CLSI Rationale Document – Doxycycline and Tetracycline Breakpoints for *Streptococcus pneumoniae*: 2013

Background/Introduction:

Doxycycline represents an alternative oral agent for ambulatory patients (especially those that are penicillin- or macrolide- allergic) with community-acquired pneumonia, and also represents an alternative to a macrolide for inpatient intravenous therapy (combined with a beta-lactam) of community-acquired pneumonia (1). Clinicians may expect doxycycline susceptibility testing of *Streptococcus pneumoniae* isolates from lower respiratory infections with doxycycline. However, only tetracycline MIC and zone diameter breakpoints have been available for testing *S. pneumoniae*. CLSI M100-S22 includes a comment in Table 2G that "Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline" (2). This results in many clinical microbiology laboratories finding it necessary to attach a footnote to the tetracycline susceptibility result that tetracycline was tested to represent doxycycline. In addition, doxycycline is a more potent agent than tetracycline (lower MICs) against *S. pneumoniae* and testing tetracycline might underestimate the activity of doxycycline. Thus, it would be preferable to test doxycycline directly against respiratory isolates of pneumococci. The CLSI M-23 document (3) defines the requirements for establishment of interpretive criteria (breakpoints) and for reassessment of existing breakpoints. Studies were undertaken over the past two years to develop specific interpretive breakpoints for doxycycline and to reevaluate tetracycline breakpoints when testing *S. pneumoniae*. Quality control ranges for doxycycline MICs and zones with *S. pneumoniae* 49619 were established earlier and have been published in M100 (2).

The studies to develop MIC and zone diameter breakpoints for doxycycline and revisions to the tetracycline breakpoints were conducted in two phases. Initially, a single center study was performed to establish provisional breakpoints using 101 strains of invasive pneumococci from the CDC Active Bacterial Core Surveillance (ABCs) program (http://www.cdc.gov/abcs). Strains were recovered from patients in 8 of the 10 ABCS surveillance sites (states) from 2009-2010. Strains were selected to represent approximately 50% tetracycline resistant isolates based upon prior testing results. A variety of serotypes were included in the strain collection. Special frozen broth microdilution panels were prepared with 3% lysed horse blood supplemented Mueller-Hinton broth (prepared using a single manufacturer's medium). Incubation proceeded at the recommended 35^{o}C in ambient air for 20-24 h

(4). Commercial Mueller-Hinton 5% sheep blood agar plates were purchased from a single commercial source. Plates with tetracycline (30 µg) and doxycycline (30 µg) disks were incubated at 35°C in 5% CO2 for 20-24 h (5). This initial study demonstrated that there was a direct relationship between tetracycline and doxycycline MICs, with the doxycycline MICs being consistently lower (Table 1). Scattergrams relating tetracycline and doxycycline MICs to their respective disk diffusion data appeared to represent acceptable correlations and the opportunity to establish doxycycline disk breakpoints and possibly revise those for tetracycline depending upon the MIC breakpoints chosen. When these initial results were presented to the staphylococcal and streptococcal working group of the CLSI Antimicrobial Susceptibility Testing Subcommittee, it was decided that a larger study was needed to include at least one more testing laboratory and additional brands and lots of media.

Table 1. Comparison of doxycycline and tetracycline MICs on 101 pneumococcal isolates.

Count of TET	Doxy MIC											
TET MIC	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	(blank)	Grand Total
(blank)												
>16							5	29	8	3		45
16							1	6	1			8
8						3	1					4
4				1	3							4
0.5			1	2								3
0.25			12									12
0.12		12	9									21
0.06	2	2										4
Grand Total	2	14	22	3	3	3	7	35	9	3		101

The same 101 strains plus 88 additional isolates also from the CDC ABCs surveillance program were tested at the CDC Streptococcal Laboratory and in the Division of Healthcare Quality Improvement Laboratory. Frozen broth microdilution panels were prepared at CDC using a different brand of Mueller-Hinton dehydrate than was used in the UTHSC lab. A second brand of commercially prepared Mueller-Hinton sheep blood agar plates was used for disk testing at CDC. There was good agreement between MICs and zone diameters determined at the UTHSC and in the CDC labs. Scattergrams were prepared from the original 101 strains tested at the UTHSC merged with the 88 strains tested at CDC. These merged data provided very few interpretive errors (Figure 1 and 2).

Figure 1. Doxycycline MICs and disk diffusion zones on 189 isolates of pneumococci tested in three laboratories.

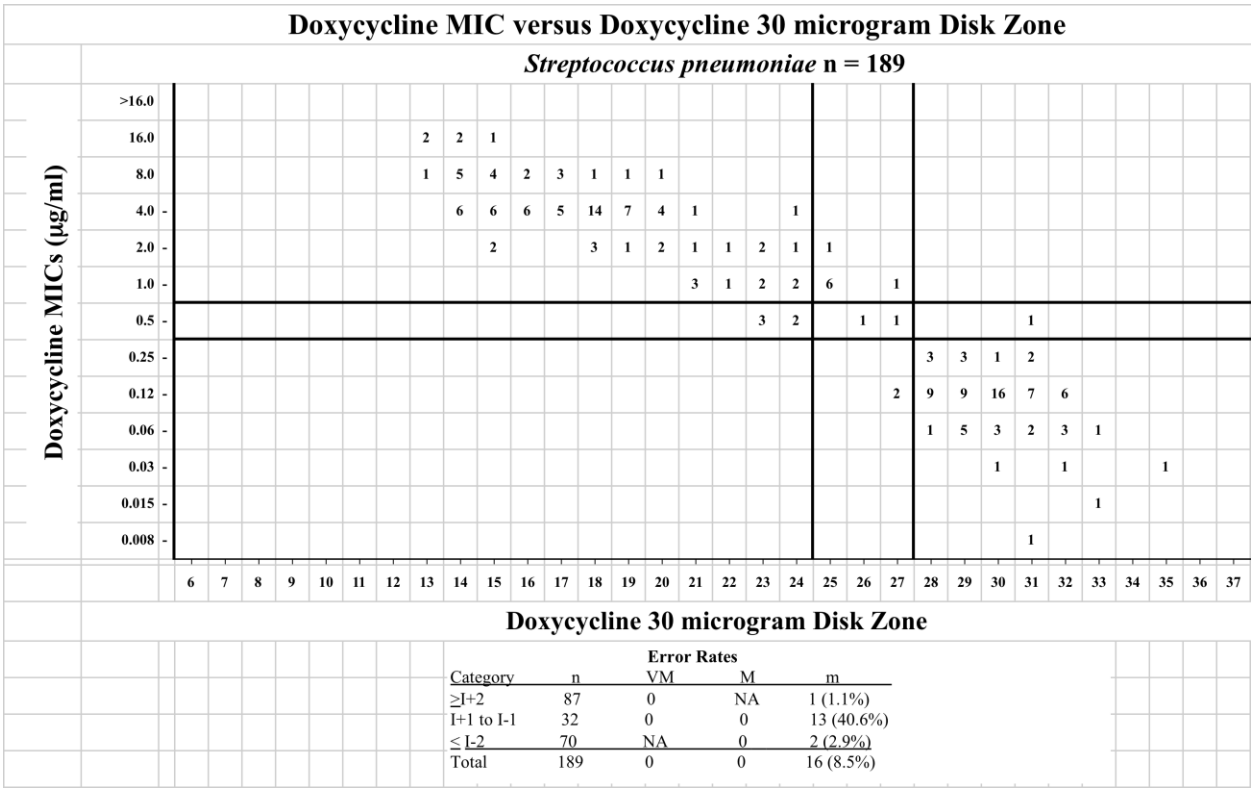
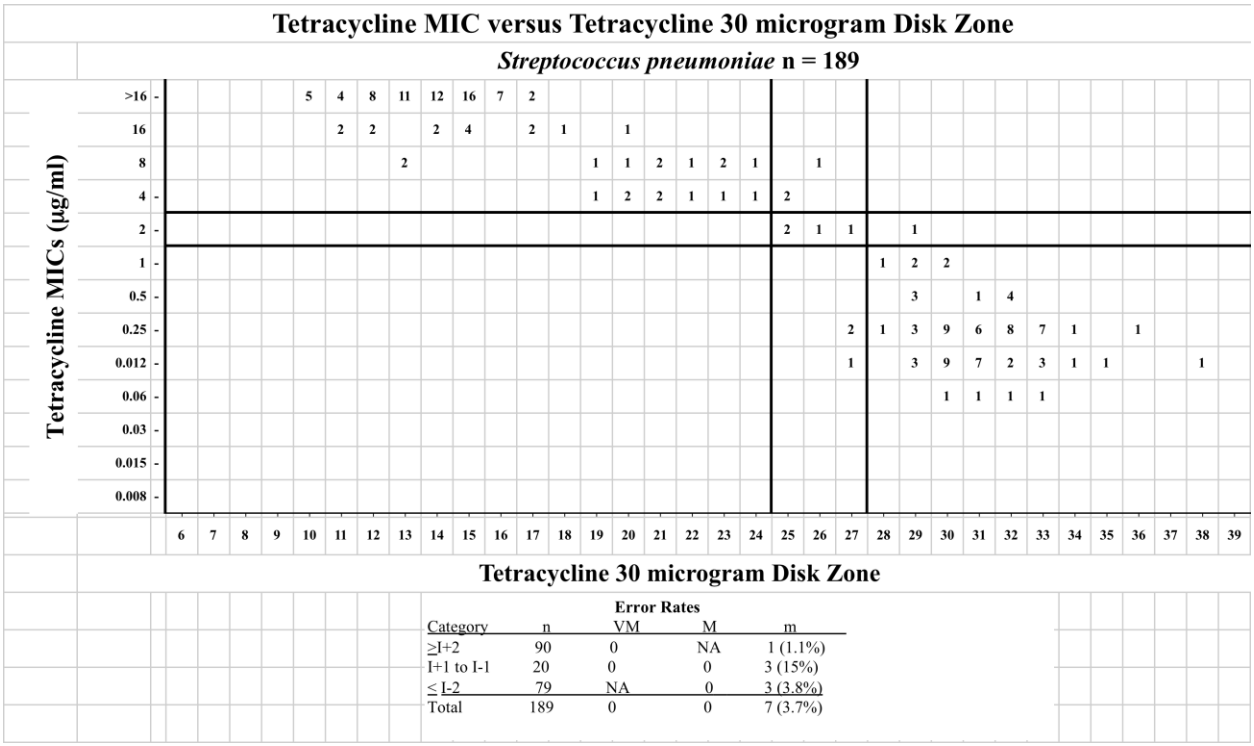


Figure 2. Tetracycline MICs and disk diffusion zones on 189 isolates of pneumococci tested in three laboratories.



Presence of the *tetM* resistance determinant

In an effort to correlate the most common mechanism of tetracycline resistance in pneumococci, PCR was performed for the *tetM* resistance determinant on selected isolates with different tetracycline and doxycycline MICs at the UTHSC and at the CDC. Strains with tetracycline MICs of 4 µg/ml or higher and doxycycline MICs of 1 µg/ml or greater contained the *tetM* determinant (96.6% and 90% respectively), whereas those with lower MICs usually did not (Table 2).

Table 2. Comparison of tetracycline and doxycycline MICs with the presence of the *tetM* resistance determinant.

Strain	**MIC** (mg/L)	**Dose** **(mg/kg/12 h)**	**Free Drug 24-h AUC/MIC**

No. isolates	Tetracycline MIC	Doxycycline MIC	Presence of tetM
5	>16	16	5 of 5
8	>16 or 16	8	8 of 8
19	>16 or 16	4	19 of 19
7	>16 or 16	2	6 of 7
1	8	4	1 of 1
6	8	2	6 of 6
4	8	1	4 of 4
1	4	2	0 of 1
9	4	0.5	9 of 9
3	2	0.5	3 of 3
4	2	1	0 of 4
2	1	1	0 of 2
3	1	0.25 or 0.5	0 of 3
1	0.5	0.5	1 of 1
11	0.5	0.12 or 0.25	2 of 11
34	≤ 0.25	≤ 0.12	0 of 34
Total of 118 isols			
	≥ 4		58 of 60 or 96.6%
	≥ 2		61 of 67 or 91%
	≤ 1		3 of 51 or 5.9%
	≤ 0.5		3 of 46 or 6.5%
		≥ 2	45 of 47 or 95.7%
		≥ 1	49 of 57 or 90%
		≤ 0.5	15 of 61 or 24.6%
		≤ 0.25	2 of 47 or 4.3%

Pharmacokinetics and Pharmacodynamics

Limited PK/PD data from the literature for doxycycline and tetracycline were reviewed (6,7,8) and from published abstracts from Dr. Bill Craig (9,10). Based upon experiments performed in neutropenic murine thigh model of infection, the 24 h AUC/MIC was the best predictor of doxycycline in vivo activity (10).

		Static	2 Log Kill	Static	2 Log Kill
ATCC 10813	0.06	12.1	33.0	52.6	174
CDC 141	0.12	9.66	43.1	20.1	110
CDC 146	0.12	6.74	42.9	14.0	109
CDC 1285	0.12	5.64	34.9	11.7	88.4
CDC 130	0.03	3.25	>320	14.8	-
3313	2	>320	-	>34.9	-
3427	2	>320	-	>34.9	-
Mean		8.54 ± 2.92	38.5 ± 5.28	22.6 ± 17.0	120 ± 37.1

Table 3. Pharmacodynamic determinations in the neutropenic mouse thigh infection model.

From references 9 and 10

The data in Table 3 derived from studies in mice suggested that a mean AUC/MIC of 120 mg/h/L would be required for a 2-log kill and 22.6 mg/h/L for a static effect. The mean AUC of doxycycline in healthy human volunteers receiving a 100 mg BID dose is ~ 160 mg/h/L. Protein binding in human serum is 82-93% (mean 87.5%). The free drug AUC would be ~20 mg/h/L. That would suggest a possible susceptible breakpoint of 1 µg/ml based upon the mean values. In order to pick up the majority of patients and to take into account the variability of AUC values of an oral drug, it was determined that the susceptibility breakpoint for doxycycline should be no higher than 0.5 µg/mL for *S. pneumoniae* (10). Since there are very few PK data available for tetracycline plus the presence of the tetM determinant at the higher MICs currently encompassed in the susceptible category, it was decided that the tetracycline breakpoints should be lowered.

Clinical Efficacy:

Relatively few clinical data were available for review with doxycycline or tetracycline (11, 12). However, expert opinion expressed in the pneumonia treatment guidelines suggested that doxycycline represented an alternative to macrolides in ambulatory outpatients with pneumococcal pneumonia or moderately ill inpatients with pneumonia when combined with an active beta-lactam (1).

Summary

The CLSI Antimicrobial Susceptibility Testing Subcommittee reviewed in vitro data generated in three different laboratories with multiple brands and lots of media determined over a two-year period. In addition MICs were correlated with the presence of the most common tetracycline resistance determinant in pneumococci, *tetM*. These data were used in conjunction with PK-PD studies and simulations to establish MIC breakpoints for doxycycline and to revise (lower) previously published tetracycline breakpoint for pneumococci (Table 3). Having established the MIC breakpoints, the error-rate bounding method was utilized to determine appropriate disk diffusion zone diameter breakpoints.

Table 3. CLSI doxycycline and tetracycline MIC (μg/mL) and disk diffusion (mm) breakpoints for *S. pneumoniae*

Antimicrobial Agent	New MIC breakpoints (S, I, R)	Previous MIC breakpoints (S, I, R)	New zone diameter breakpoints (S, I, R)	Previous zone diameter breakpoints (S, I, R)
Doxycycline	≤0.25, 0.5, ≥1	NA	≥28, 25-27, ≤24	NA
Tetracycline	≤1, 2, ≥ 4	≤ 2, 4, ≥ 8	≥28, 25-27, ≤24	≥23, 19-22, ≤18

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