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Re-evaluating the Contemporary (2007-2011) Spectrums of Tetracyclines (Minocycline, Doxycycline, and Tetracycline) Tested against Enterobacteriaceae and *Acinetobacter* spp.

RN JONES[1], MG STILWELL[1], O LOMOVSKAYA[2], MN DUDLEY[2]
[1]JMI Laboratories, North Liberty, IA, USA; [2]Rempex Pharmaceuticals, San Diego, CA, USA

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

ABSTRACT

Background: For decades tetracycline HCL (TETR) has represented its class for susceptibility (S) testing as an accurate surrogate predictor of doxycycline (DOXY) and minocycline (MINO), but not recognizing superior potencies against *S. aureus* and *Acinetobacter* spp. (ACB). Because of emerging resistance (R) among Gram-negative bacilli (GNB), especially ESKAPE pathogens, we seek better *in vitro* testing guidance for older potentially usable agents. Here we re-evaluate this class, analyzing 62,971 isolates from clinical infections in USA, Latin America (LATAM), Asia-Pacific (APAC) and Europe (EU).

Methods: All S tests used reference methods and published breakpoints. The following species were tested: *E. coli* (EC; 23,977), *Klebsiella* spp. (14,808), *Citrobacter* spp. (2,001), *Enterobacter* spp. (7,441), *Serratia* spp. (SER; 3,525) and ACB (5,478). Accuracy of surrogate TETR tests to predict S, not R was assessed; and coverage (% S) at CLSI breakpoints.

Results: Against Enterobacteriaceae (EB; 57,493), MINO showed superior potencies (MIC_{50}, 2 µg/ml; 73.7% S) over DOXY (64.2%) and TETR (60.3%). Greatest increase of MINO coverage versus TETR was among EC (+20.9%) and SER (+69.1%); also among ACB, MINO % S at 79.1% compared to 59.6 and 30.2% for DOXY and TETR, respectively. Clearly TETR can predict S to other class agents with high accuracy (>99%), but grossly underestimates their potential usefulness via expanded activity (MINO > DOXY). MINO advantages against EB was noted across the world with S highest in USA (MIC_{50}, 2 µg/ml; 78.2% S) >EU (MIC_{50}, 2 µg/ml; 75.6%) > LATAM (MIC_{50}, 2 µg/ml; 68.2%) > APAC (MIC_{50}, 4 µg/ml; 66.3%). MINO was most potent against ACB in LATAM ($MIC_{50/90}$, 0.5/4 µg/ml; 91.7%) > APAC=USA (MIC_{50}, 1-2 µg/ml; 75.1-75.3%) > EU (MIC_{50}, 2 µg/ml; 72.5%).

Conclusions: TETR class agents differ significantly in activity against GNB, especially MINO versus ACB, SER, and some MDR EC. MINO appears to be a potential treatment candidate for problematic MDR ESKAPE species, but requires direct *in vitro* S testing.

Tetracycline (no.)	% inhibited at ≤4 µg/ml[b]:						
	ACB	All EB	EC	KSP	CIT	ENT	SER
	(5,478)	(57,493)	(23,977)	(14,808)	(2,001)	(7,441)	(3,525)
MINO	**79.1**[b]	**73.7**	**78.8**	75.7	84.8	81.4	**77.7**
DOXY	59.6	64.2	61.0	73.6	81.7	81.4	52.8
TETR	30.2	60.3	57.9	74.4	84.2	81.1	8.6

a. All EB=all Enterobacteriaceae; EC=*E. coli*; KSP=*Klebsiella* spp.; CIT=*Citrobacter* spp.; ENT=*Enterobacter* spp.; SER=*Serratia* spp.; and ACB=*Acinetobacter* spp.
b. Bolded values = significantly expanded coverage (% S).

INTRODUCTION

In the mid-1940s, the tetracyclines became the first broad-spectrum antimicrobial class to be described. Derived from various *Streptomyces* species (*S. rimosus* and *S. aureofaciens*), these agents were expanded via semi-synthetic production processes to include tetracycline HCL (dehalogenation), doxycycline, and minocycline. Their mode of action targeted the bacterial ribosomes resulting in the inhibition of protein synthesis. Tetracycline HCL is considered short acting, and doxycycline and minocycline are long acting, each having extended serum half-lives and additionally possess a more potent spectrum against some species, particularly the ESKAPE pathogens *Staphylococcus aureus* (including methicillin-resistant [MRSA]) and *Acinetobacter* spp. (including multidrug-resistant [MDR] strains).

In view of limited choices for the treatment of MDR isolates of *Acinetobacter*, an intravenous formulation of minocycline (Minocin® IV) has been reintroduced into the USA market. Minocycline is among the few antimicrobial agents with FDA approval for the treatment of Acinetobacter infections. Recent publications have described clinical use of this agent in the treatment of a variety of infections due to *Acinetobacter* spp., as there is increasing interest in seeking alternatives to polymyxins in patients with isolates resistant to other antibiotic classes.

The structure for susceptibility testing of tetracyclines has dated from the earliest years of standardized methods development, with breakpoints appearing in the initial interpretive tables of the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards [NCCLS]). Over three decades ago, all tetracyclines were interpreted by a MIC breakpoint of ≤4 µg/ml for susceptibility and ≥12 µg/ml for resistance using correlate disk diffusion interpretive criteria with application to all pathogens. Today, the published criteria vary widely by the pathogen tested and the published international guidelines utilized.

To assess the contemporary differences in potency that exist for tetracyclines, we queried the large organism resistance surveillance collection of the SENTRY Antimicrobial Surveillance Program for 62,971 Gram-negative pathogens (2007-2011) tested by reference MIC methods against three tetracyclines. The data was analyzed using current CLSI breakpoint criteria to detail the potential differences in susceptibility results produced by the often-used concept of testing tetracycline HCL as the surrogate class representative to represent (predict susceptibility) doxycycline and minocycline.

METHODS

Organism collection. All strains (62,971 total) were collected between 2007-2011 from medical centers worldwide (USA, Europe, Latin America and the Asia-Pacific) and sent for reference susceptibility testing (more than 30 antimicrobials). Local identifications were confirmed by the monitoring laboratory using biochemical algorithms and Vitek® 2 under Good Laboratory Practice (GLP)/Clinical Laboratory Improvement Amendments (CLIA) -certified conditions (JMI Laboratories, North Liberty, Iowa, USA).

These organisms included: *Acinetobacter baumannii* (5,478), and 57,493 Enterobacteriaceae. Among the latter group the major species groups were *Escherichia coli* (23,977), *Klebsiella* spp. (14,808), *Enterobacter* spp. (7,441), *Serratia* spp. (3,525), *Proteus mirabilis* (2,662), *Citrobacter* spp. (2,001), Indole-positive *Proteae* (1,958), and another 1,121 isolates representing other species.

Susceptibility testing methods. These selected Gram-negative bacilli were tested for susceptibility to the tetracyclines by reference CLSI (2012) methods. The validated broth microdilution panels were produced under GMP conditions at ThermoFisher Scientific (Cleveland, Ohio, USA). Interpretations of all MIC results applied current CLSI (2013) breakpoints. Quality control (QC) was assured by using CLSI-recommended strains: *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 27853. All QC results were found within published QC ranges (CLSI, 2013).

Analyses were applied to determine i) perceived spectrums of activity (percentage susceptible) for each set of breakpoint criteria, ii) cross-susceptibility accuracy for tetracycline HCL results to predict minocycline (or doxycycline) susceptibility, and iii) cross-susceptibility and -resistance for all categories.

RESULTS

- Tetracyclines tested against *A. baumannii*.
  - Minocycline showed a 79.1% susceptibility rate ($MIC_{50/90}$, 1/8 µg/ml), significantly greater than doxycycline ($MIC_{50/90}$, 2/>8 µg/ml) and tetracycline HCL (only 30.2% susceptible; **Table 1**).
  - Across all regions, minocycline was the most active tetracycline against *A. baumannii*, with activity highest in Latin America (MIC_{50}, 0.5 µg/ml; 91.7% susceptible) and lowest against strains isolated in Europe (MIC_{50}, 2 µg/ml; 72.5% susceptible), see **Table 2**.
- Tetracyclines tested against Enterobacteriaceae.
  - **Table 3** shows that minocycline, doxycycline and tetracycline have similar rates of susceptibility when tested against *Klebsiella* spp. (73.6-75.7%), *Enterobacter* spp. (81.1-81.4%) and *Citrobacter* spp. (81.7-84.8%). However, a significantly wider spectrum/rate of susceptibility was observed for minocycline versus *E. coli* (78.8% versus 57.9-61.0%), *Serratia* spp. (77.7% versus 8.6-52.8%), and all Enterobacteriaceae (73.7% versus 60.3-64.2%).
  - The rank order of potency for the tetracyclines was minocycline > doxycycline > tetracycline HCL (**Tables 1** and **3**).
  - Minocycline was most active against enteric bacilli in the USA and Europe and only slightly less active against isolates from Latin America and the Asia-Pacific region (see **Table 2**).
- Susceptibility testing using tetracycline underestimates minocycline and doxycycline activity.
  - While over 99% of isolates susceptible to tetracycline HCL were also susceptible to minocycline, **minocycline was active at ≤4 µg/ml against an additional 49.0% of isolates that were non-susceptible to tetracycline.**
  - **Figure 1** illustrates the significant differences between these two tetracyclines when testing 5,477 *A. baumannii*. A total of 2,684 tetracycline-intermediate or -resistant isolates were minocycline-susceptible, and an additional 639 strains had an intermediate result (8 µg/ml) for minocycline (11.7%).

Figure 1. Cross-susceptibility analysis scattergram comparing minocycline MIC results to those of tetracycline, the class representative when testing 5,477 *A. baumannii*.

Minocycline MIC (µg/ml) \ Tetracycline MIC (µg/ml)	≤2	4	8	>8
>8			3*	497
8		4*	1	638*
4		5		806
2	5	6	7*	480
1	10	36	128*	339
0.5	52	180	388*	274
0.25	185	156	143*	86
0.12	381	117	17*	11
≤0.06	487	30	1*	4

Note that 2,000 (36.5%) false-resistance (major) errors (red highlight) were observed, as well as 24.3% minor interpretive errors (*).

Table 1. Comparative activity of tetracyclines tested against 5,478 *A. baumannii* strains from worldwide surveillance programs (2007-2011)[a].

Antimicrobial agent (no. tested)	Cum. % inhibited at MIC (µg/ml):							MIC	
	≤0.12	0.25	0.5	1	2	4	8	50%	90%
Minocycline (5,478)	19.1	29.5	45.9	59.2	64.3	79.1[b]	90.9	1	8
Doxycycline (5,477)	17.7	24.6	38.1	48.4	56.4	59.6[b]	61.3	2	>8
Tetracycline (5,477)	-	0.1	0.8	4.1	20.5	30.2[b]	42.8	>8	>8

a. Susceptibility to polymyxin B or colistin was >98% at ≤2 µg/ml. All other agents had very low susceptibility rates at ≤38.6% (includes amikacin [34.4% susceptible], ampicillin/sulbactam [25.9%], cefepime [21.9%], ceftazidime [20.8%], gentamicin [29.5%], imipenem [37.4%], levofloxacin [21.8%], meropenem [36.4%], piperacillin/tazobactam [17.7%] and tobramycin [38.6%]). Tigecycline inhibited 80.7% of strains at ≤1 µg/ml.
b. CLSI breakpoints (2013).

Table 2. Geographic variations of minocycline activity directed against *A. baumannii* (5,478) and all Enterobacteriaceae (57,493).

Organism/Parameter	Region			
	USA	Europe	Latin America	Asia-Pacific
A. baumannii				
(no. tested)	(760)	(1,196)	(1,498)	(2,024)
MIC (µg/ml)				
50%	1	2[a]	0.5[b]	2
90%	>8	>8	4	8
% inhibited				
≤2 µg/ml	66.1	57.3	88.2	50.2
≤4 µg/ml	75.1	72.5[a]	91.7[b]	75.3
≤8 µg/ml	89.6	85.3	95.5	91.2
Enterobacteriaceae				
(no. tested)	(18,507)	(20,430)	(7,075)	(11,481)
MIC (µg/ml)				
50%	2[b]	2	2	4[a]
90%	>8	>8	>8	>8
% inhibited				
≤2 µg/ml	64.6	61.6	52.4	45.1
≤4 µg/ml	78.2[b]	75.6	68.2	66.3[a]
≤8 µg/ml	85.8	84.3	79.0	78.6

a. Lowest activity for minocycline among the four monitored regions.
b. Minocycline had greatest activity in this region.

Table 3. Activity of minocycline and other tetracyclines tested against 57,493 Enterobacteriaceae (2007-2011 samples, worldwide).

Organism (no. tested)/ antimicrobial agent	Cum. % inhibited at MIC (µg/ml)[a]:			
	≤1	2	4	8
Enterobacteriaceae (57,493)				
Minocycline	31.7	58.1	73.7[b]	83.0
Doxycycline	30.0	54.8	64.2	73.4
Tetracycline	-	55.2	60.3	63.8
E. coli (23,977)				
Minocycline	53.2	70.3	78.8[b]	87.4
Doxycycline	41.2	56.6	61.0	72.0
Tetracycline	-	56.1	57.9	58.2
Klebsiella spp. (14,808)				
Minocycline	21.7	59.9	75.7	84.6
Doxycycline	34.3	65.4	73.6	78.9
Tetracycline	-	65.3	74.4	78.1
Enterobacter spp. (7,441)				
Minocycline	12.2	54.2	81.4	88.6
Doxycycline	12.2	63.2	81.4	87.8
Tetracycline	-	71.7	81.1	85.2
Serratia spp. (3,525)				
Minocycline	3.8	30.9	77.7[b]	94.4
Doxycycline	2.6	19.9	52.8	85.2
Tetracycline	-	1.2	8.6	34.1
Citrobacter spp. (2,001)				
Minocycline	37.5	71.9	84.8	90.5
Doxycycline	30.8	71.4	81.7	87.1
Tetracycline	-	79.6	84.2	86.8

a. Breakpoint at ≤4 µg/ml (CLSI, 2013).
b. A statistically significant greater susceptibility rate compared to peer tetracyclines (p <0.05) was noted, see underline.

CONCLUSIONS

- Minocycline activity against a contemporary collection of worldwide isolates of *A. baumannii* and Enterobacteriaceae demonstrates a significantly greater potency and spectrum than either doxycycline or tetracycline (class representative for *in vitro* susceptibility testing). In particular, Acinetobacter has a higher susceptibility rate to minocycline when compared to other commonly used, first-line drug classes such as carbapenems, fluoroquinolones, and aminoglycosides.
- As recommended by CLSI documents, **minocycline should be routinely tested by clinical microbiology laboratories against *Acinetobacter* spp.**, particularly in institutions where MDR isolates are frequent.
- Use of tetracycline susceptibility data as a surrogate agent will also significantly **underestimate** Enterobacteriaceae susceptibility to minocycline. Thus, it is recommended that laboratories test minocycline using established reference methods to determine its potential usefulness on individual clinical isolates.
- Minocycline (particularly in its IV formulation) is a potential treatment entity for several ESKAPE pathogens found in critically ill patients, particularly in patients where choices are limited due to MDR or toxicities.

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