P675
ECCMID 2012
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

Contemporary Doxycycline and Tetracycline Susceptibility Testing using CLSI and EUCAST Criteria for Gram-positive Pathogens: Results from SENTRY Program

RN JONES, MG STILWELL
JMI Laboratories, North Liberty, Iowa, USA

Abstract

Objectives: To assess the potency and intermethod agreement for doxycycline (DOXY) and tetracycline (TETRA) susceptibility (S) testing breakpoints when tested against SENTRY Antimicrobial Surveillance Program isolates of Gram-positive species (13,188 isolates) collected worldwide.

Methods: All organisms were cultured in 2010 with S testing by CLSI M07-A9 (2012) methods and results interpreted by CLSI M100-S22 (2012) and EUCAST (2012) criteria for TETRA and DOXY. A total of 9,012 *S. aureus* (SA; 44.9% MRSA); 2,325 *S. pneumoniae* (SPN); and 1,851 beta-haemolytic streptococci (BHS; 42.8% *S. pyogenes* [SPYO]) were analyzed for S and cross-S rates by the two international breakpoint sets. The tetracycline's S breakpoint MIC (mg/L) criteria differ (CLSI/EUCAST, respectively) as follows: for SA (≤4/≤1), for SPN (≤2/≤1) and for BHS (≤2/≤1). All quality control tests were within published CLSI ranges.

Results: S rates for DOXY were consistently greater than TETRA for each interpretive criteria used and for each pathogen group analyzed. The CLSI DOXY/TETRA S rates (EUCAST rates) were as follows: 99.2/94.2(96.7/93.2)% for MSSA; 96.2/91.2(93.5/88.1)% for MRSA; 75.3/73.2(73.8/73.0)% for SPN; 81.2/80.3(80.2/79.6)% for SPYO; and 15.7/14.6(15.4/14.6)% for *S. agalactiae* (SAGA). DOXY (MIC_{90}, 0.5 mg/L) was generally 2- to 4-fold more potent than TETRA (MIC_{90}, 2 mg/L) versus MRSA. Use of TETRA-S results to predict DOXY-S was excellent (>99.9 - 100.0%) for SA regardless of breakpoints used, as were predicted for SPN (99.8-100.0%), SPYO (99.6-99.9%) and SAGA (100.0%); errors usually higher applying the lower EUCAST breakpoints. Concerns persist that strains of staphylococci and streptococci having TET-R mechanisms could be categorized by CLSI as S (MICs at 2 or 4 mg/L) by current breakpoints e.g. 2.7% of MRSA tested against DOXY.

Conclusions: CLSI and EUCAST interpretive criteria for tetracyclines (TETRA and DOXY) remain discordant, but each determines DOXY to have wider spectrum against four Gram-positive pathogen species and that TETRA-S can accurately predict DOXY-S (99.93-99.86% across 13,188 isolates). Moreover, molecular test-confirmed mechanisms appear highly probably among CLSI-susceptible (MICs, 2 or 4 mg/L) strains requiring international harmonization, to also include other tetracycline or-like agents and systematically applying pharmacodynamic principles.

Introduction

The tetracyclines (particularly chlortetracycline) were the first broad-spectrum antimicrobial class to be described in 1944. Derived from various *Streptocmyces* species (rimosus, aureofaciens) these agents were expanded via semi-synthetic processes to include tetracycline (dehalogenation), doxycycline and minocycline; the latter three persisting in contemporary chemotherapy. Their mode of action targets the bacterial ribosomes resulting in the inhibition of protein synthesis. Tetracycline HCl is considered short-acting; and doxycycline and minocycline are long-acting by having extended plasma half-lives.

Tetracyclines are very active against Gram-positive bacteria, producing bimodal disk and MIC distributions of wild type (WT) susceptible strains and those with elevated MIC results indicating acquired or intrinsic resistances. These features were recognized early in the history of standardized antimicrobial susceptibility testing (Barry, 1976), and methods with interpretive criteria were proposed to separate these bacterial modes using dilution (MICs) and agar diffusion disks (zone diameters). The National Committee for Clinical Laboratory Standards (currently the Clinical and Laboratory Standards Institute [CLSI]) proposed initial susceptible category breakpoints at ≤4 mg/L and resistance at ≥12 mg/L; later adjusted to a log_2 dilution scale and to species-specific criteria:

1. For Staphylococci: Susceptible at ≤4 mg/L and resistant at ≥16 mg/L; and
2. For Streptococci: Susceptible at ≤2 mg/L and resistant at ≥8 mg/L

Re-evaluations of worldwide existing in vitro testing results, MIC population data and updated pharmacokinetics/pharmacodynamic information by the EUCAST group has led to differing breakpoints by at least two log_2 dilution steps. This presentation quantitates the level of difference between the application of these two sets of breakpoint criteria (CLSI and EUCAST) when testing a large collection of Gram-positive surveillance study pathogens from 2010 (SENTRY Antimicrobial Surveillance Program, worldwide).

Materials and Methods

Organism collection: All organisms were cultured in the year 2010 from medical centers worldwide (United States [USA], Europe, Latin America, Asia-Pacific Region) and sent for reference susceptibility testing (>30 agents) and identification confirmation by a monitoring GLP/CLIA-certified laboratory (JMI Laboratories, North Liberty, Iowa, USA). These strains included: *Staphylococcus aureus* (9,012; 44.9% methicillin-resistant [MRSA]), *Streptococcus pneumoniae* (2,325), and β-haemolytic streptococci (1,851; 42.8% *S. pyogenes*).

Antimicrobial susceptibility tests: These 13,188 Gram-positive pathogens were tested against tetracycline and doxycycline by the broth microdilution method as described in the CLSI M07-A9 (2012) document in validated panels produced under GMP conditions at ThermoFisher Scientific (formerly TREK Diagnostics) of Cleveland, Ohio, USA. Concurrent quality control (QC) used ATCC strains *S. aureus* 29213, *E. faecalis* 29212 and *S. pneumoniae* 49619. All QC results were within CLSI M100-S22 (2012) limits.

The interpretations of results were taken from those published by the CLSI (M100-S22, 2012) and EUCAST (2012); see above for CLSI breakpoints. EUCAST applies lower MIC breakpoints at ≤1 mg/L for susceptibility. Analyses also considered the use of tetracycline HCl susceptibility results to predict doxycycline susceptibility for the four tabulated species.

Molecular characterization of *tet* genes was performed by methods described by Aminov et al. (2001) for *tet* K, L, M, N and O.

Results

- Tested against MSSA and MRSA, doxycycline was four-fold more potent than tetracycline (MIC_{90} results). Futhermore, MSSA (MIC_{90}, 0.12 and 0.5 mg/L) were four-fold more susceptible to both agents than MRSA (MIC_{90}, 0.5 and 2 mg/L).
- The CLSI doxycycline/tetracycline susceptibility rates (EUCAST rates) were as follows for (Table 1):
  - MSSA 99.2/94.2% (96.7/93.2%)
  - MRSA 96.2/91.2% (93.5/88.1%)
  - *S. pneumoniae* 75.3/73.2% (73.8/73.0%)
  - *S. pyogenes* 81.2/80.3% (80.2/79.6%)
  - *S. agalactiae* 15.7/14.6% (15.4/14.6%)
- Many strains of staphylococci were resistant to tetracycline (Table 2) but susceptible or intermediate to doxycycline, regardless of breakpoints used.
- If tetracycline susceptibility was used to predict doxycycline the error rate was ≤0.1% for staphylococci, and 0.0-0.4% for streptococci (Tables 2 and 3). Greatest error rates overall were when using CLSI criteria (Table 3).
- Figure 1 shows the comparative tetracycline's potencies for MSSA with plotted breakpoints (CLSI and EUCAST). EUCAST criteria appear to minimize categorization error and the false-susceptible results for strains having resistance genes. In *S. aureus,* 13 of 50 strains having a tetracycline MIC of either 2 or 4 mg/L had detectable *tet* genes (*tet* M & K). All strains tested with tetracycline MIC values at ≥8 mg/L had one or more *tet* resistance (*tet* K, L or M).
- Even using the EUCAST doxycycline breakpoint (≤1 mg/L), staphylococci had *tet* genes among strains with MIC values at 0.25-1 mg/L.

Figure 1. Scattergram comparing tetracycline and doxycycline MIC results when testing methicillin-susceptible population of *S. aureus* (4,966) strains. The correlation co-efficient (r) was 0.87, and the regression equation was y=0.99 + 1.07x. Circled numbers are the *tet* K-positive strains among 20 tested strains with tetracycline MICs of 2 or 4 mg/L. Superscript * shows negative strains.

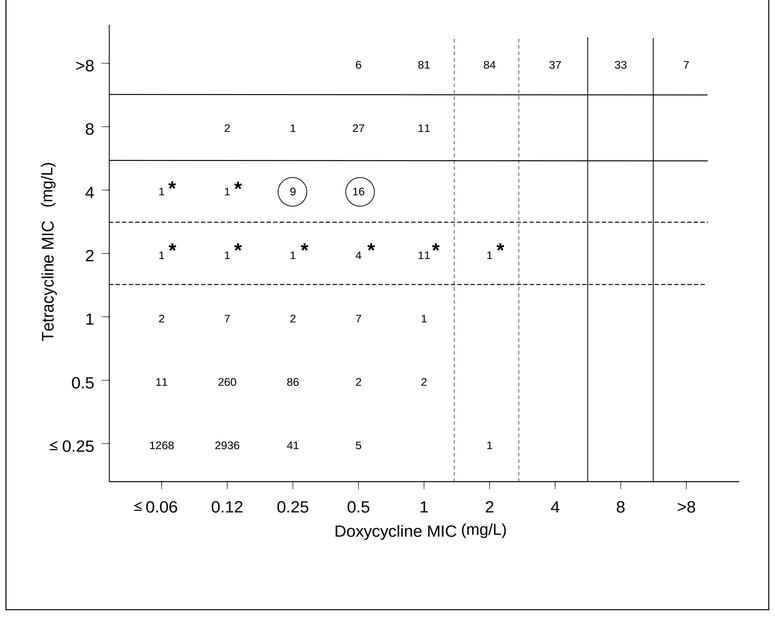

Table 1. Comparative potencies and susceptibility rate results for tetracycline and doxycycline tested against isolates of Gram-positive pathogens (13,188 strains in 2010 SENTRY Program).

Organism (no. tested)	Antimicrobial	MIC (mg/L) 50%	MIC (mg/L) 90%	% by category: (susceptible/resistant by method)[a] CLSI	% by category: (susceptible/resistant by method)[a] EUCAST
MRSA (4,046)	Tetracycline	≤0.25	2	91.2 / 8.0	88.1 / 9.0
	Doxycycline	0.12	0.5	96.2 / 0.6	93.5 / 5.5
MSSA (4,966)	Tetracycline	≤0.25	0.5	94.2 / 5.0	93.2 / 6.4
	Doxycycline	0.12	0.12	99.2 / 0.1	96.7 / 1.6
S. pneumoniae (2,325)	Tetracycline	0.5	>8	73.2 / 26.5	73.0 / 26.7
	Doxycycline	0.25	8	75.3 / 15.3	73.8 / 24.7
S. pyogenes (793)	Tetracycline	≤0.25	>8	80.3 / 19.7	79.6 / 19.7
	Doxycycline	0.12	8	81.2 / 16.0	80.2 / 18.8
S. agalactiae (1,058)	Tetracycline	>8	>8	14.6 / 84.9[b]	14.6 / 85.4[b]
	Doxycycline	8	8	15.7 / 81.3[b]	15.4 / 84.3[b]

a. Susceptibility/resistance criteria of the CLSI (2012) and EUCAST (2012).
b. Dominant resistance to tetracyclines.

Table 2. Categorical comparisons between doxycycline and tetracycline using the breakpoint criteria of the CLSI (2012) and EUCAST (2012) for over 9,000 *S. aureus* isolated in 2010.

Organism/ Antimicrobial (no. tested)	Tetracycline (no. isolates) CLSI category[a] Susc.	Interm.	Resist.	EUCAST category[b] Susc.	Interm.	Resist.
Doxycycline						
MSSA (4,966)						
Susceptible	4,677	41[c]	208	4,630	18[c]	155
Intermediate	0	0	33[c]	1	1	84[c]
Resistant	0	0	7	0	0	77
MRSA (4,046)						
Susceptible	3,689	32[c]	172	3,564	117[c]	103
Intermediate	0	0	128[c]	0	1	38[c]
Resistant	0	0	25	1	0	222

a. CLSI (2012) criteria for tetracycline: susceptible at ≤4 mg/L and resistant at ≥16 mg/L. Same categorical criteria are used for doxycycline.
b. EUCAST (2012) criteria for tetracycline: susceptible at ≤1 mg/L and resistant at >2 mg/L. Same categorical criteria are used for doxycycline.
c. Most prevalent type of minor predictive error using tetracycline results to predict doxycycline susceptibility, regardless of interpretive criteria (CLSI or EUCAST) e.g. false-intermediate or false-resistant.

Table 3. Calculated error rates for tetracycline results used to predict doxycycline susceptibility categories using CLSI (2012) and EUCAST (2012) breakpoints (cross-susceptibility testing).

Pathogen group (no. tested)	% by error type[a] Very major	Major	Minor	Total
MSSA (4,996)	0.0 (<0.1)	4.2 (3.1)	1.5 (2.1)	5.7 (5.2)
MRSA (4,046)	0.0 (<0.1)	4.2 (2.5)	4.0 (3.8)	8.2 (6.4)
S. pneumoniae (2,325)	0.0 (0.2)	1.9 (0.7)	9.6 (1.8)	11.5 (2.7)
S. pyogenes (793)	0.1 (0.4)	1.4 (1.1)	2.8 (1.5)	4.3 (3.0)
S. agalactiae (1,058)	0.0 (0.0)	0.6 (0.9)	3.6 (0.3)	4.2 (1.2)

a. Error rates for CLSI with EUCAST error rates in parenthesis.

Conclusions

- Doxycycline remains more potent than tetracycline by MIC comparisons and possesses greater spectrum of activity (% susceptible rate) by either CLSI and EUCAST breakpoints.
- EUCAST breakpoints for each Gram-positive pathogen seems better correlated to current data (PK/PD) including contemporary MIC distributions and minimizes false-susceptibility categorization of the four analyzed species.
- Further studies with other tetracyclines (minocycline or more) and disk diffusion methods to establish correlate breakpoints are urgently needed, especially for the CLSI criteria. Even EUCAST breakpoints for doxycycline will categorize as susceptible Gram-positive pathogens having detectable *tet* genes.

Acknowledgment

Co-authors are employees of JMI Laboratories and have no conflicts of interest to declare.

References

1. Aminov RI, Garrigues-Jeanjean N, Mackie RI (2001). Molecular ecology of tetracycline resistance: Development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. *Appl Environ Microbiol* 67: 22-32.
2. Barry AL (1976). The Antimicrobic Susceptibility Test: Principles and Practices. Philadelphia: Lea & Fegiber.
3. Bryskier A (2005). Antimicrobial Agents: Antibacterials and Antifungals. Washington, D.C.: ASM Press.
4. Clinical and Laboratory Standards Institute (2012). *M07-A9. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: ninth edition.* Wayne, PA: CLSI.
5. Clinical and Laboratory Standards Institute (2012). *M100-S22. Performance standards for antimicrobial susceptibility testing: 22nd informational supplement.* Wayne, PA: CLSI.
6. European Committee on Antimicrobial Susceptibility Testing (2011). Breakpoint tables for interpretation of MICs and zone diameters. Version 1.3, January 2011. Available at: http://www.eucast.org/clinical_breakpoints/. Accessed: January 1, 2012.
7. Jones RN (2003). Global epidemiology of antimicrobial resistance among community-acquired and nosocomial pathogens: A five-year summary from the SENTRY Antimicrobial Surveillance Program (1997-2001). *Semin Respir Crit Care Med* 24: 121-134.