Performance of ETEST® Telavancin for Antimicrobial Susceptibility Testing of *Staphylococcus aureus* and *Enterococcus faecalis*

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ABSTRACT

Background:
Telavancin is a lipoglycopeptide antibacterial drug with activity against select Gram-positive bacterial species. In this study, the performance of ETEST® Telavancin (TLA), an *in vitro* technique for determining the antimicrobial susceptibility of *Enterococcus faecalis* and *Staphylococcus aureus* to this agent, was evaluated against the Clinical and Laboratory Standards Institute (CLSI) broth microdilution reference method (BMD).

Method:
A total of 665 isolates (289 *E. faecalis* and 376 *S. aureus*) were tested by ETEST TLA and BMD methods. Isolates were subcultured on tryptic soy agar plates supplemented with 5% sheep blood. After overnight incubation 0.5 McFarland suspensions were prepared to inoculate ETEST TLA and BMD. Results were read after 16 – 20 hours incubation. Results were analyzed for essential agreement (EA), category agreement (CA), major and very major error rates and compared to the FDA performance criteria, EA and CA (≥ 90%), major error rate (≤ 3.0%) and very major error rate (≤ 1.5%) using the FDA breakpoints for Telavancin (*S. aureus* susceptible S ≤ 0.125 µg/mL and *E. faecalis* S ≤ 0.25 µg/mL).

Results:
ETEST TLA results including the rate of Telavancin non-susceptible results and the performance against *E. faecalis* and *S. aureus* are summarized in Tables 1 and 2 respectively. Overall, ETEST TLA met the FDA performance acceptance criteria for EA and CA (>90%), major error rate (<3%) and very major error rate (<1.5%)

Conclusion:
ETEST TLA performance for *E. faecalis* and *S. aureus* met the FDA performance criteria. When compared to the reference method, ETEST TLA proved to be a suitable test for susceptibility testing of *E. faecalis* and *S. aureus*.

INTRODUCTION

ETEST is quantitative technique for the determination the antimicrobial susceptibility of both non-fastidious Gram-negative and Gram-positive aerobic bacteria such as *Enterobacteriaceae, Pseudomonas, Staphylococcus,* and *Enterococcus* species and fastidious bacteria such as anaerobes, *N. gonorrhoeae, Streptococcus* and *Haemophilus* species. The system comprises a predefined antibiotic gradient which is used to determine the Minimum Inhibitory Concentration (MIC).

Telavancin (TLA) is a lipoglycopeptide antibacterial agent with a dual mechanism of action. Telavancin clinical utility incudes treatment of *Staphylococcus aureus* and vancomycin susceptible *Enterococcus faecalis* infections.

ETEST TLA is an *in vitro* quantitative technique for determining the susceptibility of *S. aureus* and *E. faecalis* isolates to telavancin. The device consists of a plastic strip that has an exponential gradient of Telavancin drug concentrations on one side and a MIC reading scale on the other. Application of the strip to an inoculated agar surface produces an elliptical zone of inhibited bacterial growth on the agar surface following incubation. The point at which the edge of the elliptical zone of inhibition meets the strip is interpreted as the MIC endpoint. In this study the performance of ETEST TLA was compared to the CLSI broth microdilution (BMD) reference method.

MATERIAL AND METHODS

A total of 665 fresh and stock *S. aureus* isolates were evaluated at three clinical trial sites. The test set included 38 vancomycin-resistant *E. faecalis* to provide on scale results above the susceptible breakpoint. Each isolate was subcultured on TSA blood agar. A 0.5 McFarland suspension of each isolate was prepared in saline using visual comparison to a 0.5 McFarland standard. This suspension was used to inoculate an agar plate for ETEST TLA and for broth microdilution.
ETEST methodology – Mueller Hinton agar was used for ETEST. The plates were inoculated with the 0.5 McFarland suspension by streaking the entire surface of the plates. Following inoculation the agar plates were allowed to dry then the ETEST strips were applied to the agar surface. ETEST TLA plates were incubated at 35±2°C for 16 – 20 hours. Telavancin is a bactericidal agent. Endpoints were the point at which complete inhibition of bacterial growth met the ETEST strip.
BMD methodology - Broth microdilution panels were inoculated with a 1:200 dilution of the 0.5 McFarland suspension. Panels were incubated at 35±2°C for 16 - 20 hours.
Data analysis - Results were analyzed for essential agreement (EA), category agreement (CA), major and very major error rates using the FDA interpretative breakpoints for telavancin (*S. aureus* susceptible S ≤ 0.125 µg/mL and *E. faecalis* S ≤ 0.25 µg/mL). Major errors were defined instances where the ETEST result interpretation was resistant and the reference result was susceptible. Very major errors defined instances where the ETEST result interpretation was susceptible and the reference result was resistant. Performance was compared to the FDA performance criteria, EA and CA (≥ 90%), major error rate (≤ 3.0%) and very major error rate (≤ 1.5%) using the FDA breakpoints for Telavancin.
Results including the rate of Telavancin non-susceptible results and the performance against *E. faecalis* and *S. aureus* are summarized in Tables 1 and 2 respectively. ETEST TLA performance compared to broth microdilution is presented in frequency table from in Tables 3, 4 and 5.

RESULTS

Table 1 Telavancin Non-Susceptible Result Rate

Species	Percentage Non-Susceptible Results
Staphylococcus aureus	1.3% (5*/376)
Vancomycin susceptible *E. faecalis*	0.0% (0/251)
Vancomycin resistant *E. faecalis*	100% (38/38)

* All five Telavancin non-susceptible results were vancomycin intermediate *S. aureus*.

Table 2 ETEST TLA Performance for *E. faecalis* and *S. aureus*

Species	EA	CA	Major Error Rate	Very Major Error Rate
S. aureus	98.4% (370/376)	97.9% (368/376)	2.2% (8/371)	0.0% (0/5)
E. faecalis (vancomycin susceptible)	91.6% (230/251)	97.6% (245/251)	2.4% (6/251)	0.0% (0/0)
E. faecalis (vancomycin susceptible & resistant)	92.7% (268/289)	97.9% (283/289)	2.4% (6/251)	0.0% (0/38)
E. faecalis (vancomycin resistant)	100% (38/38)	100% (38/38)	0.0% (0/0)	0.0% (0/38)

Table 3 Frequency Table ETEST TLA Compared to Broth Microdilution - *S. aureus*

	Reference Results												
ETEST Results	≤0.002 S	0.004 S	0.008 S	0.016 S	0.032 S	0.064 S	0.125 S	0.25 NS	0.5 NS	1 NS	2 NS	4 NS	≥ 8 NS
≤0.002 S													
0.004 S													
0.008 S													
0.016 S													
0.032 S				12	55	2							
0.064 S				2	206	66	1						
0.125 S					2	12	5						
0.25 NS						2	6	1					
0.5 NS								1	3				
1 NS													
2 NS													
4 NS													
≥8 NS													

Table 4 Frequency Table ETEST TLA Compared to Broth Microdilution - *E. faecalis* (Vancomycin Susceptible)

	Reference Results												
ETEST Results	≤0.002 S	0.004 S	0.008 S	0.016 S	0.032 S	0.064 S	0.125 S	0.25 S	0.5 NS	1 NS	2 NS	4 NS	≥ 8 NS
≤0.002 S													
0.004 S													
0.008 S													
0.016 S													
0.032 S					1								
0.064 S					4	5	3						
0.125 S					3	44	41						
0.25 S						15	112	17					
0.5 NS							3	3					
1 NS													
2 NS													
4 NS													
≥8 NS													

Table 5 Frequency Table ETEST TLA Compared to Broth Microdilution - *E. faecalis* (Vancomycin Susceptible and Resistant)

	Reference Results												
ETEST Results	≤0.002 S	0.004 S	0.008 S	0.016 S	0.032 S	0.064 S	0.125 S	0.25 S	0.5 NS	1 NS	2 NS	4 NS	≥ 8 NS
≤0.002 S													
0.004 S													
0.008 S													
0.016 S													
0.032 S					1								
0.064 S					4	5	3						
0.125 S					3	44	41						
0.25 S						15	112	17					
0.5 NS							3	3					
1 NS													
2 NS											1	1	
4 NS											3	7	1
≥8 NS												3	22

CONCLUSION

ETEST TLA met the FDA performance acceptance criteria for EA and CA (>90%), major error rate (<3%) and very major error rate (<1.5%). ETEST TLA was found to be an accurate and reliable method for susceptibility testing of *S. aureus* and *E. faecalis* species providing Telavancin MIC results that are comparable to broth microdilution. As a manual method ETEST is easy to perform and doesn't require any specific instrumentation; therefore adding flexibility to the clinical laboratory performing antimicrobial susceptibility.