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# Recently Approved Tigecycline Susceptibility Breakpoints for *S. pneumoniae* and *H. influenzae*: Do Broth Microdilution and Disk Diffusion Results Agree?

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## ABSTRACT

**Background**: The United States Food and Drug Administration (USA-FDA) recently added the indication for community-acquired bacterial pneumonia caused by *S. pneumoniae* (SPN) and *H. influenzae* (HI) with associated tigecycline (TIG) breakpoints for susceptibility (S) at ≤0.06 μg/ml (≥19 mm) and ≤0.25 μg/ml (≥19 mm), respectively. Earlier S breakpoints for TIG have been ≤0.25 to ≤2 μg/ml for various pathogens, all with the same disk diffusion (DD) correlate of ≥19 mm! The new SPN and HI breakpoints were compared for intermethod categorical agreement.

**Methods**: 166 SPN and 161 HI collected from USA patients in 2008 were tested for TIG by CLSI M07-A8 and M02-A10 methods. Concurrent quality control used TIG and clarithromycin reference tests, all results were within published ranges for ATCC strains 49619 and 49247.

**Results**: 48.2, 37.3, 10.2 and 4.2% of SPN had TIG MIC of ≤0.03, 0.06, 0.12 and 0.25 μg/ml, respectively (85.5% S); while 13.7, 30.4, 54.7 and 1.2% of HI were inhibited at 0.25, 0.5, 1 and 2 μg/ml of TIG, respectively (only 13.7% S). In contrast, all (100.0%) SPN and HI strains had inhibition zones of ≥19 mm and were S by the USA-FDA DD breakpoint. DD very major error rates were 14.5% for SPN and 86.3% for HI. In the overall collection of consecutively collected strains (USA, 2008), 92.3% of SPN (953 strains tested) and 5.5% of HI (574 strains) were S to TIG according to the USA-FDA MIC breakpoint criteria.

**Conclusions**: DD and MIC breakpoints for TIG recently approved by the USA-FDA for SPN and HI demonstrated unacceptable intermethod agreement (13.7% for HI and 85.5% for SPN). MIC breakpoints appear to divide wildtype populations of these pathogens. Re-evaluation of these TIG criteria appears needed.

| TIG MIC(μg/ml) | No. of isolates with zone diameter (mm) of: | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *S. pneumoniae* | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | |
| 0.25 | | | | | | | 1 | 1 | 3 | 2 | | | |
| 0.12 | | | | | | 1 | 1 | 2 | 6 | 6 | 1 | | |
| 0.06 | | | | | 1 | 3 | 3 | 17 | 19 | 10 | 6 | 3 | |
| ≤0.03 | | | 1 | | 1 | 3 | 4 | 24 | 17 | 20 | 7 | 3 | |
| *H. influenzae* | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | |
| 2 | | | 1 | | 1 | | | | | | | | |
| 1 | | 1 | 1 | 3 | 6 | 7 | 13 | 24 | 20 | 9 | 4 | | |
| 0.5 | | | 2 | | 1 | 6 | 5 | 14 | 12 | 8 | 1 | | |
| 0.25 | | | 1 | 1 | 1 | 1 | 2 | 11 | 4 | | 1 | | |

a. Bolded lines indicate breakpoints

## INTRODUCTION

Tigecycline was approved by the United States Food and Drug Administration (USA-FDA) in 2005 for treatment of complicated skin and skin-structure infections (cSSSI) and complicated intra-abdominal infections. More recently, USA-FDA added the indication for community-acquired bacterial pneumonia caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* with associated tigecycline breakpoints for susceptibility at ≤0.06 μg/ml (≥19 mm) and ≤0.25 μg/ml (≥19 mm), respectively.

Earlier susceptible breakpoints for tigecycline have been ≤0.25 μg/ml (*Enterococcus* spp. and *Streptococcus* spp. other than *S. pneumoniae*), ≤0.5 μg/ml (*Staphylococcus* spp.) and ≤2 μg/ml (Enterobacteriaceae), all with the same disk diffusion (DD) correlate of ≥19 mm.

Many studies have attempted to evaluate the in vitro activity of tigecycline against *S. pneumoniae* and *H. influenzae*, but results vary substantially even when the same methodology is used. In a series of studies when isolates were tested by broth microdilution method (BMD) according to Clinical and Laboratory Standards Institute (CLSI) guidelines, tigecycline $MIC_{50}$ results varied from 0.03 to 0.12 μg/ml for *S. pneumoniae* and from 0.12 to 4 μg/ml for *H. influenzae* (Table 1).

Previous studies have identified the necessity that tigecycline MIC testing be performed in liquid medium that has reduced oxygen content, which can be accomplish by using fresh media (<12 hours old). Furthermore, other factors may also affect the accuracy tigecycline susceptibility tests since MIC results variability is observed with some species when broth microdilution tests are performed with fresh media or by other susceptibility testing methods.

In the present study, we compare tigecycline results for *S. pneumoniae* and *H. influenzae* obtained with broth microdilution with those of disk diffusion method for intermethod categorical agreement. Due to the great variability of results reported in previous investigations, broth microdilution was performed with dry-form and frozen-form panels when testing *H. influenzae*.

## MATERIALS AND METHODS

Organism Collection: A total of 166 *S. pneumoniae* and 161 *H. influenzae* were selected for this study. The isolates were collected from patients with community-acquired respiratory tract infections from 21 USA medical centers in 2008. *S. pneumoniae* collection was enriched with isolates showing elevated tigecycline MIC values (0.12 and 0.25 μg/ml). Bacterial identification was confirmed by the central monitoring site (JMI Laboratories, Iowa, USA) using standard algorithms and an automated system, when needed (Vitek® 2; bioMerieux, Missouri, USA).

Susceptibility Test Methods: The isolates were tested for susceptibility by the reference CLSI broth microdilution method using commercially prepared, validated dry-form panels (TREK Diagnostic Systems, Ohio, USA) and frozen-form panels prepared in-house. Broth microdilution was performed according to CLSI document M07-A8. *S. pneumoniae* was tested in Mueller-Hinton broth supplemented with 3-5% lysed horse blood and *H. influenzae* was tested in Haemophilus Test Media. Fresh media was used for all tigecycline MIC tests.

Disk diffusion method was performed according to CLSI document M02-A10 using a 15-μg tigecycline disk. *S. pneumoniae* was tested in Mueller-Hinton agar supplemented with 5% lysed horse blood and *H. influenzae* was tested in Haemophilus Test Media. Tigecycline susceptible breakpoints recently established by the USA-FDA for *S. pneumoniae* (≤0.06 μg/ml and ≥19 mm) and *H. influenzae* (≤0.25 μg/ml and ≥19 mm) were applied. Concurrent quality control used tigecycline and clarithromycin reference tests, all results were within published ranges for ATCC strains 49619 and 49247.

## RESULTS

- A significant variability was observed among previous reports of the in vitro activity of tigecycline when tested by reference methods against *H. influenzae*. Tigecycline $MIC_{50}$ values ranged from 0.12 to 4 μg/ml. The highest tigecycline results were reported in 2000 and 2003 and testing may have been performed in aged media (non-fresh, >1 week old), which is known to affect tigecycline MIC results.
- We observed a great variation between tigecycline MIC results obtained by broth microdilution method using "validated" dry-form panels (MIC mode, 1 μg/ml) compared to those obtained with frozen-form panels (MIC mode, 0.25 μg/ml; Table 2).
- A poor correlation between MIC and disk diffusion results was obtained with *H. influenzae* (r = 0.047 and 0.49) and *S. pneumoniae* (r = 0.016; Figures 1 to 3).
- The number of *H. influenzae* isolates categorized as non-susceptible to tigecycline by broth microdilution (MIC, ≥0.5 μg/ml) were 139 (86.3%) when using dry-form panels and 34 (21.1%) when using frozen-form panels. In contrast, all isolates were considered susceptible according to disk diffusion results and USA-FDA criteria.
- Twenty-four of 161 (14.5%) *S. pneumoniae* strains had tigecycline MIC values of 0.12 μg/ml (17) or 0.25 μg/ml (7) and were categorized as tigecycline non-susceptible according to USA-FDA MIC susceptible breakpoint of ≤0.06 μg/ml. Like *H. influenzae* disk diffusion results, all *S. pneumoniae* isolates had tigecycline inhibition zones of ≥19 mm and would be categorized as tigecycline-susceptible according to USA-FDA disk diffusion breakpoints – 14.5% very major error rate.

**Table 1**. Summary of in vitro activity of tigecycline against *H. influenzae* and *S. pneumoniae* from published reports.

| | | | *H. influenzae* | | | *S. pneumoniae* | | |
|---|---|---|---|---|---|---|---|---|
| Author | Year | Method | No. of strains | $MIC_{50}$ | $MIC_{90}$ | No. of strains | $MIC_{50}$ | $MIC_{90}$ |
| Gales et al. | 2000 | BMD[a] | 35 | 1 | 2 | 151 | 0.03 | 0.03 |
| Zhanel et al. | 2003 | BMD | 7,566 | 4 | 4 | - | - | - |
| Bouchillon et al. | 2005 | BMD (MicroScan) | 272 | 0.12 | 0.25 | 280 | 0.06 | 0.5 |
| Fritsche et al. | 2005 | BMD (dry-form, TREK) | 4,011 | 0.5 | 1 | 2,969 | ≤0.12 | ≤0.12 |
| Hoban et al. | 2005 | BMD (MicroScan) | 425 | 0.12 | 0.25 | 435 | 0.06-0.12 | 0.25-0.5 |
| Sooko et al. | 2006 | BMD | 84 | 0.12 | 0.25 | 623 | ≤0.06 | ≤0.06 |
| Bradford et al.[b] | 2008 | BMD | 39 | 0.25 | 0.5 | 184 | 0.06 | 0.06 |
| Gales et al. | 2008 | BMD (dry-form, TREK) | 486 | 0.5 | 1 | 1,034 | ≤0.03 | ≤0.03 |
| Gonullu et al. | 2008 | Agar dilution | 140 | 0.25 | 0.5 | 97 | 0.03 | 0.12 |
| Rio et al. | 2009 | BMD (MicroScan) | 831 | 0.12 | 0.5 | 550 | 0.03 | 0.12 |
| Norskov-Lauritsen et al. | 2009 | BMD | 1,634 | 0.12 | 0.5 | 1,602 | 0.03 | 0.12 |

a. BMD = broth microdilution method.
b. This study reports tigecycline susceptibility of isolates from patients enrolled in phase 3 tigecycline clinical trials for community-acquired pneumonia.

**Table 2**. Tigecycline MIC distributions for *H. influenzae* tested by broth microdilution.

| | No. of isolates (cumulative %) inhibited at tigecycline MIC of: | | | | | | |
|---|---|---|---|---|---|---|---|
| | ≤0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 |
| *H. influenzae* | | | | | | | |
| Dry-form panel | | | | 22 (13.7) | 49 (44.1) | 88 (98.8) | 2 (100.0) |
| Frozen-form panel | | 3 (1.9) | 35 (23.6) | 89 (78.9) | 34 (100.0) | | |

**Figure 1**. Correlation between broth microdilution (dry-form panels) and disk diffusion methods when testing *H. influenzae* (161 strains). Solid bolded lines indicate breakpoints.

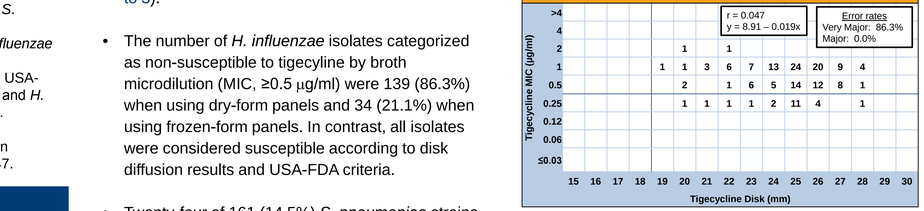



**Figure 2**. Correlation between broth microdilution (frozen-form panels) and disk diffusion methods when testing *H. influenzae* (161 strains). Solid bolded lines indicate breakpoints.

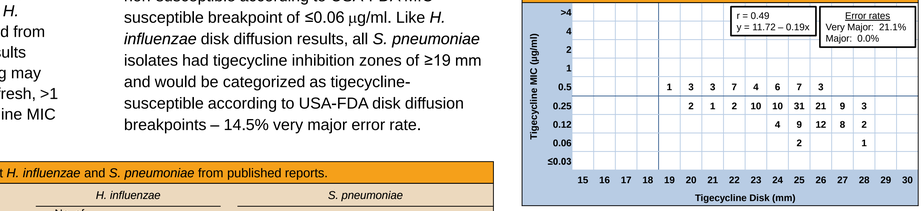
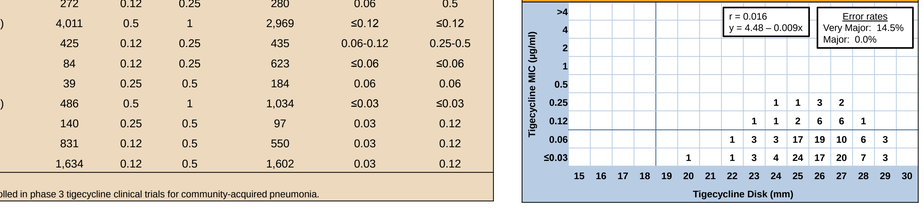



**Figure 3**. Correlation between broth microdilution (dry-form panels) and disk diffusion methods when testing *S. pneumoniae* (166 strains). Solid bolded lines indicate breakpoints.




## CONCLUSIONS

- A great variability for in vitro activity of tigecycline against *H. influenzae* has been reported in the medical microbiology/infectious disease literature. Although the use of aged (non-fresh) media could be responsible for some of this variability, other factors could affect tigecycline testing against *H. influenzae*, including the choices of breakpoint criteria and reagent product validation results.
- Disk diffusion and MIC breakpoints for tigecycline recently approved by the USA-FDA for *S. pneumoniae* and *H. influenzae* demonstrated poor intermethod agreement and serious interpretive errors. Re-evaluation of these tigecycline criteria appears needed as well as re-evaluations of some commercial products.

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