culture Collection (ATCC) strains: *Staphylococcus aureus* ATCC 29213 and *Streptococcus pneumoniae* ATCC 49619.

## Materials and Methods

A total of eight laboratories were recruited to provide data for this QC investigation. For the broth microdilution (BMD) study, four cation-adjusted Mueller-Hinton (MH) broth media included lots produced by Difco Laboratories (Detroit, MI), Becton Dickinson (BD; Sparks, MD), and Oxoid (Hampshire, United Kingdom [UK]). Four cation-adjusted MH broth lots supplemented with 2-5% lysed horse blood were also supplied by Difco, BD and Oxoid. Fusidic acid was provided by Cembra Pharmaceuticals, Inc. (Chapel Hill, NC); levofloxacin and linezolid were acquired from Sigma-Aldrich (St. Louis, MO). Panels were prepared by a certified GMP source (TREK Diagnostics Cleveland, OH). Appropriate inoculum concentrations were established by performing colony counts from the broth microdilution trays which were subcultured onto drug-free agar plates.

Three lots of agar medium were used for the disk diffusion (DD) study from BD, Remel (Lenexa, KS) and Hardy Diagnostics (Santa Maria, CA). The fusidic acid disks (10-µg) were provided by Oxoid (lot #459655) and Mast Group (Merseyside, UK; lot #207567). Internal QC was established using levofloxacin (5-µg) and linezolid (30-µg) disks obtained from Remel. Ten replicates of each of the two ATCC strains produced 960 zone diameters for fusidic acid.

## Results

Colony counts were performed on the BMD panels with the average colony counts among the participating centers ranging from  $1.4 \times 10^5$  CFU/ml to  $4.6 \times 10^5$  CFU/ml for *S. aureus* ATCC 29213 and  $1.0 \times 10^5$  CFU/ml to  $4.4 \times 10^5$  CFU/ml for *S. pneumoniae* ATCC 49619.

There was no significant difference between lots of Mueller-Hinton broth when testing fusidic acid (Table 1). The modal fusidic acid MIC was the same for all lots regardless of QC strain tested (0.12 µg/ml for *S. aureus*, 8 µg/ml for *S. pneumoniae*).

The results of the BMD *S. aureus* ATCC 29213 testing from eight laboratories are shown in Table 1. Using M23 criteria to establish MIC ranges, 97.8% of all results were within the proposed limits of 0.06 – 0.25 µg/ml.

*S. pneumoniae* ATCC 49619 MIC results are shown in Figure 1. A four dilution range of 4 – 32 µg/ml was proposed with 100.0% of all participant results.

Disk diffusion results for *S. aureus* ATCC 25923 are shown in Table 2. A proposed range of 24 – 32 mm for fusidic acid included only 92.1% of all laboratory results. Laboratory D reported *S. aureus* zone diameters that were significantly smaller than the other seven laboratories. As only seven laboratories are required to establish a QC range, the exclusion of Laboratory D produced a QC range with 99.8% of the results within the range (24 – 32 mm). All geometric mean zone diameters were within  $\leq 2.0$ mm of each other, as were the disk lots (Table 2).

Fusidic acid zone diameters for *S. pneumoniae* ATCC 49619 are shown in Table 3 and Figure 2. A proposed range of 8 – 16 mm included 97.1% of all results. However, one agar medium lot (A) produced zones 2.2mm larger and disk lot A was 1.7mm larger than lot B by geometric mean analysis; acceptable.

All results for linezolid were within the CLSI published range. Linezolid was used as a valid internal control for the study.

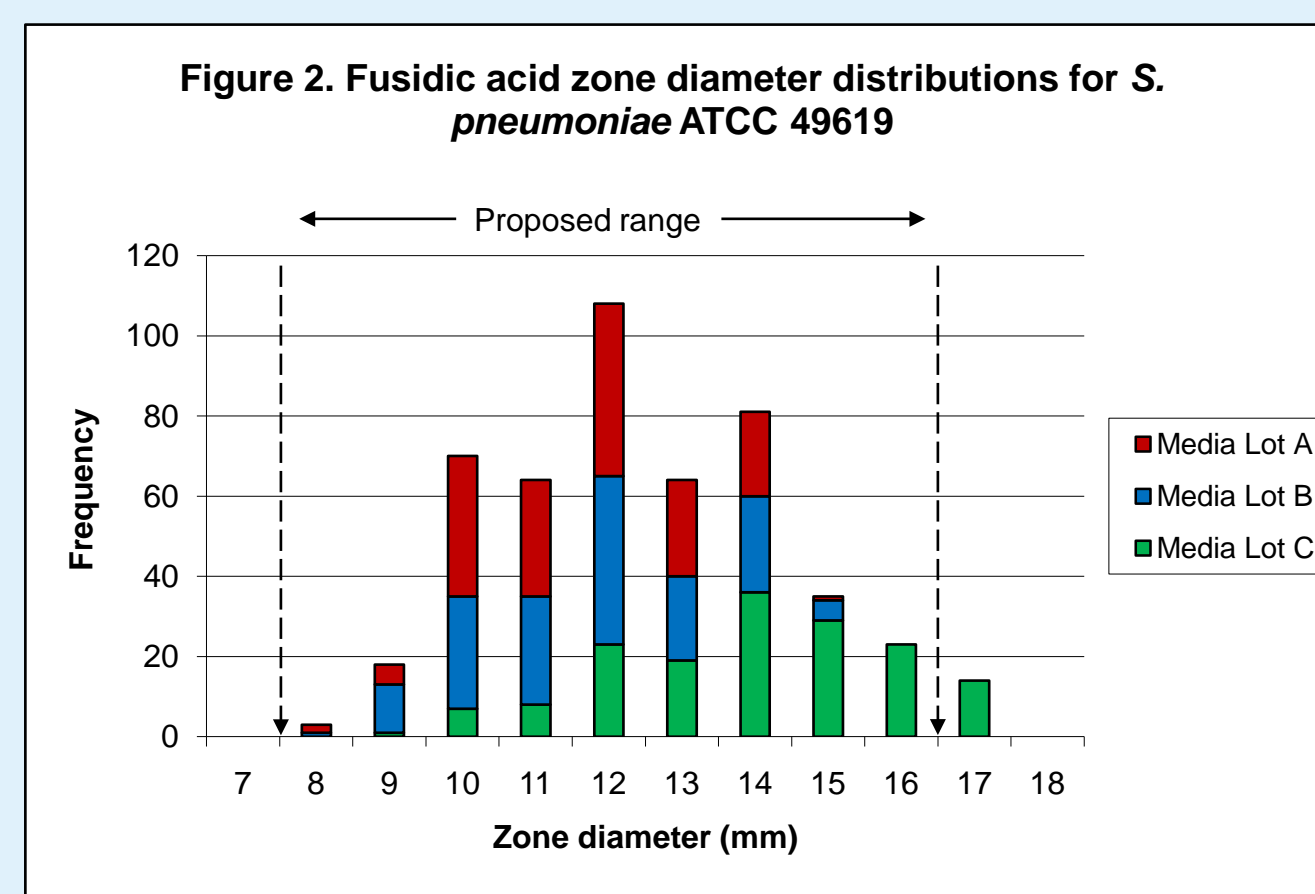
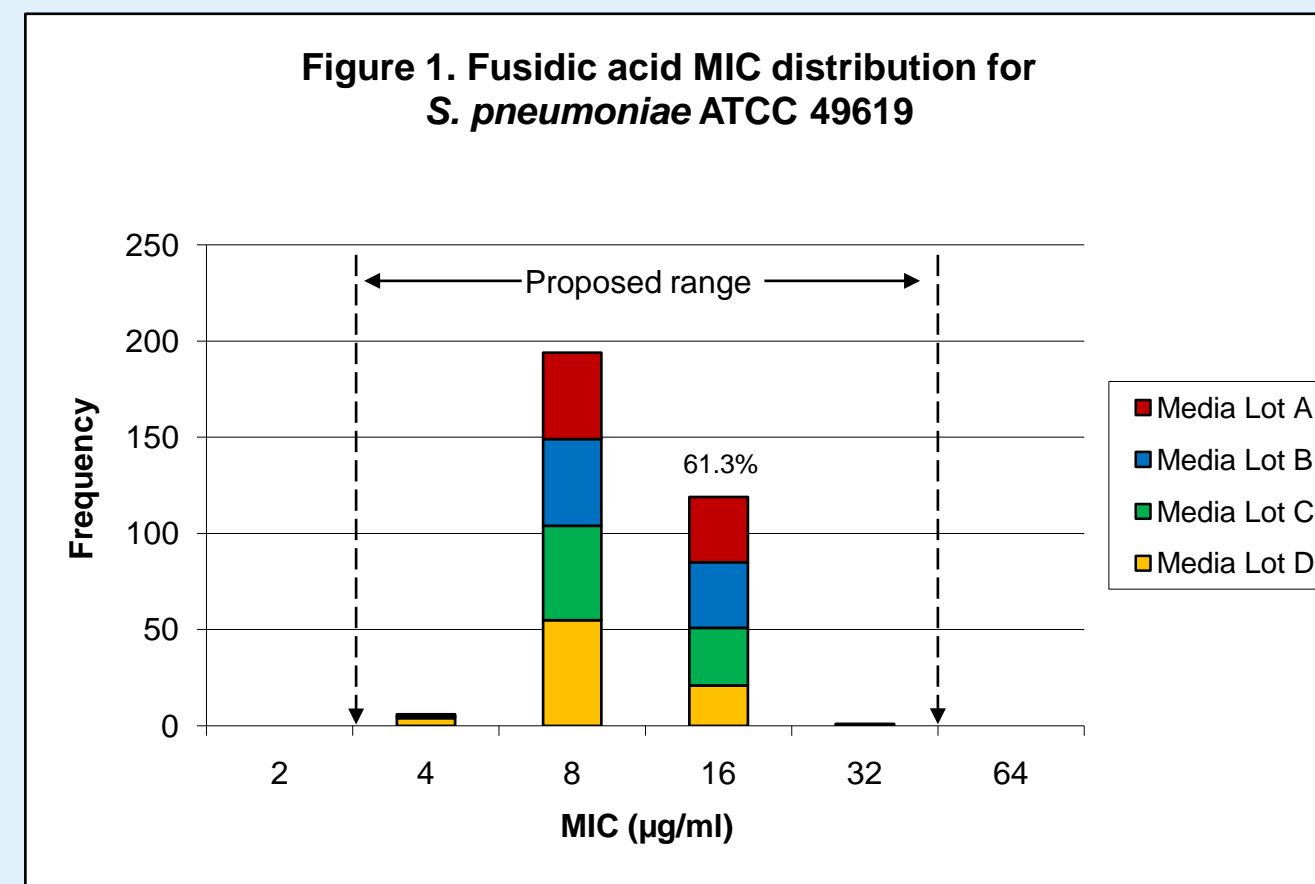


Table 1. Inter- and intra-laboratory comparisons of fusidic acid MIC results versus *S. aureus* ATCC 29213 for an eight medical center protocol meeting the study design guidelines found in CLSI M23-A3 (2008).

MIC (µg/ml)	Media lot				Laboratory code (occurrences):								Total				
	A	B	C	D	A	B	C	D	E	F	G	H					
0.015																	
0.03	4	3													7	7	
0.06	11	9	9	12	12					4	2			23	41 <sup>a</sup>		
0.12	61	66	60	61	28	36	33	37	34	36	34	10		248 <sup>a</sup>			
0.25	4	2	11	7		4	7	3	2	2	6			24 <sup>a</sup>			
0.5																	
Total	80	80	80	80	40	40	40	40	40	40	40	40	40	320			
Mode	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.06	0.12			
Geomean	0.11	0.11	0.12	0.12	0.10	0.13	0.14	0.13	0.12	0.12	0.13	0.06	0.11				
Range	4	4	3	3	2	2	2	2	3	3	2	3	4				

a. 97.8% of qualified results in proposed QC range (0.06 – 0.25 µg/ml).

Table 2. Inter- and intra-laboratory comparisons of the fusidic acid zone diameter results versus *Staphylococcus aureus* ATCC 25923 for an eight medical center protocol meeting the study design guidelines found in CLSI M23-A3 (2008).

Zone diameter (mm)	Media Lot		Disk Lot		Laboratory code (occurrences):								Total			
	A	B	A	B	A	B	C	D	E	F	G	H				
19																
20	1			0	1				1						1	(0)
21	1	3		1	3				4						4	(0)
22	5	6	5	1	15				16						16	(0)
23	5	4	7	5	11				16						16	(0)
24	2	10	2	11	3				11	1				2	14	(3) <sup>a</sup>
25	11	14	6	15	16	4	1		9	3	1	3	10	31	(22) <sup>a</sup>	
26	17	37	2	21	35	4	0	3	3	13	8	14	11	56	(53) <sup>a</sup>	
27	39	38	18	38	57	16	8	5		13	12	21	20	95	(95) <sup>a</sup>	
28	33	28	32	43	50	15	16	11		11	19	13	8	93	(93) <sup>a</sup>	
29	25	11	37	42	31	9	17	8		14	15	4	6	73	(73) <sup>a</sup>	
30	14	7	27	37	11	9	9	19		5	1	5	0	48	(48) <sup>a</sup>	
31	5	2	19	19	7	3	6	12			3		2	26	(26) <sup>a</sup>	
32	2		4	6	0		3	2			1		0	6	(6) <sup>a</sup>	
33			1	1	0								1	1	(1)	
34																
Total	160	160	160	240	240	60	60	60	60	60	60	60	60	480		
Median	27	27	29	28	27	28	29	30	23	27	28	27	27	28		
Geomean	27.2	26.4	28.2	27.9	26.7	28.0	28.9	29.3	23.1	27.5	27.9	27.2	26.9	27.3		
Range	13	11	12	13	12	7	8	7	7	7	8	6	10	14		

a. 92.1% of qualified results are in proposed QC range (24 – 32 mm), but 99.8% were within range after excluding Laboratory D (see totals in parenthesis).

Table 3. Inter- and intra-laboratory comparisons of the fusidic acid zone diameter results versus *Streptococcus pneumoniae* ATCC 49619 for an eight medical center protocol meeting the study design guidelines found in CLSI M23-A3 (2008).

Zone diameter (mm)	Media lot			Disk lot		Laboratory code (occurrences):								Total		
	A	B	C	A	B	A	B	C	D	E	F	G	H			
7																
8		1	2		3					2			1		3 <sup>a</sup>	
9	1	12	5		18	8				0			6		18 <sup>a</sup>	
10	7	28	35	4	66	12			1	15	6	13	23		70 <sup>a</sup>	
11	8	27	29	40	24	14	1	5	6	5	9	20	4		64 <sup>a</sup>	
12	23	42	43	48	60	13	12	12	19	18	14	10	10		108 <sup>a</sup>	
13	19	21	24	35	29	5	10	11	9	11	7			11	64 <sup>a</sup>	
14	36	24	21	62	19	7	15	14	5	14	8			18	81 <sup>a</sup>	
15	29	5	1	18	17	1	13	5	2	3	4			7	35 <sup>a</sup>	
16	23			19	4		3	5		1	5			9	23 <sup>a</sup>	
17	14			14			6	7						1	14	
18																
Total	160	160	160	240	240	60	60	60	60	60	60	60	60	480		
Median	14	12	12	13	12	11	14	14	12	12	12	10	14	12		
Geomean	13.8	11.6	11.6	13.2	11.5	11.2	13.9	13.6	11.5	12.3	12.2	10.5	13.7	12.3		
Range	9	8	8	8	9	7	7	8	7	9	7	5	7	10		

a. 97.1% of qualified results in proposed QC range (8 – 16 mm).

## Conclusions

The proposed QC ranges for disk diffusion and BMD methods showed that fusidic acid has generally good inter- and intra-laboratory reproducibility for the commonly utilized control isolates, *S. aureus* ATCC 29213 and *S. pneumoniae* ATCC 49619.

These studies established QC ranges that can be utilized to support accurate testing for susceptibility of fusidic acid during clinical trials and continued product development.

Fusidic acid is a potentially useful treatment option for infections caused by multidrug-resistant staphylococci, including methicillin-resistant *S. aureus* (MRSA).

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## References

- Besier S, Ludwig A, Brade V, Wichelhaus TA (2003). Molecular analysis of fusidic acid resistance in *Staphylococcus aureus*. *Molec Microbiol* 47: 463-469.
- Clinical and Laboratory Standards Institute (2009). *M02-A10. Performance standards for antimicrobial disk susceptibility tests; approved standard - tenth edition*. Wayne, PA: CLSI.
- Clinical and Laboratory Standards Institute (2009). *M07-A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - eighth edition*. Wayne, PA: CLSI.
- Clinical and Laboratory Standards Institute (2008). *M23-A3. Development of in vitro susceptibility testing criteria and quality control parameters - third edition*. Wayne, PA: CLSI.
- Clinical and Laboratory Standards Institute (2009). *M100-S19. Performance standards for antimicrobial susceptibility testing. 19th informational supplement*. Wayne, PA: CLSI.
- O'Neill AJ, Larsen AR, Henriksen AS, Chopra I (2004). A fusidic acid-resistant epidemic strain of *Staphylococcus aureus* carries the fusB determinant, whereas fusA mutations are prevalent in other resistant isolates. *Antimicrob Agents Chemother* 48: 3594-3597.
- O'Neill AJ, McLaws F, Kahlmeter G, Henriksen AS, Chopra I (2007). Genetic basis of resistance to fusidic acid in staphylococci. *Antimicrob Agents Chemother* 51: 1737-1740.
- Rennie RP (2006). Susceptibility of *Staphylococcus aureus* to fusidic acid: Canadian data. *J Cutan Med Surg* 10: 277-280.
- Turnidge J, Collignon P (1999). Resistance to fusidic acid. *Int J Antimicrob Agents* 12 Suppl 2: S35-S44.