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Contemporary Potencies of Minocycline and Tetracycline Tested Against Gram-positive Pathogens: SENTRY Program Results using CLSI and EUCAST Breakpoint Criteria

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SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT

Background: Tetracycline (TET) class agents vary widely in their activity against emerging important antimicrobial-resistant (R) pathogens such as MRSA and *Acinetobacter* spp. Also published susceptibility (S) breakpoints are discordant between CLSI and EUCAST documents. We assessed the impact of these differences for minocycline (MIN) and TET when tested against contemporary Gram-positive pathogens.

Methods: The SENTRY Antimicrobial Surveillance Program (2011) compared MIN and TET activity via CLSI reference methods (M07-A9) using a worldwide collection of *S. aureus* (SA; 4,917 strains with 1,955 MRSA), *S. pneumoniae* (SPN; 1,899), *S. pyogenes* (GRA; 246), and *S. agalactiae* (GRB; 217).

Results: Regardless of applied categorical breakpoints, MIN exhibited wider coverage (%S) than TET of 4.5-7.8 / 0.5-2.1 / 1.2-2.3 / 0.4-0.4% for MRSA/SPN/GRB/GRA, respectively. Lower EUCAST S breakpoints produced decreased %S for MIN ranging from nil (≤0.5 µg/ml) for GRA to 8.9% (≤1 µg/ml) for MRSA (97.2%S by CLSI; 88.3% by EUCAST). Use of TET-S results to predict MIN-S was very accurate (99-100.0%) with absolute categorical agreement rates ranging from 92.1-98.4% (CLSI) to 98.4-99.6% (EUCAST) for streptococci (see Table 4). Greatest cross S and R errors were noted using the CLSI breakpoints (14.7%) compared to EUCAST criteria only (5.0%; acceptable), both for MRSA testing dominated by false-R results for MIN.

Conclusions: MIN (also doxycycline, not addressed here) demonstrates continued superior in vitro activity compared to TET when testing SA (especially MRSA), and pathogenic streptococci. When testing tetracyclines, laboratories must recognize the expanded spectrum of MIN against certain pathogens, and apply methods minimizing interpretive error. We conclude that EUCAST criteria (≤0.5 or ≤1 µg/ml) represent the most conservative (recognize strains with R mechanisms with MIC values at 2 or 4 µg/ml) and accurate tetracyclines breakpoints for testing current isolates of Gram-positive cocci.

INTRODUCTION

In 1944, the tetracyclines (particularly chlortetracycline) became the first broad-spectrum antimicrobial class to be described. 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 as contemporary chemotherapies. 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 serum 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 susceptible and resistance populations using dilution (MICs) and agar diffusion disks (zone diameters). The National Committee for Clinical Laboratory Standards (currently the Clinical Laboratory and Standards Institute [CLSI]) proposed initial susceptible category breakpoints at ≤4 µg/ml and resistance at ≥12 µg/ml; later adjusted to a $\log_2$ dilution scale and to species-specific criteria:

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

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 versus EUCAST) for tetracycline and minocycline when testing a large collection of Gram-positive surveillance study pathogens from 2011 (SENTRY Antimicrobial Surveillance Program, worldwide).

MATERIALS AND METHODS

Organism collection: All organisms were cultured in the year 2011 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* (4,917; 1,955 MRSA), *Streptococcus pneumoniae* (1,899), and β-haemolytic streptococci (463; 246 *S. pyogenes*).

Antimicrobial susceptibility tests: These 7,279 Gram-positive pathogens were tested against tetracycline and minocycline 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 of *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 ≤0.5 µg/ml for minocycline susceptibility. Analyses also considered the use of tetracycline HCl susceptibility results to predict minocycline 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 & O.

RESULTS

- Tested against methicillin-susceptible *S. aureus* (MSSA) and MRSA, minocycline was generally two-to four-fold more potent than tetracycline using MIC_{90} results.
- The CLSI minocycline/tetracycline susceptibility rates (EUCAST rates in parenthesis) were as follows (Table 1), each favoring minocycline:
  - MSSA 99.6/94.4% (98.3/93.8%)
  - MRSA 97.2/85.4% (88.3/83.8%)
  - *S. pneumoniae* 74.8/72.7% (72.7/72.2%)
  - *S. pyogenes* 78.0/77.6% (78.0/77.6%)
  - *S. agalactiae* 29.5/27.2% (28.6/27.2%)
- Many strains of staphylococci were resistant to tetracycline (Table 2), but susceptible or intermediate to minocycline, regardless of breakpoints used.
- Categorical agreement between minocycline and tetracycline was greater with the streptococcal testing results, and better for the EUCAST breakpoint criteria (98.4-99.6%); see Table 3.
- If tetracycline susceptibility was used to predict minocycline susceptibility the error rate was ≤0.1% for staphylococci, and 0.0-0.2% for streptococci (Tables 2 and 3). Greatest error rates overall were observed when using CLSI criteria (Table 4).
- In *S. aureus,* 15 of 68 strains having a tetracycline MIC of either 2 or 4 µg/ml had detectable *tet* genes (*tet* M & K). All strains tested with tetracycline MIC values at ≥8 µg/ml had one or more *tet* resistances (*tet* K, L or M). Even using the lower EUCAST minocycline breakpoint (≤0.5 µg/ml), staphylococci had detectable *tet* genes among strains with MIC values at 0.25 or 0.5 µg/ml.

Table 1. Activity and spectrum of tetracycline and minocycline tested against *Staphylococcus aureus* and three streptococcal species.

Organism (no. tested)/ Antimicrobial agent	% susceptible/resistant by two sets of breakpoint criteria[a]: CLSI	EUCAST
S. aureus		
MSSA (2,962)[b]		
Tetracycline	94.4 / 5.2	93.8 / 5.9
Minocycline	99.6 / <0.1	98.3 / 1.5
MRSA (1,955)[b]		
Tetracycline	85.4 / 14.3	83.8 / 14.8
Minocycline	97.2 / <0.1	88.3 / 11.3
S. pneumoniae (1,899)		
Tetracycline	72.7 / 26.8	72.2 / 27.3
Minocycline	74.8 / 19.6	72.7 / 26.4
S. agalactiae (217)		
Tetracycline	27.2 / 71.4	27.2 / 72.8
Minocycline	29.5 / 68.7	28.6 / 71.4
S. pyogenes (246)		
Tetracycline	77.6 / 22.4	77.6 / 22.4
Minocycline	78.0 / 20.7	78.0 / 22.0

a. Interpretive criteria of the CLSI (2012) and EUCAST (2012).
b. MSSA = methicillin-susceptible *S. aureus* and MRSA = methicillin-resistant *S. aureus*.

Table 2. Correlations of tetracycline and minocycline MIC results as categorized by CLSI (2012) and EUCAST (2012) breakpoint criteria.

		Tetracycline category by method (MIC criteria):					
		CLSI			EUCAST		
S. aureus group (no. tested)	Minocycline MIC (µg/ml)	Susceptible (≤4 µg/ml)	Intermediate (8 µg/ml)	Resistant (>8 µg/ml)	Susceptible (≤1 µg/ml)	Intermediate (2 µg/ml)	Resistant (>2 µg/ml)
MRSA (1,955)	≤0.5	1664[a]	7	56	1616[b]	45	66
	1	3[a]	0	4	0	2[b]	5
	2	1[a]	0	29	1	0	29[b]
	4	0[a]	0	136	0	0	136[b]
	8	0	0[a]	54	0	0	54[b]
	>8	1	0	0[a]	1	0	0[b]
MSSA (2,962)	≤0.5	2792[c]	12	109	2776[d]	9	128
	1	3[c]	0	2	2	0[d]	3
	2	0[c]	0	6	0	0	6[d]
	4	0[c]	1	24	0	0	25[d]
	8	0	1[c]	10	0	0	11[d]
	>8	0	0	2[c]	0	0	2[d]

a. Absolute categorical agreement = only 85.3%, unacceptable.
b. Absolute categorical agreement = 94.0%.
c. Absolute categorical agreement = 94.5%.
d. Absolute categorical agreement = 95.3%.

Table 3. Correlations of tetracycline and minocycline MIC results as determined by CLSI (2012) and EUCAST (2012) breakpoint criteria.

		Tetracycline category by method (MIC criteria):					
		CLSI			EUCAST		
Organism/ (no. tested)	Minocycline MIC (µg/ml)	Susceptible (≤2 µg/ml)	Intermediate (4 µg/ml)	Resistant (>4 µg/ml)	Susceptible (≤1 µg/ml)	Intermediate (2 µg/ml)	Resistant (>2 µg/ml)
S. pneumoniae (1,899)	≤0.5	1,374[a]	3	3	1,367[b]	7	6
	1	4[a]	7	5	3	1[b]	12
	2	0[a]	0	24	0	0	24[b]
	4	1	0[a]	105	0	1	105[b]
	≥8	1	1	371[a]	1	1	371[b]
S. agalactiae (217)	≤0.5	59[c]	1	2	59[d]	0	3
	1	0[c]	0	0	0	0[d]	0
	2	0[c]	1	1	0	0	2[d]
	4	0	2[c]	2	0	2	4[d]
	≥8	0	0	149[c]	0	0	149[d]
S. pyogenes (243)	≤0.5	191[e]	0	1	191[f]	0	1
	1	0[e]	0	0	0	0[f]	0
	2	0[e]	0	0	0	0	0[f]
	4	0	0[e]	3	0	0	3[f]
	≥8	0	0	51[e]	0	0	51[f]

a. Absolute categorical agreement = 92.1%.
b. Absolute categorical agreement = 98.4%.
c. Absolute categorical agreement = 96.8%.
d. Absolute categorical agreement = 98.6%.
e. Absolute categorical agreement = 98.4%.
f. Absolute categorical agreement = 99.6%.

Table 4. Overall error rates using tetracycline to predict the minocycline categorical results (cross-susceptibility and -resistance) for CLSI (2012) and EUCAST (2012) breakpoints.

Organism/Group (no. tested)	CLSI errors (%)[a]			EUCAST errors (%)[a]		
	Very major	Major	Minor (total)	Very major	Major	Minor (total)
MSSA (2,962)	0.0	4.8	0.8 (5.6)	0.0	4.3	0.5 (4.8)
MRSA (1,955)	<0.1	11.5	3.1 (14.7)	0.1	3.4	1.5 (5.0)
S. pneumoniae (1,899)	<0.1	1.7	6.2 (7.9)	<0.1	0.3	1.2 (1.6)
S. agalactiae (217)	0.0	1.4	1.8 (3.2)	0.0	1.4	0.0 (1.4)
S. pyogenes (246)	0.0	0.4	1.2 (1.6)	0.0	0.4	0.0 (0.4)

a. Very major = false-susceptible; major = false-resistance; and minor = intermediate result for one of the two agents.

CONCLUSIONS

- Minocycline remains more potent than tetracycline by MIC comparisons and possesses a greater spectrum of activity (% susceptible rate) by either CLSI or 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 (doxycycline or more) and disk diffusion methods to establish correlate breakpoints are urgently needed, especially for the CLSI criteria. However, even EUCAST breakpoints for minocycline could categorize as susceptible a few Gram-positive isolates having detectable *tet* (k or m) genes.

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