In Vitro Activity of Omadacycline against *E. coli* Biofilms

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Abstract

The goal of the current study was to investigate the anti-biofilm properties of omadacycline (OMC), a first-in-class compound of a new class of aminomethylcycline antibiotics. OMC exhibits potent bacteriostatic activity against both Gram-negative and Gram-positive bacteria and is currently in Phase III clinical trials for multiple indications. Microbial biofilms are defined by a dense extracellular polymeric substance that can act as a physical barrier to the external environment, and results in a subpopulation of metabolically quiescent microbes, which can lead to a remarkable tolerance to antibiotics that normally kill planktonic bacteria. Given that OMC exhibits activity against a broad spectrum of organisms and has potential utility against organisms that produce biofilms, the current study aimed to establish the baseline anti-biofilm properties of OMC. Focusing on *Escherichia coli* (ATCC 25922), which is a common cause of community-acquired infections, doses of OMC above and below the minimum inhibitory concentration (MIC) for planktonic cells were assessed for biofilm prevention and/or induction, and against established biofilms using the MBEC™ 96 well plate high throughput screening device. In experiments using sub-MIC and supra-MIC doses of OMC applied to early-phase and established biofilms in the presence of planktonic bacteria, sub-MIC doses (e.g., 0.74 µg/mL) do not appear to induce further biofilm propagation above the levels observed in the absence of OMC. Further, biofilm prevention/induction assays indicate that OMC strongly inhibits biofilm formation at all doses above the MIC. At sub-MIC doses (e.g., 0.74 µg/mL), mean biofilm formation is reduced by approximately 3 to 4 log units compared to controls, in high and low inoculum conditions, respectively. In contrast to other translation inhibitors, sub-MIC doses of OMC not only failed to induce biofilm formation above the level of controls, several doses below the MIC appear to have an inhibitory effect on biofilm formation.

Overview of the MBEC™ Assay

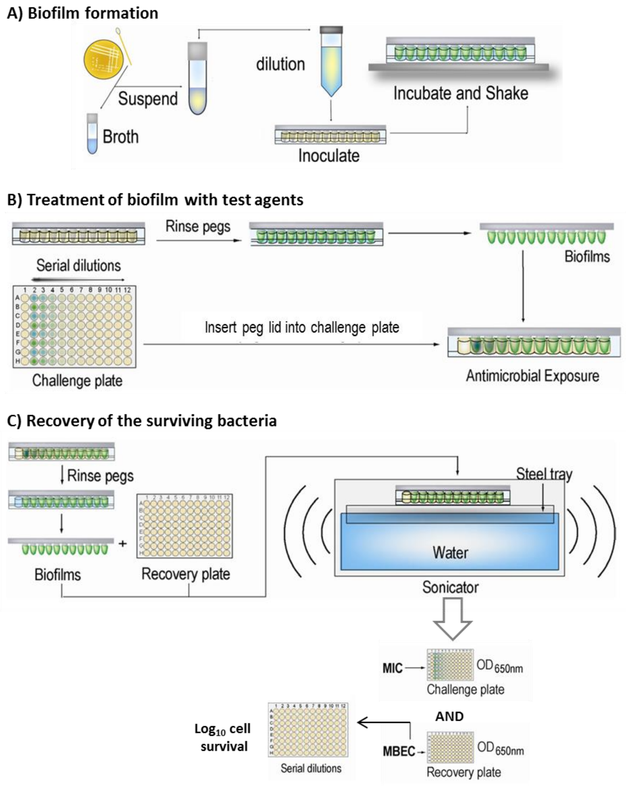

Figure 1. General workflow of the MBEC™ assay (adapted from [1]).

The MBEC™ assay (formerly the Calgary biofilm device; Innovotech Inc., Canada) is a well established high-throughput assay for assessment of anti-biofilm activity of test agents in a 96-well format. Within this assay the biofilm is formed on the plastic pegs of the plate lid. After inoculated media is introduced to the wells of the 96-well plate, the plate is placed in a shaking incubator to generate the necessary shear force for biofilm growth on the pegs (Figure 1 A). Following a suitable incubation time, the individual biofilms can be treated with varying concentrations of test agents (challenge plate, Figure 1 B). After the desired treatment time, the remaining biofilm-associated bacteria are retrieved from the pegs through sonication (recovery plate, Figure 1 C). Serial dilution and/or plating of the recovered bacterial suspension allow for the quantification of test agents' efficacy against pre-formed biofilms. Additionally, readout of the challenge plate from the biofilm treatment can be used to determine MIC values of test agents. The absence of growth in the recovery plate indicates complete biofilm eradication and the lowest concentration of a test agent that achieves this is designated as the minimum biofilm eradication concentration (MBEC). The MBEC™ assay is also amenable to assess potential synergistic effects of combination treatments, as well as efficacy of test agents in inhibiting or inducing biofilm formation.

Antimicrobial Activity of Omadacycline against *E. coli*

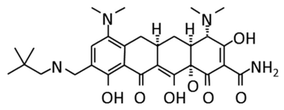

Figure 2. Structure of OMC.

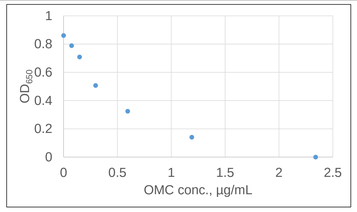

Figure 3. OD_{650} of planktonic *E. coli* ATCC 25922 after 24 hours treatment with varying concentrations of OMC.

Antimicrobial activity of OMC against *E. coli* ATCC 25922 was determined using a standard broth microdilution method [2] with slight modifications. Extrapolating from the plot (Figure 3), the minimum inhibitory concentration (**MIC**) of OMC was estimated to be **1.13 µg/mL** (reported MIC against *E. coli* 0.5-2 µg/mL [3]).

Anti-Biofilm Activity of Omadacycline

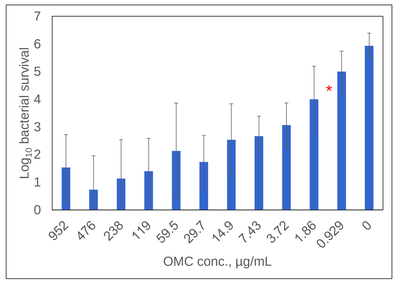

Figure 4. Activity of OMC against 24 hour *E. coli* biofilm. *denotes approx. MIC value

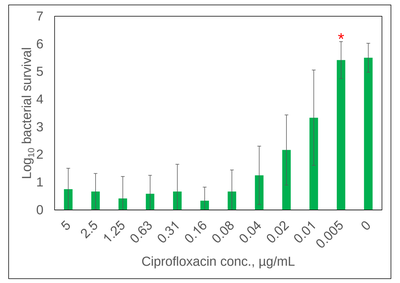

Figure 5. Activity of ciprofloxacin against 24 hour *E. coli* biofilm. *denotes approx. MIC value

The MBEC™ plates were inoculated with 10^7 CFU/mL of stationary phase *E. coli* ATCC 25922 and incubated for 24 hours to allow formation of a robust biofilm. The individual biofilms formed on the pegs were challenged with varying concentrations of antibiotics for 24 hours, followed by recovery of the surviving bacteria. Bacterial enumeration was performed using a Most Probable Number (MPN) assay [4] in a 96-well format. As a comparison to OMC efficacy, another small molecule antibiotic, ciprofloxacin, was used.

- Around MIC values (1.13 µg/mL) OMC reduced the total biofilm-associated bacteria by 1-2 log units (Figure 4). At higher concentrations the effect is more pronounced with up to 4-5 log units reduction in bioburden.
- At MIC values (5.6 ng/mL) ciprofloxacin did not show activity (Figure 5). However, at higher concentrations it elicited significant bioburden reduction (up to 5 log units).
- While both antibiotics showed significant reduction in the total number of surviving bacteria, none of the treatments resulted in complete biofilm eradication.

Sub-MIC OMC Does Not Promote Planktonic Adhesion

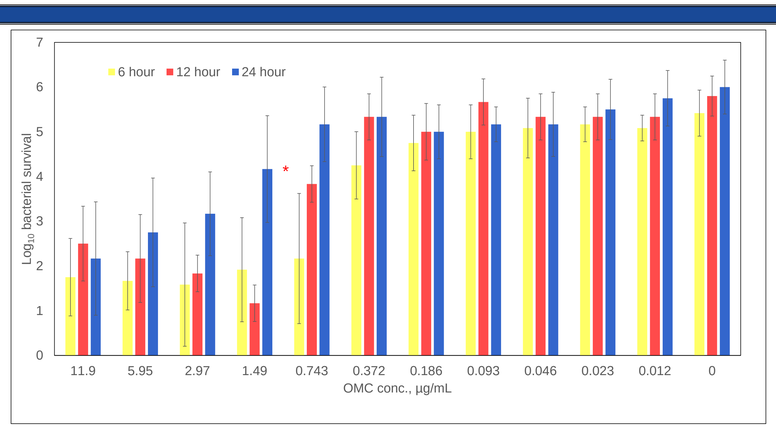

Figure 6. Effect of OMC against biofilms established for 6, 12 and 24 hours with addition of planktonic bacteria. *denotes approx. MIC value

The MBEC™ plates were inoculated with 10^7 CFU/mL of stationary phase *E. coli* ATCC 25922 and incubated for 6, 12 or 24 hours. The biofilms on the pegs were then simultaneously challenged with varying concentrations of OMC and additional 10^6 CFU/mL planktonic *E. coli*. Following 24 hour incubation, the biofilm-associated bacteria were recovered and enumerated (as above) to determine if exposure to sub-MIC doses of OMC results in additional adhesion of planktonic bacteria to the biofilms (Figure 6).

- For all biofilm ages tested (6, 12, 24 hours), mean bioburden when treated with sub-MIC concentrations of OMC was lower than that of untreated control biofilms.
- On average, 24 hour biofilms treated with doses as low as 4% of the MIC (0.046 µg/mL) were reduced by about a log in total biofilm-associated bacteria relative to controls, even in the presence of planktonic bacteria.
- Sub-MIC concentrations of OMC do not promote additional adhesion of planktonic bacteria.

Inhibition of Biofilm Formation

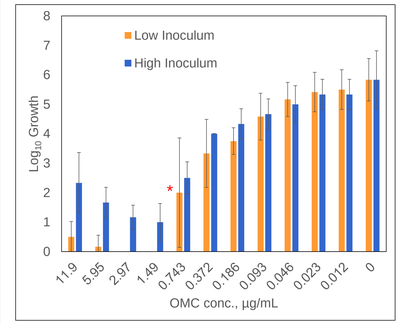

Figure 7. Biofilm growth in the presence of OMC. *denotes approx. MIC value

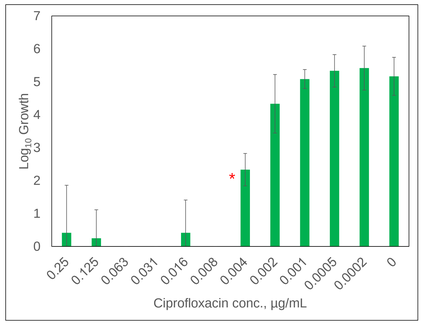

Figure 8. Biofilm growth in the presence of ciprofloxacin. *denotes approx. MIC value

The MBEC™ plates were inoculated with either 10^5 CFU/mL (low inoculum) or 10^7 CFU/mL (high inoculum) of stationary phase *E. coli* ATCC 25922 with simultaneous addition of antibiotics to evaluate the progression of biofilm formation under antibiotic challenge. After 24 hour incubation, the bacteria were recovered and enumerated (as above). Effects of ciprofloxacin were evaluated under low inoculum conditions only.

- Sub-MIC concentrations of OMC lead to a decreased number of total biofilm-associated bacteria compared to controls with mean biomass increasing in a step-wise fashion with decreasing dose. For example, 0.743 µg/mL OMC results in biofilm with approx. 3-4 log units lower total bioburden.

  Unlike other translation inhibitors shown to induce biofilm formation at sub-MIC doses [5-7], these data indicate that sub-MIC doses of OMC prevent biofilm formation.

- Ciprofloxacin (fluoroquinolone antibiotic) also did not induce biofilm formation at sub-MIC levels. However, the effects of OMC in biofilm prevention were more pronounced. I.e. at approx. 15% of the respective MIC values, OMC (0.183 µg/mL) leads to biofilm with approx. 2 log units less of a bioburden compared to control, while ciprofloxacin (1 ng/mL) shows no reduction in the total bioburden.

Conclusions

Omadacycline (OMC) shows good *in vitro* activity against *E. coli* biofilms and is capable of significantly reducing the total bioburden even at concentrations close to MIC. Perhaps more important is the information that sub-MIC concentrations of OMC do not induce *E. coli* biofilm formation, but rather seem to have a preventative effect. Sub-MIC doses of many different classes of antibiotics have been found to stimulate the biofilm growth mode of exposed bacteria. For example, treatment of *E. coli* with sub-MIC doses of five different classes of translational inhibitors, including tetracycline (chemical analogue of OMC), has been demonstrated to result in increased biofilm growth [5-7]. This finding is of particular relevance considering clinical implications of microbial biofilms and the fact that patients' sera routinely contain sub-inhibitory doses of antibiotics at the beginning and end of treatment, as well as in between doses.
Future efforts should be geared towards assessment of anti-biofilm activity against a panel of biofilm-forming pathogens and comparison of OMC performance with that of tetracycline.

References

[1] Innovotech Inc. The MBEC™ High-throughput (HTP) Assay: Instructions Manual.

[2] CLSI in *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, Approved Standard M7-A8, Clinical and Laboratory Standards Institute, Wayne, PA (USA), 2011.

[3] Macone, A. B. *In Vitro* and *In Vivo* Antibacterial Activities of Omadacycline, a Novel Aminomethylcycline. *Antimicrobial agents and Chemotherapy*, 2014, 58 (2), 1127-1135.

[4] Sutton, S. The Most Probable Number Method and Its Uses in Enumeration, Qualification, and Validation. *Journal of Validation Technology*, 2010, 16 (3), 35-38.

[5] Boehm, A., *et al.* Second messenger signaling governs *Escherichia coli* biofilm induction upon ribosomal stress. *Molecular Microbiology*, 2009, 72(6), 1500 – 1516.

[6] Hoffman, L. R., *et al.* Aminoglycoside antibiotics induce bacterial biofilm formation. *Nature*, 2005, 436 (7054), 1171-1175.

[7] Kaplan, J. B. Antibiotic-induced biofilm formation. *International Journal of Artificial Organs*, 2011 34 (9), 737-751.

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