# *In Vitro* Extracellular and Intracellular Activity of Omadacycline Against *Staphylococcus aureus*

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## Abstract

**Background: Omadacycline (OMC)** is the first aminomethylcycline in late stage clinical development for community-acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infection (ABSSSI) as once-daily oral and IV formulations. *In vitro* extracellular bacterial activities and intracellular activities using human monocytes against a variety of resistant *S. aureus* were investigated.
**Methods:** The extracellular and intracellular activity of **OMC** was compared with that of tigecycline (TI), linezolid (LI), ceftaroline (CE), levofloxacin (LE), moxifloxacin (MO) and azithromycin (AZ) against a total of 2 ATCC methicillin-susceptible (MSSA) and 2 ATCC methicillin-resistant strains of *S. aureus*. The intracellular activity was determined by exposing human monocytes, TPH-1 cell line, infected with intracellular *S. aureus* at the 1, 2, 8 or 16XMIC of antibiotic for each strain during 24 hour exposure. The extracellular activity was performed using the same antibiotic concentration and time exposure against *S. aureus* growing in cell culture medium RPMI1640. Viable bacterial cells (CFU/mL) were enumerated for all groups at time zero, 2, 6 and 24h in triplicate using the Brain Heart Infusion agar.
**Results:** Against all tested strains of *S. aureus* ATCC (MSSA or MRSA) and after 24 hour of extracellular antibiotic, **OMC** exposure at 1 to 16XMIC reduced bacterial growth ≥99.9% and was as active as CE, LE and MO. **OMC** was more active than LI which reduced growth <99.9% to ≥99%, TI <99% to ≥90% and AZ <90%. Against all tested intracellular strains of *S. aureus* ATCC (MSSA or MRSA) and after 24 hour of antibiotic exposure, **OMC** exposure at 2 to 16XMIC reduced intracellular growth ≥99% and was as active as LE and MO. **OMC** was more active than TI and LI (intracellular growth reduction of <99% to ≥90%), CE and AZ (intracellular growth reduction <90%).
**Conclusions:** This data demonstrate good bactericidal activity and human monocytes penetration of **OMC** and suggest that **OMC** may have use in infections caused by MSSA or MRSA *S. aureus* and highlights the potential utility of this oral and IV agent for the treatment of ABSSSI or CABP.

## Introduction

**Omadacycline** is the first aminomethylcycline to be developed as a once daily, oral and IV treatment of Acute Bacterial Skin and Skin Structure Infection (ABSSSI) and Community-Acquired Bacterial Pneumonia (CABP). The Phase 3 CABP development program is complete. **Omadacycline** has excellent activity against the primary pathogens associated with ABSSSI and CABP, including antibiotic resistant organisms, including *S. aureus*, β-hemolytic *streptococc*i, *S. pneumoniae*, *H. influenzae*, *Legionella* and *C. pneumoniae*.

## Objective

The goal of this study was to investigate the extracellular and intracellular activity of **omadacycline** against *Staphylococcus aureus.* We determined the minimum inhibitory concentration (MIC) and the extracellular and intracellular human monocytes activity of **omadacycline** and comparators (tigecycline, linezolid, ceftaroline, levofloxacin, moxifloxacin and azithromycin) against a variety of *S. aureus* strains from ATCC sources.

## Materials and Methods

**Strains:** A total of 4 strains of *S. aureus* strains obtained from ATCC sources: *S. aureus* ATCC 25923 (fully susceptible); *S. aureus* ATCC 29213 (β-lactamase producing MSSA); *S. aureus* ATCC 33591 (MRSA with homogeneous resistance to oxacillin); *S. aureus* ATCC 43300 (MRSA with heterogeneous resistance to oxacillin). Strains were identified by standard methods such as described by Versalovic et al. (1).

**Determination of MICs**

MICs were determined using the CLSI broth medium microdilution method using microdilution plating of the organisms onto a series of broth medium microplates of increasing concentrations from 0.004 mg/L to 16 mg/L (2, 3). Cation supplemented Mueller-Hinton broth medium (M-H) supplemented by 2% NaCl was used as broth medium against *S. aureus* ATCC strains.. *S. aureus* ATCC 29213 was included as quality control strain.

**Determination of extracellular activity**

Kill curve experiments against the 4 ATCC *S. aureus* strains was performed according to CLSI procedure (4) using 48 wells microplates. RPMI 1640 (with 10% foetal calf serum) with antimicrobial concentration of 1, 2, 8 or 16 times the MIC and each ATCC *S. aureus (*4-5 X $10^5$ CFU/ml) have been prepared in a final volume of 1 ml or equivalent. The bacterial cultures were maintained under stationary conditions for 24 hours at 37ºC in 5% $CO_2$ and 95% air. Counts of CFU/ml were performed on all bacterial cultures at time zero (H0), after 2h (H2), after 6h (H6) and after 24h (H24) of incubation in triplicate using the Brain Heart Infusion (BHI) agar.

**Determination of Intracellular Human Monocytes Activity**

The intracellular activity of **omadacycline** was compared against the 4 ATCC *S. aureus* strains. The in vitro method using the mononuclear cells described by Paul M. Tulkens (4) was performed using 48 wells microplates. RPMI 1640 medium (with 10% foetal calf serum), mononuclear cells (TPH-1 cell line; 1-2 X$10^6$ cells/ ml) and *S. aureus* (4-5 X$10^5$ CFU/ml) have been used. After a 1 hour's exposure in a shaking incubator, the infected cultures were centrifuged to eliminate non-phagocytized bacteria, and were re-suspend in RPMI medium. The antimicrobials (1, 2, 8 or 16 times the MIC of each ATCC *S. aureus* ) have been added at time zero (H0) and cultures were maintained under stationary conditions thereafter for 24 hours at 37ºC in 5% $CO_2$ and 95% air. Counts of CFU/ml at time: H0, H2, H6 and H24 were performed on all bacterial cell cultures in triplicate using BHI agar.

## Results

**Table 1. Susceptibility of *S. aureus ATCC* strains**

| Organism tested | MIC (mg/L) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **omadacycline** | tigecycline | linezolid | ceftaroline | levofloxacin | moxifloxacin | azithromycin |
| *S. aureus* ATCC 25923 (fully susceptible) | **0.5** | 0.12 | 1 | 0.12 | 0.25 | 0.06 | 0.5 |
| *S. aureus* ATCC 29213 (β-lactamase producing MSSA) | **0.5** | 0.12 | 2 | 0.12 | 0.25 | 0.06 | 0.5 |
| *S. aureus* ATCC 33591 (MRSA with homogeneous resistance to oxacillin) | **0.25** | 0.25 | 2 | 0.5 | 8 | 2 | ≥16 |
| *S. aureus* ATCC 43300 (MRSA with heterogeneous resistance to oxacillin) | **0.5** | 0.5 | 4 | 2 | ≥16 | 4 | ≥16 |

## Results continued

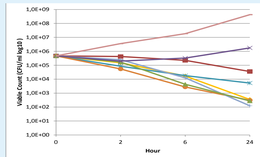


**Figure 1A.** ***In vitro*** **extracellular activity against *S. aureus* (all tested strains: 2 MSSA: (ATCC29213 & 25923) & 2 MRSA: (ATCC33591 & 43300)) with omadacycline and comparators at 1XMIC from 0 Hour until 24 Hours of incubation**

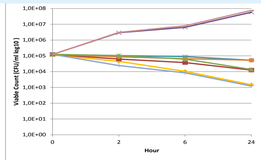


**Figure 1B.** ***In vitro*** **intracellular activity against *S. aureus* (all tested strains: 2 MSSA: (ATCC29213 & 25923) & 2 MRSA: (ATCC33591 & 43300)) with omadacycline and comparators at 1XMIC from 0 Hour until 24 Hours of incubation**

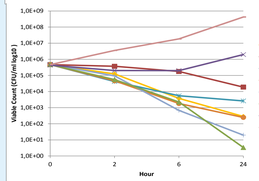


**Figure 2A.** ***In vitro*** **extracellular activity against *S. aureus* (all tested strains: 2 MSSA: (ATCC29213 & 25923) & 2 MRSA: (ATCC33591 & 43300)) with omadacycline and comparators at 2XMIC from 0 Hour until 24 Hours of incubation**

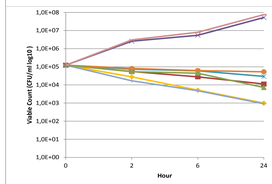


**Figure 2B.** ***In vitro*** **intracellular activity against *S. aureus* (all tested strains: 2 MSSA: (ATCC29213 & 25923) & 2 MRSA: (ATCC33591 & 43300)) with omadacycline and comparators at 2XMIC from 0 Hour until 24 Hours of incubation**

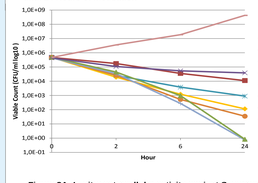


**Figure 3A.** ***In vitro*** **extracellular activity against *S. aureus* (all tested strains: 2 MSSA: (ATCC29213 & 25923) & 2 MRSA: (ATCC33591 & 43300)) with omadacycline and comparators at 16XMIC from 0 Hour until 24 Hours of incubation**

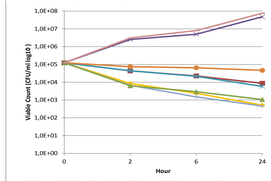


**Figure 3B.** ***In vitro*** **intracellular activity against *S. aureus* (all tested strains: 2 MSSA: (ATCC29213 & 25923) & 2 MRSA: (ATCC33591 & 43300)) with omadacycline and comparators at 16XMIC from 0 Hour until 24 Hours of incubation**

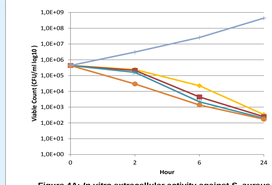


**Figure 4A: *In vitro* extracellular activity against *S. aureus* (2 MSSA strains: (ATCC29213 & 25923)) with omadacycline from 0 Hour until 24 Hours of incubation**

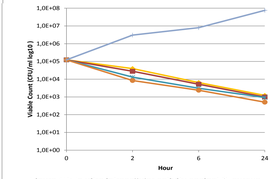


**Figure 4B: *In vitro* intracellular activity against *S. aureus* (2 MSSA strains: (ATCC 29213 & 25923)) with omadacycline from 0 Hour until 24 Hours of incubation**

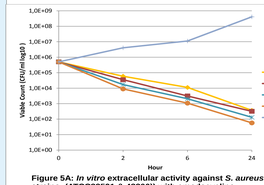


**Figure 5A: *In vitro* extracellular activity against *S. aureus* (2 MRSA strains: (ATCC33591 & 43300)) with omadacycline from 0 Hour until 24 Hours of incubation**

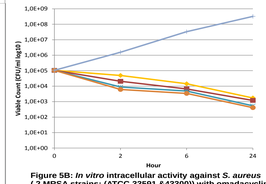


**Figure 5B: *In vitro* intracellular activity against *S. aureus* ( 2 MRSA strains: (ATCC 33591 &43300)) with omadacycline from 0 Hour until 24 Hours of incubation**

## Discussion

- Bactericidal activity (mean growth reduction of ≥ 3 $\log_{10}$ CFU/ml (≥99.9%) is reached at H24 of antibiotic exposure by **omadacycline,** ceftaroline, levofloxacin and moxifloxacin at extracellular MIC increasing from 1XMIC to 16XMIC against *S. aureus* ATCC strains (MSSA or MRSA)
- At H6, an important growth reduction (≥ 2 $\log_{10}$ CFU/ml or ≥99%) of *S. aureus* ATCC strains (MSSA and MRSA) is also detected at 1X to 16XMIC by both: **omadacycline** and moxifloxacin, by ceftaroline at 1X to 8XMIC and by linezolid at 8X to 16XMIC.
- At H24, an important growth reduction of *S. aureus* (MSSA and MRSA) is also detected by linezolid at 2X to 16XMIC. Among the tested antibiotics, tigecycline and azithromycin demonstrate only a bacteriostatic activity (growth reduction <2 $\log_{10}$ CFU/ml or <99%) against tested *S. aureus* (MSSA and MRSA)
- Significant intracellular activity (a mean intracellular growth reduction of ≥2 $\log_{10}$ CFU/ml or ≥99%) is achieved at H24 by **omadacycline,** levofloxacin and moxifloxacin at MIC increasing from 2X to 16XMIC against intracellular *S. aureus* ATCC strains (MSSA and MRSA).
- At H24, an important intracellular activity (a mean intracellular growth reduction of ≥1 $\log_{10}$ CFU/ml or ≥90% but <2 $\log_{10}$ CFU/ml or <99%) against intracellular *S. aureus* (MSSA and MRSA) is also detected by **omadacycline** at 1XMIC, by levofloxacin at 1XMIC, by moxifloxacin at 1XMIC and 2XMIC, by tigecycline at 2XMIC or more and by linezolid at 8XMIC or more.
- Unlike **omadacycline**, the intracellular growth reduction of intracellular *S. aureus* (MSSA and MRSA) is not modified by increasing MICs from 1X to 16XMIC of ceftaroline or azithromycin.

## Conclusion

Based on the *in vitro* results of this study, **omadacycline** exhibits potent extracellular and intracellular activity against *S. aureus* ATCC (MSSA or MRSA) and warrants further study as a potential antimicrobial agent for the treatment of ABSSSI, CABP or hospital-acquired bacterial pneumonia caused by *S. aureus* MRSA.

## References

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5. Paul M. Tulkens, Journal of Antimicrobial Chemotherapy (2005) 55, 897–904,