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Potency and Bactericidal Activity of Iclaprim against Recent Clinical Gram-Positive Isolates▽

Helio S. Sader,[1,2]* Thomas R. Fritsche,[1]† and Ronald N. Jones[1,3]

JMI Laboratories, North Liberty, Iowa[1]; Universidade Federal de São Paulo, São Paulo, Brazil[2]; and Tufts University School of Medicine, Boston, Massachusetts[3]

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The in vitro activity of iclaprim, a novel diaminopyrimidine derivative, was evaluated against 5,937 recent gram-positive clinical isolates collected in the United States and Europe. Iclaprim demonstrated potent activity against *Staphylococcus aureus* (including methicillin-resistant *S. aureus* [MRSA]), beta-hemolytic *Streptococcus* spp., and *Enterococcus faecalis* strains tested. In addition, iclaprim exhibited bactericidal activity against all *S. aureus* strains tested, including MRSA.

Staphylococcus aureus strains, including methicillin-resistant *S. aureus* (MRSA) strains, beta-hemolytic streptococci (most commonly *Streptococcus pyogenes* and *Streptococcus agalactiae*), and *Enterococcus* spp. are the principal gram-positive pathogens responsible for complicated skin and skin-structure infections (cSSSI). The increasing prevalence of MRSA in hospital and community settings (5, 20), as well as the potential for resistance or emergence of resistance during therapy to drugs such as vancomycin (1, 19), linezolid (9), and daptomycin (12), underscores the urgent need for additional well-differentiated therapeutic agents (17). Iclaprim is a new-generation diaminopyrimidine that potently and selectively inhibits bacterial dihydrofolate reductase (DHFR) (11, 18, 21). It was designed by a rational drug design approach based on structural information available on trimethoprim (TMP), the best known compound of the diaminopyrimidine class which, either alone or in its synergistic 1:19 combination with sulfamethoxazole, has been widely used in medical practice for over four decades. Iclaprim has been shown to have very potent activity against gram-positive bacteria that are susceptible to TMP. Iclaprim inhibits bacterial DHFR in a similar manner to TMP but possesses higher affinity due to increased hydrophilic interactions between iclaprim and DHFR (15). Thus, iclaprim may retain activity against some TMP-resistant isolates, but it would be lower than that against TMP-susceptible strains.

Unlike TMP, the spectrum of activity of iclaprim is more focused against gram-positive pathogens, including MRSA strains. Iclaprim has been shown to be rapidly bactericidal against these strains and to possess a low potential for resistance development when used on its own, without the synergistic combination of a sulfonamide agent (8). For these reasons, iclaprim is under development as a monotherapy, and an intravenous formulation of iclaprim has completed two phase 3 trials for the treatment of cSSSI caused by gram-positive pathogens (11, 16). In addition, an oral formulation of iclaprim as a step-down therapy for patients with cSSSI is ongoing (16).

We evaluated the potency and bactericidal activity of iclaprim against a large collection of contemporary gram-positive isolates from hospitalized patients in the United States (26 centers), the European Union (22 centers), Israel (1 center), and Turkey (1 center) from 2004 to 2006. In total, 5,937 clinical isolates representative of predominant gram-positive pathogens in cSSSI were tested. All organisms were collected from skin and soft tissue, bloodstream, and respiratory clinical specimens. The numbers of individual strains for each species tested are shown in Tables 1 and 2. Susceptibility testing by broth microdilution, including the appropriate quality controls, was performed according to the documents CLSI M7-A7 (2) and CLSI M100-S18 (3). Antimicrobial agents tested included iclaprim (Arpida Ltd., Reinach, Switzerland) and comparators. Minimum bactericidal concentration (MBC) values were determined for 101 randomly selected strains. MBC experiments were performed by plating the broth from wells from at least five $\log_2$ dilutions from the MIC (13, 14) onto growth medium. The lowest concentration of the antimicrobial that killed ≥99.9% of the starting test inoculum was defined as the MBC endpoint. Cidality was defined as an MBC/MIC ratio of ≤4. In all cases, the thymidine content of the test medium was assessed to ensure that no artifactual inhibition of iclaprim activity occurred (2, 6).

Numbers of strains and activity summaries of all drugs tested are shown in Table 1. Whereas the vast majority of methicillin-sensitive *S. aureus* (MSSA; 1,513 strains) strains were susceptible to most of the compounds tested in this study, a large proportion of MRSA strains were resistant to erythromycin (84.3%), clindamycin (47.0%), and both quinolones tested (83.1% and 82.4% for ciprofloxacin and levofloxacin, respectively) (Table 1). Iclaprim was highly active against both MSSA and MRSA (MIC_{50}/MIC_{90}, 0.06/0.12 μg/ml for both) (Tables 1 and 2), with 98.7% of MSSA strains and 94.6% of MRSA strains being inhibited at a MIC of ≤2 μg/ml (Table 2). For group A streptococci (GAS), resistance rates to erythromycin, clindamycin, and tetracycline were higher in the European Union isolates (26.1%, 5.9%, and 14.2%, respectively) than those isolated in the United States (6.0%, 0.7%, and 5.0%, respectively; data not shown). Iclaprim inhibited 100% of GAS

* Corresponding author. Mailing address: JMI Laboratories, 345 Beaver Kreek Centre, Suite A, North Liberty, IA 52317. Phone: (319) 665-3370. Fax: (319) 665-3371. E-mail: helio-sader@jmilabs.com.
† Present address: Marshfield Clinic, 1000 N. Oak Ave., Marshfield, WI 54449.
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TABLE 1. Activity of iclaprim and comparator agents tested against *Staphylococcus aureus* isolates from the United States and the European Union

Organism and antimicrobial agent (no. of isolates tested)	MIC (μg/ml)			% Susceptible/ resistant isolates[a]
	50%	90%	Range	
S. aureus (4,516)				
Methicillin-sensitive (1,513)				
Iclaprim	0.06	0.12	0.008–8	−/−
Trimethoprim	1	1	0.06–>64	98.7/1.3
Trimethoprim-sulfamethoxazole	0.06	0.06	0.015–>8	99.3/0.7
Erythromycin	0.25	>4	≤0.12–>4	77.9/21.1
Clindamycin	≤0.12	≤0.12	≤0.12–>4	96.4/3.4
Tetracycline	≤0.5	≤0.5	≤0.5–>16	95.8/4.0
Ciprofloxacin	0.5	1	≤0.12–>4	92.0/5.9
Levofloxacin	0.25	0.5	≤0.12–>4	94.2/5.6
Linezolid	2	2	0.5–2	100.0/−
Vancomycin	1	1	≤0.5–2	100.0/0.0
Methicillin-resistant (3,003)				
Iclaprim	0.06	0.12	≤0.004–8	−/−
Trimethoprim	1	2	0.06–>64	92.9/7.1
Trimethoprim-sulfamethoxazole	0.06	0.25	0.015–>8	96.1/3.9
Erythromycin	>4	>4	≤0.12–>4	14.6/84.3
Clindamycin	≤0.12	>4	≤0.12–>4	52.7/47.0
Tetracycline	≤0.5	>16	≤0.5–>16	86.5/12.8
Ciprofloxacin	>4	>4	≤0.12–>4	16.3/83.1
Levofloxacin	>4	>4	≤0.12–>4	16.8/82.4
Linezolid	2	2	≤0.25–2	100.0/−
Vancomycin	1	1	≤0.5–2	100.0/0.0
Beta-hemolytic streptococci (808)				
Group A (604)				
Iclaprim	0.015	0.03	≤0.004–0.12	−/−
Trimethoprim	0.25	0.5	≤0.03–2	−/−
Trimethoprim-sulfamethoxazole	0.06	0.12	0.015–0.25	−/−
Erythromycin	≤0.12	>4	≤0.12–>4	83.3/16.1
Clindamycin	≤0.12	≤0.12	≤0.12–>4	96.4/3.3
Tetracycline	≤0.5	≤0.5	≤0.5–>16	90.2/9.6
Levofloxacin	0.5	1	0.25–2	100.0/0.0
Vancomycin	≤0.5	≤0.5	≤0.5–1	100.0/−
Linezolid	1	1	0.5–2	100.0/−
Penicillin	≤0.06	≤0.06	≤0.06	100.0/−
Group B (204)				
Iclaprim	0.12	0.25	0.015–0.5	−/−
Trimethoprim	1	4	0.25–8	−/−
Trimethoprim-sulfamethoxazole	0.06	0.12	0.03–0.25	−/−
Erythromycin	≤0.12	>4	≤0.12–>4	73.0/26.0
Clindamycin	≤0.12	>4	≤0.12–>4	88.7/10.3
Tetracycline	>16	>16	≤0.5–>16	21.6/77.9
Levofloxacin	0.5	1	0.25–>4	99.5/0.5
Vancomycin	≤0.5	≤0.5	≤0.5	100.0/−
Linezolid	1	1	0.5–2	100.0/−
Penicillin	≤0.06	≤0.06	≤0.06	100.0/−
E. faecalis (310)				
Iclaprim	0.015	4	≤0.004–>8	−/−
Trimethoprim	0.25	>64	≤0.03–>64	−/−
Trimethoprim-sulfamethoxazole	0.03	>8	0.008–>8	−/−
Erythromycin	>4	>4	≤0.12–>4	13.5/52.9
Clindamycin	>4	>4	≤0.12–>4	−/−
Tetracycline	>16	>16	≤0.5–>16	28.4/71.3
Ciprofloxacin	1	>4	0.25–>4	64.8/34.2
Levofloxacin	1	>4	0.25–>4	65.2/34.2
Teicoplanin	≤0.5	≤0.5	≤0.5–>16	98.4/1.0
Vancomycin	1	2	≤0.5–>16	97.7/1.9
Linezolid	1	2	0.5–2	100.0/0.0
Ampicillin	≤4	≤4	≤4	100.0/0.0
E. faecium (303)				
Iclaprim	2	>8	≤0.004–>8	−/−
Trimethoprim	32	>64	≤0.03–>64	−/−
Trimethoprim-sulfamethoxazole	>8	>8	≤0.004–>8	−/−
Erythromycin	>4	>4	≤0.12–>4	3.0/84.5
Clindamycin	>4	>4	≤0.12–>4	−/−
Tetracycline	≤0.5	>16	≤0.5–>16	67.3/32.0
Ciprofloxacin	>4	>4	0.25–>4	6.9/86.5
Levofloxacin	>4	>4	0.5–>4	14.2/82.2
Teicoplanin	≤0.5	>16	≤0.5–>16	63.4/34.0
Vancomycin	1	>16	≤0.5–>16	55.8/43.2
Linezolid	1	2	0.5–>8	99.3/0.7
Ampicillin	>16	>16	≤4–>16	11.9/88.1

[a] Criteria as published by the CLSI (1). β-Lactam susceptibility should be directed by the oxacillin test results. −, no criteria have been established by the CLSI (1).

TABLE 2. Iclaprim, trimethoprim, and trimethoprim-sulfamethoxazole MIC distributions for MSSA, MRSA, beta-hemolytic streptococcal strains, and enterococci evaluated by reference methods

Organism and antimicrobial agent (no. of isolates tested)[a]	No. of isolates (cumulative %) inhibited at indicated MIC (μg/ml)[b]														
	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>[c]
MSSA (1,513)															
Iclaprim	1 (0.1)	3 (0.3)	88 (6.1)	797 (58.8)	557 (95.6)	45 (98.5)	1 (98.6)	1 (98.7)	1 (98.7)	17 (99.9)	2 (100.0)	–	–	–	
Trimethoprim	–	–	0 (0.0)	2 (0.1)	2 (0.3)	32 (2.4)	483 (34.3)	845 (90.2)	116 (97.8)	11 (98.5)	2 (98.7)	0 (98.7)	1 (98.7)	0 (98.7)	19 (100.0)
TMP-SMX	0 (0.0)	3 (0.2)	174 (11.7)	1,238 (93.5)	69 (98.1)	12 (98.9)	2 (99.0)	3 (99.2)	2 (99.3)	2 (99.5)	1 (99.5)	–	–	–	7 (100.0)
MRSA (3,003)															
Iclaprim	2 (0.1)	11 (0.4)	370 (12.8)	1,707 (69.6)	646 (91.1)	35 (92.3)	20 (92.9)	26 (93.8)	23 (94.6)	99 (97.9)	64 (100.0)	–	–	–	
Trimethoprim	–	–	0 (0.0)	1 (<0.1)	5 (0.2)	166 (5.7)	1,260 (47.7)	1,226 (88.5)	109 (92.1)	11 (92.5)	12 (92.9)	22 (93.6)	15 (94.1)	13 (94.6)	163 (100.0)
TMP-SMX	0 (0.0)	2 (0.1)	580 (19.4)	1,824 (80.1)	246 (88.3)	164 (93.8)	22 (94.5)	22 (95.2)	25 (96.1)	17 (96.6)	33 (97.7)	–	–	–	68 (100.0)
GAS (604)															
Iclaprim	184 (30.5)	285 (77.6)	104 (94.9)	27 (99.3)	4 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	–	–	–	
Trimethoprim	–	–	5 (0.8)	47 (8.6)	225 (45.9)	234 (84.6)	84 (98.5)	6 (99.5)	3 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	
TMP-SMX	0 (0.0)	3 (0.5)	58 (10.1)	369 (71.2)	153 (96.5)	21 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	–	–	–	
GBS (204)															
Iclaprim	0 (0.0)	1 (0.5)	0 (0.5)	10 (5.4)	102 (55.4)	73 (91.2)	18 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	–	–	–	
Trimethoprim	–	–	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	11 (5.9)	92 (51.0)	78 (89.2)	21 (99.5)	1 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	
TMP-SMX	0 (0.0)	0 (0.0)	7 (3.4)	101 (52.9)	92 (98.0)	4 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	–	–	–	
E. faecalis (310)															
Iclaprim	79 (29.0)	100 (57.7)	19 (63.9)	10 (67.1)	6 (69.0)	5 (70.6)	0 (70.6)	1 (71.0)	3 (71.9)	77 (96.8)	6 (1.9)	–	–	–	4 (100.0)
Trimethoprim	–	–	2 (0.6)	11 (4.2)	79 (29.7)	83 (56.5)	20 (62.9)	5 (64.5)	10 (67.7)	7 (70.0)	2 (70.6)	0 (70.6)	1 (71.0)	6 (72.9)	84 (100.0)
TMP-SMX	1 (0.3)	43 (14.2)	130 (56.1)	32 (66.5)	17 (71.9)	11 (75.5)	4 (76.8)	4 (78.1)	9 (81.0)	4 (82.3)	4 (83.5)	–	–	–	–
E. faecium (303)															
Iclaprim	102 (33.7)	8 (36.3)	4 (37.6)	0 (37.6)	1 (38.0)	1 (38.3)	3 (39.3)	9 (42.2)	48 (58.1)	51 (74.9)	2 (75.6)	–	–	–	–
Trimethoprim	–	–	42 (13.9)	50 (30.4)	17 (36.0)	4 (37.3)	1 (37.6)	1 (38.0)	1 (38.3)	2 (38.9)	8 (41.6)	17 (47.2)	24 (55.1)	17 (60.7)	119 (100.0)
TMP-SMX	4 (1.3)	10 (4.6)	40 (17.8)	47 (33.3)	10 (36.6)	5 (38.3)	0 (38.3)	0 (38.3)	1 (38.6)	1 (38.9)	14 (43.6)	–	–	–	171 (100.0)

[a] TMP-SMX, trimethoprim-sulfamethoxazole.
[b] –, concentration not tested.
[c] MICs greater than the highest concentration tested, which was 8 μg/ml for iclaprim and trimethoprim-sulfamethoxazole and 64 μg/ml for trimethoprim.

TABLE 3. MBC/MIC ratios for iclaprim and vancomycin against 101 organisms

Organism (no. of isolates) and MBC/MIC ratio	No. of isolates at MBC/MIC ratio for indicated antimicrobial agent	
	Iclaprim	Vancomycin
Staphylococcus aureus		
Methicillin-susceptible (21)		
1	2	14
2	7	2
4	9	2
8	1	1
16	0	0
≥32	2	2
Methicillin-resistant (20)		
1	4	8
2	13	1
4	3	3
8	0	4
16	0	1
≥32	0	3
Beta-hemolytic streptococci		
Group A (20)		
1	1	0
2	6	0
4	2	0
8	0	0
16	0	0
≥32	11	18[a]
Group B (20)		
1	1	0
2	10	0
4	2	0
8	1	0
16	0	0
≥32	6	20
Enterococcus faecalis (15)		
1	0	0
2	1	0
4	2	0
8	0	0
16	1	0
≥32	11	15
Enterococcus faecium (5)		
1	0	0
2	1	0
4	1	0
8	1	0
16	0	0
≥32	2	5

[a] MBC could not be evaluated for two strains because both MIC and MBC values were beyond the dilution range tested (≤0.5 μg/ml).

at an MIC of ≤0.12 μg/ml and an MIC_{50}/MIC_{90} of 0.015/0.03 μg/ml (Tables 1 and 2). After penicillin (MIC_{50}/MIC_{90}, ≤0.06/≤0.06 μg/ml) (Table 1), iclaprim was the most active among all the antibacterial agents tested. Unlike GAS, group B streptococci (GBS) from the United States showed higher rates of resistance to erythromycin (39.2% versus 12.7%), clindamycin (14.7% versus 5.9%), and tetracycline (82.4% versus 73.5%) than those isolated in the European Union (data not shown). Iclaprim was also highly potent against GBS (MIC_{50}/MIC_{90}, 0.12/0.25 μg/ml; MIC_{100}, 0.5 μg/ml) (Tables 1 and 2), and its activity was not affected by the organisms' resistance to erythromycin, clindamycin, or tetracycline. As expected, *E. faecalis* isolates were generally more susceptible than *Enterococcus faecium* isolates to the drugs tested (Tables 1 and 2), and resistance rates for *E. faecalis* did not vary much between the United States and European Union isolates (data not shown). Resistance rates were generally high among *E. faecium* isolates and differed significantly between those from the United States and European Union. Most notably, resistance to vancomycin was 70.8% among isolates in the United States compared to 14.8% among non-United States isolates. Iclaprim demonstrated the typical bimodal activity of its class against enterococci (Table 2). However, it showed high potency (MIC_{50}/MIC_{90}, 0.015/4 μg/ml) (Table 1) against *E. faecalis*, with approximately 72% and 97% of the isolates being inhibited at MICs of ≤2 and ≤4 μg/ml, respectively (Table 2). Iclaprim was also active (MIC_{50}/MIC_{90}, 2/>8 μg/ml) against *E. faecium* (Table 1), and its activity was not affected by resistance to vancomycin; approximately 58% and 75% of the isolates were inhibited at iclaprim MICs of ≤2 and ≤4 μg/ml, respectively (Table 2).

Iclaprim was bactericidal against the *S. aureus* strains tested (Table 3). Against MRSA, iclaprim demonstrated MBC/MIC ratios of ≤4 for 100% of strains compared to 60% of strains for vancomycin. Against MSSA, iclaprim and vancomycin demonstrated MBC/MIC ratios of ≤4 for 86% of strains (Table 3). Iclaprim exhibited bactericidal activity against 45.0% of GAS and 65.0% of GBS, compared to ratios of ≥32 for vancomycin for most GAS and 100.0% of GBS tested (Table 3). Against enterococci, MBC/MIC ratios of ≤4 were seen in two out of five *E. faecium* strains and 3 out of 15 *E. faecalis* strains for iclaprim, whereas all the enterococcal strains tested exhibited MBC/MIC ratios of ≥32 for vancomycin (Table 3). The bactericidal activity of iclaprim when tested against staphylococci corroborates the results of previous studies (8, 10). In contrast, the fact that iclaprim demonstrated higher bactericidal activity against staphylococci than against streptococci and enterococci has not been previously reported and warrants further evaluation.

Results from this study support previously reported nonclinical susceptibility data (4, 7, 21) and demonstrate the potent bactericidal activity of iclaprim in vitro against both MSSA and MRSA. This finding, together with the high activity of iclaprim against beta-hemolytic streptococci and enterococcal species, confirms iclaprim as an important addition to the existing panel of therapies for the treatment of cSSSI caused by gram-positive organisms, including MRSA.

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REFERENCES

1. **Chang, S., D. M. Sievert, J. C. Hageman, M. L. Boulton, F. C. Tenover, F. P. Downes, S. Shah, J. T. Rudrik, G. R. Pupp, W. J. Brown, D. Cardo, and S. K. Fridkin.** 2003. Infection with vancomycin-resistant *Staphylococcus aureus* containing the *vanA* resistance gene. N. Engl. J. Med. **348:**1342–1347.
2. **Clinical and Laboratory Standards Institute.** 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed. Approved standard CLSI M7-A7. Clinical and Laboratory Standards Institute, Wayne, PA.
3. **Clinical and Laboratory Standards Institute.** 2008. Performance standards

for antimicrobial susceptibility testing; 18th informational supplement. CLSI M100-S18. Clinical and Laboratory Standards Institute, Wayne, PA.
4. **Clinical and Laboratory Standards Institute.** 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed. Approved standard CLSI M7-A7. Clinical and Laboratory Standards Institute, Wayne, PA.
5. **Eisenstein, B. I.** 2008. Treatment challenges in the management of complicated skin and soft-tissue infections. Clin. Microbiol. Infect. **14**(Suppl. 2)**:** 17–25.
6. **Ferone, R., S. R. Bushby, J. J. Burchall, W. D. Moore, and D. Smith.** 1975. Identification of Harper-Cawston factor as thymidine phosphorylase and removal from media of substances interfering with susceptibility testing to sulfonamides and diaminopyrimidines. Antimicrob. Agents Chemother. **7:**91–98.
7. **Hawser, S., S. Lociuro, and K. Islam.** 2006. Dihydrofolate reductase inhibitors as antibacterial agents. Biochem. Pharmacol. **71:**941–948.
8. **Hawser, S., L. Weiss, M. Fischer, J. Jaeger, S. Greiveldinger, D. Gillessen, I. M. Kompis, and K. Islam.** 2002. AR-100, a novel diaminophyrimidine compound: bactericidal activity and post-antibiotic effect on gram-positive pathogens. Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., San Diego, CA, 27 to 30 September 2002.
9. **Hentschke, M., B. Saager, M. Horstkotte, S. Scherpe, M. Wolters, H. Kabisch, R. Grosse, P. Heisig, M. Aepfelbacher, and H. Rohde.** 2008. Emergence of linezolid resistance in a methicillin resistant *Staphylococcus aureus* strain. Infection **36:**85–87.
10. **Jones, M. E., D. C. Draghi, P. K. Grover, S. Hawser, K. Islam, and F. Sahm.** 2007. Bactericidal activity and post antibiotic effect of iclaprim (ICL) against *Staphylococcus aureus* (SA). Abstr. 47th Intersci. Conf. Antimicrob. Agents Chemother., Chicago, IL, 17 to 20 September 2007.
11. **Kohlhoff, S. A., and R. Sharma.** 2007. Iclaprim. Expert Opin. Investig. Drugs **16:**1441–1448.
12. **Mangili, A., I. Bica, D. R. Snydman, and D. H. Hamer.** 2005. Daptomycin-resistant, methicillin-resistant *Staphylococcus aureus* bacteremia. Clin. Infect. Dis. **40:**1058–1060.
13. **Moody, J., and C. Knapp.** 2004. Tests to assess bactericidal activity, p. 5.10.31–36. *In* H. D. Isenberg (ed.), Clinical microbiology procedures handbook. ASM Press, Washington, DC.
14. **National Committee for Clinical Laboratory Standards.** 1999. Methods for determining bactericidal activity of antibacterial agents; approved guideline M26-A. National Committee for Clinical Laboratory Standards, Wayne, PA.
15. **Oefner, C., M. Bandera, A. Haldimann, H. Laue, H. Schulz, S. Mukhija, S. Parisi, L. Weiss, S. Lociuro, and G. E. Dale.** 2009. Increased hydrophobic interactions of iclaprim with *Staphylococcus aureus* dihydrofolate reductase are responsible for the increase in affinity and antibacterial activity. J. Antimicrob. Chemother. **63:**687–698.
16. **Peppard, W. J., and C. D. Schuenke.** 2008. Iclaprim, a diaminopyrimidine dihydrofolate reductase inhibitor for the potential treatment of antibiotic-resistant staphylococcal infections. Curr. Opin. Investig. Drugs **9:**210–225.
17. **Raghavan, M., and P. K. Linden.** 2004. Newer treatment options for skin and soft tissue infections. Drugs **64:**1621–1642.
18. **Schneider, P., S. Hawser, and K. Islam.** 2003. Iclaprim, a novel diaminopyrimidine with potent activity on trimethoprim sensitive and resistant bacteria. Bioorg. Med. Chem. Lett. **13:**4217–4221.
19. **Steinkraus, G., R. White, and L. Friedrich.** 2007. Vancomycin MIC creep in non-vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-susceptible clinical methicillin-resistant *S. aureus* (MRSA) blood isolates from 2001–05. J. Antimicrob. Chemother. **60:**788–794.
20. **Stevens, D. L., A. L. Bisno, H. F. Chambers, E. D. Everett, P. Dellinger, E. J. Goldstein, S. L. Gorbach, J. V. Hirschmann, E. L. Kaplan, J. G. Montoya, and J. C. Wade.** 2005. Practice guidelines for the diagnosis and management of skin and soft-tissue infections. Clin. Infect. Dis. **41:**1373–1406.
21. **Then, R. L., H. H. Locher, and P. Angehrn.** 2002. AR-100, a novel diaminopyrimidine compound: in vitro activity against gram-positive and gram-negative bacterial pathogens. Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., San Diego, CA, 27 to 30 September 2002.