Research Article

In Vitro and Intracellular Activities of Omadacycline against Staphylococcus aureus Isolates

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Abstract

Purpose: Omadacycline is a once-daily Intravenous (IV) and oral aminomethylcycline antibiotic that exhibits *in vitro* activity against Gram-positive and Gram-negative aerobes, anaerobes and atypical bacteria including many drug-resistant strains. This study investigated the *in vitro* activity of omadacycline and comparators against 239 resistant Staphylococcus aureus strains.

Methods: The *in vitro* activity of omadacycline and comparators was tested against *S. aureus* strains including methicillin-resistant (mecA), macrolide-resistant (ermA, B or C) and ciprofloxacin-resistant (gyrA and parC) isolates. In addition, the intracellular human monocyte activity of omadacycline and comparators was determined against ATCC *S. aureus* strains.

Results: Against all resistant strains of *S. aureus*, the *in vitro* activity of omadacycline (MIC90 0.25 mg/L) was lower than that of other tested antibiotics. Bactericidal activity, defined as a mean growth reduction of ≥3 log10 CFU/mL (≥99.9%), was attained at 24 hours of antibiotic exposure with omadacycline, ceftaroline, levofloxacin, and moxifloxacin at extracellular MICs increasing from 1X MIC to 16X MIC against both Methicillin-Sensitive (MSSA) and Resistant (MRSA) strains of *S. aureus*. Mean intracellular growth reduction of ≥2 log10 CFU/mL (≥99%) was achieved at 24 hours by omadacycline, levofloxacin, and moxifloxacin at MICs increasing from 2X to 16X MIC against intracellular *S. aureus* strains (MSSA and MRSA).

Conclusion: Based on the results of this study, omadacycline exhibits potent extracellular and intracellular activity against *S. aureus* isolates including methicillin- and ciprofloxacin-resistant strains.

Keywords: Omadacycline, Staphylococcus aureus, MIC, Intracellular activity, Tetracycline

Introduction

Staphylococcus aureus is a frequent cause of serious bacterial infections worldwide [1, 2]. Skin and skin structure infections have increased in recent years, and the most frequent bacteria were *S. aureus* or other Gram-positive bacteria [2, 3]. Effective management of serious skin infections often is complicated by antibiotic resistance, in particular Methicillin-Resistant Staphylococcus Aureus (MRSA), which accounts for nearly half of all isolates from skin and skin structure infections in the United States [4]. Community-Acquired Pneumonia (CAP) is the most common infectious disease leading to hospitalization and mortality among all age groups, especially the elderly [5, 6]. While *S. aureus* only is isolated in approximately 2% of cases of CAP, identification of *S. aureus* as a cause of CAP is associated with poor outcomes and increased mortality [1] and has been reported to be the cause of co-infection in 39%-45% of patients hospitalized with influenza [7].

Infections due to *S. aureus* often are slow to respond to antibiotics with frequent recurrences and higher mortality [8]. While often thought of as an extracellular pathogen, with inherent problems of antibiotic resistance, new evidence indicates that *S. aureus* is a facultative intracellular pathogen [8, 9], and the combination of antibiotic-resistance and intracellular activity can result in incomplete eradication of *S. aureus* even with recommended treatment. Intracellular antimicrobial activity may be markedly impaired compared to *in vitro* activity observed in broth or extracellular media. Thus, assessing both the intracellular and extracellular activity of antibiotics against *S. aureus* is essential to fully characterize its potential use to treat infections.

Omadacycline, a novel once-daily Intravenous (IV) and oral aminomethylcycline antibiotic, is a semisynthetic tetracycline derivative that exhibits *in vitro* activity against a range of Gram-positive and Gram-negative aerobes, anaerobes, and atypical bacteria [10, 11]. *In vitro* and *in vivo* studies demonstrated that omadacycline circumvents the efflux and ribosomal protection mechanisms of tetracycline resistance and has activity against pathogens common in community-acquired infections, including MRSA [12-14]. In addition, evidence from healthy subjects showed that alveolar cell concentrations of omadacycline exceeded plasma concentrations [15].

This study investigated the *in vitro* activity of omadacycline and comparators against 239 resistant *S. aureus* including methicillin-resistant (mecA), macrolide-resistant (ermA, B or C) and ciprofloxacin-resistant (gyrA and parC) strains. In addition, the extracellular and intracellular human monocyte activity of omadacycline and comparators against a variety of ATCC *S. aureus* strains including drug-resistant isolates was determined.

Methods

Drugs

Standard antimicrobial reference powders were provided by the following sources: omadacycline (Lot #CA16-0193) from Carbogen Amcis AG, Bubendorf, Switzerland; ceftaroline from IRIX Pharmaceuticals, Durham, North Carolina; telithromycin from Sanofi Aventis, Montréal, Québec, Canada; doxycycline, tigecycline, linezolid, levofloxacin, moxifloxacin, azithromycin and erythromycin from Sigma Chemicals, Mississauga, Ontario, Canada.

Strains

The strain collection represents a phenotypically and genotypically well-characterized variety of resistant community and hospital-acquired *S. aureus* strains isolated from 1995 to 2016. Twenty of theses 239 (8%) strains were collected between 1995-2001 and the remainders have been more recently collected during the 2000s.

All strains were grown on Trypticase soy agar (with 5% sheep blood) to produce pure cultures. Genomic DNA was isolated as previously described [16] and multiplex polymerase chain reaction was performed with primers specific for mecA, ermA, ermB, ermC, and mefE [17] or for gyrA and parC [18]. Four ATCC *S. aureus* strains (two methicillin-sensitive *S. aureus* (MSSA) strains (ATCC 29213, ATCC 25923) and two methicillin-resistant (MRSA) strains (ATCC 33591, ATCC 43300)) were also used to assess the extracellular and intracellular activity.

In Vitro Activity

The *in vitro* activity of omadacycline was compared with that of doxycycline, tigecycline, linezolid, ceftaroline, levofloxacin, moxifloxacin, telithromycin, azithromycin, and erythromycin against a total of 239 resistant *S. aureus* by broth microdilution according to Clinical and Laboratory Standards Institute (CLSI) guidelines [19, 20]. The tested strains included *S. aureus* that were methicillin-resistant (mecA [150]), macrolide-resistant (ermA, B or C [50]), and ciprofloxacin-resistant (gyrA and parC [39]).

Freshly cation-adjusted Mueller-Hinton broth (Becton Dickinson, Cockeysville, MD, USA) supplemented by 2% NaCl (MH) was used as broth medium against resistant *S. aureus* strains, ATCC *S. aureus* strains and Quality Control (QC) strain. MIC microplates, containing approximately $5\pm3 \times 10^5$ CFU/mL in MH broth and drug dilutions were incubated at $35\pm2°C$ in aerobic conditions and were read after 20-24 hours of incubation. Exclusively to simulate the extracellular and phagolysosomal environments, MIC microplates of MH broth were prepared at pH 7.4 ± 0.1 (original pH of medium) and 5.5 ± 0.1 (modified pH of medium adjusted with 2 N HCl solution) and were tested only against *S. aureus* ATCC strains (ATCC 29213, ATCC 25923, ATCC 33591 and ATCC 43300)).

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of drug that completely inhibited visible growth after incubation. *S. aureus* ATCC 29213 was included as a QC strain. For extracellular and intracellular activity, MICs obtained at pH 7.4 ± 0.1 were considered for choosing the tested concentrations.

Determination of extracellular activity

Kill curve experiments against 4 ATCC *S. aureus* strains (ATCC 25923, ATCC 29213 and ATCC 33591, ATCC 43300) were performed in duplicate by broth microdilution methodology modified from CLSI procedure [21] using flat cell culture microplates. One hundred and fifty microliters of RPMI 1640 medium (with 10% fetal calf serum) with antimicrobial concentrations of 1 to 6 times their MIC was inoculated with log-phase culture of each ATCC *S. aureus* to final bacterial density of $5\pm1 \times 10^5$ CFU/mL) into each well of culture microplates for a final volume of 300 µL. The bacterial cultures were maintained under stationary conditions for 24 hours at $37\pm2°C$ in 5% CO_2 and 95% air. Counts of CFU/mL were perf¬ormed on all bacterial cultures at time 0, 2, 6, and 24 hours of incubation in triplicate using Brain Heart Infusion (BHI) agar.

Determination of intracellular human monocyte activity

The intracellular activity of omadacycline was compared against 4 ATCC *S. aureus* strains (ATCC 25923, ATCC 29213, ATCC 33591 and ATCC 43300). The *in vitro* method using mononuclear cells [22-24] was performed in duplicate using 48-flat cell well culture microplates using RPMI 1640 medium (with 10% fetal calf serum) and mononuclear cells (THP-1 (ATCC TIB-202) cell line; $2\pm1 \times 10^6$ cells/ mL). Logarithmic-phase culture in BHI broth pelleted down at 14000 r.p.m. for 4 min and opsonized by suspending pellets in RPMI 1640 supplemented with non-decomplemented 10% fresh human serum for 30 min at $37\pm2°C$. Opsonized *S. aureus* were adjusted to $5\pm1 \times 10^5$ CFU/mL in RPMI 1640 and phagocytized at a 4:1 ratio of bacteria to THP-1 monocytes. After a 1 hour exposure at $37\pm2°C$ in a shaking incubator, the infected cultures were centrifuged (1300 rpm; 8 min) to eliminate non-phagocytized bacteria and were re-suspended in RPMI medium. One hundred and fifty microliters of RPMI with diluted antibiotics at 1, 2, 8 or 16 times the MIC of each ATCC *S. aureus* strain were added at time 0 into each well of infected culture microplates for a final volume of 300µL. Cultures were maintained under stationary conditions thereafter for 24 hours at $37\pm2°C$ in 5% CO_2 and 95% air. Monocytes in a 20µl sample taken at each time point from each well were diluted by 10-fold dilutions and lysed with distilled water. Counts of CFU/mL at time 0, 2, 6, and 24 hours were performed on all bacterial cell cultures in triplicate using BHI agar.

Cytotoxicity

The cytotoxicity of omadacycline and comparators was assessed in THP-1 monocytes. After 24 h exposure to antibiotics, even the highest tested concentration (16XMIC) resulted in <1% cells being stained with tryptan blue. This observation suggested that all of the tested antibiotics were non-cytotoxic to THP-1 monocytes.

Results

In vitro Activity

Against all resistant strains of *S. aureus*, the activity of omadacycline (MIC90 0.25mg/L) was more potent than other tested antibiotics (Table 1). An MIC90 of 0.25mg/L was obtained against methicillin-resistant *S. aureus* (mecA genotype group) with omadacycline that was

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Table 1: Susceptibility of resistant *S. aureus* strains: methicillin-resistant (*mec*A), macrolide-resistant (*erm*A, B, C), and ciprofloxacin-resistant (*gyr*A and *par*C) strains using MH broth.

Organism (no. tested)	Antibiotic	MICᵃ (mg/L)		
		Range	50%	90%
S. aureus All resistant tested strains (239)	**Omadacycline**	**0.016-1**	**0.25**	**0.25**
	Doxycycline	0.06-≥16	0.5	1
	Tigecycline	0.25-1	0.5	0.5
	Linezolid	0.5-4	1	2
	Ceftaroline	0.06-2	0.5	2
	Levofloxacin	0.5-≥16	4	≥16
	Moxifloxacin	0.25-≥16	4	≥16
	Telithromycin	0.016-≥16	0.12	4
	Azithromycin	0.016-≥16	2	≥16
	Erythromycin	0.06->16	1	≥16
S. aureus methicillin-resistant *mec*A genotype (150)	**Omadacycline**	**0.016-0.25**	**0.25**	**0.25**
	Doxycycline	0.06-≥16	0.5	1
	Tigecycline	0.25-2	0.5	0.5
	Linezolid	0.5-4	1	2
	Ceftaroline	0.06-2	0.5	1
	Levofloxacin	1-≥16	4	≥16
	Moxifloxacin	0.25-≥16	2	4
	Telithromycin	0.016-≥16	0.06	0.12
	Azithromycin	1-≥16	2	≥16
	Erythromycin	0.5-≥16	1	≥16
S. aureus Macrolide-resistant *erm*A, B & C genotype (50)	**Omadacycline**	**0.06-0.25**	**0.25**	**0.25**
	Doxycycline	0.25-1	1	1
	Tigecycline	0.25-1	0.5	0.5
	Linezolid	1-4	2	2
	Ceftaroline	0.12-2	1	1
	Levofloxacin	0.5-4	2	4
	Moxifloxacin	0.25-4	1	4
	Telithromycin	0.12-≥16	2	4
	Azithromycin	4-≥16	≥16	≥16
	Erythromycin	8-≥16	≥16	≥16
S. aureus Ciprofloxacin- Resistant *gyr*A & *par*C genotype (39)	**Omadacycline**	**0.06-0.25**	**0.25**	**0.25**
	Doxycycline	0.5-1	1	1
	Tigecycline	0.25-0.5	0.5	0.5
	Linezolid	1-4	2	4
	Ceftaroline	0.06-1	0.5	1
	Levofloxacin	8-≥16	≥16	≥16
	Moxifloxacin	4-≥16	≥16	≥16
	Telithromycin	0.016-4	0.06	0.25
	Azithromycin	0.016-≥16	0.12	≥16
	Erythromycin	0.12-≥16	1	≥16

ᵃ MICs determined by broth microdilution according to CLSI guidelines in antibiotic concentrations from 0.004 to 16 mg/L. Geometric mean value (mg/L) for MIC.

MIC: Minimal Inhibitory Concentration

comparable to tigecycline (MIC90 0.5mg/L), and more potent than doxycycline (MIC90 1mg/L), ceftaroline (MIC90 1mg/L), linezolid (MIC90 2mg/L), and moxifloxacin (MIC90 4mg/L). An MIC90 of ≥16mg/L was observed with azithromycin, erythromycin, and levofloxacin against methicillin-resistant *S. aureus* (mecA genotype group). Against macrolide-resistant *S. aureus* (ermA, B, C genotype group) strains, omadacycline (MIC90 0.25 mg/L) was the most active agent and was more active than telithromycin, azithromycin, and erythromycin (MIC90 ≥4mg/L) or levofloxacin and moxifloxacin (MIC90 4mg/L). Against ciprofloxacin-resistant *S. aureus* (gyrA and parC genotype group), an MIC90 >16mg/L was observed with levofloxacin, moxifloxacin, azithromycin, and erythromycin. While, the MIC90 for omadacycline (0.25mg/L) remained lower than that of linezolid (MIC90 4mg/L), doxycycline (MIC90 1mg/L), and ceftaroline (MIC90 1mg/L), this was comparable to telithromycin (MIC90 0.25mg/L) and tigecycline (MIC90 0.5mg/L).

At pH 7.4, the MICs obtained for omadacycline against ATCC *S. aureus* strains (ATCC 29213, ATCC 25923, ATCC 33591 and ATCC 43300) were from 0.25 to 0.5 mg/L (Table 2). Against the four tested ATCC *S. aureus* strains, the MICs of omadacycline were comparable to tigecycline (MIC range: 0.12 to 0.5 mg/L) and ceftaroline (MIC range: 0.12 to 2 mg/L). At a pH of 5.5 (phagolysosomal environments), omadacycline MICs were one to two 2-fold serial dilutions higher against ATCC *S. aureus* strains, which was less active than ceftaroline but comparable to tigecycline, linezolid, levofloxacin, and moxifloxacin.

Extracellular and Intracellular Activity

Bactericidal activity, defined as mean growth reduction of ≥3 log10 CFU/mL (≥99.9%), was reached at 24 hours of antibiotic exposure with omadacycline, ceftaroline, levofloxacin, and moxifloxacin at increasing extracellular MIC concentrations from 1X to 16X MIC against both MSSA and MRSA ATCC strains (Figures 1-4). At 6 hours, growth reduction (≥2 log10 CFU/mL or ≥99%) of *S. aureus* (MSSA and MRSA) was detected at 1X to 16X MIC with omadacycline and moxifloxacin, at 1X to 8X (data not shown) MIC with ceftaroline, and at 8X (data not shown) to 16X MIC with linezolid. At 24 hours, growth reduction of *S. aureus* (MSSA and MRSA) was detected with linezolid at 2X to 16X MIC. Among the tested antibiotics, tigecycline and azithromycin only demonstrated a bacteriostatic activity (growth reduction <2 log10 CFU/mL or <99%) against tested MSSA and MRSA strains.

Important intracellular activity, mean intracellular growth reduction of ≥2 log10 CFU/mL (≥99%), was achieved at 24 hours by omadacycline and levofloxacin, at increasing concentrations from 2X

Table 2: Susceptibility of *S. aureus* ATCC 25923 and ATCC 33591 strains at pH 7.4 and pH 5.5 in MH broth.

Organism tested	pH	MICᵃ (mg/L)						
		Omadacycline	Tigecycline	Linezolid	Ceftaroline	Levofloxacin	Moxifloxacin	Azithromycin
S. aureus ATCC 25923	7.4	0.5	0.12	2	0.12	0.25	0.06	0.5
	5.5	2	0.5	4	0.12	1	0.25	>16
S. aureus ATCC 33591	7.4	0.25	0.25	2	0.5	8	2	>16
	5.5	1	2	2	0.25	>16	4	>16

ᵃ MICs determined by broth microdilution according to CLSI guidelines in antibiotic concentrations from 0.004 to 16 mg/L. Geometric mean value (mg/L) for MIC.

Only data for strains, *S. aureus* ATCC 25923 and ATCC 33591 is shown due to similar data for strains *S. aureus* ATCC 29213 and ATCC 43300.

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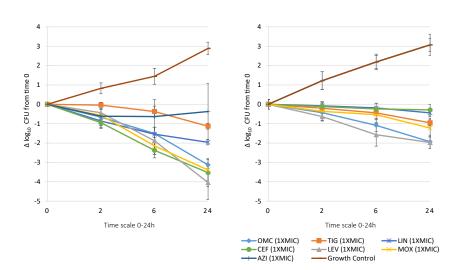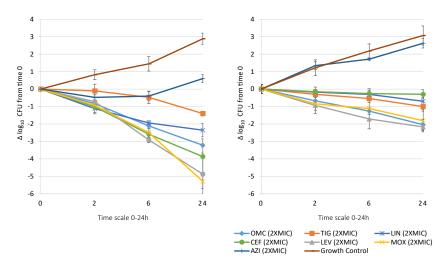

Figure 1: *In vitro* extracellular (left) and intracellular (right) activity against *S. aureus* all tested strains: 2 MSSA (ATCC 29213 & 25923) and 2 MRSA (ATCC 33591 & 43300) with omadacycline (OMC) and comparators (tigecycline (TIG), linezolid (LIN), ceftaroline (CEF), levofloxacin (LEV), moxifloxacin (MOX), azithromycin (AZI)) at 1XMIC from 0-24 hours of incubation. Note that each point corresponds to the mean value of all tested strains determined by triplicate independent counts.
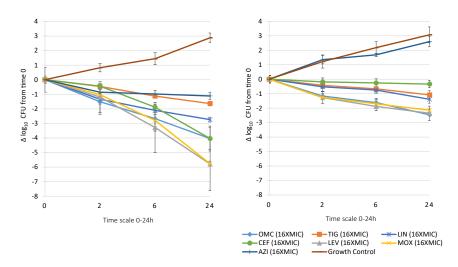

Figure 2: *In vitro* extracellular (left) and intracellular (right) activity against S. aureus all tested strains: 2 MSSA (ATCC 29213 & 25923) and 2 MRSA (ATCC 33591 & 43300) with omadacycline (OMC) and comparators (tigecycline (TIG), linezolid (LIN), ceftaroline (CEF), levofloxacin (LEV), moxifloxacin (MOX), azithromycin (AZI)) at 2XMIC from 0-24 hours of incubation. Note that each point corresponds to the mean value of all tested strains determined by triplicate independent counts.
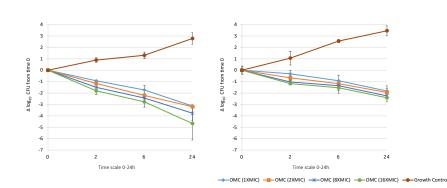

Figure 3: *In vitro* extracellular (left) and intracellular (right) activity against S. aureus all tested strains: 2 MSSA (ATCC 29213 & 25923) and 2 MRSA (ATCC 33591 & 43300) with omadacycline (OMC) and comparators (tigecycline (TIG), linezolid (LIN), ceftaroline (CEF), levofloxacin (LEV), moxifloxacin (MOX), azithromycin (AZI)) at 16XMIC from 0-24 hours of incubation. Note that each point corresponds to the mean value of all tested strains determined by triplicate independent counts.

Figure 4: *In vitro* extracellular activity (left) and intracellular activity (right) against 2 MRSA strains (ATCC 33591 & 43300) with omadacycline (OMC) at 1X to 16X MIC from 0-24 hours of incubation. Note that each point corresponds to the mean value of 2 MRSA strains determined by triplicate independent counts. Only data of strains MRSA ATCC 33591 and ATCC 43300 is shown due to similar data of strains MSSA ATCC 29213 and ATCC 25923.

to 16X MIC against intracellular MSSA and MRSA (Figures 1-4). At 24 hours, intracellular activity, mean intracellular growth reduction of ≥1 log10 CFU/mL (≥90%) but <2 log10 CFU/mL (<99%), against intracellular MSSA and MRSA was detected with omadacycline at 1X MIC, levofloxacin at 1X MIC, moxifloxacin at 1X MIC and 2X MIC, tigecycline at 2X MIC or greater, and linezolid at 8X MIC or greater (data not shown). Unlike omadacycline, growth reduction of intracellular MSSA and MRSA was not modified by increasing concentrations of ceftaroline or azithromycin from 1X to 16X MIC.

Discussion

Results from this study showed that omadacycline exhibits *in vitro* activity against resistant strains of *S. aureus*. Omadacycline appeared to have more predominant activity than other older tetracyclines, ketolide, macrolides, quinolones, oxazolidinone or third generation cephalosporin's against the most resistant isolates such as β-lactam-resistant, erythromycin-resistant or ciprofloxacin-resistant *S. aureus*. Further, these results revealed that omadacycline has potent extracellular and intracellular activity that was comparable to levofloxacin and moxifloxacin and was higher than tigecycline, linezolid, ceftaroline and azithromycin.

Previously, it was thought that *S. aureus* only infected the extracellular space, and treatment failure was caused by resistance mechanisms to many antibiotics that were inherent in *S. aureus* [8]. However, in recent years, *S. aureus* were discovered to exist in the intracellular space (phagolysosomes) in macrophages, monocytes, and other human cells as well as the extracellular space, which may provide a more complete explanation for failed antibiotic treatment of infections due to *S. aureus* [8, 9, 25]. Importantly, the results from this study showed that omadacycline demonstrated not only bactericidal extracellular activity (99.9% of growth reduction) but also produced an intracellular activity (99% of growth reduction) in human monocytes infected with resistant *S. aureus*. This finding supports the potential clinical activity of omadacycline against a broad variety of *S. aureus* isolates.

The pharmacokinetics of omadacycline has been studied extensively in healthy volunteers after IV and oral administration [15, 26-29].

Recent studies also provided evidence for the presence of omadacycline in alveolar macrophages of animals and humans [15, 25, 30]. The pharmacokinetics of omadacycline and tigecycline

were evaluated in plasma, epithelial lining, and alveolar cells of healthy subjects. Subjects received omadacycline 100 mg IV every 12 hours for 2 doses, then 100 mg IV every 24 hours for 3 doses, and concentrations were measured in pulmonary tissues during bronchopulmonary lavage [15]. At the time of bronchoscopy, a mean area under the concentration-time curve (AUC0-24) value of 17.23 mg ⋅ h /L and 302.42 mg ⋅ h /L was observed respectively in Epithelial Lining Fluid (ELF) or in alveolar cells (AC). Combining the observed mean MIC value (0.5mg/L) at 24 hours to obtain a mean intracellular growth reduction of ≥2 log10 CFU/mL, with the observed mean ELF and AC AUC0–24 values, the estimated AUC0–24/MIC ratio in ELF and AC would be ~35 and ~605 for tested ATCC strains of *S. aureus*, respectively. Results from these studies indicate potent intracellular omadacycline concentrations and confirm that omadacycline produces AUC0-24 in ELF or AC suggesting achievable level at infection site that far exceed the MICs or the potential intracellular activity for a broad variety of *S. aureus* including resistant isolates included in this study.

Based on the *in vitro* results of the study reported here, omadacycline exhibits potent extracellular and intracellular activity against *MSSA* or MRSA. These results combined with extensive *in vitro* susceptibility studies and pharmacokinetic studies demonstrating consistent systemic exposure after IV and oral dosing are consistent with results from Phase 3 studies. Omadacycline demonstrates consistent efficacy in patients hospitalized with serious infections due to acute Bacterial Skin and Skin Structure Infections (ABSSSI) and CAP caused by *S. aureus* and other causative pathogens [31, 32].

Declarations

Conflicts of Interest

The author, Jacques Dubois declare conflicts of interest relevant to this study. The authors, Maïtée Dubois, and Jean-François Martel declare no conflicts of interest relevant to this study.

Funding

Authors' Contributions

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