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A Multi-Site Study of an 18-24h Susceptibility System to the CLSI Microdilution Method for *Acinetobacter* spp., *Pseudomonas* spp. and *Pseudomonas aeruginosa* vs. Colistin (COL) and Polymyxin B (POL)

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ABSTRACT

Background: (COL) and (POL) (Sigma-Aldrich, St. Louis Missouri) are lipopeptides, effective in treating multi drug resistant gram negative infections. An evaluation was undertaken to determine the accuracy and reproducibility of the Sensititre® dried susceptibility system (TREK Diagnostic Systems, Cleveland, OH) using both automated and manual reading methodologies with COL and POL compared to the CLSI M7 reference broth microdilution method (BMD).
Materials and Methods: COL and POL (0.06 - 8 µg/ml) were tested against 188 fresh clinical isolates (82 *Acinetobacter* spp. and 106 *Pseudomonas* species) and 25 reproducibility isolates. Dried plates were inoculated as per manufacturers' instructions, and the BMD was performed as per CLSI M7. Recommended CLSI quality control (QC) organisms were tested daily and were within the CLSI expected QC ranges.
Results: Comparisons of COL and POL MIC results on the Sensititre system to the CLSI M7 BMD for both automated and manual reads, resulted in 97.6/98.8% essential agreement for COL and 99.9/99.7% for POL (+/- one $\log_2$ dilution). Categorical agreements (CA) for both the autoread and manual read methods for COL were 97.2/97.1% and 97.2/98.1% for POL. Regarding CA, the results did not show very major or major errors and had a <10% minor error rate for all isolates. Reproducibility was calculated as the percentage of results within +/- one $\log_2$ dilution of the modal MIC. Overall agreement for the reproducibility, autoread and manually read, for COL was 97/97% and 97/97% for POL.
Conclusions: The results of COL and POL indicates that the Sensititre susceptibility system for *Acinetobacter* spp., *Pseudomona*s spp. and *Pseudomonas aeruginosa* gave reliable results using either the automated/manual read method compared to the reference BMD.

***Essential and Categorical agreements are subject to change**

INTRODUCTION

Colistin (polymyxin E) and **Polymyxin B** are polypeptide antibiotics. These are the only two polymyxins that can be used clinically. The main therapeutic use of polymyxins is for healing infections of gram negative bacteria (except for *Proteus* spp.) including *Pseudomonas aeruginosa* and *Acinetobacter* species. They are not active against gram positive bacteria.

PURPOSE OF THE STUDY

A multi-site study to evaluate the performance of Colistin and Polymyxin B on the Sensititre® 18 – 24 hour automated/manual susceptibility system compared to the CLSI Microdilution Method System.

MATERIALS & METHODS

Antimicrobials Tested

Antimicrobials Tested	Range Tested µg/ml	Supplied By
Colistin (COL)	0.06-8 µg/ml	Sigma-Aldrich (St. Louis Missouri)
Polymyxin B (POL)	0.06-8 µg/ml	Sigma-Aldrich (St. Louis Missouri)

Organisms Tested

	Colistin/Polymyxin B
Clinical Isolates (combined sites)	188
CDC challenge isolates (one site)	18
Reproducibility isolates (combined sites)	25
CLSI Quality Control Strains (combined sites)	40

Quality Control

Recommended CLSI quality control (QC) organisms were tested daily and were within the CLSI expected QC ranges.

Quality Control Strains	CLSI MIC Ranges (µg/ml)	
	Colistin	Polymyxin B
Escherichia coli ATCC 25922	0.25-1	0.25-2
Pseudomonas aeruginosa 27853	0.25-2	0.25-2

SUSCEPTIBILITY TESTING METHODS

- Indications for use: The Sensititre 18-24 hour MIC or breakpoint susceptibility system is an *in vitro* diagnostic product for clinical susceptibility testing of non-fastidious organisms.
- Each isolate was tested using a Sensititre 18 – 24 susceptibility plate containing **Colistin** and **Polymyxin B**. The plates were set-up and tested according to the manufacturers' instructions.
- The CLSI reference microdilution plate was prepared and tested according to the Clinical Laboratory Standards Institute (CLSI, M7).
- Testing consisted of 188 fresh non-fermenter clinical isolates; approximately 62 supplied by each site and 18 non-fermenter challenge isolates supplied to a single testing site.
- CLSI interpretive criteria for both manual and auto read methods were used to establish categorical agreement rates for *Acinetobacter* spp. and other Non-Enterobacteriaciae.
- Reproducibility testing consisted of 25 gram negative isolates tested at all 3 sites on the Sensititre 18-24 hour susceptibility plate. The test plate results were compared to the CLSI reference microdilution plate results.
- Quality control consisted of 20 replicates performed at each site of ATCC strains *E.coli* 25922 and *Ps. aeruginosa* 27853.
- Colony counts were performed on the QC on each day of testing.

RESULTS

Essential agreement for the clinical and challenge isolates were calculated using the +/- one $\log_2$ dilution standard for comparison studies. The categorical agreement was calculated using FDA interpretations. The essential agreement rates for the clinical and challenge isolates were as follows:

Essential Agreement of Clinical and Challenge Isolates using the Automated Read Methodology for Colistin

Test Results \ Reference Results	0.06	0.12	0.25	0.5	1	2	4	8	>8
0.06		4	1						
0.12									
0.25		2	5	12	1				
0.5			11	20	16				
1				17	24	4			
2					42	27	3		
4					4	2	4	1	
8							1	1	
>8									3
TOTALS	**0**	**6**	**17**	**49**	**87**	**33**	**8**	**2**	**3**

Essential Agreement 97.1%
CLSI Interpretive Criteria for *Acinetobacter* spp. Sensitive <=2 Resistant >=4
CLSI Interpretive Criteria for other Non-Enterobacteriaciae Sensitive <=2 Intermediate 4 Resistant >=8

Essential Agreement of Clinical and Challenge Isolates using the Manual Read Methodology for Colistin

Test Results \ Reference Results	0.06	0.12	0.25	0.5	1	2	4	8	>8
0.06		2	1						
0.12		2	2						
0.25		1	5	13					
0.5			9	19	15				
1				18	25	5			
2					42	27	4		
4					4	1	4	2	
8						1			
>8									3
TOTALS	**0**	**5**	**17**	**50**	**86**	**34**	**8**	**2**	**3**

Essential Agreement 97.1%
CLSI Interpretive Criteria for *Acinetobacter* spp. Sensitive <=2 Resistant >=4
CLSI Interpretive Criteria for other Non-Enterobacteriaciae Sensitive <=2 Intermediate 4 Resistant >=8

RESULTS cont.

Essential Agreement of Clinical and Challenge Isolates using the Automated Read Methodology for Polymyxin B

Test Results \ Reference Results	0.06	0.12	0.25	0.5	1	2	4	8	>8
0.06		3	1						
0.12			1						
0.25		2	2	12	1				
0.5			6	25	19				
1				9	20	4			
2					37	54	6		
4							1		
8									3
>8									
TOTALS	**0**	**5**	**10**	**46**	**77**	**58**	**7**	**0**	**3**

Essential Agreement 99%
CLSI Interpretive Criteria for *Acinetobacter* spp. Sensitive <=2 Resistant >=4
CLSI Interpretive Criteria for other Non-Enterobacteriaciae Sensitive <=2 Intermediate 4 Resistant >=8

Essential Agreement of Clinical and Challenge Isolates using the Manual Read Methodology for Polymyxin B

Test Results \ Reference Results	0.06	0.12	0.25	0.5	1	2	4	8	>8
0.06		3	1						
0.12			1						
0.25		1	1	9					
0.5			6	25	18				
1				12	18	2			
2					42	57	4		
4							2		
8							1		
>8									3
TOTALS	**0**	**4**	**9**	**46**	**78**	**59**	**7**	**0**	**3**

Essential Agreement 99.5%
CLSI Interpretive Criteria for *Acinetobacter* spp. Sensitive <=2 Resistant >=4
CLSI Interpretive Criteria for other Non-Enterobacteriaciae Sensitive <=2 Intermediate 4 Resistant >=8

Interlaboratory Reproducibilty % Essential (+/- one $\log_2$ dilution of the modal MIC) and Categorical Agreements

POLYMYXIN B	Auto Read	Manual Read
Between-site total isolates tested	75	75
Between-site isolates within +/- 1 well from mode	73	73
Between-site reproducibility ratio	73/75	73/75
Between-site reproducibility %	97%	97%
Total Essential Agreement	73	73
Essential Agreement %	97%	97%
Total Categorical Agreement	15	15
Categorical Agreement	15	15
Categorical Agreement %	100%	100%

COLISTIN	Auto Read	Manual Read
Between-site total isolates tested	75	75
Between-site isolates within +/- 1 well from mode	75	75
Between-site reproducibility ratio	74/75	75/75
Between-site reproducibility %	99%	100%
Total Essential Agreement	74	72
Essential Agreement %	99%	96%
Total Categorical Agreement	15	15
Categorical Agreement	15	15
Categorical Agreement %	100%	100%

CONCLUSION

The Sensititre 18 – 24 hour susceptibility system when compared to the CLSI M7 reference microdilution plate demonstrated an equivalent level of performance when testing **Colistin and Polymyxin B** vs. gram negative clinical and challenge isolates.

• Clinical and CDC Challenge Isolates

The overall essential agreement for **Colistin** and Gram Negative isolates, within +/- one $\log_2$ dilution, was 97.1% for the manual method and 97.1% for the autoread method.

The overall essential agreement for **Polymyxin B** and Gram Negative isolates, within +/- one $\log_2$ dilution, was 99.5% for the manual method and 99% for the autoread method.

The overall categorical agreement for **Colistin** vs. *Acinteobacter spp.* for both clinical and challenge isolates was 99.4% for the manual method and 100% for the autoread method.

The overall categorical agreement for **Polymyxin B** vs. *Acinteobacter spp.* for both clinical and challenge isolates was 100% for the manual method and 100% for the autoread method.

• Reproducibility:

Reproducibility testing results, within +/- one $\log_2$ dilution from the expected result for **Colistin** was, 99% for the autoread method and 100% for the manual read method.

Reproducibility testing results, within +/- one $\log_2$ dilution from the expected result for **Polymyxin B** was, 97% for the autoread method and 97% for the manual read method.

REFERENCES

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