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Role of an Older Tetracycline for Treatment of Multidrug-resistant *Acinetobacter* spp. Infections: Understanding the Enhanced Potency of Minocycline!

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ABSTRACT

Background: An intravenous formulation of minocycline (MINO) has recently become available for clinical use. In contrast to numerous older tetracyclines, MINO has high measurable activity against *Acinetobacter* spp. (ACB). To quantify MINO activity against contemporary (2007-2011) isolates and its potential therapeutic role, a collection of 5,478 ACB were analyzed compared to tetracycline (TETR), doxycycline (DOXY) and numerous other agents.

Methods: ACB collections (year 2007-2011) from the USA (760 strains), Europe (EU; 1,196), Latin America (LATAM; 1,498) and Asia-Pacific (APAC; 2,024) were susceptibility (S) tested using reference CLSI broth microdilution methods and CLSI/USA-FDA breakpoints (BPs). Cross-S calculations using TETR as a surrogate marker for MINO- and DOXY-S were also applied (target accuracy at >95%).

Results: Activity of tetracyclines rank MINO ($MIC_{50/90}$, 1/8 µg/ml) > DOXY ($MIC_{50/90}$, 2/>8 µg/ml) > TETR ($MIC_{50/90}$, >8/>8 µg/ml). MINO inhibited 64.3, 79.1 and 90.9% of ACB at ≤2, ≤4 (CLSI/USA-FDA BP), and ≤8 µg/ml, respectively; 19-49% greater than other class agents. MINO and tigecycline had comparable inhibition rates (46-48%) at ≤0.5 µg/ml. MINO coverage of ACB strains was higher (% ≤4 µg/ml in LATAM [MIC_{50}, 0.5 µg/ml; 91.7%] > APAC=USA [MIC_{50}, 1-2 µg/ml; 75.1-75.3%] > EU [MIC_{50}, 2 µg/ml; 72.5%]). DOXY and TETR only inhibited (≤4 µg/ml) 30.2 to 59.6% of all ACB. The most potent agents tested were colistin (MIC_{90}, 1 µg/ml) and polymyxin B (MIC_{90}, 0.5 µg/ml), all other S rates ranged from only 17.7% (piperacillin/tazobactam [P/T]) to 39.4% (imipenem [IMP]); see **Table**. Finally, TETR used as a surrogate to predict S to DOXY and MINO using a ≤4 µg/ml BP was highly accurate (99.76% [MINO] and 99.70% [DOXY]), but those results grossly underestimated the potential MINO use against ACB isolates (**Table**).

Conclusions: Current surveillance data identifies MINO as a strong candidate for infections caused by ACB among USA-FDA-approved agents; and coverages (% S) potentially ranging from 64.3-90.9% (≤2 to ≤8 µg/ml) as dictated by ECOFF values and CLSI/USA-FDA BPs. MINO should be considered for intravenous treatment of serious ACB infections; but guided by direct MINO testing, not surrogate TETR marker S results.

Antimicrobial (no.= 5,478)	Cum.% inhibited at MIC (µg/ml):								
	≤0.06	0.12	0.25	0.5	1	2	4	8	16
MINO	10	19	30	46	55	64	**79[a]**	91	.[b]
DOXY	-	18	25	38	48	56	**60[a]**	61	-
TETR	-	-	-	-	-	21	**30[a]**	43	-
AMK[c]	-	-	<1	<1	3	14	26	32	**34[a]**
A/S[c]	-	-	-	-	-	12	19	**26[a]**	37
P/T[c]	-	-	-	6	8	9	12	15	**18[a]**
IMI[c]	-	3	14	22	28	34	**37[a]**	40	-

a. Breakpoint concentrations (CLSI and USA-FDA), bolded.
b. "-" = untested concentration.
c. 10 comparison drugs (amikacin [AMK], ampicillin/sulbactam [A/S], cefepime, ceftazidime, ciprofloxacin, gentamicin, IMI, levofloxacin, meropenem, and P/T) had S rates at ≤37.4%, like the TETR-S rate at ≤4 µg/ml.

INTRODUCTION

The tetracyclines during the 1940s became the first broad-spectrum antimicrobial class to be described. These compounds were derived from *Streptomyces* species (*S. rimosus* and *S. aureofaciens*), and this class was expanded by semi-synthetic processes to include tetracycline HCL, doxycycline, and minocycline. Tetracycline HCL is considered short-acting, and doxycycline and minocycline are long-acting each having extended serum half-lives, and additionally possess more potent spectrums against some species, particularly non-fermentative Gram-negative bacilli such as *Acinetobacter* spp. (including multidrug-resistant strains).

In view of limited choices for the treatment of MDR isolates of Acinetobacter, an intravenous formulation of minocycline (Minocin® IV) has been reintroduced onto the United States (USA) market. Minocycline is among the few antimicrobial agents with USA-Food and Drug Administration (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 antimicrobial 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 activity of tetracyclines and other antimicrobial agents against non-fermentative Gram-negative pathogens other than Pseudomonas, we queried the large organism resistance surveillance collection of the SENTRY Antimicrobial Surveillance Program (2007-2011) for over 5,000 *Acinetobacter baumannii*, *Stenotrophomonas maltophilia* (1,706 strains), and *Burkholderia cepacia* (191 strains). Each organism was tested by reference MIC methods against three tetracyclines, as well as other comparison agents. The data was analyzed using current CLSI breakpoint criteria where available.

METHODS

Organism collections. All strains (7,374 total) were collected between 2007-2011 from medical centers worldwide (USA, Europe, Latin America [LATAM] and the Asia-Pacific [APAC]) and sent for reference susceptibility testing (more than 30 antimicrobials were assessed). 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: *A. baumannii* (5,478) and two other species. Among the latter group the major species groups were *S. maltophilia* (1,706 strains) and *B. cepacia* (191 strains).

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: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 27853. All QC results were observed to be within published QC ranges (CLSI, 2013).

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

RESULTS

- Minocycline activity was compared to peer tetracyclines when tested against three non-fermentative Gram-negative bacilli (**Table 1**). Minocycline potencies (MIC_{50} range, 0.5-2 µg/ml) were two- to four-fold and ≥ eight-fold greater than doxycycline and tetracycline HCL against these three species, respectively. At CLSI susceptibility breakpoints, minocycline susceptibility was 79.1, 83.3 and 98.9% for *A. baumannii*, *B. cepacia* and *S. maltophilia,* respectively. Overall, minocycline coverage for *A. baumannii* (79.1% susceptible) was 29.5% more than doxycycline (59.6%), and 58.9% more than tetracycline HCL (**Table 1**).
- *A. baumannii* isolates were generally less susceptible to other classes of agents listed in the current CLSI interpretive tables (**Table 2**). Minocycline was the second most active (79.1% susceptible) agent, only exceeded by colistin (98.8% susceptibility). All other classes of agents had susceptibility less than 42.0% (tobramycin).
- For *B. cepacia*, only a limited number of agents had susceptibility rates exceeding 80.0%: (minocycline [83.3%], ceftazidime [89.0%], TMP/SMX [94.8%]; see **Table 2**). Similarly, only two agents (minocycline [98.9% susceptible], TMP/SMX [96.7-98.5%]) demonstrated broad coverage of *S. maltophilia.*
- **Table 3** compares the differing rates of minocycline susceptibility and potencies across the four sampled geographic regions. For *A. baumannii*, lowest and highest coverage was noted for Europe (72% susceptible to ≤4 µg/ml) and Latin America (92% susceptible), respectively. For *B. cepacia*, minocycline was most active against USA isolates (88% susceptible to ≤4 µg/ml). Susceptibility to *S. maltophilia* (≤4 µg/ml) was similar and exceeded 98% across all regions. Across all regions and pathogens analyzed (**Table 3**), the minocycline MIC_{50} only varied from 0.5 to 2 µg/ml.

Table 1. Comparative activity of minocycline and other tetracyclines tested against *A. baumannii*, *S. maltophilia* and *B. cepacia* strains from a worldwide surveillance program (2007-2011).

Species (no. tested) / Antimicrobial agent	Cum. % inhibited at MIC (µg/ml):							MIC (µg/ml)	
	≤0.12	0.25	0.5	1	2	4	8	50%	90%
A. baumannii (5,478)[a]									
Minocycline	19.1	29.5	45.9	59.2	64.3	79.1[b]	90.9	1	8
Doxycycline	17.7	24.6	38.1	48.4	56.4	59.6[b]	61.3	2	>8
Tetracycline	-	0.1	0.8	4.1	20.5	30.2[b]	42.8	>8	>8
B. cepacia (191)									
Minocycline	2.1	3.1	10.5	31.4	54.2	83.3[b]	92.2	2	8
Doxycycline	2.1	7.3	12.6	21.5	37.7	64.9	81.2	4	>8
Tetracycline	-	0.0	1.1	1.8	10.0	15.8	16.8	>8	>8
S. maltophilia (1,706)									
Minocycline	7.6	33.7	67.5	87.3	96.0	98.9[b]	99.9	0.5	2
Doxycycline	0.1	0.8	5.1	31.1	75.5	94.7	98.5	2	4
Tetracycline	-	<0.1	<0.1	0.1	0.5	3.1	22.0	>8	>8

a. All other agents had very low susceptibility rates at ≤41.9% (includes amikacin [34.4% susceptible], cefepime [21.9%], ceftazidime [20.8%], gentamicin [29.5%], imipenem [37.4%], levofloxacin [21.8%], meropenem [36.4%], piperacillin/tazobactam [17.7%] and tobramycin [41.9%], see Table 2; and tigecycline inhibited 80.7% of strains at ≤1 µg/ml.
b. CLSI breakpoints (2013).

Table 2. Minocycline activity compared to selected agents tested against three species of non-fermentative Gram-negative bacilli, (2007-2011); interpretations by CLSI and EUCAST criteria.

Pathogen (no. tested)/ Antimicrobial agent	MIC (µg/ml):		% susceptible by:	
	50%	90%	CLSI	EUCAST[a]
A. baumannii (5,478)				
Minocycline	1	8	79.1	-
Doxycycline	2	>8	59.6	-
Tetracycline HCL	>8	>8	30.2	-
Piperacillin/Tazobactam	>64/4	>64/4	17.7	-
Ceftazidime	>16	>16	20.8	-
Cefepime	>16	>16	21.9	-
Ceftriaxone	>8	>8	7.2	-
Imipenem	>8	>8	37.4	34.3
Meropenem	>8	>8	36.4	32.8
Amikacin	>32	>32	34.4	31.7
Gentamicin	>8	>8	29.5	29.5
Tobramycin	>16	>16	41.9	41.9
Ciprofloxacin	>4	>4	20.5	20.5
Levofloxacin	>4	>4	21.8	21.0
TMP/SMX[b]	>2/38	>2/38	28.5	28.5
Colistin	≤0.5	1	98.8	98.8
B. cepacia (191)				
Minocycline	2	8	83.3	-
Doxycycline	4	>8	-	-
Tetracycline HCL	>8	>8	-	-
Ticarcillin/Clavulanate	>128	>128	6.8	6.8
Ceftazidime	2	16	89.0	89.0
Levofloxacin	2	>4	67.0	35.6
TMP/SMX[b]	≤0.5/9.5	2/38	94.8	-
S. maltophilia (1,706)				
Minocycline	0.5	2	98.9	-
Doxycycline	2	4	-	-
Tetracycline HCL	>8	>8	-	-
Ticarcillin/Clavulanate	32	>128	35.9	35.9
Ceftazidime	16	>16	41.2	41.2
Levofloxacin	1	>4	79.1	56.5
TMP/SMX[b]	≤0.5/9.5	1	96.7	98.5

a. Breakpoint criteria for EUCAST were as published for *Acinetobacter* spp. and TMP/SMX for *S. maltophilia*; all others were those used for *Pseudomonas* spp., but applied to *B. cepacia* and *S. maltophilia* (no breakpoints are found in EUCAST version 3.0).
b. TMP/SMX = trimethoprim/sulfamethoxazole.

- Testing tetracycline HCL as a surrogate for other tetracyclines fails to recognize the potential utility of minocycline (**Table 4**) among contemporary *A. baumannii*. For the 5,478 strains tested, only 1,654 were susceptible to tetracycline HCL, **but an additional 2,684 isolates were minocycline-susceptible** (**Table 4**). The number of *A. baumannii* strains resistant (MICs, >8 µg/ml) was markedly different between tetracycline HCL (3,135), doxycycline (2,119) and minocycline (only 500; see **Table 4**).

Table 3. Geographic variations of minocycline activity directed against *A. baumannii*, *B. cepacia* and *S. maltophilia* (SENTRY Program; 2007-2011).

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
B. cepacia				
(no. tested)	(34)	(29)	(37)	(91)
MIC (µg/ml)				
50%	1[b]	2	2[a]	2
90%	8	8	>8	8
% inhibited				
≤2 µg/ml	70.6	65.5	59.5	52.8
≤4 µg/ml	88.2[b]	82.8	78.4[a]	83.5
≤8 µg/ml	94.1	90.0	86.5	94.5
S. maltophilia				
(no. tested)	(607)	(479)	(183)	(437)
MIC (µg/ml)				
50%	0.5	0.5	0.5[b]	0.5[a]
90%	2	2	1	2
% inhibited				
≤2 µg/ml	96.4	97.1	96.7	93.8
≤4 µg/ml	99.5	99.0	100.0[b]	97.7[a]
≤8 µg/ml	100.0	99.8	100.0	99.8

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

Table 4. Correlations (accuracy) of tetracycline MIC results to predict minocycline or doxycycline susceptibility when testing *A. baumannii* (5,478 strains)[a].

Antimicrobial agent predicted	MIC (µg/ml)	Tetracycline MIC (µg/ml):			
		≤2	4	8	>8
Minocycline	>8			3	497
	8		4	1	638
	4		5[b]	0	806[c]
	2	5[b]	6[b]	7[c]	480[c]
	1	10[b]	36[b]	128[c]	339[c]
	≤0.5	1,105[b]	483[b]	549[c]	375[c]
Doxycycline	>8	1	4	1	2,113
	8		0	4	90
	4		5[b]	2[c]	166[c]
	2	6[b]	3[b]	16[c]	414[c]
	1	9[b]	27[b]	231[c]	298[c]
	≤0.5	1,104[b]	495[b]	433[c]	54[c]

a. Horizontal and vertical lines show the breakpoint concentrations for each agent (≤4 µg/ml = susceptible; >8 µg/ml = resistant).
b. Number of strains having tetracycline MIC values at ≤4 µg/ml (susceptible) and also susceptible to minocycline (99.76% accuracy) or doxycycline (99.70% accuracy).
c. False non-susceptible strains for minocycline (2,684 occurrences; 49.0%) and doxycycline (1,624 occurrences; 29.7%).

CONCLUSIONS

- Minocycline among tetracycline agents exhibits broad coverage of important non-*P. aeruginosa* non-fermentative species.
  - *A. baumannii* ($MIC_{50/90}$, 1/8 µg/ml; 79.1% susceptible to ≤4 µg/ml)
  - *B. cepacia* ($MIC_{50/90}$, 2/8 µg/ml; 83.3% susceptible to ≤4 µg/ml)
  - *S. maltophilia* ($MIC_{50/90}$, 0.5/2 µg/ml; 98.9% susceptible to ≤4 µg/ml)
- Among antimicrobials considered as candidate regimens for these studied species, only minocycline had susceptibility rates exceeding 79% (range, 79.1-98.9%) versus all three organisms. This is in contrast to colistin susceptibility at ≤2 µg/ml at only 2.1 and 33.4% against *B. cepacia* and *S. maltophilia*, respectively. Also TMP/SMX was not active versus *A. baumannii*, and ceftazidime only had significant activity against *B. cepacia* (89.0% susceptible).
- Minocycline should not be tested by a surrogate class representative (tetracycline HCL), but should be tested directly by CLSI reference methods using the appropriate interpretive criteria to guide treatment caused by these non-fermentative species, where there are often limited choices of antimicrobials.

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