

Antistaphylococcal Activity of CG400549, a New Experimental FabI Inhibitor, Compared with That of Other Agents[∇]

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Received 25 April 2007/Returned for modification 2 July 2007/Accepted 10 July 2007

Among 203 strains of *Staphylococcus aureus*, the MICs of CG400549 were 0.06 to 1.0 µg/ml, with MIC₅₀ and MIC₉₀ values of 0.25 µg/ml each. All strains were susceptible to linezolid and quinupristin-dalfopristin (MICs, 0.25 to 2.0 µg/ml). The daptomycin MICs were 0.25 to 2.0 µg/ml for methicillin-susceptible and 0.25 to 4.0 µg/ml against methicillin-resistant strains (including vancomycin-intermediate strains). Single-passage selection testing showed low resistance frequencies with CG400549, but multistep analysis showed that CG400549 yielded resistant mutants after 14 to 17 days in all strains tested.

Although in the past most *Staphylococcus aureus* infections have been nosocomial, the past few years have witnessed a worrisome emergence of community-acquired strains, many of which produce Panton-Valentine leukocidin (PVL) and cause serious and life-threatening infections (4, 11, 12, 14, 16, 17). Most community and nosocomial strains are methicillin-resistant *S. aureus* (MRSA) and quinolone-resistant, nosocomially acquired MRSA strains are often multiresistant (18).

Since the first description of vancomycin-intermediate *Staphylococcus aureus* (VISA) strains in Japan, these organisms have been reported all over the world (21). The recent lowering by the Clinical Laboratory Standards Institute (CLSI) of the vancomycin susceptibility breakpoint from ≤4.0 µg/ml to ≤2.0 µg/ml (10) will surely lead to a much wider appreciation of the clinical importance of these strains. In 2002, the first report of vancomycin-resistant *S. aureus* (VRSA) producing *vanA* occurred (3); to date, seven VRSA strains have been reported (8, 22; M. Rybak, personal communication).

It seems clear that the widespread use of vancomycin or teicoplanin in the community and hospital settings produces the selective pressure for VISA and VRSA strains: in most cases, prior glycopeptide use has been found in individual patients. The further spread of virulent community-acquired *S. aureus* strains (9, 14) will only exacerbate this problem. There is therefore a need for new antistaphylococcal agents with unique mechanisms of action.

The last step in fatty acid synthesis is performed by enoyl-acyl carrier protein (ACP) reductase (FabI), which is responsible for the reduction of the double bond in the enoyl-ACP derivative (1, 5). In *S. aureus* and *Escherichia coli*, this enzyme has been shown to be the antibacterial target of triclosan and diazaborines, thereby showing the essentiality of FabI in these organisms (17, 20). FabI is absent in some organisms, and an alternative enoyl-ACP reductase, FabK, is present in some pathogens, such as *Streptococcus pneumoniae*. Both FabI and

FabK have been found in *Enterococcus faecalis* and *Pseudomonas aeruginosa* (19).

The current study investigates (i) the activity of CG400549, a new experimental FabI inhibitor (Fig. 1), in comparison with the activities of vancomycin, teicoplanin, linezolid, quinupristin-dalfopristin, daptomycin, amoxicillin-clavulanate, azithromycin, and levofloxacin against 203 *S. aureus* strains as determined by agar dilution MIC testing and (ii) the abilities of CG400549, vancomycin, linezolid, quinupristin-dalfopristin, and daptomycin to select for resistance in 10 strains by single- and multistep resistance selection techniques.

One hundred and three methicillin-susceptible *Staphylococcus aureus* (MSSA) and 100 MRSA strains were studied. Among MRSA strains, 54 were community-acquired and 47 of these produced PVL. The MRSA strains included five VISA strains (including a recently isolated VISA strain from a patient at Hershey Medical Center which developed the VISA phenotype while the patient was on vancomycin therapy) (15) and the first three VRSA strains described (from Michigan, Pennsylvania, and New York). The single- and multistep testing used 10 *S. aureus* strains of various phenotypes: two MSSA, four vancomycin-susceptible MRSA, two VISA, and two VRSA.

CG400549 susceptibility testing powder was obtained from Crystalgenomics, Inc., Seoul, Republic of Korea. Other antimicrobials were obtained from their respective manufacturers. Agar dilution testing was performed by the CLSI methodology, with added calcium for the daptomycin testing (10). The vancomycin MICs were read after a full 24-h incubation (10).

For the single-step resistance studies, two MSSA and eight MRSA (including two VRSA and two VISA) strains were tested as previously described (6). Because colonies obtained at these MICs often occurred on a background of confluent growth, retesting by the agar dilution MIC method was performed. To ensure the reproducibility and reliability of the results, resistance in the single-step studies was defined as a MIC more than four times greater than that against the parent.

In the multistep resistance selection tests, 10 *S. aureus* strains, including 8 MRSA (two VRSA and two VISA) and 2 MSSA strains, were examined. Serial passages were performed daily for each strain in subinhibitory concentrations of all an-

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[∇] Published ahead of print on 17 September 2007.

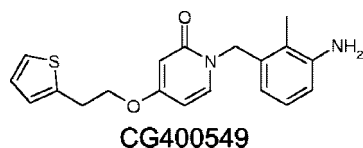


FIG. 1. Structure of CG400549.

timicrobials. The methods were described previously (6). The identities of the mutants obtained and of their respective parents were confirmed at the end of the study by pulsed-field gel electrophoresis using a CHEF DR III apparatus (Bio-Rad, Hercules, CA) (2).

Among 203 methicillin-susceptible and -resistant strains of *S. aureus*, including strains with reduced susceptibility to vancomycin and teicoplanin, the MICs for CG400549 were all between 0.06 and 1.0 $\mu\text{g/ml}$, with MIC₅₀ and MIC₉₀ values of 0.25 $\mu\text{g/ml}$ for each. All strains were susceptible to linezolid and quinupristin-dalfopristin (MICs between 0.25 and 2.0 $\mu\text{g/ml}$ for both compounds). The daptomycin MICs ranged between 0.25 and 2.0 $\mu\text{g/ml}$ for methicillin-susceptible and 0.25 and 4.0 $\mu\text{g/ml}$ against methicillin-resistant strains. When vancomycin-nonsusceptible strains were excluded, the highest daptomycin MIC was 1.0 $\mu\text{g/ml}$. Methicillin-susceptible strains were amoxicillin-clavulanate susceptible, and resistance to levofloxacin and azithromycin was found (Table 1).

The results of single-step studies are presented in Table 2. The MICs ($\mu\text{g/ml}$) against the parental strains were as follows: for CG400549, 0.125 to 1; for vancomycin, 1 to >64; for linezolid, 0.5 to 2; for quinupristin-dalfopristin, 0.5 to 1; and for daptomycin, 0.5 to 8. The mutation frequencies with CG400549 ranged from 1.9×10^{-7} to $<1.4 \times 10^{-10}$ at 2 \times MIC to 1.5×10^{-8} to 8×10^{-10} at 8 \times MIC; the maximal CG400549 MIC for the recovered clones was 16 $\mu\text{g/ml}$. For vancomycin, the mutation frequencies at 2 \times and 8 \times MIC were 1.3×10^{-5} to $<3.7 \times 10^{-10}$ and 1.4×10^{-6} to $<3.7 \times 10^{-10}$, respectively. The linezolid mutation frequencies were 1.1×10^{-4} to $<3.7 \times 10^{-10}$ at 2 \times MIC and $<1.3 \times 10^{-10}$ to $<3.7 \times 10^{-10}$ at 8 \times MIC. The daptomycin mutation frequencies ranged from 1.2×10^{-6} to $<2 \times 10^{-10}$ at 2 \times MIC to $<3.6 \times 10^{-9}$ to $<2.6 \times 10^{-10}$ at 8 \times MIC. For quinupristin-dalfopristin, the mutation frequencies were in the range of 3.3×10^{-6} to 4.1×10^{-10} at 2 \times MIC and 1.1×10^{-10} to $<3.3 \times 10^{-10}$ at 8 \times MIC. Single-step mutants were verified by MIC (6).

The results of the multistep selection studies are presented in Table 3. The MIC ($\mu\text{g/ml}$) ranges for parent strains were as follows: for CG400549, 0.125 to 1; for vancomycin, 2 to >64; for linezolid, 1 to 4; for quinupristin-dalfopristin, 0.125 to 0.5; and for daptomycin, 1 to 16.

As can be seen, CG400549 selected for resistant mutants in all 10 strains after 14 to 17 days (parents, 0.125 to 1 $\mu\text{g/ml}$, and mutants, 2 to >64 $\mu\text{g/ml}$). By comparison, daptomycin selected mutants in 8 of the 10 strains after 14 to 24 days (parents, 1 $\mu\text{g/ml}$, and mutants, 8 $\mu\text{g/ml}$). Linezolid selected for resistant mutants in 7 of 10 strains after 15 to 48 days (parents, 1 to 4 $\mu\text{g/ml}$, and mutants, 8 to 32 $\mu\text{g/ml}$). Quinupristin-dalfopristin selected for resistant mutants in all 10 strains tested after 14 to 23 days (parents, 0.125 to 0.5 $\mu\text{g/ml}$, and mutants, 1 to 4 $\mu\text{g/ml}$). None of the eight vancomycin-susceptible and vancomycin-intermediate strains showed increased resistance to vancomycin even after 50 days of subculture. The two VRSA strains (MIC, ≥ 64 $\mu\text{g/ml}$) were not tested with vancomycin in single- or multistep studies. No cross-reactivity between drug groups was observed. Three mutants (one each with CG400549, linezolid, and quinupristin-dalfopristin) had daptomycin MICs that were two to three dilutions lower than those of the parent strains, and nine mutants (three with CG400549, two with daptomycin, and four with quinupristin-dalfopristin) had vancomycin MICs that were two to more than five dilutions lower than those of the parent strains (Table 3). Additionally, two CG400549 mutants and two daptomycin mutants had MICs that were two dilutions lower after 10 daily subcultures on antibiotic-free media than they had been before the subcultures (Table 3). All resistant clones were identical to their parent strains by pulsed-field analysis.

Previous studies have demonstrated elevated CG400549 MICs for FabI-overexpressing *S. aureus* strains; animal studies showed promising results, and the drug was safe at up to 1 g/kg of body weight of subcutaneous or oral use (C. Kim, J. Kwak, C. Lee, H. Park, S. Kang, J. Kim, Y. Song, S. Ro, T. Lee, and J. Cho, presented at the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy, December 2005). The CG400549 MIC₉₀ values were all 0.5 $\mu\text{g/ml}$ against 1 VISA strain, 215 MRSA strains, and 41 coagulase-negative methicillin-resistant staphylococci (C. Kim, J. Kwak, Y. Kim, H. Yoon, H. Park, S. Ro, T. Lee, and J. Cho, presented at the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy, December 2005). CG400549 was bactericidal at 8 \times

TABLE 1. MICs of CG400549 compared with those of other agents against *S. aureus*

Drug	MIC(s) ($\mu\text{g/ml}$) against:					
	MSSA strains ($n = 103$)			MRSA strains ($n = 100$)		
	Range	MIC ₅₀	MIC ₉₀	Range	MIC ₅₀	MIC ₉₀
CG400549	0.06–1.0	0.25	0.25	0.06–1.0	0.25	0.25
Vancomycin	1.0–2.0	1.0	2.0	1.0–>64.0	1.0	2.0
Teicoplanin	0.125–8.0	1.0	2.0	0.25–>64.0	1.0	2.0
Linezolid	0.25–2.0	1.0	2.0	0.25–2.0	1.0	2.0
Quinupristin-dalfopristin	0.25–2.0	1.0	2.0	0.25–2.0	1.0	2.0
Daptomycin	0.25–2.0	1.0	1.0	0.25–4.0	0.5	0.5
Amoxicillin-clavulanate	0.125–4.0	1.0	2.0	0.5–>64.0	>64.0	>64.0
Levofloxacin	≤ 0.06 –32.0	0.25	4.0	0.125–>32.0	1.0	>32.0
Azithromycin	0.25–>64.0	1.0	>64.0	0.5–>64.0	>64.0	>64.0

TABLE 2. Results of single-step resistance selection studies

Strain; description	Drug	Mutation frequency at:		
		2× MIC	4× MIC	8× MIC
SA510; MRSA, VRSA	CG400549	$\sim 6.8 \times 10^{-9}$	$\sim 3.4 \times 10^{-9}$	$\sim 6.8 \times 10^{-9}$
	Vancomycin	NT ^a	NT	NT
	Linezolid	$< 3.3 \times 10^{-10}$	$< 3.3 \times 10^{-10}$	$< 3.3 \times 10^{-10}$
	Quinupristin-dalfopristin	$\sim 3.3 \times 10^{-6}$	$\sim 3.3 \times 10^{-8}$	$< 3.3 \times 10^{-10}$
	Daptomycin	$\sim 2.6 \times 10^{-7}$	$< 2.6 \times 10^{-10}$	$< 2.6 \times 10^{-10}$
SA509; MRSA, VRSA	CG400549	$\sim 3.0 \times 10^{-7}$	$\sim 1.5 \times 10^{-8}$	$\sim 1.5 \times 10^{-8}$
	Vancomycin	NT	NT	NT
	Linezolid	$< 2.6 \times 10^{-10}$	$< 2.6 \times 10^{-10}$	$< 2.6 \times 10^{-10}$
	Quinupristin-dalfopristin	$\sim 3.9 \times 10^{-9}$	$< 2.6 \times 10^{-10}$	$< 2.6 \times 10^{-10}$
	Daptomycin	$< 1.7 \times 10^{-10}$	$< 1.7 \times 10^{-10}$	$< 1.7 \times 10^{-10}$
SA555; MRSA, VISA	CG400549	$\sim 2.0 \times 10^{-9}$	$< 1.0 \times 10^{-10}$	$< 1.0 \times 10^{-10}$
	Vancomycin	$< 1.6 \times 10^{-10}$	$< 1.6 \times 10^{-10}$	$< 1.6 \times 10^{-10}$
	Linezolid	$\sim 4 \times 10^{-6}$	$< 2 \times 10^{-10}$	$< 2 \times 10^{-10}$
	Quinupristin-dalfopristin	$< 2 \times 10^{-10}$	$< 2 \times 10^{-10}$	$< 2 \times 10^{-10}$
	Daptomycin	$< 2 \times 10^{-10}$	$< 2 \times 10^{-10}$	$< 2 \times 10^{-10}$
SA504; MRSA, VISA	CG400549	$< 1.4 \times 10^{-10}$	$< 1.4 \times 10^{-10}$	$< 1.4 \times 10^{-10}$
	Vancomycin	$< 3.7 \times 10^{-10}$	$< 3.7 \times 10^{-10}$	$< 3.7 \times 10^{-10}$
	Linezolid	$< 3.7 \times 10^{-10}$	$< 3.7 \times 10^{-10}$	$< 3.7 \times 10^{-10}$
	Quinupristin-dalfopristin	$< 1.6 \times 10^{-10}$	$< 1.6 \times 10^{-10}$	$< 1.6 \times 10^{-10}$
	Daptomycin	$\sim 4 \times 10^{-6}$	$\sim 2 \times 10^{-8}$	$\sim 3.6 \times 10^{-9}$
SA481; MRSA	CG400549	$\sim 3.0 \times 10^{-9}$	$\sim 3.0 \times 10^{-9}$	$\sim 8.0 \times 10^{-10}$
	Vancomycin	$< 1.7 \times 10^{-10}$	$< 1.7 \times 10^{-10}$	$< 1.7 \times 10^{-10}$
	Linezolid	$\sim 1.7 \times 10^{-6}$	$< 1.7 \times 10^{-10}$	$< 1.7 \times 10^{-10}$
	Quinupristin-dalfopristin	$< 1.2 \times 10^{-10}$	$< 1.2 \times 10^{-10}$	$< 1.2 \times 10^{-10}$
	Daptomycin	$< 1.9 \times 10^{-10}$	$\sim 4.7 \times 10^{-8}$	$\sim 3.9 \times 10^{-9}$
SA490; MRSA	CG400549	$\sim 1.9 \times 10^{-9}$	$\sim 1.4 \times 10^{-9}$	$< 1.4 \times 10^{-10}$
	Vancomycin	$< 1.3 \times 10^{-10}$	$< 1.3 \times 10^{-10}$	$< 1.3 \times 10^{-10}$
	Linezolid	$< 1.4 \times 10^{-10}$	$< 1.4 \times 10^{-10}$	$< 1.4 \times 10^{-10}$
	Quinupristin-dalfopristin	$< 1.3 \times 10^{-10}$	$< 1.3 \times 10^{-10}$	$< 1.3 \times 10^{-10}$
	Daptomycin	$\sim 7.6 \times 10^{-8}$	$\sim 2.6 \times 10^{-8}$	$\sim 6.4 \times 10^{-9}$
SA525; MRSA	CG400549	$\sim 5.3 \times 10^{-8}$	$\sim 7.3 \times 10^{-9}$	$\sim 2.3 \times 10^{-9}$
	Vancomycin	$< 2.1 \times 10^{-10}$	$\sim 3.2 \times 10^{-9}$	$< 2.1 \times 10^{-10}$
	Linezolid	$\sim 1.1 \times 10^{-4}$	$< 2.1 \times 10^{-10}$	$< 2.1 \times 10^{-10}$
	Quinupristin-dalfopristin	$\sim 1.1 \times 10^{-9}$	$< 1.1 \times 10^{-10}$	$< 1.1 \times 10^{-10}$
	Daptomycin	$\sim 2.7 \times 10^{-8}$	$< 1.3 \times 10^{-10}$	$< 1.3 \times 10^{-10}$
SA547; MRSA	CG400549	$\sim 1.1 \times 10^{-8}$	$\sim 2.2 \times 10^{-9}$	$\sim 1.3 \times 10^{-9}$
	Vancomycin	$< 1.7 \times 10^{-10}$	$< 1.7 \times 10^{-10}$	$< 1.7 \times 10^{-10}$
	Linezolid	$\sim 6.3 \times 10^{-6}$	$< 1.6 \times 10^{-10}$	$< 1.6 \times 10^{-10}$
	Quinupristin-dalfopristin	$\sim 4.1 \times 10^{-10}$	$< 1.4 \times 10^{-10}$	$< 1.4 \times 10^{-10}$
	Daptomycin	$< 1.9 \times 10^{-10}$	$< 1.9 \times 10^{-10}$	$< 1.9 \times 10^{-10}$
SA540; MSSA	CG400549	$\sim 1.9 \times 10^{-7}$	$\sim 2.5 \times 10^{-9}$	$\sim 1.3 \times 10^{-9}$
	Vancomycin	$< 1.2 \times 10^{-10}$	$< 1.2 \times 10^{-10}$	$< 1.2 \times 10^{-10}$
	Linezolid	$< 1.3 \times 10^{-10}$	$< 1.3 \times 10^{-10}$	$< 1.3 \times 10^{-10}$
	Quinupristin-dalfopristin	$\sim 1.2 \times 10^{-10}$	$\sim 2.4 \times 10^{-10}$	$< 1.2 \times 10^{-10}$
	Daptomycin	$\sim 1.2 \times 10^{-6}$	$< 1.2 \times 10^{-10}$	$\sim 4.9 \times 10^{-9}$
SA543; MSSA	CG400549	$\sim 2.2 \times 10^{-7}$	$\sim 1.2 \times 10^{-8}$	$\sim 5.2 \times 10^{-9}$
	Vancomycin	$\sim 1.3 \times 10^{-5}$	$\sim 4.7 \times 10^{-5}$	$\sim 1.4 \times 10^{-6}$
	Linezolid	$< 1.4 \times 10^{-10}$	$< 1.4 \times 10^{-10}$	$< 1.4 \times 10^{-10}$
	Quinupristin-dalfopristin	$< 1.1 \times 10^{-10}$	$< 1.1 \times 10^{-10}$	$< 1.1 \times 10^{-10}$
	Daptomycin	$< 2 \times 10^{-10}$	$< 2 \times 10^{-10}$	$< 2 \times 10^{-10}$

^a NT, not tested.

MIC after 24 h, with a postantibiotic effect of 1 h against MRSA. The development of resistance to this drug in *S. aureus* was rare, with a frequency of 2.1×10^{-8} (C. Kim, Y. Kim, H. Yoon, J. Kim, S. Ro, T. Lee, and J. Cho, presented at the 45th

Interscience Conference on Antimicrobial Agents and Chemotherapy, December 2005).

The results of single-step studies showed low frequencies of resistant mutants with CG400549, either comparable to or

TABLE 3. Results of multistep resistance selection studies^a

Strain; description	Antibiotic	Initial MIC (μg/ml)	MIC (μg/ml) after resistance selection (no. of passages)	MIC after 10 antibiotic-free subcultures for:				
				CG400549	Daptomycin	Linezolid	Quinupristin-dalfopristin	Vancomycin
SA481; CAMRSA, PVL neg	CG400549	0.25	2 (14)	2	1	4	0.25	2
	Daptomycin	1	8 (14)	0.25	8	4	0.25	4
	Linezolid	4	32 (37)	0.125	1	32	0.5	2
	Quinupristin-dalfopristin	0.25	2 (16)	0.25	1	4	2	2
	Vancomycin	2	8 (50)	NT	NT	NT	NT	NT
SA490; CAMRSA, PVL pos	CG400549	0.25	8 (17)	8	1	4	0.25	2
	Daptomycin	1	8 (14)	0.25	4	4	0.125	2
	Linezolid	4	32 (22)	0.25	1	32	0.25	2
	Quinupristin-dalfopristin	0.25	4 (23)	0.125	1	4	4	2
	Vancomycin	2	8 (50)	NT	NT	NT	NT	NT
SA504; VISA	CG400549	0.25	2 (14)	2	1 ^b	2	0.5	2 ^b
	Daptomycin	4	16 (50)	NT	NT	NT	NT	NT
	Linezolid	1	8 (22)	0.25	1 ^b	8	0.5	8
	Quinupristin-dalfopristin	0.25	2 (16)	0.5	2	2	2	2 ^b
	Vancomycin	8	8 (50)	NT	NT	NT	NT	NT
SA509; VRSA, from Michigan	CG400549	1	>64 (14)	>64	1	2	0.5	16 ^b
	Daptomycin	1	8 (20)	1	4	2	0.5	32 ^b
	Linezolid	2	16 (28)	0.5	2	32	1	>64
	Quinupristin-dalfopristin	0.5	4 (18)	1	1	4	4	4 ^b
	Vancomycin	>64	NT	NT	NT	NT	NT	NT
SA510; VRSA, from Hershey	CG400549	0.25	4 (14)	4	1	2	0.25	16 ^b
	Daptomycin	1	8 (18)	0.125	4	4	0.25	2 ^b
	Linezolid	4	16 (50)	NT	NT	NT	NT	NT
	Quinupristin-dalfopristin	0.25	4 (14)	0.25	0.5	4	4	2 ^b
	Vancomycin	64	NT	NT	NT	NT	NT	NT
SA525; MRSA	CG400549	0.25	4 (14)	4	1	4	0.25	1
	Daptomycin	1	8 (20)	0.125	2 ^c	4	0.25	2
	Linezolid	4	16 (50)	NT	NT	NT	NT	NT
	Quinupristin-dalfopristin	0.25	2 (16)	0.125	1	4	2	1
	Vancomycin	2	8 (50)	NT	NT	NT	NT	NT
SA547; MRSA	CG400549	0.125	32 (14)	8 ^c	1	4	0.25	2
	Daptomycin	1	8 (21)	0.125	4	4	0.25	4
	Linezolid	4	32 (48)	0.125	2	32	0.5	2
	Quinupristin-dalfopristin	0.25	2 (21)	0.125	1	4	2	2
	Vancomycin	2	8 (50)	NT	NT	NT	NT	NT
SA555; VISA, from Hershey	CG400549	0.25	2 (14)	2	8	2	0.06	8
	Daptomycin	16	32 (50)	NT	NT	NT	NT	NT
	Linezolid	2	4 (50)	NT	NT	NT	NT	NT
	Quinupristin-dalfopristin	0.125	1 (20)	0.25	2 ^b	4	0.5	2 ^b
	Vancomycin	8	8 (50)	NT	NT	NT	NT	NT
SA540; MSSA	CG400549	0.125	16 (14)	4 ^c	1	4	0.25	2
	Daptomycin	1	8 (24)	0.125	2 ^c	4	0.25	4
	Linezolid	4	32 (36)	0.125	2	16	0.5	4
	Quinupristin-dalfopristin	0.25	2 (16)	0.125	2	4	2	2
	Vancomycin	4	8 (50)	NT	NT	NT	NT	NT
SA543; MSSA	CG400549	0.125	8 (14)	8	1	4	0.25	1
	Daptomycin	1	8 (17)	0.125	4	4	0.25	2
	Linezolid	4	32 (15)	0.125	2	32	0.25	2
	Quinupristin-dalfopristin	0.25	2 (18)	0.125	2	4	2	4
	Vancomycin	2	8 (50)	NT	NT	NT	NT	NT

^a CAMRSA, community-acquired MRSA; NT, not tested. neg, negative; pos, positive.^b MIC for mutant was ≥2 dilutions lower than MIC for parent.^c MIC for mutant was 2 dilutions lower than its MIC after resistance selection was performed.

lower than those with other compounds tested, including against strains nonsusceptible to vancomycin. However, multi-step resistance selection studies showed that in all 10 strains, CG400549 yielded resistant mutants with MICs of 2 to >64 µg/ml. The clinical significance of the low yield of resistant mutants by CG400549 in single-step testing compared with the high yield of resistant mutants by multistep testing remains to be elucidated.

Fourteen detected mutants selected by vancomycin or daptomycin showed unstable resistance phenotypes (Table 3). This phenomenon has been described in previous studies. Boyle-Vavra et al. described uniform decreases in the MICs of vancomycin and teicoplanin after 15 days of serial passage on nonselective medium; the presence of vancomycin in the medium prevented such reversion (7). Kaatz et al. reported that continuous exposure to daptomycin appears to be necessary to select stable mutants (13). Instability of vancomycin resistance has also been demonstrated in VRSA strains carrying the *van(A)* gene (22). The clinical significance of the single- and multistep findings obtained in our study for possible resistance selection in the potential future clinical use of CG400549 remains to be elucidated.

Experimental animal and pharmacokinetic/pharmacodynamic studies are necessary before the clinical usefulness of CG400549 in the treatment of staphylococcal infections can be investigated.

This study was supported by a grant from Crystalgenomics Inc., Seoul, Republic of Korea.

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