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An Evaluation of Daptomycin Using the Sensititre® Dried Susceptibility Plate for its Effectiveness in Detecting Non-Susceptible Isolates

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ABSTRACT

Background: Daptomycin (Cubist Pharmaceuticals, Lexington, MA) is a cyclic lipopeptide for treatment of complicated skin and skin structure infections. This study was undertaken to evaluate the performance of the Sensititre (TREK Diagnostic Systems, Cleveland, OH) dried susceptibility plates in detecting non-susceptible isolates with daptomycin (DAP) using the frozen CLSI (M7) broth microdilution reference method as the standard. The Sensititre plates were tested using both the autoread and manual reading methods. The drug range tested for daptomycin was (0.25 – 8 µg/ml) for the Sensititre plate and (0.12 –128 µg/ml) for the reference method.

Materials and Methods: The evaluation was conducted by Lab Services at TREK Diagnostic Systems and consisted of 32 challenge isolates and 9 paired clinical gram-positive isolates that became non-susceptible. The isolates included *S. aureus, E. faecalis,* and *E. faecium.* The recommended quality control organisms were tested daily and were within the CLSI expected quality control ranges.

Results: All of the 32 challenge isolates when tested using both the automated and manual read methods had an essential agreement (+/- one $\log_2$ dilution) of 100% when compared to the frozen reference. The 9 paired clinical isolates where both methods detected the shift from susceptible to non-susceptible, had an essential agreement (+/- one $\log_2$ dilution) of 100%.

Conclusions: This multi-site evaluation indicates that the Sensititre 18-24 hour susceptibility system, using either the autoread or manual reading methodology, was equivalent to the CLSI broth microdilution reference method for detecting non-susceptible isolates to daptomycin.

PURPOSE OF THE STUDY

With the continued concern of the ability of automated susceptibility testing systems used in the clinical lab to detect resistance to new antimicrobials, a study was undertaken to evaluate the performance of the Sensititre® GPN3F (standard format) 18 – 24 hour susceptibility plate in detecting daptomycin non-susceptible isolates using CLSI broth microdilution as the reference method (M7–A6).

MATERIALS & METHODS

Organisms: The testing conducted at TREK Lab Services consisted of the following:

- 9 Paired Gram-positive clinical isolates (provided by Cubist Pharmaceuticals)
  - Each pair of isolates consisted of 1 daptomycin susceptible isolate and 1 daptomycin-non-susceptible isolate that had been confirmed to be clonally related by pulsed-field gel electrophoresis (PFGE)
- 32 Gram-positive challenge isolates (provided by Cubist Pharmaceuticals)
  - 17 *S. aureus* (daptomycin MIC value range of 0.5 to 32 µg/mL)
  - 14 *Enterococci* (daptomycin MIC value range of 2 to >32 µg/mL)
- 2 Quality control strains
  - *Staphylococcus aureus* ATCC 29213
  - *Enterococcus faecalis* ATCC 29212

Number of Isolates Tested

Organism Species	Challenge Tested	Clinical Tested (Pairs)
Staphylococcus aureus	17	14 (7)
Enterococcus faecalis	2	0
Enterococcus faecium	13	4 (2)
Total	**32**	**18 (9)**

Antimicrobial Powder

Antimicrobial	Range Tested	Supplied By
Daptomycin (DAP)	0.25-8µg/ml (dried) 0.12-128µg/ml (reference)	**Cubist Pharmaceuticals** Lexington, MA

SUSCEPTIBILITY TESTING METHODS

- Each isolate was tested using a Sensititre 18 – 24 GPN3F (standard format) susceptibility plate. The plates were set-up and tested according to the manufacturer's instructions.
- The reference plate was tested according to the microdilution methods published by the Clinical and Laboratory Standards Institute (CLSI, M7-A6).
- All quality control testing results were within the CLSI-approved ranges for daptomycin (CLSI, M100-S15).

Interpretive criteria for Daptomycin (CLSI, M100-S15)

Pathogen	Minimal inhibitory concentration (µg/ml)[a] S	I	R
Staphylococcus spp.	≤1	--	--
Enterococcus spp.	≤4	--	--

*For some organism/antimicrobial agent combinations, the absence of resistant strains precludes defining any results category other than "susceptible." For strains yielding results suggestive of a "non-susceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed. Subsequently, the isolates should be saved and submitted to a reference laboratory that will confirm results using a CLSI (NCCLS) reference dilution method.

RESULTS

Staphylococcus aureus
Manual Read and CLSI Reference Method MIC Distribution for Paired Clinical Isolates vs. Daptomycin

CLSI Reference Method

Manual Read	≤0.12	0.12	0.25	0.5	1	2	4	8	>8
≤0.12									
0.12									
0.25			1						
0.5			5	1					
1									
2					1				
4					3	3			
8									
>8									
TOTALS	0	0	6	1	0	4	3	0	0

% Essential Agreement of Total = 100%
% Categorical Agreement of Total = 100%

Staphylococcus aureus
Auto Read and CLSI Reference Method MIC Distribution for Paired Clinical Isolates vs. Daptomycin

CLSI Reference Method

Auto Read	≤0.12	0.12	0.25	0.5	1	2	4	8	>8
≤0.12									
0.12									
0.25			1						
0.5			5	1					
1									
2					1				
4					3	2			
8						1			
>8									
TOTALS	0	0	6	1	0	4	3	0	0

% Essential Agreement of Total = 100%
% Categorical Agreement of Total = 100%

RESULTS cont.

Staphylococcus aureus
Manual Read and CLSI Reference Method MIC Distribution for Challenge Isolates vs. Daptomycin

CLSI Reference Method

Manual Read	≤0.12	0.12	0.25	0.5	1	2	4	8	>8
≤0.12	1								
0.12									
0.25									
0.5					1	1			
1						1	5		
2							1	2	
4								1	1
8									1
>8									1
TOTALS	1	0	0	1	3	6	3	2	1

% Essential Agreement of Total = 100%
% Categorical Agreement of Total = 94%

Staphylococcus aureus
Auto Read and CLSI Reference Method MIC Distribution for Challenge Isolates vs. Daptomycin

CLSI Reference Method

Auto Read	≤0.12	0.12	0.25	0.5	1	2	4	8	>8
≤0.12	1								
0.12									
0.25									
0.5					1	1			
1						1	5		
2							1	2	
4								1	2
8									1
>8									1
TOTALS	1	0	0	1	2	7	3	2	1

% Essential Agreement of Total = 100%
% Categorical Agreement of Total = 94%

***Enterococcus* Species**
Manual Read and CLSI Reference Method MIC Distribution for Paired Clinical Isolates vs. Daptomycin

CLSI Reference Method

Manual Read	≤0.12	0.12	0.25	0.5	1	2	4	8	>8
≤0.12									
0.12									
0.25									
0.5									
1									
2					1				
4					1				
8									2
>8									2
TOTALS	0	0	0	0	0	2	0	0	2

% Essential Agreement of Total = 100%
% Categorical Agreement of Total = 100%

***Enterococcus* Species**
Auto Read and CLSI Reference Method MIC Distribution for Paired Clinical Isolates vs. Daptomycin

CLSI Reference Method

Auto Read	≤0.12	0.12	0.25	0.5	1	2	4	8	>8
≤0.12									
0.12									
0.25									
0.5									
1									
2						1			
4						1			
8									2
>8									2
TOTALS	0	0	0	0	0	2	0	0	2

% Essential Agreement of Total = 100%
% Categorical Agreement of Total = 100%

***Enterococcus* Species**
Manual Read and CLSI Reference Method MIC Distribution for Challenge Isolates vs. Daptomycin

CLSI Reference Method

Manual Read	≤0.12	0.12	0.25	0.5	1	2	4	8	>8
≤0.12									
0.12									
0.25			1						
0.5									
1									
2						2			
4						2	3		
8								5	2
>8									2
TOTALS	0	0	1	0	0	4	3	5	2

% Essential Agreement of Total = 100%
% Categorical Agreement of Total = 100%

***Enterococcus* Species**
Auto Read and CLSI Reference Method MIC Distribution for Challenge Isolates vs. Daptomycin

CLSI Reference Method

Auto Read	≤0.12	0.12	0.25	0.5	1	2	4	8	>8
≤0.12									
0.12									
0.25			1						
0.5									
1						2			
2						2	2		
4						1	1		
8							4		
>8									2
TOTALS	0	0	1	0	0	5	3	0	2

% Essential Agreement of Total = 100%
% Categorical Agreement of Total = 93%

Paired Clinical Isolates vs. Daptomycin for *S. aureus*

Read Method	Total Isolates Tested	Essential Agreement of Total	% Essential Agreement	Categorical Agreement of Total	% Categorical Agreement
Manual	14	14	100	14	100
Auto	14	14	100	14	100

RESULTS cont.

Challenge Isolates vs. Daptomycin for *S. aureus*

Read Method	Total Isolates Tested	Essential Agreement of Total	% Essential Agreement	Categorical Agreement of Total	% Categorical Agreement
Manual	17	17	100	16	94
Auto	17	17	100	16	94

Paired Clinical Isolates vs. Daptomycin for *Enterococcus spp.*

Read Method	Total Isolates Tested	Essential Agreement of Total	% Essential Agreement	Categorical Agreement of Total	% Categorical Agreement
Manual	4	4	100	4	100
Auto	4	4	100	4	100

Challenge Isolates vs. Daptomycin for *Enterococcus spp.*

Read Method	Total Isolates Tested	Essential Agreement of Total	% Essential Agreement	Categorical Agreement of Total	% Categorical Agreement
Manual	15	15	100	15	100
Auto	15	15	100	14	93

CONCLUSION

The Sensititre dried GPN3F (standard format) susceptibility plate (containing daptomycin with a range of 0.25-8µg/ml) demonstrated a high level of agreement and was very reproducible when using either the autoread or manual reading method and the GPN3F standard plates against the CLSI frozen reference method in detecting daptomycin non-susceptible isolates.

Paired Clinical Isolates:
The overall essential agreement for daptomycin within a +/- one $\log_2$ dilution was 100% for both manual and autoread methods. The overall categorical agreement for daptomycin was 100% for both manual and autoread methods.

Challenge Isolates:
The overall essential agreement for daptomycin within +/- one $\log_2$ dilution was 100% for both manual and autoread methods. The overall categorical agreement for daptomycin was 97% and 94% for the manual and autoread methods, respectively.

REFERENCES

1. National Committee for Clinical Laboratory Standards. 2003. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard sixth edition.* Approved document M7-A6. Wayne, PA:NCCLS.

2. National Committee for Clinical Laboratory Standards. 2005. *Performance standards for antimicrobial susceptibility testing, 15th informational supplement* M100-S15.Wayne, PA: NCCLS.