

Revised Abstract

In 2009, an apparent discordance in tigecycline activity against *Haemophilus influenzae* in a large surveillance study was noted by our laboratory. MIC₉₀ values had increased from 0.25 mcg/ml in the years 2004-07 to 1 mcg/ml in 2008-09. Quality control MIC values also increased one to two doubling dilutions during this time. This increase was contradicted by in-house re-testing of isolates, as well as other published data. The current study was undertaken to assess potential variability in different lots and manufacturers of Haemophilus Test Medium (HTM). **Methods:** One hundred clinical isolates of *H. influenzae* were tested in duplicate on dried Sensititre panels (Trek Diagnostics) containing 11 antibiotics, using HTM broth from two different manufacturers: Trek and Hardy Diagnostics (Hardy). We also evaluated 8 replicates of *H. influenzae* ATCC 49247 using different manufacturers (Trek, Hardy and Siemens) and lots of HTM broth. **Results:** The MIC₉₀, MIC₅₀, and mode (mcg/ml) of tigecycline were 1, 1, 1 using the Trek broth, and 0.5, 0.25, and 0.25 using the Hardy broth. Minocycline, the parent compound of tigecycline, also showed different activity in the two broths (MIC₉₀, MIC₅₀, and mode in Trek HTM: 2, 2, 2; in Hardy HTM: ≤0.5, ≤0.5, ≤0.5). There were no differences between the two broths for the other drugs tested. The MICs (mcg/ml) of eight replicates of *H. influenzae* ATCC 49247 were Trek lot 149861SA: 1; Trek lot 160570SA: 0.5; Hardy: 0.25; Siemens: 0.25. **Conclusions:** The increase of one to two doubling dilutions between the different media shifted the majority of the clinical isolates from susceptible when tested in Hardy HTM to non-susceptible when tested in Trek HTM. These inconsistencies highlight variability of tigecycline MICs in different lots or manufacturers of HTM, and suggest that if QC results are at the high end of the 4-dilution QC range, further investigation is warranted before reporting clinical results.

Introduction

The Clinical and Laboratory Standards Institute (CLSI) recommends the use of Haemophilus Test Medium (HTM) for routine susceptibility testing of *H. influenzae* [1]. This medium has a relatively short shelf life, and susceptibility tests need to be controlled carefully for adequate growth as well as for test values of quality control (QC) strains. While evaluating data from a multiyear surveillance study, we noted that both the MIC₉₀ value and the number of repeat tests dictated by quality assurance (QA) flags had increased for tigecycline when tested against *H. influenzae*. In this study, QA flags are triggered when the tigecycline MIC for an individual *H. influenzae* isolate is ≥0.5 mcg/ml. During the period in which clinical isolates showed this increase in MIC₉₀, the MIC₅₀ and MIC₉₀ values from routine quality control (QC) testing of tigecycline against *H. influenzae* ATCC 49247 done by external study investigators also increased one doubling dilution, but were still within the acceptable CLSI QC range of 0.06 – 0.5 mcg/ml [2]. Other compounds on the panel showed no increase in MIC. Investigators had been supplied with HTM manufactured by PML for Siemens (Siemens Medical Solutions Diagnostics, West Sacramento, CA, USA) from 2004 to 2007, and Sensititre HTM from Trek (TREK Diagnostic Systems, Cleveland, OH, USA) from 2008 to 2009. QA retests of isolates done in our laboratory were set up using Hardy HTM (Hardy Diagnostics, Santa Maria, CA, USA). Based on these variable results, we compared several different lots and manufacturers of HTM broth.

Materials & Methods

One hundred *H. influenzae* strains were tested on Research Use Only Trek panels using Trek and Hardy HTM. Panels were inoculated according to the manufacturer's instructions. Inocula were prepared by suspending 3-5 colonies from chocolate agar plates incubated overnight (20 to 24h) into 5 ml cation-adjusted Mueller Hinton broth (CAMHB) and adjusted to a 0.5 McFarland standard. 50mcl of this inoculum was then pipetted into 11ml tubes of Hardy and Trek HTM. MICs were determined according to CLSI criteria [2]. Cumulative QC data was gathered from investigator reports from the surveillance study. In-house QC evaluated 8 replicates of *H. influenzae* ATCC 49247 using different manufacturers and lots of HTM broth.

References

1. Clinical Laboratory Standards Institute, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition*, in Document M7-A7. 2007: Clinical Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
2. Performance Standard for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement, M100-S19, 2009. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

Results

Table 1. Investigator QC testing of tigecycline against *H. influenzae* ATCC 49247 2004-2009.

	2004	2005	2006	2007	2008	2009
N	106	181	263	341	225	138
MIC ₅₀	0.12	0.12	0.12	0.25	0.25	0.25
MIC ₉₀	0.25	0.25	0.25	0.5	0.5	0.5

Table 2. MIC_{50/90} (mcg/ml) of nine antibiotics against 100 *H. influenzae* isolates in Hardy and Trek HTM broth.

	Hardy HTM Broth		Sensititre HTM Broth	
	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀
TIG*	0.25	0.5	1	1
P/T	≤0.06	≤0.06	≤0.06	≤0.06
LEV	0.015	0.015	0.015	0.03
CAX	≤0.06	≤0.06	≤0.06	≤0.06
AUG	0.5	2	0.5	2
AMP	≤0.5	>32	≤0.5	>32
AMK	4	8	4	8
MER	≤0.06	0.12	≤0.06	0.12
MIN	≤0.5	≤0.5	2	2

*TIG=tigecycline, P/T=piperacillin/tazobactam, LEV=levofloxacin, CAX=ceftriaxone, AUG=amoxicillin/clavulanate, AMP=ampicillin, AMK=amikacin, MER=meropenem, MIN=minocycline.

Figure 1. Distribution of tigecycline MICs of 100 *H. influenzae* tested in Hardy HTM and Trek HTM.

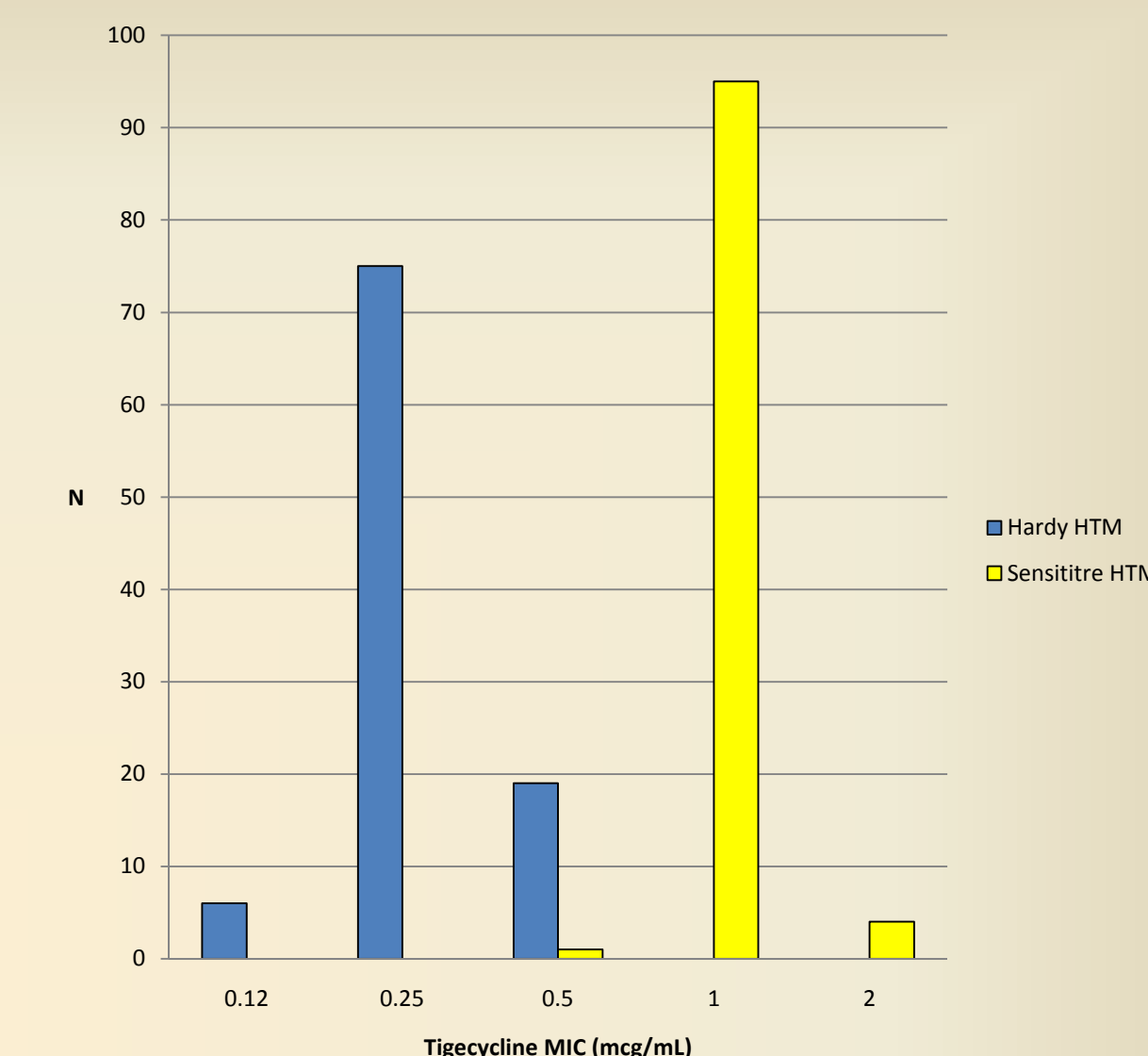


Table 3. Tigecycline MICs (mcg/ml) of eight replicates of *H. influenzae* ATCC 49247 in HTM broth from different manufacturers and different lots*.

Replicate:	MIC (mcg/ml)							
	1	2	3	4	5	6	7	8
Trek lot A	1	1	1	1	1	1	1	1
Trek lot B	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Siemens	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Hardy	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

*CLSI range for tigecycline is 0.06 – 0.5 mcg/ml

Conclusions

- Use of HTM broth from different manufacturers or even lots from the same manufacturer can impact *H. influenzae* tigecycline MICs by 1 to 2 doubling dilutions.
- Based on our testing sample of 100 clinical isolates, this broth variability affected the interpretation of tigecycline MICs, with 82% changing from susceptible (≤0.25 mcg/ml) to non-susceptible (≥0.5 mcg/ml) depending on the broth used.
- Since the CLSI QC range for *H. influenzae* ATCC 49247 is four-dilutions wide (0.06–0.5 mcg/ml), the majority of QC results should theoretically fall at 0.12 or 0.25, and consistent results at the high end of the range may suggest a broth problem requiring further investigation.
- These data highlight the variability in performance of different lots of HTM in susceptibility testing of tigecycline against *H. influenzae*, and suggest that QC results at the high end of the range correlate to higher MICs in clinical isolates. Since tigecycline only has a susceptible breakpoint (≤0.25) for *H. influenzae*, such a shift upward of one to two dilutions has the potential to cause a very significant number of false resistant or “non-susceptible” reports.