Background: Omadacycline (OMC) is the first aminomethylcyclohexyl in late stage clinical development for community-acquired bacterial pneumonia (CAPB) and acute streptococcal pharyngitis (SSP). To determine activity of OMC in vitro against homogenous resistance to oxacillin (MRSA) and other organisms, including MSSA and MRSA. Methods: The extracellular and intracellular activity of OMC was compared with that of tigecycline (TIG), teicoplanin (TEI), linezolid (LIN), levofloxacin (LEV), moxifloxacin (MOX) and azithromycin (AZ) against a total of 2 ATCC susceptible (MISSA and 2 ATCC methicillin-resistant strains of S. aureus. The extracellular activity was determined by exposing human monocytes, THP-1 cell line, infected with intracellular S. aureus at the 1, 2, or 4XMIC of antibiotics for each compound. The extracellular activity was performed using the same antibiotic concentration and time points as above. Viable bacterial cells (CFU/mL) were enumerated for all groups at zero time, 2, 4 and 24h using the Brain Heart Infusion (BHI) agar. Results: Against all tested strains of S. aureus ATCC (MISSA or MSSA) and after 24 hours of extracellular antibiotic, OMC exposure at 1 to 16X MIC reduced bacterial growth ≥99.9% and was as active as CE, LE and MO. The goal of this study was to investigate the extracellular and intracellular activity of omadacycline against Staphylococcus aureus. We determined the minimum inhibition concentration (MIC) and the extracellular and intracellular human monocyte activity of omadacycline and comparators (teicoplanin, linezolid, ceftaroline, levofloxacin, moxifloxacin and azithromycin) against a variety of S. aureus strains from ATCC sources. Determination of extracellular activity Hill curve fitting the strains against the 4 ATCC S. aureus strains was performed according to CLSI procedure (1). The MICs were determined using the CLSI broth medium microdilution method with 2% NaCl was used as broth medium against S. aureus ATCC strains. Extracellular activity was performed using the same antibiotic concentration and time points as above. Viable bacterial cells (CFU/mL) were enumerated for all groups at zero time, 2, 4 and 24h using the Brain Heart Infusion (BHI) agar. Conclusion: This data demonstrates good bacterial activity and human monocytes activity of OMC against S. aureus. The in vitro method using the mononuclear cells described by Paul M. Tulenko (4) was performed using 48 well microplates. RPMI 1640 medium (with 10% fetal calf serum), mononuclear cells (THP-1 cell line; 1:2 X10^6 cells/ml) and S. aureus (4-5 X 10^5 CFU/ml) have been used. After a 1 hour exposure in a shaking incubator, the infected cultures were centrifuged to eliminate non-phagocytized bacteria, and were re-suspended in RPMI medium. The antimicrobials (1, 2, or 24h MIC of each ATCC S. aureus strain) have been added at time zero (H0) and cultures were maintained under standard conditions thereafter for 24 hours at 37°C in 5% CO2 and 95% air. Counts of CFU/ml at time: H0, H2, H6, H24, and CFU/ml at 16XMIC of S. aureus (MISSA and MRSA) is also detected by omadacycline at 1MIC, by levofloxacin at 1MIC, by moxifloxacin at 1MIC, by tigecycline at 1MIC, and by linezolid at 1MIC. Viable Count (CFU/ml log10 ) Figure 1B. Viable Count (CFU/ml log10 ) Figure 3B. Viable Count (CFU/ml log10 ) Figure 5B. Viable Count (CFU/ml log10 ) Figure 5A. Viable Count (CFU/ml log10 ) Figure 4A.