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Zone breakpoint by the CLSI disk method for 15 µg tigecycline disks corresponding to EUCAST MIC breakpoints

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ABSTRACT

Introduction: Tigecycline is a new broad-spectrum antibiotic, recently lisensed by the European Medicines Agency (EMEA). The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has defined MIC breakpoints, in some cases lower than those of the FDA, whilst the CLSI has not yet set breakpoints. This creates issues for the growing number of European labs that use the CLSI disk method tests but grade results vs. EUCAST breakpoints. We defined the zone breakpoints for CLSI disk tests corresponding to EUCAST MIC breakpoints. **Method:** Organisms (n 357) were from the 2004 British Society for Antimicrobial Chemotherapy bacteremia survey, supplemented with isolates with tigecycline MICs >1 mg/L from other survey collections and reference submission. Zones and agar dilution MICs were determined by CLSI methods, and reviewed vs. EUCAST breakpoints. **Results:** Disc zone breakpoints for α/β-haemolytic streptococci (EUCAST MIC bpt ≤0.25, >0.5 mg/L), were S ≥21 mm, R ≤18 mm; for staphylococci (MIC bpt ≤0.5, >0.5 mg/L) S ≥22mm, and R ≤21 mm and for *Enterococcus* spp. (MIC bpt ≤0.25, >0.5 mg/L) S ≥ 22 mm, R ≤18 mm. These gave three very major, 12 major and 28 minor errors based on 194 tests. Assigning breakpoints for Enterobacteriaceae was more complex because MICs of 2 mg/L (intermediate on EUCAST/EMEA criteria) fall within the upper end of the normal distribution for *Klebsiella* spp. Error minimization indicated that zone breakpoints of S ≥21 mm, R ≤17 mm produced the best agreement with MIC categorisation (MIC bpt ≤1 >2 mg/L), with only 1/33 resistant isolates misidentified as susceptible, and 1/90 susceptible isolates as resistant. However, 38/163 isolates gave minor errors. **Conclusion:** The 15 µg tigecycline discs adequately differentiated between S/I/R isolates for most Gram-positive and Enterobacteriaceae species, although differentiation of the S/I border was difficult for Enterobacteriaceae. It may be prudent to do MIC determination (e.g. by Etest) on isolates giving zones around this border.

INTRODUCTION

- Tigecycline is a new broad-spectrum antibiotic, recently licensed in the EU and the USA.
- Acquired ribosomal protection (*tet* (M)) and efflux (*tet* (A-E)) mechanisms affecting other tetracyclines do not compromise tigecycline,[1-3] which therefore may be useful in infections due to multi-resistant Enterobacteriaceae.
  - This is important because Enterobacteriaceae with CTX-M ESBLs and quinolone resistance are a rapidly increasing problem in Europe and Asia [4-6]
- A 15-µg tigecycline disc has been proposed for routine susceptibility testing worldwide, irrespective of disc method.
- We determined provisional zone breakpoints, corresponding to EUCAST MIC breakpoints, for these discs in the CLSI disc method, which uses confluent inocula on Mueller-Hinton agar.[7]
- This is relevant because in Europe, many labs use CLSI methods but EUCAST breakpoints.

METHOD

- Isolates (n=357) were from the 2004 BSAC bacteremia survey,[8] which collects 10 isolates of each of 12 species group per annum from 25 hospital laboratories in the UK and Ireland. These were supplemented with additional isolates with tigecycline MICs >1 mg/L from other survey collections and reference submission.
- Zones and agar dilution MICs were determined by CLSI methods, and reviewed *vs.* the European Committee on Antimicrobial Susceptibility Testing European Medicines Agency (EUCAST/EMEA) MIC breakpoints:
  - *Streptococcus* spp. and *Enterococcus* spp.: S ≤0.25 mg/L and R >0.5 mg/L
  - *Staphylococcus* spp.: S ≤0.5 mg/L and R >0.5 mg/L
  - Enterobacteriaceae: S ≤1 mg/L and R >2 mg/L

RESULTS

Based on error minimization we propose the following disc zone breakpoints for Gram-positive species:

- α/β-haemolytic streptococci (Table 1),
  - S ≥21 mm, R ≤18 mm
- *S. aureus* and coagulase-negative staphylococci (Table 2)
  - S ≥22mm, R ≤21 mm
- *Enterococcus* spp. (Table 3)
  - S ≥22 mm, R ≤18 mm

These gave three very major, 12 major and 28 minor errors based on 194 tests.

- Assigning breakpoints for the Enterobacteriaceae excluding Proteeae (Table 4) was more complex because MICs of 2 mg/L (intermediate by EUCAST/EMEA criteria) fall within the upper end of the normal distribution for *Klebsiella* spp. Error minimization analysis indicated that zone breakpoints of S ≥21mm, R ≤17 mm produced the best agreement with MIC categorisation, with only 1/33 (3%) resistant isolates misidentified as susceptible, and 1/90 (1.1 %) susceptible isolates as resistant. However, 38/163 (23.3 %) isolates gave minor errors.

Table 1. Tigecycline zone MIC calibration plot for α/β-haemolytic streptococci

Zone diameter (mm)	MIC (mg/L)								Disc Susceptibility Category
	≤0.03	0.05	0.06	0.125	0.25	0.5	1	2	
43			1						Susceptible
42									
41									
40									
39									
38									
37									
36			1						
35			1						
34				1					
33									
32	1		1	2					
31			1						
30			1						
29				1					
28			3						
27				3					
26			2		1				
25			2	2	2				
24			2	7					
23			1	5	4				
22			1	8	3				
21				2	5	2			
20				3	1	7			Intermediate
19		1		2	4	3			
18									Resistant
17									
16				1		1			

Key: Colour indicates susceptibility category based on MIC values

- susceptible
- intermediate
- resistant

Table 2. Tigecycline zone MIC calibration plot for staphylococci

Zone diameter (mm)	MIC (mg/L)								Disc Susceptibility Category
	≤0.03	0.05	0.06	0.125	0.25	0.5	1	2	
27					3				Susceptible
26					2	1			
25					5	5			
24					2	3			
23					5	5	**1**		
22					7	2	**2**		
21					7	1	**1**		Resistant
20					1	1	**3**	**1**	
19					1	1	**4**		
18							**1**		

Table 3. Tigecycline zone MIC calibration plot for enterococci.

Zone diameter (mm)	MIC (mg/L)								Disc Susceptibility Category
	≤0.03	0.05	0.06	0.125	0.25	0.5	1	2	
25				1	1				Susceptible
24				1					
23			1	6	2	2			
22				2	2	8			
21					2	7			Intermediate
20					1	2			
19						2			
18									Resistant
17									

Table 4. Tigecycline zone MIC calibration plot for Enterobacteriaceae (excludes Proteeae)

Zone diameter (mm)	MIC (mg/L)						Disc Susceptibility Category
	0.25	0.5	1	2	4	8	
27		1					Susceptible
26	2						
25	1						
24	2	3					
23	2	10	2				
22		9	3	2			
21		8	21	3	**1**		
20		8	10	10	**2**		Intermediate
19			6	11	**1**	**1**	
18			1	13	**3**		
17				1	**9**		Resistant
16					**3**	**2**	
15			1		**3**	**4**	
14					**1**	**3**	

CONCLUSION

- The 15-µg tigecycline discs adequately differentiated between S/I/R for most Gram-positive and for Enterobacteriaceae isolates.
- Differentiation of the S/I border was difficult for some species e.g. *Klebsiella* spp. It is prudent to do MIC determination (e.g. by Etest) on isolates giving zones around this border.

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