

A Multi-Site Study of Gemifloxacin MIC Results on the Sensititre® 18-24 hour Automated/Manual Susceptibility Plate to the CLSI Broth Microdilution Method

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ABSTRACT

Background: A multi-site comparison was performed to evaluate Gemifloxacin (GEM) (Oscient Pharmaceuticals, Waltham, MA) MIC results on the Sensititre dried 18-24 hour susceptibility plate (TREK Diagnostic Systems, Cleveland, OH). Both autoread and manual reading methodologies were tested against the CLSI broth microdilution reference method (BMD). **Methods:** GEM (0.002-16 µg/ml) was tested against 302 fresh clinical gram-positive isolates and 73 CDC gram-positive challenge strains. GEM (0.002-4 µg/ml) was tested against 305 recent clinical gram-negative isolates and 75 CDC gram-negative challenge isolates. Sensititre plates were inoculated and read per the manufactures instructions. BMD was performed according to CLSI M7-A6. The recommended CLSI quality control organisms were tested daily and were within the expected GEM quality control ranges.

Results: Comparisons of GEM MIC results with the BMD method for both autoread and manual reads on the Sensititre dried susceptibility plate were performed. Percent essential agreement (EA) rates within ± one log₂ dilution were determined. The EA for the gram-positive isolates on the autoread and manual methods was 97.0%/99.7% for clinical and 98.6%/100% for challenge isolates, respectively. The categorical agreement (CA) rates were also determined. The CA for both the gram-positive clinical and challenge isolates were 100% for manual and autoread methods. The EA for the gram-negative isolates on the autoread and manual methods was 98.7%/99.3% for clinical and 100%/100% for the challenge isolates, respectively. The CA for the gram-negative isolates on the autoread and manual methods was 95.7%/97.8% for clinical and 100%/100% for challenge isolates, respectively.

Conclusions: This multi-site comparison of GEM on the Sensititre dried 18-24 hour susceptibility plate for both gram-positive and gram-negative organisms gave reliable results using either the automated or manual read methodology compared to the reference BMD method.

INTRODUCTION

Gemifloxacin is a novel naphthridone compound in the fluoroquinolone class of antimicrobial agents. Gemifloxacin acts by inhibiting DNA synthesis through the inhibition of both DNA gyrase and topoisomerase IV (TOPO IV) both of which are essential for bacterial growth.

PURPOSE OF THE STUDY

To evaluate the performance of Gemifloxacin on the Sensititre 18 – 24 hour susceptibility plate compared to the CLSI Broth Microdilution Method (M7 – A6).

MATERIALS & METHODS

Organisms: The testing at 3 sites consisted of the following:

- 607 total fresh clinical isolates (combined sites)
 - (305 gram negative and 302 gram positive isolates)
- 148 CDC challenge isolates tested at one site
- 50 interlaboratory reproducibility isolates (25 gram positive and 25 gram negative)
- 4 Quality Control strains (run 20 times each per site)

Antimicrobial		
Antimicrobial Tested	Range Tested	Supplied By:
Gemifloxacin (GEM)	0.002 – 16µg/ml	Oscient Pharmaceuticals Waltham, PA

SUSCEPTIBILITY TESTING METHODS

- Each isolate was tested using a Sensititre 18 – 24 susceptibility plate. The plates were set-up and tested according to the manufacturers' instructions.
- The reference plate was tested according to the broth microdilution methods published by the Clinical Laboratory Standards Institute (CLSI, M7-A6).
- Interlaboratory reproducibility testing consisted of 25 gram positive and 25 gram negative isolates provided to each laboratory. The results were compared to the reference method results, and to the test plate results from each laboratory.

Clinical Isolates Tested	
Gram Positive Organism Species	Number Tested
<i>Staphylococcus aureus</i>	92
Coagulase Negative Staph. spp.	86
<i>Enterococcus faecalis</i>	63
<i>Enterococcus faecium</i>	30
<i>Streptococcus</i> species Group B	21
<i>Enterococcus</i> species Group A	10
Total	302

SUSCEPTIBILITY TESTING METHODS cont.

Gram Negative Organism Species	Number Tested
<i>Escherichia coli</i>	46
<i>Klebsiella spp.</i>	48
<i>Enterobacter spp.</i>	32
<i>Providencia spp.</i>	10
<i>Proteus spp.</i>	44
<i>Citrobacter spp.</i>	34
<i>Morganella morganii</i>	12
<i>Serratia spp.</i>	17
<i>Acinetobacter spp.</i>	15
<i>Pseudomonas aeruginosa</i>	35
<i>Pseudomonas spp.</i>	11
<i>Alcaligenes spp.</i>	1
Total	305

RESULTS

Quality Control Testing						
Quality Control Organism Tested	QC Range (µg/ml)	and Frequency of Results				
<i>S. aureus</i> ATCC 29213	<0.008	(0.008	0.015	0.03)	>0.03	
Autoread		26	34			
Manual read		42	18			
<i>E. faecalis</i> ATCC 29212	<0.015	(0.015	0.03	0.06	0.12)	>0.12
Autoread		2	56	1	1	
Manual read		23	37			
<i>E. coli</i> ATCC 25922	<0.004	(0.004	0.008	0.015)	>0.015	
Autoread		24	37			
Manual read		16	45			
<i>P. aeruginosa</i> 27853	<0.25	(0.25	0.5	1)	>1	
Autoread		37	21	3	1	
Manual read		43	16	2	1	

Gram Negative Clinical Isolates Tested - Autoread Method

Organisms	Total Isolates Tested	Isolates in Agreement	% Essential Agreement	% Categorical Agreement
<i>Escherichia coli</i>	46	46	100	N/A
<i>Klebsiella spp.</i>	48	48	100	92
<i>Citrobacter spp.</i>	34	34	100	N/A
<i>Enterobacter spp.</i>	32	30	94	N/A
<i>Proteus spp.</i>	44	43	98	100
Other Enterobacteriaceae	39	39	100	N/A
Non Enterics	62	61	98	N/A
Totals	305	301	98.7	95.7

Gram Negative Clinical Isolates Tested - Manual Read Method

Organisms	Total Isolates Tested	Isolates in Agreement	% Essential Agreement	% Categorical Agreement
<i>Escherichia coli</i>	46	46	100	N/A
<i>Klebsiella spp.</i>	48	48	100	96
<i>Citrobacter spp.</i>	34	34	100	N/A
<i>Enterobacter spp.</i>	32	30	94	N/A
<i>Proteus spp.</i>	44	44	100	100
Other Enterobacteriaceae	39	39	100	N/A
Non Enterics	62	62	100	N/A
Totals	305	303	99.3	97.8

RESULTS cont.

Gram Positive Clinical Isolates Tested - Autoread Method

Organisms	Total Isolates Tested	Isolates in Agreement	% Essential Agreement	% Categorical Agreement
Methicillin Sensitive				
<i>Staph. aureus</i>	45	41	91	100
Methicillin Resistant				
<i>Staph. aureus</i>	47	47	100	N/A
Coag. Neg. Staph. spp.	86	85	98.8	N/A
<i>Streptococcus spp.</i>	31	29	93.5	N/A
<i>Enterococcus faecalis</i>	63	61	96.8	N/A
<i>Enterococcus faecium</i>	30	30	100	N/A
Totals	302	293	97	100

Gram Positive Clinical Isolates Tested - Manual Read Method

Organisms	Total Isolates Tested	Isolates in Agreement	% Essential Agreement	% Categorical Agreement
Methicillin Sensitive				
<i>Staph. aureus</i>	45	45	100	100
Methicillin Resistant				
<i>Staph. aureus</i>	47	47	100	N/A
Coag. Neg. Staph. spp.	86	86	100	N/A
<i>Streptococcus spp.</i>	31	30	96.8	N/A
<i>Enterococcus faecalis</i>	63	63	100	N/A
<i>Enterococcus faecium</i>	30	30	100	N/A
Totals	302	301	99.4	100

Gram Negative Challenge Isolates Tested - Autoread Method

Organisms	Total Isolates Tested	Isolates in Agreement	% Essential Agreement	% Categorical Agreement
<i>Escherichia coli</i>	13	13	100	N/A
<i>Klebsiella spp.</i>	10	10	100	100
<i>Citrobacter spp.</i>	N/A			
<i>Enterobacter spp.</i>	10	10	100	N/A
<i>Proteus spp.</i>	6	6	100	100
Other Enterobacteriaceae	19	19	100	N/A
Non Enterics	17	17	100	N/A
Totals	75	75	100	100

Gram Negative Challenge Isolates Tested - Manual Read Method

Organisms	Total Isolates Tested	Isolates in Agreement	% Essential Agreement	% Categorical Agreement
<i>Escherichia coli</i>	13	13	100	N/A
<i>Klebsiella spp.</i>	10	10	100	100
<i>Citrobacter spp.</i>	N/A			
<i>Enterobacter spp.</i>	10	10	100	N/A
<i>Proteus spp.</i>	6	6	100	100
Other Enterobacteriaceae	19	19	100	N/A
Non Enterics	17	17	100	N/A
Totals	75	75	100	100

RESULTS cont.

Gram Positive Challenge Isolates Tested - Autoread Method

Organisms	Total Isolates Tested	Isolates in Agreement	% Essential Agreement	% Categorical Agreement
Methicillin Sensitive				
<i>Staph. aureus</i>	11	11	100	100
Methicillin Resistant				
<i>Staph. aureus</i>	7	7	100	N/A
Coag. Neg. Staph. spp.	20	20	100	N/A
<i>Streptococcus spp.</i>	3	3	100	N/A
<i>Enterococcus faecalis</i>	14	14	100	N/A
<i>Enterococcus faecium</i>	14	14	100	N/A
<i>Enterococcus spp.</i>	4	4	100	N/A
Totals	73	73	100	100

Gram Positive Challenge Isolates Tested - Manual Read Method

Organisms	Total Isolates Tested	Isolates in Agreement	% Essential Agreement	% Categorical Agreement
Methicillin Sensitive				
<i>Staph. aureus</i>	11	11	100	100
Methicillin Resistant				
<i>Staph. aureus</i>	7	7	100	N/A
Coag. Neg. Staph. spp.	20	20	100	N/A
<i>Streptococcus spp.</i>	3	3	100	N/A
<i>Enterococcus faecalis</i>	14	14	100	N/A
<i>Enterococcus faecium</i>	14	14	100	N/A
<i>Enterococcus spp.</i>	4	4	100	N/A
Totals	73	73	100	100

CONCLUSION

This investigation compared the 18 – 24 hour Sensititre susceptibility plate with the Broth Microdilution plate (M7–A6). The Sensititre plate demonstrated a high level of agreement and was very reproducible.

- **Clinical Isolates:** The overall essential agreement for Gemifloxacin, within +/- one log₂ dilution, was 99.5% for the manual method and 97.9% for the autoread method.

- **CDC Challenge Organisms:** The overall essential agreement for Gemifloxacin, within +/- one log₂ dilution, was 100% for the manual method and 99.3% for the autoread method.

- **Interlaboratory Reproducibility:** Interlaboratory reproducibility testing results, within +/- one log₂ dilution from the expected result was, 98.7% for the autoread method and 98% for the manual read method.

Interlaboratory reproducibility testing essential agreement, within +/- one log₂ dilution of the reference method result, was 98% for the manual read method and 97.3% for the autoread method.

- **The Sensititre® 18-24 hour Automated/Manual Susceptibility Plate** has been cleared by the FDA Center for Devices and Radiological Health for the following with the EA agreement rates for manual reads and autoreads respectively as follows:

Enterobacteriaceae	99% and 99%
<i>Acinetobacter lwoffii</i>	100% and 93.3%
<i>Streptococcus spp.</i>	97% and 94%
Methicillin Susceptible <i>Staph. aureus</i>	100% and 91%

- The approved primary "Indications for Use" and clinical significance of Gemifloxacin is for:

Klebsiella pneumoniae
Gemifloxacin exhibits *in vitro* minimum inhibitory concentrations (MIC's) of (0.25µg/ml) or less against most (>90%) of the following microorganisms:

- * *Staphylococcus aureus* (Methicillin-susceptible strains only)
- * *Streptococcus pyogenes*
- * *Acinetobacter lwoffii*
- * *Klebsiella oxytoca*
- * *Proteus vulgaris*

*The safety and effectiveness of gemifloxacin in treating clinical infections due to these microorganisms has not been established in adequate and well controlled clinical trials.

REFERENCES

1. Clinical and Laboratory Standards Institute. 2006. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-sixth edition*. Approved document M7-A7. Wayne, PA: CLSI.
2. Clinical and Laboratory Standards Institute. 2006. *Performance standards for antimicrobial susceptibility testing, 16th informational supplement* M100-S16. Wayne, PA: CLSI.

Gram Negative Interlaboratory Reproducibility Isolates vs. Gemifloxacin Essential Agreement Within +/- 1 log₂ Dilution of the Reference MIC

Site	# Agreement		Total Isolates Tested	Manual % Autoread%	
	Manual	Autoread		Manual %	Autoread%
1	24	25	25	96	100
2	25	25	25	100	100
3	24	24	25	96	96
Combined	73	74	75	97	99

Gram Positive Interlaboratory Reproducibility Isolates vs. Gemifloxacin Essential Agreement Within +/- 1 log₂ Dilution of the Reference MIC

Site	# Agreement		Total Isolates Tested	Manual % Autoread%	
	Manual	Autoread		Manual %	Autoread%
1	25	25	25	100	100
2	24	24	25	96	96
3	25	23	25	100	92
Combined	74	72	75	99	96