Preclinical evaluation of omadacycline for potential as a drug to treat Acinetobacter baumannii and Klebsiella pneumoniae infections in combat wounds

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Abstract

New drug treatment options are urgently needed for effective clinical management of multidrug-resistant (MDR) bacterial infections in combat injuries. *Acinetobacter baumannii* and *Klebsiella pneumoniae,* along with *Staphylococcus aureus* and *Pseudomonas aeruginosa*, are among the most common drug-resistant bacterial strains isolated from U.S. military trauma-related infections. A study from the U.S. Army Institute of Surgical Research Burn Center from 2003 to 2008 found that MDR prevalence rates among these isolates were 53% for *A. baumannii* and 17% for *K. pneumoniae.* Treatment options for MDR strains of *A. baumannii* and *K. pneumoniae* are limited, but available treatments include colistin/polymyxins, minocycline, and tigecycline. Unfortunately, many of these therapies are associated with toxicities, gastrointestinal side effects, and poor oral bioavailability. To increase treatment options for combat wound infections with MDR bacteria, we evaluated omadacycline's potential to inhibit growth of military-relevant pathogens through *in vitro* and *in vivo* studies. Omadacycline is an aminomethylcycline that inhibits protein synthesis and was designed to overcome most common tetracycline resistant mechanisms. Omadacycline is an ideal candidate for the treatment of combat wound infections due to its broad-spectrum activity, oral and intravenous formulations, and attractive safety profile relative to other standard-of-care antibiotics. Omadacycline is currently approved by the U.S. Food and Drug Administration (FDA) to treat community acquired bacterial pneumonia and acute bacterial skin and skin structure infections. As a first step to evaluate omadacycline's potential as a drug to treat combat wound infections, we assessed omadacycline activity in comparison to standard-of-care antibiotics for *in vitro* potency against military-relevant clinical isolates of *A. baumannii* and *K. pneumoniae.* We then tested *in vivo* efficacy of omadacycline, relative to a standard-of-care antibiotic, to reduce bacterial load in an MDR *A. baumannii* neutropenic thigh infection model.

Rationale

Omadacycline is a semisynthetic tetracycline derivative with the same mechanism of action as the tetracycline class but designed to overcome tetracycline resistance mechanisms. Activity of omadacycline is mediated through binding to the primary tetracycline binding site on the 30S subunit of the bacterial ribosome. Structural modifications incorporated in the design of omadacycline allow the molecule to overcome common resistance mechanisms, such as tetracycline-specific efflux pumps and ribosomal protection, which make other tetracyclines ineffective. We evaluated the *in vitro* activity of omadacycline relative to four standard-of-care comparator antibiotics (meropenem, doxycycline, tigecycline, and minocycline) against clinical isolates of *A. baumannii* and *K. pneumoniae* collected from Military Health System patients worldwide. Previous *in vivo* assessments of omadacycline activity demonstrated efficacy against various Gram-positive and Gram-negative pathogens. Herein, we report the first *in vivo* evaluations of omadacycline against MDR *A. baumannii* utilizing a neutropenic murine thigh infection model.

Diversity panel sample information for *A. baumannii* 5075

Origins	Year	Sample Type
USA	2008	Wound

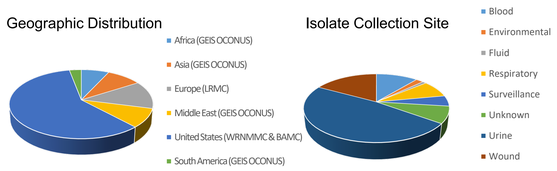
AMK	CAZ	CIP	COL	CRO	FEP	GEN	IPM	LVX	MEM	SXT	TOB	SAM	TET
R	R	R	S	R	R	R	R	R	R	R	R	R	S

All resistance Genes
aac(6')-Ib3, aadA2, ant(2'')-Ia, aph(3'')-Ib, aph(3')-VIa, aph(6)-Id, blaADC-25, blaGES-11, blaOXA-23, blaOXA-69, cmlA1, dfrA7, sul1

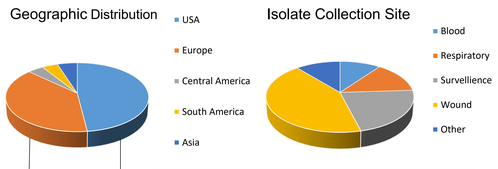
Methods

The Multidrug-Resistant Organism Repository and Surveillance Network (MRSN) assembled panels of 100 clinical strains each of *A. baumannii* and *K. pneumoniae* generated from isolates collected through surveillance or healthcare facilities from Military Health System (MHS) beneficiaries and other individuals located worldwide. Each strain within the panel has been characterized with regard to antibiotic susceptibility (antibiogram) and antimicrobial resistance gene content. We used these panels to assess activity of omadacycline and the standard-of-care comparator antibiotics against bacterial strains present in military healthcare facilities. Panel strains were plated on blood agar from freezer stocks, incubated overnight at 37 ˚C, and cultivated in cation-adjusted Mueller-Hinton broth to determine the minimum inhibitory concentrations (MIC)s of omadacycline, doxycycline, tigecycline, and levofloxacin by broth microdilution. The MIC profile for each isolate was compared to the genotype provided by the MRSN. To assess omadacycline activity against MDR *A. baumannii* in a neutropenic thigh infection model, 8-week-old male and female ICR-CD1 mice (5 each) were rendered neutropenic by pre-infection treatment with cyclophosphamide. Mice were infected on day 0 with 1.89 x 10^6 colony forming units (CFU) of *A. baumannii* 5075 in a 25 µL bacterial suspension. Two and 14 hours post-infection, mice were treated with either omadacycline (10 mg/kg – group 1, 25 mg/kg – group 2) or tigecycline (10 mg/kg – group 3, 25 mg/kg – group 4) and vehicle control (VC). Mice were humanely euthanized 24 hr post infection and infected thigh tissues were harvested, weighed, homogenized, and diluted for plating and colony counts.

MRSN *K. pneumoniae* Diversity Panel (Isolates collected 2003 – 2017)

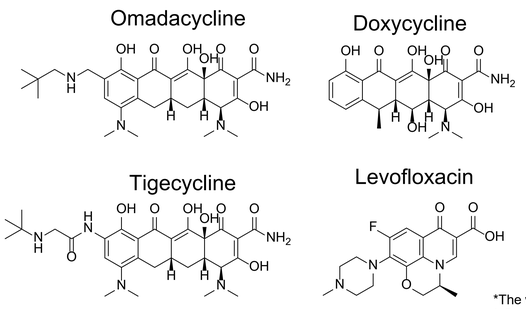


MRSN *A. baumannii* Diversity Panel (Isolates collected 2003 – 2017)



A number of isolates collected in the US and Europe are from service members Who served in Iraq, Afghanistan, and other countries.

Results



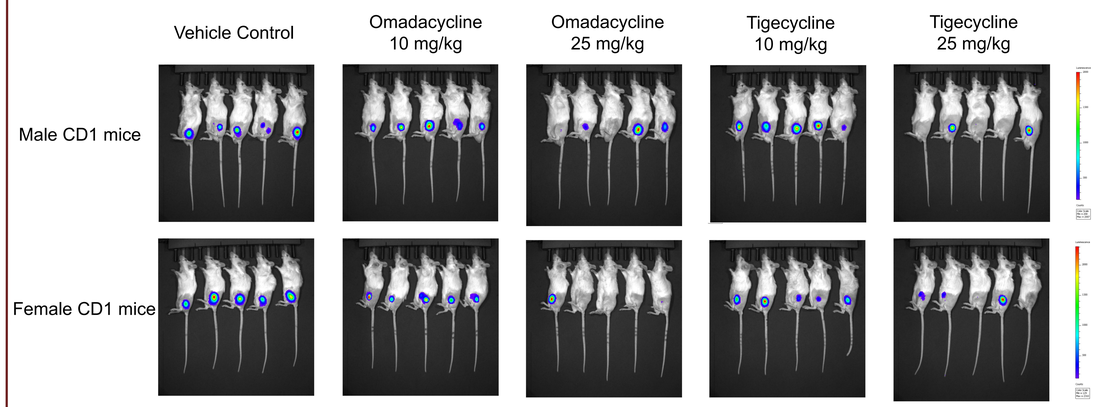
In vitro susceptibilities of MRSN *K. pneumoniae* strains.

Antibiotics	MIC range (µg/mL)	MIC (µg/mL)	
		MIC_{50}	MIC_{90}
Omadacycline*	0.5 - 64	2	8
Doxycycline	0.5 - ≥128	8	64
Tigecycline	≤0.25 - 8	0.5	2
Levofloxacin**	≤0.25 - 128	0.5	64

In vitro susceptibilities of MRSN *A. baumannii* strains.

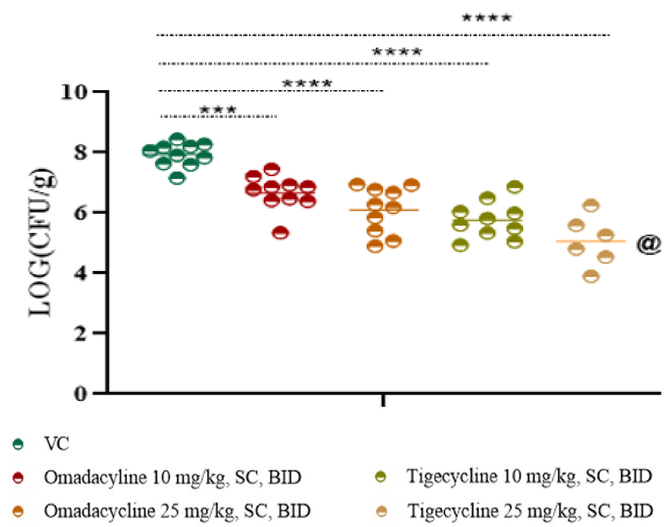
Antibiotics	MIC range (µg/mL)	MIC (µg/mL)	
		MIC_{50}	MIC_{90}
Omadacycline*	≤0.25 - 16	0.5	2
Doxycycline	≤0.25 - 64	0.25	32
Tigecycline	≤0.25 - 8	0.5	2
Levofloxacin**	≤0.25 - 128	2	16

*The working stock concentration of omadacycline was calculated using the drug potency of 74.3%.
**Standard of care lexofloxacin used as quality control (QC) MIC in diversity panel.



IVIS images of mouse thigh model infected with *A. baumannii* and dosed as shown

Efficacy of Omadacycline vs. Tigecycline in Neutropenic CD1 mice infected with *A. baumannii* 5075



One-way ANOVA: ****p<0.0001 and ***p=0.003

@ There was no bacteria growth (0 colony forming units per gram of tissue (CFUs/g tissue) at any of the dilutions (10-10^{12}) in thigh muscle homogenate obtained from 4 (four) ICR-CD1 mice belonging to the subcutaneous (SC), BID (twice a day), at 25 mg/kg tigecycline-treated group. These four "zero" values have not been included in data analysis.

In addition to not having bacteria growth detected, 75% of these mice in the tigecycline 25 mg/kg group had no detectable bioluminescence signal in the thigh muscle, which suggests that these mice significantly cleared their bacterial burden.

Conclusions

- Omadacycline showed potent *in vitro* activity against a geographically and genetically diverse panel of MDR *A. baumannii* and *K. pneumoniae* clinical isolates collected largely from MHS beneficiaries.
- Omadacycline produced a dose-response reduction in bacterial load in the 24-hour neutropenic mouse thigh infection model using the *A. baumannii* 5075 MDR strain. The efficacy of omadacycline in this model was similar to that of the control comparator antibiotic, tigecycline, as the bacterial load reduction mediated by omadacycline at 25 mg/kg (1.82-log CFU/g) and tigecycline at 25 mg/kg (2.16-log CFU/g) were not significantly different.
- Upcoming studies will assess omadacycline in preclinical wound infection models to further assess potential clinical use expansion of omadacycline as a treatment for *A. baumannii* infections in combat wounds