Microdilution Testing Methodology for Tigecycline: Addition of A Biocatalytic Oxygen-Reducing Agent May Be Required in The Absence of Fresh Media

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ABSTRACT

Background: Tigecycline (TGC) is a new glycylcycline antimicrobial in development. TGC has been evaluated in vitro and quality control ranges were presented to NCCLS. Tentative quality control ranges were determined in fresh broth (<12 hours old). This study looks at the addition of a biocatalytic oxygen-reducing agent (BORA) to aged broth (>12 hours old) as an alternative when performing microdilution testing. **Methods**: Testing was done on approximately 3,400 *Enterobacteriaceae*, enterococci; *H. influenzae (HFL); M. catarrhalis*; *S. aureus (SA); S. pneumoniae (SP); S. pyogenes* and other streptococci. Microdilution panels and broth were prepared by Microscan. Organisms were prepared in water or saline to achieve a 0.5 McFarland standard prior to adding to a broth inoculum. Two identical sets of aged broth tubes, containing appropriate organism media, were used in the study. To one set of broth was added 2% Oxyrase® (OXY) for Broth. Each corresponding tube was inoculated with the identical test organism. Quality controls were performed using the following ATCC strains: *E. coli* 25922, *P. aeruginosa* 27853, *E. faecalis* 29212, *S. aureus* 29213, *S.pneumoniae* 49619, and *H.influenzae* 49247. **Results**: Combined test results showed a 67.02% higher MIC value (1-4 $\log_2$ dilutions) in the non-OXY panels versus the OXY panels. The impact on MIC_{90} values is significant for some organisms such as: *A. baumannii*; *SA*; *SP*; *HFL*; *C. freundii*; and *E. faecalis* where non-OXY panels produced results higher than OXY panels – 2/1, 0.5/0.25, 0.12/0.06, 2/0.12; 1/0.5; and 0.25/0.12, respectively. Quality controls were out of range 29.3% of the time in non-OXY panels and in compliance 100% of the time with OXY panels. **Conclusion**: The addition of a BORA in aged broth is a viable alternative to performing TGC microdilution testing in fresh broth.

INTRODUCTION

Tigecycline is a novel antimicrobial with an expanded broad-spectrum of activity from a new class of compounds, glycylcyclines. Tigecycline inhibits protein synthesis by binding to the 30S ribosomal subunit. Although it is perceived to be bacteriostatic, its anti-bacterial activity is significant and has shown some bactericidal activity against key targeted pathogens [1,2].. Currently, tigecycline is being developed for use in a broad range of infections and against both Gram-positive and –negative bacteria.

Recently, it was noted that freshly prepared or frozen panels using aged broth (>12 hours old) may produce MICs higher than those seen with panels using fresh broth (<12 hours old). This has led to a recommendation that tigecycline be tested using fresh broth to ensure MICs are correctly reported. Work has been performed by Wyeth Pharmaceuticals on the addition of a biocatalytic oxygen-reducing agent (BORA) to aged broth in an effort to compensate for the tigecycline MIC discrepancies between aged and fresh broth.

Extensive in vitro testing is now underway to evaluate the activity of this new compound using dried microdilution panels. The impact of aged broth on dried panels is unknown and is the reason this study was commissioned. However, the testing of tigecycline in dried panels using fresh broth is somewhat problematic. This study was design to measure the impact of aged broth with and without the addition of a BORA when dried panels are used to test tigecycline.

All organisms were tested concurrently using aged broth with or without the addition of a BORA. A subset of selected organism groups were also tested against tigecycline in fresh broth without the addition of a BORA and compared to aged broth.

MATERIALS & METHODS

- Over 3,400 clinical isolates were tested using microdilution panels by Dade Microscan (Dade Behring Inc., Sacramento, CA. USA) according to NCCLS guidelines [3] and manufacturers instructions.
- Each individual isolate was inoculated into the appropriate broth medium.
- Each individual isolate was also inoculated in the appropriate broth medium with the addition of 2% Oxyrase® (a biocatalytic oxygen-reducing agent).
- The individual broth with the 2% Oxyrase was incubated at 35°C for ½ hour before the organism was inoculated into the broth.
- Each pair of broths (with Oxyrase and without Oxyrase) was inoculated with a single 0.5 McFarland standard of organism.
- Quality Control was performed using the following ATCC strains: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212, and *S. aureus* ATCC 29213, *S.pneumoniae* ATCC 49619, and *H.influenzae* ATCC 49247 [3].

ACKNOWLEDGEMENTS

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REFERENCES

1 Sum, P.E. and P. Petersen, Synthesis and structure-activity relationship of novel glycylcycline derivatives leading to the discovery of GAR-936. Bioorg Med Chem Lett, 1999. 9(10): p. 1459-62.

2 Abbanat, D., M. Macielag, and K. Bush, Novel antibacterial agents for the treatment of serious Gram-positive infections. Expert Opin Investig Drugs, 2003. 12(3): p. 379-99.

3 National Committee for Clinical Laboratory Standards (NCCLS). Performance Standards for Antimicrobial Susceptibility Testing; Fourteenth Informational Supplement. NCCLS document M100-S14. Wayne, PA, 2004.

RESULTS

Table 1 provides an overview of the organisms evaluated in this study.

Table 1: Organisms collected October 1, 2001 from 15 countries and evaluated against tigecycline in broth containing BORA or no BORA .

Organisms	Number	Organisms	Number	Organisms	Number
Streptococcus agalactiae	100	*Staphylococcus epidermidis*		*Klebsiella pneumoniae*	
Streptococcus pyogenes	107	MSSE	89	ESBL	150
Streptococcus pneumoniae		MRSE	150	Non-ESBL	150
PenS	100	*Corynebacterium jeikeium*	43	*Escherichia coli*	
PenI	100	*Enterococcus faecium*	150	ESBL	150
PenR	100	*Enterococcus faecalis*	150	Non-ESBL	160
MacS	60	*Acinetobacter baumanii*	120	*Citrobacter freundii*	140
MacR	60	*Acinetobacter lwoffii*	60	*Citrobacter diversus*	15
Staphylococcus aureus		*Enterobacter cloacae*	151	*Moraxella catarrhalis*	175
MSSA	150	*Enterobacter aerogenes*	150	*Haemophilus influenzae*	
MRSA	140	*Klebsiella oxytoca*		Beta lactamase +	100
Serratia marcescens	150	ESBL	120	Beta lactamase -	100
		Non-ESBL	120	*Citrobacter koserii*	

Preliminary Evaluation Results:

Previous to the initiation of this study, an evaluation of the impact of BORA on fresh and aged media was conducted using freshly prepared panels. It was concluded from this study that the addition of a BORA to aged broth provided similar results to those seen when fresh broth was used. These results are shown in Graph 1.

Graph 1. Evaluation of oxyrase against ATCC strains prior to performing study on 3700 isolates.

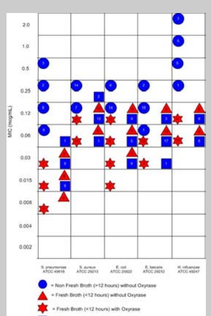


Table 2 reflects the range, MIC_{90} and mean dilution difference for tigecycline when organisms are tested in aged broth with or without BORA.

Organisms	Range (mcg/mL)		MIC_{90} (mcg/mL)		Mean Dilution Difference
	W/O BORA	W/BORA	W/O BORA	W/BORA	(log$_2$)
Acinetobacter baumannii (n=120)	0.06-4	0.03-2	2	1	0.778
Acinetobacter lwoffii (n=60)	0.03-2	0.03-1	0.5	0.25	0.967
Citrobacter diversus (n=15)	0.06-1	0.06-1	0.5	0.25	0.667
Citrobacter freundii (n=140)	0.12-2	0.12-2	2	0.5	0.774
Enterobacter cloacae (n=151)	0.12-1	0.12-0.25	0.5	0.25	0.758
Enterobacter aerogenes (n=150)	0.03-0.25	0.03-0.25	0.25	0.12	0.767
Enterobacter cloacae (n=151)	0.12-8	0.06-8	2	2	0.331
Enterococcus faecalis (n=150)	0.03-0.5	0.06-0.25	0.25	0.12	0.77
Enterococcus faecium (n=150)	0.06-0.25	0.03-0.25	0.25	0.12	0.443
Escherichia coli – ESBL (n=150)	0.12-2	0.06-2	0.5	0.5	0.691
Escherichia coli – non-ESBL (n=160)	0.12-2	0.06-1	0.5	0.25	0.756
Haemophilus influenzae (n=186)	0.25-4	0.03-0.5	2	0.12	1.311
Klebsiella oxytoca (n=240)	0.12-2	0.06-2	0.5	0.5	0.355
Klebsiella pneumoniae -ESBL (n=120)	0.25-4	0.25-4	2	2	0.517
Klebsiella pneumoniae -non-ESBL (n=150)	0.25-4	0.25-4	1	0.5	0.543
Moraxella catarrhalis (n=175)	≤0.008-0.25	≤0.008-0.12	0.12	0.06	2.226
Serratia marcescens (n=150)	0.25-4	0.12-4	1	1	0.797
Staphylococcus aureus - MRSA (n=140)	0.12-1	0.06-0.25	0.5	0.25	0.627
Staphylococcus aureus - MSSA (n=150)	0.12-0.25	0.06-0.1	0.25	0.12	0.708
Staphylococcus epidermidis (n=239)	0.12-2	0.03-1	0.5	0.5	0.766
Streptococcus pneumoniae (n=100)	0.06-0.5	0.06-0.12	0.25	0.12	1.031
Streptococcus pneumoniae (n=200)	0.015-0.25	≤0.008-0.12	0.12	0.06	0.946
Streptococcus pyogenes (n=107)	0.015-1	0.015-0.25	0.12	0.06	0.598

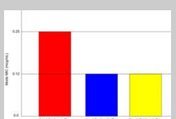
Quality control results for tigecycline varied based on the organisms tested and the use of a BORA in aged broth. Table 3 gives all quality control results from this study:

Table 3: Quality control results for tigecycline when tested in fresh broth, aged broth without a BORA and aged broth with a BORA.

MIC (mcg/mL)	≤0.008	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2
S. aureus ATCC 29213 (0.03-0.25 mcg/mL)										
Fresh Broth						6				
Aged w/o BORA						1	14			
Aged with BORA						1	18	2		
E. coli ATCC 25922 (0.03-0.25 mcg/mL)										
Fresh Broth						6				
Aged w/o BORA						12	6			
Aged with BORA						5	13			
E. faecalis ATCC 29212 (0.03-0.12 mcg/mL)										
Fresh Broth					2	4				
Aged w/o BORA					1	16	3			
Aged with BORA					1	17	3			
S. pneumoniae ATCC 49619 (0.008-0.01 mcg/mL)										
Fresh Broth					6					
Aged w/o BORA						6	6	2	3	
Aged with BORA					18	1				
H. influenzae ATCC 49247 (0.06-0.25 mcg/mL)										
Fresh Broth					3	3				
Aged w/o BORA							1	5	5	2
Aged with BORA					1	7	6			

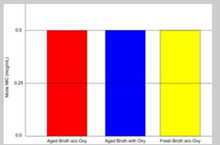
A subset of organisms from the *E. coli* group, *K. pneumoniae* group, *M. catarrhalis* group, *S. epidermidis* group and *S. pneumoniae* groups were further compared. The mode MIC for each group was determined on aged broth with out BORA, aged broth with BORA and fresh broth containing no BORA. The following graphs provide view of the impact BORA in aged broth when testing tigecycline.

Graph 2: *E. coli* non-ESBL (n=20).

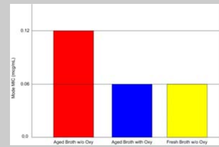


Graph 3: *E. coli* ESBL (n=28)

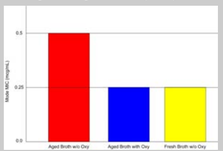
Graph 4: *K. pneumoniae* ESBL (n=20)



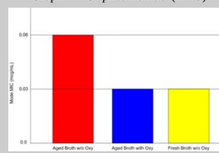
Graph 5: *M. catarrhalis* (n=17)



Graph 6: *S. epidermidis* (n=20)



Graph 7: *S. pneumoniae* (n=13)



CONCLUSIONS

- Tigecycline is a new promising agent to treat both Gram-positive and –negative bacteria. It is important to the microbiologist and physician to have an accurate in vitro evaluation of the activity of tigecycline against known and suspected pathogens. Many laboratories will evaluate tigecycline using dried panels with broth purchased from various suppliers.
- This study concludes that dried panels using aged broth can be used when a biocatalytic oxygen-reducing agent (BORA) is added to the broth. The addition of a BORA is especially important when testing organisms like *S. pneumoniae* and *H. influenzae*. The testing of tigecycline against fastidious organisms appears to be impacted more by aged broth than some other organisms.
- The results seen in this study demonstrate a good correlation between tigecycline testing when fresh broth or aged broth containing a BORA is used. It is unlikely laboratories will obtain accurate quality control results for some organisms without the addition of a BORA if aged broth is used.
- It appears that the use of a BORA in aged broth is a viable alternative to using fresh broth each time the testing of tigecycline is performed using broth microdilution panels.