

# Activity of AFN-1252, a Novel FabI Inhibitor, Against Methicillin-Susceptible and Methicillin-Resistant *Staphylococcus epidermidis* from Canadian Intensive Care Units

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## ABSTRACT

**Background:** API-1252, recently renamed by Affinium Pharmaceuticals as AFN-1252 (API), is a potent inhibitor of enoyl-ACP reductase (FabI), an essential enzyme in bacterial fatty acid synthesis. API is being developed as an oral and intravenous specific spectrum agent for treatment of Staphylococcal infections. The purpose of this study was to assess the in vitro activity of API versus Methicillin-Susceptible (MS) and Methicillin-Resistant *Staphylococcus epidermidis* (MRSE).

**Methods:** MSSE and MRSE were obtained from 19 medical centres across Canada as part of the Canadian Intensive Care Unit (CAN-ICU) study. Susceptibility testing was performed using the CLSI (2006) dilution method. API MIC (range  $\leq 0.002$  -  $\geq 2$   $\mu\text{g/ml}$ ) was compared to cefazolin, ciprofloxacin, meropenem, vancomycin, linezolid and tigecycline.

**Results:** The activity of API versus comparators against 50 MSSE and 50 MRSE is summarized below:

Antimicrobial	MIC <sub>50</sub> /MIC <sub>90</sub> (μg/ml)	
	MSSE (n=50)	MRSE (n=50)
AFN-1252	0.015/0.06	0.015/0.03
Cefazolin	1/2	16/128
Ciprofloxacin	4/>16	>16/>16
Meropenem	1/8	16/32
Vancomycin	1/2	1/2
Linezolid	1/2	1/2
Tigecycline	0.12/0.25	0.12/0.25

The API MIC range was 0.008-0.03  $\mu\text{g/ml}$  with both MSSE and MRSE. The MIC<sub>90</sub> values for FN-1252 were 0.06  $\mu\text{g/ml}$  and 0.03  $\mu\text{g/ml}$  with the MSSE and MRSE, respectively, which were 1 to 2 orders of magnitude lower than the MIC<sub>90</sub> values of the comparators active against MSSE or that maintain activity against MRSE (vancomycin, linezolid, tigecycline). **Conclusions:** AFN-1252 is highly active against both MSSE and MRSE and is a promising novel agent against drug resistant Staphylococcal infections.

## PURPOSE

The purpose of this study was to assess the in vitro activity of AFN-1252 versus clinical isolates *S. epidermidis*, including both methicillin-susceptible (MSSE) and methicillin-resistant *S. epidermidis* (MRSE).

## MATERIALS AND METHODS

**Isolate Collection:** 50 methicillin-susceptible *S. epidermidis* (MSSE) and 50 MRSE were collected and characterized as part of an ongoing study assessing antimicrobial resistance in Canadian intensive care units (CAN-ICU) [www.canr.ca]. Isolates were obtained from all geographic regions of Canada and represented various antimicrobial resistance phenotypes. All isolates collected were deemed clinically significant by that site. Isolate inclusion was not dependent on patient age. All organisms were identified at each site using local site criteria and at the reference site (Health Sciences Centre, Winnipeg, Manitoba, Canada), where isolates were subcultured on appropriate solid media and incubated overnight. Amies semi-solid transport media containing charcoal (Difco Laboratories, Detroit, MI) was inoculated with the isolate and sent to the coordinating laboratory (Health Sciences Centre, Winnipeg, Manitoba, Canada), where isolates were subcultured on appropriate media and stocked in skim milk at -70°C.

Isolates of *S. epidermidis* displayed a negative coagulase test followed by identification on a Vitek 2 instrument (bioMérieux, Hazelwood, MO). Specimen sources for isolates of MSSE and MRSE were: blood (66.0%, 56.0% of isolates); wound (30.0%, 32.0%); respiratory (0%, 2.0%); and urine (4.0%, 10.0%), respectively.

**Antimicrobial Susceptibility Testing:** A custom designed microtitre panel with AFN-1252, cefazolin, ciprofloxacin, linezolid, meropenem, tigecycline and vancomycin was created. These agents were obtained as laboratory grade powders from their respective manufacturers. Stock solutions were prepared and dilutions made as described by the Clinical Laboratory Standards Institute (CLSI, 2005).<sup>5</sup> Following two subcultures from frozen stock the MICs of the antimicrobial agents for the isolates were determined by the CLSI 2005 approved broth microdilution method. Briefly, 96-well custom designed microtitre plates containing doubling antibiotic dilutions in 100  $\mu\text{l}$  well of cation-adjusted Mueller-Hinton broth were inoculated to achieve a final concentration of approximately  $5 \times 10^5$  CFU/ml and incubated in ambient air for 24 hours prior to reading. Colony counts were performed periodically to confirm inocula. Quality control were performed using a variety of ATCC QC organisms including; *S. aureus* 29213, *E. faecalis* 29212, *E. coli* 25922 and *P. aeruginosa* 27853.

## INTRODUCTION

Infections caused by antibiotic resistant pathogens is a Canadian and global crisis. Antibiotic resistant pathogens including methicillin-resistant *S. aureus* (MRSA), methicillin-resistant *S. epidermidis* (MRSE), vancomycin-resistant *Enterococcus* species (VRE), penicillin-resistant *Streptococcus pneumoniae* (PRSP), extended spectrum  $\beta$ -lactamase (ESBL) producing Enterobacteriaceae and fluoroquinolone-resistant and carbapenem-resistant Enterobacteriaceae and *P. aeruginosa* are increasing in prevalence in all provinces in Canada as well as in other countries. Available therapeutic options available for the treatment of these antibiotic resistant organisms are increasingly severely limited as these organisms frequently display a multidrug resistant (MDR) phenotypes. Nosocomial infections caused by *S. epidermidis*, including both methicillin-susceptible (MSSE) and methicillin-resistant *S. epidermidis* (MRSE), have increased in prevalence in the last two decades as a result of increased use of prosthetics and indwelling devices and growing numbers of immunocompromised patients.

Fatty acid biosynthesis is an essential process in both bacteria and mammals, however, because fatty acid biosynthetic systems are organized differently in bacteria and mammals there is potential for selective inhibition of the bacterial system. Previous study of the inhibition of bacterial fatty acid biosynthesis, specifically mycolic acid biosynthesis in *M. tuberculosis*, has demonstrated it to be a potential target for the development of antimicrobial agents and resulted in the commercialization of isoniazid. Enzymes within the bacterial fatty acid biosynthesis pathway such as bacterial enoyl-acyl carrier protein (ACP) reductase (FabI) have been identified as potential antibacterial targets.<sup>1-3</sup> AFN-1252 is an investigational inhibitor of staphylococcal FabI, an essential enzyme that catalyzes the reduction of trans-2-enoyl-ACP to acyl-ACP, the final step in each elongation cycle of bacterial fatty acid biosynthesis. AFN-1252 is being developed by Affinium Pharmaceuticals, Inc. (Toronto, Canada), in both oral and intravenous formulations, for treatment of antimicrobial-susceptible and -resistant staphylococcal infections, particularly *Staphylococcus aureus* infections.<sup>4</sup>

FabI inhibitors disrupt both saturated and unsaturated fatty acid biosynthesis and inhibit bacterial growth.<sup>1-4</sup> FabI appears to be a selective antibacterial target for *S. aureus*, *S. epidermidis*, *H. influenzae*, *M. catarrhalis*, and other bacterial species in which it is the sole enoyl-ACP reductase present.<sup>3,4</sup> Both FabI and FabK have been identified in *E. faecalis* and *P. aeruginosa* while FabK is the sole enoyl-ACP reductase present in *S. pneumoniae*.<sup>2</sup> AFN-1252 was optimized against the *S. aureus* FabI enzyme, resulting in a unique and specific spectrum of activity. The current study was undertaken to assess the in vitro activity of AFN-1252 against recent clinical isolates of *S. epidermidis*, including both MSSE and MRSE.

## CONCLUSIONS

- AFN-1252 is highly active against methicillin-susceptible *Staphylococcus epidermidis* (MSSE).
- AFN-1252 is highly active against MRSE.
- No difference in AFN-1252 activity was observed between MSSE and MRSE.
- AFN-1252 was 10 to 2 orders of magnitude more potent than all the comparators tested.
- AFN-1252 is a promising new agent against drug resistant staphylococcal infections.

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## RESULTS

Tables 1 and 2 depict MIC<sub>50</sub>s, MIC<sub>90</sub>s, and MIC ranges for AFN-1252 and comparative agents for MSSE and MRSE. The AFN-1252 MIC<sub>90</sub>s for MSSE and MRSE were 0.06 and 0.03  $\mu\text{g/ml}$ , respectively; all isolates of *S. epidermidis* were inhibited by AFN-1252 at a concentration of 0.5  $\mu\text{g/ml}$ . AFN-1252 MIC distributions did not change for MSSE versus MRSE strains (Table 3). Multidrug resistant phenotypes of *S. epidermidis* had similar MIC<sub>90</sub>s for AFN-1252 when compared to pan-susceptible isolates (Table 4).

Table 1. Activity of AFN-1252 and Comparators Against 50 MSSE

Antibiotic	MIC <sub>50</sub> (μg/ml)	MIC <sub>90</sub> (μg/ml)	MIC Range (μg/ml)	% of Isolates Per Category <sup>a</sup>		
				S	I	R
AFN-1252	0.015	0.06	$\leq 0.008$ - 0.25	-	-	-
Cefazolin	1	2	$\leq 0.5$ - 4	100	0	0
Ciprofloxacin	4	>16	$\leq 0.06$ - >16	44	4	52
Linezolid	1	2	0.25 - 4	100	0	0
Meropenem	1	8	$\leq 0.12$ - 32	88	8	4
Tigecycline	0.12	0.25	$\leq 0.03$ - 0.5	100	0	0
Vancomycin	1	2	$\leq 0.25$ - 2	100	0	0

<sup>a</sup>S, susceptible; I, intermediate; R, resistant.

Table 2. Activity of AFN-1252 and Comparators Against 50 MRSE

Antibiotic	MIC <sub>50</sub> (μg/ml)	MIC <sub>90</sub> (μg/ml)	MIC Range (μg/ml)	% of Isolates Per Category <sup>a</sup>		
				S	I	R
AFN-1252	0.015	0.03	$\leq 0.008$ - 0.5	-	-	-
Cefazolin	16	128	$\leq 1$ - 128	0	0	100 <sup>b</sup>
Ciprofloxacin	>16	>16	0.12 - >16	20	0	80
Linezolid	1	2	0.25 - 4	100	0	0
Meropenem	16	32	$\leq 0.12$ - 32	0	0	100 <sup>b</sup>
Tigecycline	0.12	0.25	0.12 - 0.5	100	0	0
Vancomycin	1	2	1 - 2	100	0	0

<sup>a</sup>S, susceptible; I, intermediate; R, resistant.  
<sup>b</sup>as per oxacillin disk

Table 4. Activity of AFN-1252 Against *S. epidermidis* with Different Phenotypic Profiles

Phenotype (no. of isolates tested)	AFN-1252 MIC <sub>90</sub> (μg/ml)		
	MIC Range	MIC <sub>50</sub>	MIC <sub>90</sub>
Pan-susceptible <i>S. epidermidis</i> (17)	0.008-0.5	0.015	0.015
Multidrug-resistant <i>S. epidermidis</i> (23)	0.008-0.06	0.015	0.03

<sup>a</sup>Pan-susceptible was defined as susceptible to oxacillin, ciprofloxacin, gentamicin, and vancomycin.

<sup>b</sup>Multidrug-resistant was defined as resistant to ciprofloxacin and gentamicin, and included both methicillin-susceptible and methicillin-resistant isolates.

## Participating Sites and Investigators

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