The Impact of Non-Standard Test Conditions on the *In Vitro* Activity of Omadacycline by Broth Microdilution

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ABSTRACT

Background: As part of antibiotic development, it is important to understand the effect of modification of standard testing parameters on the in vitro activity observed during broth microdilution susceptibility testing. Omadacycline (OMC) is a novel broad spectrum aminomethylcycline under development for the treatment of severe community-acquired infections, including acute bacterial skin and skin structure infections and community acquired bacterial pneumonia. In this study, the impact of non-standard test medium, pH, cation concentration, atmosphere of incubation, incubation time, and inoculum size on the activity of OMC was evaluated relative to standard test conditions specified by the Clinical and Laboratory Standards Institute (CLSI).

Methods: Broth microdilution susceptibility testing of OMC and the comparator levofloxacin (LVX) was conducted under standard conditions in accordance with CLSI M7-A10 and M100-S26. In parallel, concurrent inocula of each test organism were tested with the following modifications: altered medium pH (pH 5.0, 6.0, and 8.0), incubation in 5% CO2, altered divalent cation concentration (varied [Ca2+] and [Mg2+]), low (5 x 10^4 CFU/mL) and high (5 x 10^6 CFU/mL) inoculum size, prolonged incubation (24 to 48 hr), testing in the presence of surfactant (0.002% v/v polysorbate-80), and testing in non-standard media. Test organisms included isolates from the American Type Culture Collection: *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619, *E. coli* ATCC 25922, and *H. influenzae* ATCC 49247. When compared, differences in minimal inhibitory concentration (MIC) between the non-standard and standard test condition greater than 2-fold were considered indicative of a variation in activity.

Results: MIC values for OMC and LVX were within quality control limits under standard conditions during testing. There was no apparent impact on the in vitro activity of OMC or LVX under the following non-standard test conditions with OMC or LVX MIC values at or within 2-fold of those observed under standard conditions: altered divalent cation concentration, altered atmosphere of incubation, prolonged incubation, testing at high pH, testing with low inoculum density, and supplementation of test medium with polysorbate-80. Like LVX, OMC MIC values were at least 4-fold higher when tested at low pH relative to standard pH and at high inoculum density relative to standard inoculum density for *S. aureus*. OMC MIC values were also higher in *Haemophilus* test medium (HTM) when used to test non-*Haemophilus* isolates relative to standard media.

Conclusions: Overall, the activity of OMC was largely stable by broth microdilution under non-standard test parameters, with the exception of low pH and increased inoculum size where MIC values were higher relative to standard conditions. Similar results were observed with LVX. These results highlight the importance of conducting broth microdilution susceptibility testing of OMC in accordance with CLSI guidelines using media at the correct pH and the correct inoculum size.

BACKGROUND

- Omadacycline (OMC) is an aminomethylcycline currently undergoing phase 3 clinical development by Paratek Pharmaceuticals as a once daily oral or IV treatment of community acquired skin and respiratory infections.
- Prior to widespread disseminated susceptibility testing, it is important to evaluate the impact of potential variations to the standard CLSI susceptibility testing methodology on the perceived *in vitro* activity of a new agent.
- Standard susceptibility test parameters that can potentially effect MIC test results when varied include:
  - Medium pH, cation concentration, supplementation
  - Inoculum size
  - Incubation duration and atmosphere
- Prior studies have demonstrated that media age effects the *in vitro* activity of omadacycline[1] and tigecycline[2], thus the use of freshly made media is specified for the susceptibility testing of both of these agents[3,4].

PURPOSE

To determine the impact, if any, on the *in vitro* activity of omadacycline when standard CLSI susceptibility test conditions are modified.

METHODS

- Test organisms included standard quality control isolates from the American Type Culture Collection (ATCC): *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619, *E. coli* ATCC 25922, and *H. influenzae* ATCC 49247.
- Antibiotics tested included OMC and levofloxacin (LVX).
- MICs were determined for duplicate independent inocula by broth microdilution in accordance with Clinical and Laboratory Standards Institute (CLSI) guideline M7-A10[5] and M100-S26[3] using freshly prepared cation-adjusted Mueller-Hinton broth (CAMHB) under standard and non-standard conditions in parallel.
- Testing conditions were modified as follows:
  - Media pH adjusted to 5, 6, and 8 (standard = 7.2-7.4)
  - Media with altered divalent cation concentration (standard = 10-12.5 mg/L Mg^{2+}, 20-25 mg/L Ca^{2+})
  - Media supplemented with 0.002% v/v polysorbate-80
  - Use of non-standard test media
  - Low (~5 x 10^4 CFU/mL) and high (~5 x 10^6 CFU/mL) inoculum size (standard = (~5 x 10^5 CFU/mL)
  - Prolonged incubation and altered atmosphere

RESULTS

Table 1. Impact of media pH

Organism	Drug	MIC (mg/L)			
		pH 5.0	pH 6.0	pH 7.4	pH 8.0
S. aureus ATCC 29213	OMC	16, 16	1, 1	0.5, 0.5	0.5, 0.5
	LVX	4, 2	0.25, 0.25	0.25, 0.25	0.25, 0.25
E. faecalis ATCC 29212	OMC	1, 1	0.5, 0.5	0.12, 0.12	0.12, 0.12
	LVX	8, 8	2, 2	1, 1	1, 1
S. pneumoniae ATCC 49619	OMC	NG	0.03, 0.03	0.03, 0.015	0.015, 0.015
	LVX	NG	1, 1	0.5, 0.5	0.5, 0.5
E. coli ATCC 25922	OMC	16, 16	2, 2	0.5, 0.5	1, 1
	LVX	1, 1	0.12, 0.12	0.015, 0.015	0.03, 0.015
H. influenzae ATCC 49247	OMC	NG	1, 1	2, 1	2, 1
	LVX	NG	0.015, NG	0.015, 0.015	0.015, 0.015

MICs in orange font indicate a ≥4-fold increase in MIC relative to standard conditions (grey shaded cells)
NG = no growth

Table 2. Impact of divalent cation concentration (mg/L)

Organism	Drug	MIC (mg/L)				
		5 Ca^{2+} 5 Mg^{2+}	25 Ca^{2+} 5 Mg^{2+}	5 Ca^{2+} 12.5 Mg^{2+}	25 Ca^{2+} 12.5 Mg^{2+}	50 Ca^{2+} 25 Mg^{2+}
S. aureus ATCC 29213	OMC	0.5, 0.5	0.5, 0.5	0.5, 0.5	0.5, 0.5	0.5, 1
	LVX	0.25, 0.25	0.25, 0.25	0.25, 0.25	0.25, 0.25	0.25, 0.25
E. faecalis ATCC 29212	OMC	0.12, 0.12	0.12, 0.12	0.12, 0.12	0.25, 0.25	0.25, 0.25
	LVX	1, 1	1, 1	1, 1	1, 1	1, 1
S. pneumoniae ATCC 49619	OMC	0.03, 0.03	0.03, 0.03	0.03, 0.03	0.06, 0.06	0.06, 0.06
	LVX	1, 1	1, 1	1, 1	1, 1	1, 1
E. coli ATCC 25922	OMC	0.5, 0.5	0.5, 0.5	0.5, 0.5	1, 0.5	1, 1
	LVX	0.03, 0.03	0.03, 0.03	0.03, 0.03	0.03, 0.03	0.03, 0.03
H. influenzae ATCC 49247	OMC	2, 4	2, 2	2, 2	2, 2	2, 2
	LVX	0.03, 0.03	0.015, 0.015	0.015, 0.015	0.03, 0.03	0.015, 0.03

MICs as determined under standard conditions are shown in the grey shaded cells

Table 3. Impact of testing in media with polysorbate-80

Organism	Drug	MIC (mg/L)	
		w/out polysorbate-80	w/ 0.002% (v/v) polysorbate-80
S. aureus ATCC 29213	OMC	0.5, 0.5	1, 1
	LVX	0.25, 0.25	0.25, 0.25
E. faecalis ATCC 29212	OMC	0.25, 0.12	0.12, 0.12
	LVX	1, 1	1, 1
S. pneumoniae ATCC 49619	OMC	0.015, 0.015	0.015, 0.015
	LVX	0.5, 0.5	0.5, 0.5
E. coli ATCC 25922	OMC	0.5, 0.5	0.5, 0.5
	LVX	0.015, 0.015	0.015, 0.015
H. influenzae ATCC 49247	OMC	2, 2	2, 2
	LVX	0.015, 0.015	0.015, 0.015

MICs as determined under standard conditions are shown in the grey shaded cells

Table 4. Impact of testing in non-standard media

Organism	Drug	MIC (mg/L)		
		CAMHB	CAMHB + 3% LHB	HTM
S. aureus ATCC 29213	OMC	0.5, 0.5	0.25, 0.25	1, 1
	LVX	0.25, 0.25	0.25, 0.25	0.25, 0.25
E. faecalis ATCC 29212	OMC	0.25, 0.12	0.12, 0.12	1, 1
	LVX	1, 1	1, 1	1, 1
S. pneumoniae ATCC 49619	OMC	0.03, 0.03	0.015, 0.015	0.5, 0.5
	LVX	1, 0.5	0.5, 0.5	0.5, 0.5
E. coli ATCC 25922	OMC	0.5, 0.5	0.5, 0.5	2, 2
	LVX	0.015, 0.015	0.03, 0.015	0.03, 0.015
H. influenzae ATCC 49247	OMC	0.12, 0.25[1]	0.5, 0.5	2, 2
	LVX	0.008, 0.008	0.015, 0.015	0.015, 0.015

[1] poor growth of the test isolate in the panel was observed under this test condition
MICs in orange font indicate a ≥4-fold increase in MIC relative to standard conditions (grey shaded cells)
MICs in blue font indicate a ≥4-fold decrease in MIC relative to standard conditions (grey shaded cells)
CAMHB = cation-adjusted Mueller-Hinton broth; LHB = lysed horse blood; HTM = *Haemophilus* test medium

Table 5. Impact of inoculum size (CFU/mL)

Organism	Drug	MIC (mg/L)		
		~ 5 x 10^4	~ 5 x 10^5	~ 5 x 10^6
S. aureus ATCC 29213	OMC	0.25, 0.25	0.5, 0.5	>16, >16 (0.25)[1]
	LVX	0.12, 0.25	0.25, 0.25	>8, >8 (1)[1]
E. faecalis ATCC 29212	OMC	0.12, 0.12	0.25, 0.12	0.25, 0.25
	LVX	1, 1	1, 1	1, 1
S. pneumoniae ATCC 49619	OMC	0.03, 0.03	0.015, 0.015	0.03, 0.03
	LVX	0.5, 0.5	0.5, 0.5	0.5, 1
E. coli ATCC 25922	OMC	0.5, 0.5	0.5, 0.5	1, 1
	LVX	0.03, 0.03	0.015, 0.015	0.5, 0.5 (0.03)[1]
H. influenzae ATCC 49247	OMC	1, 1	2, 2	4, 4
	LVX	0.008, 0.015	0.015, 0.015	0.03, 0.03

[1] MIC shown in parenthesis interpreted based on >80% inhibition of growth
MICs in orange font indicate a ≥4-fold increase in MIC relative to standard conditions (grey shaded cells)

Table 6. Impact of incubation duration and atmosphere

Organism	Drug	MIC (mg/L)				
		incubation time			incubation atmosphere	
		18 hr	24 hr	48 hr	ambient air	5% CO_2
S. aureus ATCC 29213	OMC	0.5, 0.5	0.5, 0.5	1, 1	0.5, 0.5	0.25, 0.25
	LVX	0.25, 0.25	0.25, 0.25	0.25, 0.25	0.25, 0.25	0.12, 0.12
E. faecalis ATCC 29212	OMC	0.12, 0.12	0.12, 0.12	0.25, 0.25	0.25, 0.12	0.25, 0.12
	LVX	1, 1	1, 1	2, 1	1, 1	1, 1
S. pneumoniae ATCC 49619	OMC	0.03, 0.015	0.03, 0.015	0.06, 0.03	0.015, 0.015	0.03, 0.03
	LVX	0.5, 0.5	0.5, 0.5	1, 1	0.5, 0.5	0.5, 0.5
E. coli ATCC 25922	OMC	0.5, 0.5	0.5, 0.5	0.5, 0.5	0.5, 0.5	1, 1
	LVX	0.015, 0.015	0.03, 0.015	0.03, 0.03	0.015, 0.015	0.03, 0.03
H. influenzae ATCC 49247	OMC	2, 1	2, 1	2, 2	2, 2	2, 2
	LVX	0.015, 0.015	0.015, 0.015	0.015, 0.015	0.015, 0.015	0.015, 0.015

MICs as determined under standard conditions are shown in the grey shaded cells

- MICs were within established quality control ranges[3] for both omadacycline and levofloxacin during testing under standard conditions (grey shaded cells).
- Based on MICs that were identical or within 2-fold of those observed under standard conditions, there was no impact on the activity of omadacycline and levofloxacin for the following non-standard conditions: higher than standard media pH (**Table 1**), varied divalent cation concentration in test media (**Table 2**), supplementation of test media with polysorbate-80 (**Table 3**), inoculation with lower than standard inoculum size (**Table 5**), prolonged incubation (**Table 6**), and incubation in 5% CO_2 (**Table 6**).
- Both omadacycline and levofloxacin MICs were higher when testing *S. aureus* ATCC 29213 with an elevated inoculum size of approximately 5 x 10^6 CFU/mL but this effect was not observed with omadacycline for other isolates (**Table 5**).
- Both omadacycline and levofloxacin MIC values were several-fold higher when testing in media at pH 5.0 relative to standard media for the isolates which were able to grow at that pH (**Table 1**). This increase was also apparent to a lesser degree at pH 6.0 for *E. faecalis* ATCC 29212 and *E. coli* ATCC 25922.
- There was a trend towards higher omadacycline MICs for non-*Haemophilus* isolates when tested in HTM (**Table 4**); for *H. influenzae* lower MICs were observed in CAMHB and CAMHB with blood though this could be due to less than optimal growth of *H. influenzae* in these media compared to HTM.

CONCLUSIONS

- The activity of omadacycline was largely stable by broth microdilution for the evaluated isolates even with variation to standard test parameters. Similar results were observed with levofloxacin.
- However, omadacycline MICs were negatively impacted when testing in media with lower than standard pH and, in the case of *S. aureus*, when testing with a larger than standard inoculum size.
- These results highlight the importance of adhering to CLSI guidelines[3,6], in particular media pH and inoculum density but also using freshly made media[1], for the broth microdilution susceptibility testing of omadacycline.

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