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In Vitro Evaluation of PTK796 Activity Tested against *Staphylococcus aureus*, Including Hospital- and Community-Associated MRSA Strains from the USA and Europe

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ABSTRACT

Background: PTK796 (PTK) is a novel aminomethylcycline currently under clinical development. PTK activity was evaluated against methicillin-susceptible (MSSA) and -resistant *S. aureus* (MRSA), including multidrug-resistant strains from documented hospital-acquired (HA) infections and community-associated (CA) infection isolates.

Methods: 325 *S. aureus* were collected from bloodstream, skin and skin structure, CA and HA pneumonia in USA (35 sites) and European (EU, 31) laboratories. The CLSI broth microdilution method (M07-A8) was utilized (9 comparators) applying fresh Mueller-Hinton media when testing PTK and tigecycline. Cefoxitin disks were used to confirm MRSA. CA-MRSA genotypes (USA300 [120] and USA400 [5]) were identified by PFGE and SCC*mec* typing, and PVL genes.

Results: PTK MIC values for all strains were ≤2 μg/ml. PTK ($MIC_{50/90}$, 0.25/0.5 μg/ml), tigecycline ($MIC_{50/90}$, 0.12/0.25 μg/ml; 100% susceptible) and daptomycin ($MIC_{50/90}$, 0.25/0.5 μg/ml; 100% susceptible) exhibited similar activity against SA, while doxycycline ($MIC_{50/90}$, 0.12/2 μg/ml; 92.6% susceptible) showed elevated MIC values. Overall, the tetracycline derivatives were more active than vancomycin ($MIC_{50/90}$, 1/1 μg/ml; 99.7% susceptible) and linezolid ($MIC_{50/90}$, 2/2 μg/ml; 100% susceptible). PTK inhibited 100.0 and 90.9% of EU and USA HA-MRSA at ≤0.5 μg/ml, respectively. All EU CA-MRSA were inhibited by PTK of 0.5 μg/ml, except for one strain; while USA300/400 MRSA strains were very susceptible to PTK ($MIC_{50/90}$, 0.12/0.25 μg/ml).

Organism group (no. tested) / Continent	Cumulative % inhibited at PTK MIC (μg/ml) of: ≤0.12	0.25	0.5	1	2
All *S. aureus* (325)	34.5	84.3	99.1	99.4	100.0
MSSA (54)	22.2	85.2	100.0	-	-
USA (27)	14.8	85.2	100.0	-	-
Europe (27)	29.6	85.2	100.0	-	-
HA-MRSA (47)	14.9	63.8	95.7	95.7	100.0
USA (22)	13.6	63.6	90.9	90.9	100.0
Europe (25)	16.0	64.0	100.0	-	-
CA-MRSA (224)	41.5	88.4	99.6	100.0	-
USA300/400 (125)	60.8	99.2	100.0	-	-
Europe (99)	17.2	74.8	99.0	100.0	-

Conclusions: PTK was active against *S. aureus*, displaying a narrow potency range (0.12 - 2 μg/ml). Consistent PTK activity was observed, regardless of geographic origin, and genotypic or resistance subsets.

INTRODUCTION

Resistance to currently available antimicrobial classes has increased markedly; and safe and effective treatment options are needed for clinical use against numerous Gram-positive and -negative bacterial pathogens. *Staphylococcus aureus* is the most common Gram-positive species associated with human infections, including serious and invasive diseases. Strains of methicillin-resistant *S. aureus* (MRSA) are commonly resistant to other antimicrobials and this often causes difficult decisions for clinical practitioners when using empiric treatment.

Healthcare-associated (HA-MRSA) infections have long been known as a serious problem among very ill patients and have been very problematic regarding treatment. Some therapeutic options have required intensive monitoring of adverse side effects such as ototoxicity, nephrotoxicity, severe colitis and muscle weakness. In more recent years, community-associated MRSA (CA-MRSA) has come to the forefront of clinical consideration and has also become a serious therapeutic concern. CA-MRSA commonly causes wound infections and it is recognized as the most problematic pathogen associated with complicated skin and skin-structure infections (cSSSI), which may advance to bacteremia.

This study was performed to evaluate the in vitro antimicrobial activity of PTK796, a novel tetracycline-class (aminomethylcycline) antimicrobial agent, against methicillin-susceptible *S. aureus* (MSSA) and MRSA from medical centers located in Europe and the United States (USA). PTK796 is a broad-spectrum agent with proven efficacy in animal models for treating clinically prevalent infections caused by Gram-positive and Gram-negative bacteria, including those with multi-drug resistance (MDR). Phase III trials will determine the safety and efficacy of PTK796 in the treatment of cSSSI, many of which will be caused by MSSA and MRSA.

MATERIALS AND METHODS

Bacterial isolates. A total of 325 non-duplicate isolates of *S. aureus* from documented patient infections were collected from medical centers located in the USA and Europe. Approximately equal numbers of isolates were collected from each region and included MSSA (54), HA-MRSA (47) and CA-MRSA (224). The primary medical center provided the species identification and a reference laboratory (JMI Laboratories, North Liberty, Iowa, USA) confirmed the speciation using BactiStaph® latex and tube coagulase agglutination tests (Remel, Lenexa, KS, USA), when needed.

Antimicrobial susceptibility testing. All isolates were tested for antimicrobial susceptibility using reference frozen-form panels with cation-adjusted Mueller-Hinton broth produced by JMI Laboratories. Tests were performed according to the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI; M07-A8, 2009) using freshly prepared Mueller-Hinton broth (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). PTK796 and nine comparator agents, including direct or related class agents (doxycycline and tigecycline) were tested. Interpretive criteria for MIC values of comparison agents were those of the CLSI (M100-S20-U; 2010) or USA-FDA criteria (tigecycline). Concurrent quality control (QC) testing of *S aureus* ATCC 29213 was performed per M07-A8 [2009] and ranges found in M100-S20-U (2010).

Molecular characterization of CA-MRSA. PCR amplification of Panton-Valentine leukocidin (PVL) genes (*lukF-PV* and *lukS-PV*) was performed with primers published elsewhere. The isolates were characterized for the type of SCC*mec* gene cassette using a multiplex PCR strategy. The *mecA* gene was amplified as part of the multiplex PCR to serve as an internal control. PCR products were separated on 2% agarose gel in TAE buffer on Criterion Sub-cell GT system (Bio-Rad, Hercules, CA) and stained with ethidium bromide. SCC*mec* types were assigned based on the number and sizes of the amplicons obtained. Pulsed-field gel electrophoresis (PFGE) band patterns of strains were compared to those of USA300, USA400 and other USA clones previously published.

RESULTS

- Overall, 99.1% of the isolates were inhibited by ≤0.5 μg/ml of PTK796, including all MSSA isolates, HA-MRSA from Europe and CA-MRSA from the USA. The MIC values for PTK796 were ≤2 μg/ml for isolates tested in this collection (Table 1).
- Slightly higher PTK796 MIC values were noted among HA-MRSA isolates from USA compared to European isolates (Table 1). The highest PTK796 MIC values (2 μg/ml) were observed among two HA-MRSA strains from two different medical centers in the USA.
- PTK796 was slightly more active against strains from the USA ($MIC_{50/90}$, 0.25/0.25 μg/ml) compared to those from Europe ($MIC_{50/90}$, 0.25/0.5 μg/ml), as shown in Table 1. In this collection, doxycyline MIC values were notably higher in the European collection (MIC_{90}, 8 μg/ml; 88.1% susceptible) compared to the USA sample (MIC_{90}, 2 μg/ml; 96.6% susceptible).
- PTK796 exhibited in vitro activity similar to that of daptomycin with MIC_{50} and MIC_{90} values of 0.25 and 0.5 μg/ml, respectively (Table 2). The tetracycline derivatives were more active than vancomycin and linezolid, which had MIC_{90} values of 1 and 2 μg/ml, respectively.
- Against all strains, tigecycline was two-fold more active (MIC_{90}, 0.25 μg/ml) and doxycyline was four-fold less active (MIC_{90}, 2 μg/ml) than PTK796 (Table 2).
- Although resistance to other antimicrobial classes was much higher among the HA-MRSA, compared to CA-MRSA isolates, similar PTK796 MIC values were observed among these two populations ($MIC_{50/90}$, 0.25/0.5 μg/ml; Tables 3 and 4).

Table 1. MIC frequency distributions of the investigational agent PTK796 tested against 325 *S. aureus* isolates.

Collection group (no. tested)	Cumulative % of strains inhibited at MIC (μg/ml) of: ≤0.06	0.12	0.25	0.5	1	2
MSSA[a] (54)	0.0	22.2	85.2	100.0	-	-
USA (27)	0.0	14.8	85.2	100.0	-	-
Europe (27)	0.0	29.6	85.2	100.0	-	-
HA-MRSA[b] (47)	0.0	14.9	63.8	95.7	95.7	100.0
USA (22)	0.0	13.6	63.6	90.9	90.9	100.0
Europe (25)	0.0	16.0	64.0	100.0	-	-
CA-MRSA[c] (224)	0.0	41.5	88.4	99.6	100.0	-
USA (125)	0.0	60.8	99.2	100.0	-	-
Europe (99)	0.0	17.2	74.8	99.0	100.0	-
All MRSA (271)	0.0	36.9	84.1	98.9	99.3	100.0
USA (147)	0.0	53.7	93.9	98.6	98.6	100.0
Europe (124)	0.0	16.9	72.6	99.2	100.0	-
All isolates (325)	0.0	34.5	84.3	99.1	99.4	100.0
USA (174)	0.0	47.7	92.5	98.9	98.9	100.0
Europe (151)	0.0	19.2	74.8	99.3	100.0	-

a. MSSA reads methicillin-susceptible *S. aureus*.
b. HA-MRSA reads hospital-acquired methicillin-resistant *S. aureus*.
c. CA-MRSA reads community-acquired methicillin-resistant *S. aureus*.

Table 2. Comparison of in vitro activity of selected antimicrobial agents tested against *S. aureus* (325 strains).

Antimicrobial agent	MIC_{50}	MIC_{90}	Range	% susceptible/ resistant[a]
PTK796	0.25	0.5	0.12 – 2	- / -
Oxacillin	>8	>8	≤0.5 – >8	16.6 / 83.4
Doxycycline	0.12	2	0.06 – 32	92.6 / 1.8
Tigecycline[b]	0.12	0.25	0.03 – 0.5	100.0 / -
Azithromycin	>32	>32	0.5 – >32	23.1 / 76.6
Clindamycin	≤0.5	>16	≤0.5 – >16	72.3 / 27.7
Levofloxacin	0.5	32	0.12 – >32	54.2 / 44.6
Trimethoprim/sulfamethoxazole	≤0.25	≤0.25	≤0.25 – >8	96.0 / 4.0
Linezolid	2	2	0.5 – 2	100.0 / -
Daptomycin	0.25	0.5	0.12 – 1	100.0 / -
Vancomycin	1	1	≤0.5 – 4	99.7 / 0.0

a. Criteria as published by the CLSI [2010].
b. USA-FDA breakpoints were applied [Tygacil Product Insert, 2005].

Table 3. Comparison of in vitro activity of selected antimicrobial agents tested against HA-MRSA (47 strains).

Antimicrobial agent	MIC_{50}	MIC_{90}	Range	% susceptible/ resistant[a]
PTK796	0.25	0.5	0.12 – 2	- / -
Oxacillin	>8	>8	8 – >8	0.0 / 100.0
Doxycycline	0.25	8	0.12 – 32	76.6 / 6.4
Tigecycline[b]	0.12	0.25	0.03 – 0.5	100.0 / -
Azithromycin	>32	>32	>32	0.0 / 100.0
Clindamycin	>16	>16	≤0.5 – >16	17.0 / 83.0
Levofloxacin	16	>32	0.5 – >32	2.1 / 93.6
Trimethoprim/sulfamethoxazole	≤0.25	4	≤0.25 – >8	83.0 / 17.0
Linezolid	2	2	0.5 – 2	100.0 / -
Daptomycin	0.5	0.5	0.12 – 0.5	100.0 / -
Vancomycin	1	2	≤0.5 – 2	100.0 / 0.0

a. Criteria as published by the CLSI [2010].
b. USA-FDA breakpoints were applied [Tygacil Product Insert, 2005].

Table 4. Comparison of in vitro activity of selected antimicrobial agents tested against CA-MRSA (224 strains).

Antimicrobial agent	MIC_{50}	MIC_{90}	Range	% susceptible/ resistant[a]
PTK796	0.25	0.5	0.12 – 1	- / -
Oxacillin	>8	>8	8 – >8	0.0 / 100.0
Doxycycline	0.12	1	0.06 – 16	95.5 / 1.3
Tigecycline[b]	0.12	0.25	0.03 – 0.5	100.0 / -
Azithromycin	>32	>32	0.5 – >32	13.8 / 86.2
Clindamycin	≤0.5	>16	≤0.5 – >16	77.7 / 22.3
Levofloxacin	0.25	32	0.12 – >32	54.0 / 45.1
Trimethoprim/sulfamethoxazole	≤0.25	≤0.25	≤0.25 – >8	97.8 / 2.2
Linezolid	2	2	0.5 – 2	100.0 / -
Daptomycin	0.25	0.5	0.12 – 1	100.0 / -
Vancomycin	1	1	≤0.5 – 4	99.6 / 0.0

a. Criteria as published by the CLSI [2010].
b. USA-FDA breakpoints were applied [Tygacil Product Insert, 2005].

CONCLUSIONS

- Overall, PTK796 was very active against all *S. aureus* isolates tested in this investigation ($MIC_{50/90}$, 0.25/0.5 μg/ml), regardless of isolate origin (geographic or hospital/ community) or resistance phenotype to other antimicrobial agents.
- Among MRSA isolates combined, strains from Europe had slightly higher PTK796 $MIC_{50/90}$ values (two-fold) compared to isolates from the USA. These results appear to be associated with the decreased susceptibility to doxycycline documented among MRSA isolates from Europe.
- The in vitro activity established in this study coupled with pharmacokinetic/pharmacodynamic and target attainment results would suggest that PTK796 is a promising antimicrobial agent for the treatment of serious MDR *S. aureus* infections.

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