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Activity of API-1252, a Novel FabI Inhibitor, Against Healthcare-Associated and Community-Associated Methicillin-Resistant *Staphylococcus aureus* from Canadian Intensive Care Units

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ABSTRACT

Background: API-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 *S.aureus* infections. The purpose of this study was to assess the in-vitro activity of API versus Healthcare-Associated (HA) and Community-Associated (CA) Methicillin-Resistant *S. aureus* (MRSA).

Methods: MRSA were obtained from 20 medical centres across Canada as part of the Canadian Intensive Care Unit (CAN-ICU) study. MRSA were confirmed using multiplex PCR for *mec*A, *nuc*, and PVL and were subtyped by PFGE. Susceptibility testing was performed using the CLSI (2005) dilution methods. API MIC (range ≤0.002 - ≥2µg/ml) was compared to cefazolin, ciprofloxacin, meropenem, vancomycin and linezolid.

Results: Against ATCC reference strains *S. aureus* 29213, MRSA 33592, *E.faecalis* 29212, *E. coli* 25922, and *P. aeruginosa* 27853, API MICs (µg/ml) were 0.015, 0.008, >2, >2 and >2, respectively. The activity of API versus comparators against 154 HA and CA MRSA is summarized below:

Antimicrobial	MIC_{50}/MIC_{90} (µg/ml)
API-1252	0.008/0.015
Cefazolin	128/>128
Meropenem	16/64
Ciprofloxacin	>16/>16
Vancomycin	1/1
Linezolid	1/2

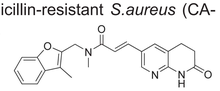
The API MIC range was 0.002 - 0.125µg/ml. No difference in activity was observed between HA and CA MRSA.

Conclusions: API-1252 is highly active against both HA and CA MRSA and is a promising new agent against drug resistant Staphylococcal infections.

INTRODUCTION

Infections caused by antibiotic resistant pathogens is a Canadian and global crisis. Antibiotic resistant pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *S. epidermidis* (MRSE), vancomycin-resistant *Enterococcus* species (VRE), penicillin-resistant *Streptococcus pneumoniae* (PRSP), extended spectrum β-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella* species and fluoroquinolone-resistant and carbapenem-resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa* are increasing in prevalence in all provinces in Canada as well as in other countries. Available therapeutic options for the treatment of these antibiotic resistant organisms are severely limited as these organisms frequently display a multidrug resistant (MDR) phenotype. A major problem both in Canada as well as globally is MRSA. These pathogens can cause both Healthcare-Associated (HA) MRSA infections as well as Community-Associated (CA) MRSA infections.

API-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 both community-associated (CA) and healthcare-associated (HA) methicillin-resistant *S.aureus* (CA-MRSA and HA-MRSA) infections.



Molecular Structure of API-1252

PURPOSE

The purpose of this study was to assess the in-vitro activity of API versus Healthcare-Associated (HA) and Community-Associated (CA) Methicillin-Resistant *S. aureus* (MRSA).

MATERIALS AND METHODS

Isolate Collection: 350 methicillin-susceptible *S. aureus* (MSSA) and 154 MRSA were collected and characterized as part of an ongoing study assessing antimicrobial resistance in Canadian intensive care units (CAN-ICU) [Zhanel and Hoban AMMI 2006]. 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 indicated. At study sites, 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.

Methicillin-Resistant *Staphylococcus aureus* (MRSA) Identification: Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates were confirmed with the disk diffusion method (cefoxitin 30µg) as described by CLSI 2005. *mec* and *nuc* genes from MRSA isolates were coamplified with a multiplex PCR assay (1). *lukF*-PV and *lukS*-PV detection was carried out by PCR with primers and protocols previously described (1). Isolates were subtyped using pulsed-field gel electrophoresis (PFGE) after digestion with *Sma*I following the Canadian-standardized Protocol as described previously (1). PFGE-generated DNA fingerprints were digitized and analyzed with BioNumerics Ver 3.5 (Applied Maths, Sint-Martens-Latem, Belgium). Relatedness was determined following established criteria with major strain clusters (designated by a letter), grouped with banding patterns of <7 band changes. Fingerprints were compared to those in the national MRSA database. Isolates with specific DNA profiles were grouped into 1 of 6 Canadian epidemic strains of MRSA (eg. CMRSA 1, CMRSA 2, CMRSA 3 etc) (1).

Antimicrobial Susceptibility Testing: A custom designed microtitre panel with API-1252, cefazolin, ceftriaxone, meropenem, gentamicin, vancomycin, ciprofloxacin, levofloxacin, linezolid and piperacillin/tazobactam 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 (2005 CLSI). 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 100µl/well of cation-adjusted Mueller-Hinton broth were inoculated to achieve a final concentration of approximately 5×10^5 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.

RESULTS

Table 1. Activity of API-1252 and Comparators Against 350 MSSA

Antibiotic	MIC_{50}	MIC_{90}	MIC Range	% of Isolates Per Category[a]		
				S	I	R
API-1252	0.008	0.015	0.004 - 0.125	-	-	-
Cefazolin	≤0.5	≤0.5	≤0.5 - 8	100.0	0.0	0.0
Ceftriaxone	4	4	≤1 - 32	97.7	2.3	0.0
Ciprofloxacin	0.5	2	≤0.06 - >16	89.7	0.9	9.4
Gentamicin	≤0.25	0.5	≤0.25 - 64	97.7	0.3	2.0
Levofloxacin	0.25	0.5	≤0.06 - >32	91.1	0.3	8.6
Meropenem	≤0.12	0.25	≤0.12 - 4	100.0	0.0	0.0
Pip/Tazo	≤1	≤1	≤1 - 8	100.0	-	0.0
Vancomycin	1	1	≤0.25 - 2	100.0	0.0	0.0

[a]S, susceptible; I, intermediate; R, resistant.
Pip/Tazo-Piperacillin/tazobactam.

Table 2. Activity of API-1252 and Comparators Against 154 MRSA

Antibiotic	MIC_{50}	MIC_{90}	MIC Range	% of Isolates Per Category[a]		
				S	I	R
API-1252	0.008	0.015	0.002 - 0.125	-	-	-
Cefazolin	128	>128	1 - >128	0.0	0.0	100.0[b]
Ceftriaxone	>256	>256	16 - >256	0.0	0.0	100.0[b]
Ciprofloxacin	>16	>16	0.25 - >16	8.4	0.0	91.6
Gentamicin	0.5	>64	≤0.25 - >64	82.5	0.0	17.5
Levofloxacin	>32	>32	0.12 - >32	8.4	0.0	91.6
Meropenem	16	64	0.25 - >64	0.0	0.0	100.0[b]
Pip/Tazo	64	128	4 - 256	0.0	-	100.0[b]
Vancomycin	1	1	0.5– 2	100.0	0.0	0.0

[a]S, susceptible; I, intermediate; R, resistant.
[b]based upon oxacillin susceptibility.
Pip/Tazo-Piperacillin/tazobactam.

Table 3. Activity of API-1252 and Comparators Against 138 HA MRSA

Antibiotic	MIC_{50}	MIC_{90}	MIC Range	% of Isolates Per Category[a]		
				S	I	R
API-1252	0.008	0.015	0.002 - 0.125	-	-	-
Cefazolin	128	>128	2 - >128	0.0	0.0	100.0[b]
Ceftriaxone	>256	>256	16 - >256	0.0	0.0	100.0[b]
Ciprofloxacin	>16	>16	0.25 - >16	5.1	0.0	94.9
Gentamicin	0.5	>64	0.25 - >64	81.2	0.0	18.8
Levofloxacin	>32	>32	0.12 - >32	5.1	0.0	94.9
Meropenem	16	64	0.5 - >64	0.0	0.0	100.0[b]
Pip/Tazo	64	256	4 - 256	0.0	-	100.0[b]
Vancomycin	1	1	0.5 - 2	100.0	0.0	0.0

[a]S, susceptible; I, intermediate; R, resistant.
[b]based upon oxacillin susceptibility.
Pip/Tazo-Piperacillin/tazobactam.
CMRSA 1 (8/138 = 5.8%), CMRSA 2 (93/138 = 67.4%), CMRSA 4 (5/138 = 3.6%), CMRSA 6 (21/138 = 15.2%), CMRSA 8 (6/138 = 4.3%), CMRSA 9 (1/138 = 0.7%)

Table 4. Activity of API-1252 and Comparators Against 16 CA MRSA

Antibiotic	MIC_{50}	MIC_{90}	MIC Range	% of Isolates Per Category[a]		
				S	I	R
API-1252	0.008	0.008	0.004 - 0.015	-	-	-
Cefazolin	16	32	1 - >64	0.0	0.0	100.0[b]
Ceftriaxone	64	256	16 - >256	0.0	0.0	100.0[b]
Ciprofloxacin	16	16	0.25 - 16	37.5	0.0	62.5
Gentamicin	0.25	0.5	0.25 - 32	93.8	0.0	6.2
Levofloxacin	4	8	0.12 - 8	37.5	0.0	62.5
Meropenem	4	4	0.25 - 8	0.0	0.0	100.0[b]
Pip/Tazo	16	32	8 - 64	0.0	-	100.0[b]
Vancomycin	1	1	0.5 - 1	100.0	0.0	0.0

[a]S, susceptible; I, intermediate; R, resistant.
[b]based upon oxacillin susceptibility.
Pip/Tazo-Piperacillin/tazobactam.
CMRSA 7 (10/16 = 62.5 %), CMRSA 10 (6/16 = 37.5%)

Table 5. Characteristics of 16 CA MRSA in Canada

Stock #	Organism	City	Specimen Source	Age	Gender	PVL	CDN Designation	Ciprofloxacin	Gentamicin	Vancomycin	Linezolid	API-1252
58173	MRSA	Winnipeg	Respiratory	1	F	pos	CMRSA 7	0.25	0.5	1	1	0.008
62697	MRSA	Saskatoon	Blood	5	M	pos	CMRSA 7	0.25	</= 0.25	1	1	0.008
62825	MRSA	Regina	Blood	25	F	pos	CMRSA 7	0.5	</= 0.25	1	1	0.008
63307	MRSA	Winnipeg	Respiratory	58	M	pos	CMRSA 7	0.5	</= 0.25	1	1	0.008
64914	MRSA	Saskatoon	Respiratory	39	M	pos	CMRSA 7	0.5	</= 0.25	1	1	0.008
66065	MRSA	Saskatoon	Respiratory	67	M	pos	CMRSA 7	0.5	32	0.5	2	0.008
61592	MRSA	Vancouver	Blood	39	M	pos	CMRSA 10	16	</= 0.25	1	1	0.004
62996	MRSA	Victoria	Blood	21	F	pos	CMRSA 10	16	</= 0.25	1	2	0.004
64195	MRSA	Dartmouth	Blood	75	F	pos	CMRSA 10	16	</= 0.25	0.5	1	0.008
65226	MRSA	Victoria	Respiratory	56	M	pos	CMRSA 10	16	</= 0.25	1	1	0.008
65667	MRSA	Victoria	Blood	48	M	pos	CMRSA 10	16	</= 0.25	1	1	0.008
66122	MRSA	Halifax	Blood	69	F	pos	CMRSA 10	16	</= 0.25	1	0.5	0.015
66303	MRSA	Vancouver	Respiratory	38	M	pos	CMRSA 10	16	</= 0.25	0.5	1	0.008
67442	MRSA	Victoria	Respiratory	36	M	pos	CMRSA 10	16	0.5	0.5	1	0.008
67878	MRSA	Victoria	Wound	39	M	pos	CMRSA 10	16	0.5	1	1	0.008
67884	MRSA	Victoria	Blood	39	M	pos	CMRSA 10	16	0.5	1	1	0.008

CMRSA 7 (USA 400) = Aboriginals
CMRSA 10 (USA 300) = IV Drug abusers

CONCLUSIONS

1. API-1252 is highly active against methicillin-susceptible *Staphylococcus aureus* (MSSA).
2. API-1252 is highly active against both HA and CA MRSA.
3. No difference in API-1252 activity was observed between HA and CA MRSA.
4. API-1252 is a promising new agent against drug resistant Staphylococcal infections.

REFERENCES

1. Mulvey M, MacDougall L, Cholin B et al. Community-associated methicillin-resistant *Staphylococcus aureus,* Canada. Emerg Inf Dis 2005;11: 844-850.

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