

Activity of the Novel Anti-Staphylococcal Agent AFN-1252 against Genotypically Unique Strains of *Staphylococcus aureus*

Françoise Perdreau-Remington¹, George G. Zhanel², David Vaughan³, Nachum Kaplan³
¹University of California, San Francisco; ²University of Manitoba, Winnipeg, Canada; ³Affinium Pharmaceuticals Inc., Toronto, Canada

REVISED ABSTRACT

Activity of the Novel Anti-staphylococcal Agent AFN-1252 against Genotypically Unique Strains of *Staphylococcus aureus*
F. Perdreau-Remington¹, G. G. Zhanel², D. Vaughan³ and N. Kaplan³
University of California, San Francisco; USA; University of Manitoba², National Microbiology Laboratory, Winnipeg, Canada; Affinium Pharmaceuticals Inc. 3, Toronto, Canada
Background: AFN-1252, recently renamed AFN-1252 by Affinium Pharmaceuticals, targets the key component of bacterial fatty acid synthesis, the enoyl-ACP reductase (FabI). AFN-1252 is being developed as a new class of oral and intravenous agent. We selected 100 unique *Staphylococcus aureus* (SA) strains and compared the activity of AFN-1252 to other drugs used in the treatment of serious staphylococcal infections.
Methods: Strains were selected according to their sequence type (ST), presence or absence of *mecA* and *PVL* genes, SCC_{mec} type, pulsed field gel electrophoresis (PFGE) profiles and antimicrobial phenotypes. MICs were determined using current CLSI guidelines.
Results: The 79 MRSA strains belonged to 12 ST^s (SCC_{mecIV} n=62; SCC_{mecII} n=17; PVL₊ n=35) and 47 unique PFGE profiles including USA100-100. The 21 MSSA strains represented nine ST^s and 20 unique PFGE profiles. Each strain represented a unique genotype/phenotype profile. The range of AFN-1252 MIC₅₀ (mg/L) were 0.004-0.125 for MRSA and 0.004-0.015 for MSSA. For MRSA, the MIC₉₀ for AFN-1252 was 0.015mg/L for all SCC_{mecIV} USA300 strains as well as all SCC_{mecIV} USA300 strains (n=26) while all other SCC_{mecIV} (n=36) needed an MIC₉₀ of 0.03mg/L. All MSSA strains independent of ST or phenotype had an MIC₉₀ of 0.015mg/L. The AFN-1252 MIC₉₀ values against MRSA strains were 1 to 2 orders of magnitude more potent than the newer anti-MRSA agents vancomycin, linezolid, daptomycin and tigecycline) or than the agents that maintained activity (>90% susceptibility) against SCC_{mecIV} strains (clindamycin, gentamicin, and trimethoprim-sulfamethoxazole).
Conclusions: AFN-1252 proved highly potent *in-vitro* against the diverse genetic backgrounds of SA included in this study. The potent activity against all of the community and hospital acquired MRSA strains tested further supports the development of AFN-1252 for treatment of challenging staphylococcal infections.

BACKGROUND

•Enoyl-ACP reductase (FabI) is an essential enzyme in bacterial fatty acid synthesis and the target of the novel antibiotic API-1252, recently renamed AFN-1252 by Affinium Pharmaceuticals (Figure 1). By inhibiting this enzyme, AFN-1252 disrupts bacterial fatty acid synthesis eventually leading to cell death.

METHODS

•One hundred *S. aureus* isolates were selected according to their sequence type (ST), presence or absence of *mecA* and Panton Valentine Leucocidine (PVL) genes, staphylococcal chromosomal cassette element (SCC_{mec}), pulsed field gel electrophoresis (PFGE) profiles (Figure 2) and antimicrobial phenotypes.

•MIC determinations were performed for the Affinium compound API-1252 and 16 comparators by broth microdilution according to CLSI defined methodology (M7-A7)

RESULTS

Table 1. Genotypic characterization of the 100 Unique *S. aureus* strains

	CDC- PFT	MLST	Strains n	PFGE subtypes	SCC mec	PVL(+)
CA-MRSA N = 62	USA300	ST8	26	10	IV	26
	USA400	ST1	4	2	IV	4
	USA500	ST8	6	4	IV	0
	USA700	ST72	5	2	IV	0
	USA1100	ST30	11	5	IV	11
	USA1000	ST59	3	1	IV	0
	ST12, ST729	4	2		0	
	ST87, ST97, ST231	3			0	
HA-MRSA N = 17	USA100	ST5	12	11	II	0
	USA200	ST36	4	3	II	0
	USA600	ST45	1	1	II	0
MSSA N=21	ST5 (n=2), ST8 (9), ST12 (1), ST45 (3), ST36 (1), ST88 (1), ST730 (1), ST188 (1), ST474 (1), ST731(1)					0

Figure 2. PFGE profiles of MRSA USA 300 strains.

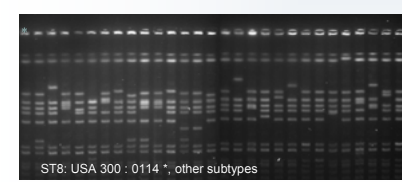


Table 2. MIC (µg/ml) of AFN-1252 and Comparators Against CA-MRSA and HA-MRSA

Antimicrobials		USA300 n=26	Other CA-MRSA n=36	All CA-MRSA n=62	HA-MRSA n=17	All MRSA n=79	All MSSA n=21	<i>S. aureus</i> Total n=100
AFN-1252	Range	0.008 - 0.06	0.004 - 0.06	0.004 - 0.06	0.008 - 0.125	0.004 - 0.125	0.004 - 0.015	0.004 - 0.125
	MIC50	0.015	0.008	0.008	0.008	0.008	0.008	0.008
	MIC90	0.015	0.03	0.03	0.015	0.015	0.015	0.015
Ciprofloxacin	Range	0.5 - >16	0.25 - >16	0.25 - >16	0.5 - >16	0.25 - >16	0.25 - >16	0.25 - >16
	MIC50	0.5	1	1	>16	1	0.5	1
	MIC90	16	>16	16	>16	>16	2	>16
Levofloxacin	Range	0.25 - 8	0.12 - >32	0.12 - >32	0.25 - >32	0.12 - >32	0.12 - 32	0.12 - >32
	MIC50	0.25	0.25	0.25	16	0.25	0.25	0.25
	MIC90	8	16	8	>32	32	0.5	16
Gentamicin	Range	≤0.25	≤0.25 - 32	≤0.25 - 32	≤0.25 - 64	≤0.25 - 64	≤0.25	≤0.25 - 64
	MIC50	≤0.25	≤0.25	≤0.25	0.25	≤0.25	≤0.25	≤0.25
	MIC90	≤0.25	≤0.25	≤0.25	32	≤0.25	≤0.25	≤0.25
Vancomycin	Range	0.5 - 0.5	0.5 - 1	0.5 - 1	0.5 - 1	0.5 - 1	0.5 - 1	0.5 - 1
	MIC50	0.5	0.5	0.5	1	0.5	0.5	0.5
	MIC90	0.5	1	1	1	1	1	1
Clarithromycin	Range	≤0.25 - >16	≤0.25 - >16	≤0.25 - >16	≤0.25 - >16	≤0.25 - >16	≤0.25 - >16	≤0.25 - >16
	MIC50	>16	>16	>16	>16	>16	0.25	>16
	MIC90	>16	>16	>16	>16	>16	>16	>16
Clindamycin	Range	≤0.25 - >8	≤0.25 - >8	≤0.25 - >8	≤0.25 - >8	≤0.25 - >8	≤0.25 - >8	≤0.25 - >8
	MIC50	≤0.25	≤0.25	≤0.25	>8	≤0.25	≤0.25	≤0.25
	MIC90	>8	>8	>8	>8	>8	>8	>8
Daptomycin	Range	0.12 - 0.25	0.12 - 0.25	0.12 - 0.25	0.12 - 0.5	0.12 - 0.5	0.06 - 0.5	0.06 - 0.5
	MIC50	0.25	0.12	0.12	0.12	0.12	0.12	0.12
	MIC90	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Linezolid	Range	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	0.5 - 2	0.5 - 2
	MIC50	2	1	2	2	2	1	2
	MIC90	2	2	2	2	2	2	2
Moxifloxacin	Range	≤0.06 - 2	≤0.06 - 8	≤0.06 - 8	≤0.06 - 8	≤0.06 - 8	≤0.06 - 8	≤0.06 - 8
	MIC50	0.06	0.06	0.06	4	0.06	0.06	0.06
	MIC90	2	2	2	8	8	0.12	4
Tigecycline	Range	0.12 - 0.25	0.06 - 0.5	0.06 - 0.5	0.12 - 1	0.06 - 1	0.12 - 0.25	0.06 - 1
	MIC50	0.12	0.12	0.12	0.12	0.12	0.12	0.12
	MIC90	0.25	0.12	0.25	0.5	0.25	0.25	0.25
Trimethoprim/sulfamethoxazole	Range	≤0.12 - ≤0.12	≤0.12 - 16	≤0.12 - 16	≤0.12 - 2	≤0.12 - 16	≤0.12 - 16	≤0.12 - 16
	MIC50	0.12	0.12	0.12	0.12	0.12	0.12	0.12
	MIC90	0.12	0.12	0.12	0.25	0.12	16	2

Figure 1. Chemical structure of AFN-1252

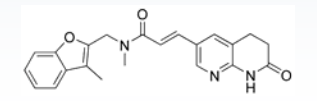


Table 3. Susceptibility (%) of AFN-1252 and Comparators Against CA-MRSA & HA-MRSA

Antimicrobials	USA300 n=26	Other CA-MRSA n=36	All CA-MRSA n=62	HA-MRSA n=17	All MRSA n=79	MSSA n=21	All <i>S.aureus</i> n=100
AFN-1252	100	100	100	100	100	100	100
Ciprofloxacin	50	75	65	12	53	81	59
Levofloxacin	50	81	68	12	56	90	63
Moxifloxacin	50	81	68	12	56	90	63
Gentamicin	100	94	97	76	92	100	94
Clarithromycin	8	36	24	6	20	67	30
Clindamycin	77	89	84	18	70	100	76
Trimethoprim/sulfamethoxazole	100	92	95	100	96	67	90
Daptomycin	100	100	100	100	100	100	100
Linezolid	100	100	100	100	100	100	100
Tigecycline	100	100	100	94	99	100	99
Vancomycin	100	100	100	100	100	100	100

Table 4. MIC (µg/ml) of AFN-1252 and Comparators Against MSSA

Antimicrobials	All MSSA (n = 21)	
	MIC (µg/ml)	Susceptibility(%)
AFN-1252	Range	0.004 - 0.015
	MIC50	0.008
	MIC90	0.015
Cefazolin	Range	≤0.5 - 8
	MIC50	0.5
	MIC90	1
Cefepime	Range	≤1 - 32
	MIC50	2
	MIC90	4
Ceftriaxone	Range	2 - 32
	MIC50	4
	MIC90	4
Piperacillin /tazobactam	Range	≤1 - 16
	MIC50	≤1
	MIC90	≤1
Meropenem	Range	≤0.12 - 2
	MIC50	≤0.12
	MIC90	≤0.12

CONCLUSIONS: AFN- 1252 is highly active against all unique 100 genotypes/phenotypes of community- and hospital-acquired MRSA and MSSA strains causing infections today. The AFN-1252 MIC₉₀ was 1 to 2 orders of magnitude lower than all comparator antimicrobials. Its novel mechanism of action and high potency support further clinical progression of AFN-1252 as a novel anti-staphylococcal agent.