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Activity of Debio1452, a FabI Inhibitor with Potent Activity against *Staphylococcus aureus* and Coagulase-Negative *Staphylococcus* spp., Including Multidrug-Resistant Strains

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Staphylococcus aureus **and coagulase-negative staphylococci (CoNS) are responsible for a wide variety of human infections. The investigational antibacterial Debio1450 (previously AFN-1720), a prodrug of Debio1452 (previously AFN-1252), specifically targets staphylococci without significant activity against other Gram-positive or Gram-negative species. Debio1452 inhibits FabI, an enzyme critical to fatty acid biosynthesis in staphylococci. The activity of Debio1452 against CoNS, methicillin-susceptible *S. aureus* (MSSA), and methicillin-resistant *S. aureus* (MRSA), including significant clones, was determined. A globally diverse collection of 574 patient isolates from 35 countries was tested that included CoNS (6 species, 103 strains), MSSA (154 strains), MRSA (163 strains), and molecularly characterized strains (including *spa*-typed MRSA clones; 154 strains). The isolates were tested for susceptibility by CLSI broth microdilution methods against Debio1452 and 10 comparators. The susceptibility rates for the comparators were determined using CLSI and EUCAST breakpoint criteria. All *S. aureus* and CoNS strains were inhibited by Debio1452 concentrations of ≤0.12 and ≤0.5 μg/ml, respectively. The MIC_{50}s for MSSA, MRSA, and molecularly characterized MRSA strains were 0.004 μg/ml, and the MIC_{90}s ranged from 0.008 to 0.03 μg/ml. The MICs were higher for the CoNS isolates ($MIC_{50/90}$, 0.015/0.12 μg/ml). Among *S. aureus* strains, resistance was common for erythromycin (61.6%), levofloxacin (49.0%), clindamycin (27.6%), tetracycline (15.7%), and trimethoprim-sulfamethoxazole (7.0%). Debio1452 demonstrated potent activity against MSSA, MRSA, and CoNS. Debio1452 showed significantly greater activity overall (MIC_{50}, 0.004 μg/ml) than the other agents tested against these staphylococcal species, which included dominant MRSA clones and strains resistant to currently utilized antimicrobial agents.**

Inhibitors of the fatty acid biosynthetic pathway have emerged as part of a potential approach to developing antibacterial agents (1–6). Among those inhibitors, Debio1452 (previously designated AFN-1252) was characterized for its specific activity against FabI, an essential enzyme involved in the final step of the elongation cycle of bacterial fatty acid biosynthesis (7, 8). Debio1452 is a novel FabI inhibitor that specifically targets *Staphylococcus* species (7–11). This compound has demonstrated a lack of activity against other species of bacteria, including streptococci, enterococci, *Enterobacteriaceae*, and nonfermentative Gram-negative species (9, 11).

The narrow targeted spectrum (staphylococci) exhibited by Debio1452 provides the benefit of minimizing the effect on normal bacterial flora and hence the potential for reduced antibiotic-associated adverse events, such as overgrowth of resistant commensals, diarrhea, and candidiasis. Further, its unique mode of action lessens the likelihood that resistance development to Debio1452 would lead to cross-resistance with currently available antimicrobial agents. A phase 2a study has been completed with Debio1452 used in acute bacterial skin and skin structure infections, with an overall cure rate of 93% (12). Debio1450, the prodrug of Debio1452, is currently in clinical development.

In the present study, Debio1452 was evaluated for its activity against a large collection of *Staphylococcus aureus* and coagulase-negative *Staphylococcus* species (CoNS) isolates. Included in the collection were *S. aureus* and CoNS clinical isolates from North America, Latin America, Europe, and the Asia-Pacific region, as well as a collection of genetically characterized isolates representing major circulating clones.

MATERIALS AND METHODS

Susceptibility testing methods. Debio1452 was supplied by Debiopharm International, SA, and was tested over 12 $\log_2$ dilutions (0.001 to 2 μg/ml). Rifampin, acquired from Sigma Chemical Co. (St. Louis, MO, USA), was used as a control agent (12 $\log_2$ dilutions [0.001 to 2 μg/ml]). The additional comparator antimicrobial agent data included those from oxacillin, erythromycin, clindamycin, daptomycin, vancomycin, linezolid, levofloxacin, tetracycline, and trimethoprim-sulfamethoxazole. These data were previously validated SENTRY MIC results. Broth microdilution frozen-form panels were supplied by Thermo Scientific (formerly TREK Diagnostics, Cleveland, OH, USA) using cation-adjusted Mueller-Hinton broth. The study design was conducted according to the CLSI M07-A9 (13) guidelines. The quality control (QC) ranges and interpretive criteria for the comparator compounds were as published in CLSI M100-S24 (14) and for Debio1452 as approved by CLSI (CLSI meeting minutes, January 2011 [http://clsi.org/standards/micro/microbiology-files/]); the tested QC strains included *S. aureus* strain ATCC 29213 and *Enterococcus faecalis* strain ATCC 29212.

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TABLE 1 Debio1452 MIC frequency distributions when tested against 574 isolates of *Staphylococcus* spp.

Species/phenotype (no. tested)	No. (cumulative %) of isolates inhibited at Debio1452 MIC (μg/ml) of:								
	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5
S. aureus (471)	4 (0.9)	297 (63.9)	142 (94.1)	9 (96.0)	6 (97.2)	11 (99.6)	2 (100.0)		
MSSA (154)	2 (1.3)	88 (58.4)	48 (89.6)	0 (89.6)	6 (93.5)	9 (99.4)	1 (100.0)		
MRSA (163)	1 (0.6)	102 (63.2)	52 (95.1)	8 (100.0)					
Molecularly characterized (154)[a]	1 (0.7)	107 (70.1)	42 (97.4)	1 (98.1)	0 (98.1)	2 (99.4)	1 (100.0)		
Coagulase-negative *Staphylococcus* spp. (103)[b]	0 (0.0)	4 (3.9)	23 (26.2)	28 (53.4)	20 (72.8)	12 (84.5)	12 (96.1)	3 (99.0)	1 (100.0)

[a] *spa* types included ST239 (Hungarian/Brazilian clone; SCC*mec* III), ST8 (USA300; SCC*mec* IV), ST22 (EMRSA-15; SCC*mec* IV), ST5 (Cordoba/Chilean clone, SCC*mec* I), USA100 to USA1100 clones, linezolid- and tigecycline-resistant strains, vancomycin-intermediate strains (VISA), and strains positive for several toxin genes, including the Panton-Valentine leukocidin (PVL) gene.

[b] Includes *S. epidermidis* (56 strains), *S. haemolyticus* (20 strains), *S. hominis* (11 strains), *S. saprophyticus* (6 strains), *S. warneri* (5 strains), and *S. xylosus* (5 strains).

Organism collection. A collection of 574 isolates was used to determine the activity of Debio1452. These included 317 geographically dispersed *S. aureus* isolates collected in 2010. This collection included methicillin-susceptible *S. aureus* (MSSA)/methicillin-resistant *S. aureus* (MRSA) isolates (154/163) from North America (60/66), Latin America (30/32), Europe (32/31), and the Asia-Western Pacific region (32/34). In addition, 154 genetically defined *S. aureus* isolates, including strains representative of major circulating global clones (details in Table 1), were used. Approximately 65% of the isolates were from bloodstream infections, 25% were from wound infections, and 10% were from miscellaneous infections. The strains were obtained from the JMI Laboratories bacterial strain collection and the Network on Antimicrobial Resistance in *S. aureus* (NARSA) (31 strains, including eight which were vancomycin-intermediate *S. aureus* [VISA]).

The coagulase-negative staphylococci ($n = 103$) collected from the SENTRY antimicrobial surveillance program were selected to include the following species: *Staphylococcus epidermidis* ($n = 56$), *Staphylococcus haemolyticus* ($n = 20$), *Staphylococcus hominis* ($n = 11$), *Staphylococcus xylosus* ($n = 5$), *Staphylococcus warneri* ($n = 5$), and *Staphylococcus saprophyticus* ($n = 6$). All strains were identified to the species level using the BactiStaph latex agglutination test, followed by the use of a confirmatory tube coagulase plasma test (Remel, Lenexa, KS, USA) and the Vitek II identification system (bioMérieux, Hazelwood, MO, USA). The resistance phenotypes were determined by reference broth microdilution tests, followed by confirmation as required or specified by the CLSI M100-S24 criteria (14).

RESULTS

Debio1452 was very active against *S. aureus* (Table 1). All *S. aureus* isolates were inhibited by Debio1452, with MICs of ≤0.12 μg/ml and MIC_{50} and MIC_{90} values of 0.004 μg/ml and 0.008 μg/ml, respectively. Against the "all *S. aureus*" isolate collection ($n = 471$), Debio1452 was 64-, 128-, and 256-fold more active than were daptomycin (MIC_{90}, 0.5 μg/ml), vancomycin (MIC_{90}, 1 μg/ml), and linezolid (MIC_{90}, 2 μg/ml), respectively. Among all *S. aureus* isolates, resistance to various antimicrobial classes according to CLSI and EUCAST criteria (15) (Table 2) was high, including for erythromycin (61.6 to 62.2%), levofloxacin (49.0%), clindamycin (27.6 to 28.0%), tetracycline (15.7 to 17.0%), and trimethoprim-sulfamethoxazole (6.6 to 7.0%). The daptomycin, linezolid, and vancomycin susceptibility rates were high, at 98.7, 99.2, and 98.3%, respectively.

The collection of global clinical isolates of *S. aureus* ($n = 317$), excluding the molecularly characterized strains, included 51.4% MRSA, against which Debio1452 ($MIC_{50/90}$, 0.004/0.008 μg/ml) was 2-fold more active than was rifampin ($MIC_{50/90}$, 0.008/0.015 μg/ml) and significantly more potent than were the other comparator agents (Table 2). For the MRSA isolates, the rates of susceptibility to clindamycin, erythromycin, and levofloxacin were very low, at 47.9, 18.4, and 19.6%, respectively (Table 2). For rifampin and trimethoprim-sulfamethoxazole, the susceptibility rates were 92.0 and 93.3%, respectively. All MRSA isolates among the global clinical isolates of *S. aureus* (excluding the molecularly characterized strains) were susceptible to linezolid, daptomycin, and vancomycin (Table 2). The susceptibility rates were higher for MSSA than for MRSA for rifampin, oxacillin, erythromycin, clindamycin, levofloxacin, tetracycline, and trimethoprim-sulfamethoxazole (Table 2).

The molecularly characterized *S. aureus* isolates were nearly all (95.5%) resistant to oxacillin (Table 2). Debio1452 retained similar potency compared to the non-molecularly characterized strains (MIC_{50} and MIC_{90}, 0.004 and 0.008 μg/ml, respectively, for both collections; Table 2). The resistances to erythromycin (81.8%), clindamycin (23.4 to 24.7%), levofloxacin (61.0%), tetracycline (21.4 to 22.7%), and trimethoprim-sulfamethoxazole (13.0%) were elevated (Table 2). The rates of susceptibility to daptomycin, linezolid, and vancomycin ranged from 94.8 to 97.4% (Table 2). Included in the molecularly characterized strains were isolates representing major MRSA clonal types, such as sequence type 239 (ST239) (Hungarian/Brazilian clone; staphylococcal cassette chromosome *mec* element type III [SCC*mec* III]), ST8 (USA300; SCC*mec* IV), ST22 (epidemic MRSA clone 15 [EMRSA-15]; SCC*mec* IV), ST5 (Cordoba/Chilean clone; SCC*mec* I), the USA100 to USA1100 clones, and strains positive for several toxin genes, including the Panton-Valentine leukocidin gene (PVL). The PVL- and non-PVL-producing strains demonstrated similar susceptibilities to Debio1452.

The CoNS isolates tended to have higher Debio1452 MIC values than those for *S. aureus* (Tables 2 and 3). The Debio1452 MIC_{50} and MIC_{90} values for CoNS were 0.015 μg/ml (4-fold higher than those for *S. aureus*) and 0.12 μg/ml (16-fold higher than those for *S. aureus*), respectively, with the highest reproducible MIC observed at 0.5 μg/ml (one *S. epidermidis* isolate) (Table 1). The MIC distributions for methicillin-resistant CoNS (MR-CoNS) and methicillin-susceptible CoNS (MS-CoNS) were similar (data not shown). All CoNS isolates were susceptible to daptomycin, linezolid, and vancomycin (Table 3).

DISCUSSION

S. aureus is a ubiquitous pathogen that has the ability to disseminate and cause severe morbidity and mortality (16–21). It is a major cause of many types of infections that occur both in com-

TABLE 2 Activities of Debio1452 and comparator antimicrobial agents when tested against isolates of *S. aureus*

Antimicrobial agent (no. tested)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)	% susceptible/% resistant according to[a]: CLSI	EUCAST
All *S. aureus* isolates (471)					
Debio1452	0.004	0.008	0.002 to 0.12	−/−	−/−
Rifampin	0.008	0.015	0.004 to >2	93.0/4.5	−/−
Oxacillin	>2	>2	≤0.25 to >2	34.2/65.8	34.2/65.8
Erythromycin	>2	>2	≤0.25 to >2	37.6/61.6	37.6/62.2
Clindamycin	≤0.25	>2	≤0.25 to >2	72.0/27.6	71.5/28.0
Daptomycin	0.25	0.5	≤0.06 to 4	98.7/−	98.7/1.3
Vancomycin	1	1	≤0.12 to 8	98.3/0.0	98.3/1.7
Linezolid	1	2	0.5 to >8	99.2/0.8	99.2/0.8
Levofloxacin	2	>4	≤0.5 to >4	49.7/49.0	49.7/49.0
Tetracycline	≤2	>8	≤2 to >8	83.4/15.7	82.0/17.0
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5 to >2	93.0/7.0	93.0/6.6
MSSA (154)					
Debio1452	0.004	0.03	0.002 to 0.12	−/−	−/−
Rifampin	0.008	0.008	0.004 to >2	98.1/0.6	−/−
Oxacillin	≤0.25	≤0.25	≤0.25 to 0.5	100.0/0.0	100.0/0.0
Erythromycin	≤0.25	>4	≤0.25 to >4	77.3/22.1	77.3/22.1
Clindamycin	≤0.25	≤0.25	≤0.25 to >2	94.2/5.8	94.2/5.8
Daptomycin	0.25	0.5	≤0.06 to 1	100.0/−	100.0/0.0
Vancomycin	1	1	0.25 to 2	100.0/0.0	100.0/0.0
Linezolid	1	1	0.5 to 2	100.0/0.0	100.0/0.0
Levofloxacin	≤0.5	≤0.5	≤0.5 to >4	92.2/7.1	92.2/7.1
Tetracycline	≤0.25	2	≤0.25 to >8	90.3/8.4	89.6/9.7
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5 to >4	98.7/1.3	98.7/1.3
MRSA (163)					
Debio1452	0.004	0.008	0.002 to 0.015	−/−	−/−
Rifampin	0.008	0.015	0.004 to >2	92.0/6.7	−/−
Oxacillin	>2	>2	>2	0.0/100.0	0.0/100.0
Erythromycin	>4	>4	≤0.25 to >4	18.4/79.8	18.4/81.6
Clindamycin	>2	>2	≤0.25 to >2	47.9/52.1	47.9/52.1
Daptomycin	0.25	0.5	0.12 to 1	100.0/−	100.0/0.0
Vancomycin	1	1	0.5 to 2	100.0/0.0	100.0/0.0
Linezolid	1	1	0.5 to 2	100.0/0.0	100.0/0.0
Levofloxacin	>4	>4	≤0.5 to >4	19.6/77.3	19.6/77.3
Tetracycline	≤0.25	>8	≤0.25 to >8	81.6/17.2	79.1/18.4
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5 to >4	93.3/6.7	93.3/5.5
Molecularly characterized *S. aureus* (154)					
Debio1452	0.004	0.008	0.002 to 0.12	−/−	−/−
Rifampin	0.008	2	0.004 to >2	89.0/5.8	−/−
Oxacillin	>2	>2	≤0.25 to >2	4.5/95.5	4.5/95.5
Erythromycin	>2	>2	≤0.25 to >2	18.2/81.8	18.2/81.8
Clindamycin	≤0.25	>2	≤0.25 to >2	75.3/23.4	74.0/24.7
Daptomycin	0.5	0.5	0.25 to 4	96.1/−	96.1/3.9
Vancomycin	1	1	≤0.12 to 8	94.8/0.0	94.8/5.2
Linezolid	1	2	0.5 to >8	97.4/2.6	97.4/2.6
Levofloxacin	4	>4	≤0.5 to >4	39.0/61.0	39.0/61.0
Tetracycline	≤2	>8	≤2 to >8	78.6/21.4	77.3/22.7
Trimethoprim-sulfamethoxazole	≤0.5	>2	≤0.5 to >2	87.0/13.0	87.0/13.0

[a] Susceptibility criteria are as published by the CLSI (14) and EUCAST (15). –, no interpretive criteria exist for this category.

munity and health care settings (17, 18). The distinction between community isolates and health care-associated isolates has blurred. Thus, drug resistance, including high MRSA rates, may occur for isolates from either inpatients or outpatients (17, 18, 22–25). Especially concerning is the increased morbidity and mortality that have been suggested to occur with MRSA infections compared to those with MSSA infections (18–21).

Coagulase-negative staphylococci (CoNS) tend to be of lesser virulence than *S. aureus*; however, they may be pathogens in a variety of infections, including those in immunocompromised

TABLE 3 Activities of Debio1452 and comparator antimicrobial agents when tested against 103 isolates of coagulase-negative staphylococci[a]

Antimicrobial agent used	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)	% susceptible/% resistant according to[b]:	
				CLSI	EUCAST
Debio1452	0.015	0.12	0.004 to 0.5	−/−	−/−
Rifampin	0.008	0.015	≤0.001 to >2	97.1/2.9	−/−
Oxacillin	≤0.25	1	≤0.25 to >2	83.5/16.5	83.5/16.5
Erythromycin	≤0.25	>2	≤0.25 to >2	51.5/48.5	51.5/48.5
Clindamycin	≤0.25	>2	≤0.25 to >2	88.3/11.7	85.4/11.7
Daptomycin	0.25	0.5	≤0.06 to 1	100.0/−	100.0/0.0
Vancomycin	1	2	0.25 to 2	100.0/0.0	100.0/0.0
Linezolid	0.5	1	≤0.12 to 1	100.0/0.0	100.0/0.0
Levofloxacin	≤0.5	>4	≤0.5 to >4	76.7/22.3	76.7/22.3
Tetracycline	≤2	>8	≤2 to >8	86.3/12.7	82.4/13.7
Trimethoprim-sulfamethoxazole	≤0.5	>2	≤0.5 to >2	86.4/13.6	86.4/10.7

[a] Includes *S. epidermidis* (56 strains), *S. haemolyticus* (20 strains), *S. hominis* (11 strains), *S. saprophyticus* (6 strains), *S. warneri* (5 strains), and *S. xylosus* (5 strains).
[b] Susceptibility criteria are as published by the CLSI (14) and EUCAST (15). –, no interpretive criteria exist for this category.

hosts (26). Drug resistance in *S. aureus* and CoNS is a continuing problem (18, 26). There is a need for a novel class of agents with high potency and clinical efficacy against *S. aureus* and CoNS.

In this study, Debio1452 was shown to have significant activity against a diverse collection of staphylococcal pathogens, including MRSA strains that are endemic in hospital and community environments worldwide (18, 27–30). The isolates tested in this study included isolates from North America, Europe, Latin America, and the Asia-Pacific region. The clinical isolates of *S. aureus* were highly susceptible to Debio1452, exhibiting an MIC_{50} and MIC_{90} of 0.004 and 0.008 μg/ml, respectively. These values were similar to those shown for *S. aureus* clinical isolates collected during 2005 to 2006 as part of the Canadian Intensive Care Unit study (MIC_{50} and MIC_{90}, 0.008 and 0.015 μg/ml, respectively) and for clinical isolates of *S. aureus* collected during 2007 as part of the Canadian Ward Surveillance (CANWARD) program (MIC_{50} and MIC_{90}, ≤0.008 μg/ml) (9). This indicated that the activity of Debio1452 tested against isolates collected from various regions of the world was similar to that noted in the previously conducted Canadian studies (9, 10). The CoNS isolates in our study were highly susceptible to Debio1452, with slightly higher MIC_{50} and MIC_{90} values observed than those for *S. aureus*. CoNS from the Canadian Intensive Care Unit study and the CANWARD program also exhibited slightly higher MIC values than those for *S. aureus* (9, 10).

In summary, Debio1452 exhibited a high level of potency against molecularly characterized *S. aureus* isolates, with MIC_{50} and MIC_{90} values identical to those of *S. aureus* (including MRSA), from a global collection of surveillance isolates (collected from North America, Europe, Latin America, and the Asia-Pacific region). The remarkably consistent activities against these common staphylococcal pathogens, including those with increasingly prevalent resistance mechanisms, are a promising feature of this novel agent and warrant its further development for the treatment of serious staphylococcal infections.

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