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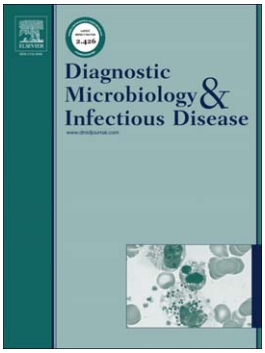

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Surveillance of Iclaprim Activity: In Vitro Susceptibility of Gram-positive Pathogens Collected from 2012-2014 From the United States, Asia Pacific, Latin American and Europe

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Abstract

Iclaprim is a novel diaminopyrimidine, which inhibits bacterial dihydrofolate reductase, and it is highly active against Gram-positive pathogens including emerging drug-resistant pathogens. *In vitro* activity of iclaprim and comparators against 2,814 Gram-positive clinical isolates from the United States, Asia Pacific, Latin American and Europe collected between 2012-2014 were tested. Susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Minimum inhibitory concentration (MIC) interpretations were based on CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. MIC_{50}/MIC_{90} for all *S.aureus*, methicillin susceptible *S. aureus*, methicillin resistant *S. aureus*, beta-hemolytic streptococci, and *Streptococcus pneumoniae* were 0.06/0.12, 0.06/0.12, 0.06/0.5, 0.06/0.25, and 0.06/2 µg/mL, respectively. Iclaprim was 8 to 32-fold more potent than trimethoprim, the only FDA approved dihydrofolate reductase inhibitor, against all Gram-positive isolates including resistant phenotypes. The MIC_{90} of iclaprim was also lower than most of the comparators including linezolid and vancomycin against Gram-positive pathogens. Iclaprim demonstrated potent activity against a contemporary collection (2012-2014) of Gram-positive clinical isolates from the United States, Asia Pacific, Latin America and Europe.

Keywords: iclaprim, surveillance, *in vitro*

1. Introduction

Iclaprim represents a novel diaminopyrimidine, which inhibits bacterial dihydrofolate reductase (DHFR) and is active against emerging drug-resistant pathogens (Sader et al., 2009; Schneider et al., 2003). Trimethoprim is the only FDA approved dihydrofolate reductase inhibitor. Iclaprim was designed to be more potent and to overcome trimethoprim resistance among Gram-positive pathogens (Oefner et al., 2009). In addition, iclaprim does not need to be combined with a sulfonamide, which is commonly associated with adverse events including (possibly severe) allergic reactions. Iclaprim is in Phase 3 clinical development for the treatment of skin and skin structure infections (SSSI). Iclaprim exhibits potent in vitro activity against Gram-positive pathogens such as *Staphylococcus aureus* and beta-hemolytic streptococci (BHS) including resistant phenotypes that cause SSSI and *S. aureus* and *S. pneumoniae* that cause pneumonia (Sader et al., 2009; Morrissey et al., 2009). Iclaprim demonstrates rapid in vitro bactericidal activity in time kill studies in human plasma (Laue et al., 2009). In a Phase 2 clinical trial among patients treated for skin and skin structure infections, clinical cure rates in the intent to treat population were 92.9% (26 of 28), 90.3% (28 of 31), and 26 of 28 (92.9%) at the test of cure visit in the iclaprim 0.8 mg/kg IV q12h, iclaprim 1.6 mg/kg IV q12h, and vancomycin 1 g IV q12h groups, respecively (Krievens et al, 2009). In a Phase 2 clinical trial among patients treated for nosocomial pneumonia, clinical cure rates in the intent to treat population were 73.9% (17 of 23), 62.5% (15 of 24), and 52.2% (12 of 23) at the test of cure visit in the iclaprim 0.8 mg/mg IV q12h, iclaprim 1.2 mg/kg IV q8h, and vancomycin 1 g IV q12h groups, respectively (Huang et al, submitted). Because of these findings, iclaprim is

potentially well suited for treating patients with SSSI and nosocomial pneumonia caused by or suspected Gram-positive bacteria, including multidrug resistant pathogens. We report contemporary (2012-2014) surveillance data on 2,814 *S. aureus*, BHS, and *S. pneumoniae* isolated from patients with Gram-positive infections in the United States (US), Asia Pacific (AP), Latin America (LA) and Europe (EU).

2. Methods

2.1 Collection of bacterial isolates

Antibacterial susceptibility testing was conducted by JMI Laboratories (North Liberty, Iowa, USA). A total of 2,814 nonduplicative, nonconsecutive isolates of *S. aureus,* BHS, and *S. pneumoniae* isolated from 2012 to 2014 were collected from multiple locations in the US, AP, LA and EU, including isolates from SSSI (n=776) and nosocomial pneumonia (n=860). The distribution of pathogens by country are shown in Table 1. Of the 2,814 isolates, 943 (33.5%) were collected from US, 981 (34.9%) EU, 432 (15.4%) AP, and 458 (16.3%) LA.

2.2 Susceptibility testing

Clinical isolates were identified by the submitting laboratories and confirmed by JMI Laboratories using standard bacteriologic algorithms and methodologies, including Matrix-Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry (MALDI-TOF MS). When necessary, MALDI-TOF MS was performed using the Bruker Daltonics MALDI Biotyper (Billerica, Massachusetts, USA), following manufacturer's instructions. Isolates were not genotyped (e.g., Panton Valentine Leukocidin, alpha hemolysin, toxic shock syndrome toxin).

Susceptibility testing was performed by broth microdilution in accordance with the Clinical and Laboratory and Standards Institute (CLSI) guidelines M07-A10 (2015) and the standard operating procedures at JMI laboratories. Minimum inhibitory concentration (MIC) values were interpreted using CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (2015). To date, there are no published clinical breakpoints for iclaprim. However, based on a number of factors (e.g., MRSA distribution of MICs, assessment of the pharmacokinetics/pharmacodynamics of iclaprim, and the study of the clinical outcomes of MRSA infections when iclaprim was used in Phase 2 and 3 studies) outlined in the CLSI M23 guideline, an iclaprim MIC ≤1 µg/mL for *S. aureus*, including MRSA, has been proposed to FDA. *S. aureus*, both methicillin-susceptible and methicillin-resistant, were tested in cation-adjusted Mueller-Hinton broth (CA-MHB) and BHS and *S. pneumoniae* were tested in CA-MHB supplemented with 2.5-5% lysed horse blood. Quality control and interpretation of results were performed in accordance with CLSI M100-S25 (2015) methods. Unlike trimethoprim (dilution scheme of 0.03 to 64 µg/mL), the MIC dilution scheme selected for iclaprim was 0.008 to 8 µg/mL because concentrations >2 µg/mL are not physiologically achievable with the therapeutic fixed dose of iclaprim used in pivotal clinical trials. Iclaprim and comparator antibiotic MIC results were within the CLSI published ranges against *S. aureus* ATCC 29213 and *S. pneumoniae* ATCC 49619. Isolates were tested with MIC panels (ThermoFisher Scientific, Cleveland, OH, USA) of comparator antibiotics (trimethoprim, trimethoprim-sulfamethoxazole, ceftriaxone, erythromycin, levofloxacin, oxacillin, meropenem, tetracycline, tigecycline, vancomycin, linezolid, and daptomycin).

3. Results

3.1 Iclaprim and comparator activity from 2012-2014

The MIC_{50} and MIC_{90} for iclaprim were 0.06 µg/mL and 0.12 µg/mL, respectively, against key Gram-positive pathogens, including strains with resistant phenotypes, isolated from patients with SSSI and nosocomial pneumonia. Table 2 shows the in vitro activity of iclaprim and comparators against *S. aureus*, MSSA, MRSA, BHS, and *S. pneumoniae*. The MIC_{90} of iclaprim was lower than most of the comparators including linezolid and vancomycin, which are considered standard of care therapies for Gram-positive hospital infections. Among the Gram-positive pathogens, the MIC_{90} for tigecycline were lower than MIC_{90} for iclaprim. For streptococci, the MIC_{90} for beta-lactams (ceftriaxone and oxacillin) were either equivalent or more potent than MICs for iclaprim. Table 3 shows the cumulative percentage of isolates inhibited at each iclaprim MIC value. The MIC_{50} / MIC_{90} of iclaprim was identical to trimethoprim-sulfamethoxazole (MIC_{50}, 0.06 µg/mL and MIC_{90}, 0.12 µg/mL) for all Gram-positive pathogens. However, trimethoprim-sulfamethoxazole had lower MIC_{90} values for MSSA and MRSA. Based on both MIC_{50} and MIC_{90}, both iclaprim and trimethoprim-sulfamethoxazole were 8 to 32-fold more potent than trimethoprim (MIC_{50} and MIC_{90} were 1 and 2 µg/mL, respectively), currently the only FDA approved dihydrofolate reductase inhibitor. For isolates with a MIC for trimethoprim of >1 µg/mL (resistance; n=366), 153 (41.9%), 15 (4.1%), 28 (7.7%), and 168 (46.0%) isolates had a MIC for iclaprim of ≤0.25, 1, 4 and ≥8 µg/mL, respectively. For isolates with a MIC for trimethoprim-sulfamethoxazole of >4 µg/mL (resistance; n=112), 0, 3 (2.7%), 16 (4.4%), and 91 (82.0%) isolates had a MIC for iclaprim of ≤0.25, 1, 4 and ≥8 µg/mL, respectively. A total of 134 (5.6%) isolates had reduced susceptibility to iclaprim with MICs >8 µg/mL; none of these isolates were resistant to trimethoprim. These isolates were not clustered in time and/or place. Future studies are planned to examine the

genotype and phenotype of these isolates.

3.2 Iclaprim and comparator activity against S. aureus

Table 2 shows iclaprim exhibited highly potent activity against all 1,178 *S. aureus* isolates. The MIC_{50} and MIC_{90} values were 0.06 and 0.12 µg/mL, respectively. For trimethoprim, the MIC_{50} and MIC_{90} were 1 and 2 µg/mL, respectively. For trimethoprim-sulfamethoxazole, the MIC_{50} and MIC_{90} were 0.06 and 0.12 µg/mL, respectively. Iclaprim was active against *S. aureus* that were resistant to erythromycin, clindamycin, levofloxacin and trimethoprim.

Iclaprim maintained activity against *S. aureus* regardess of methicillin susceptibility. For MSSA, the MIC_{50} and MIC_{90} were 0.06 and 0.12 µg/mL, respectively. For MRSA, the MIC_{50} and MIC_{90} were 0.06 and 0.5 µg/mL (89.5% of isolates inhibited at MIC values 0.12 µg/mL), respectively. In comparison, for MSSA, trimethoprim MIC_{50} and MIC_{90} were 1 and 2 µg/mL, respectively, and for MRSA, trimethoprim MIC_{50} and MIC_{90} were 2 and 8 µg/mL, respectively. In comparison, for MSSA, trimethoprim-sulfamethoxazole MIC_{50} and MIC_{90} were 0.06 and 0.06 µg/mL, respectively, and for MRSA, trimethoprim-sulfamethoxazole MIC_{50} and MIC_{90} were 0.06 and 0.25 µg/mL, respectively.

Iclaprim also maintained activity against *S. aureus* regardless of isolation from SSSI or nosocomial pneumonia. For SSSI, the MIC_{50} and MIC_{90} were 0.06 and 1 µg/mL, respectively. For nosocomial pneumonia, the MIC_{50} and MIC_{90} were 0.06 and 0.12 µg/mL, respectively (data not shown). The MIC_{90} were higher among *S. aureus* associated with SSSI compared to nosocomial pneumonia because 14.7% (n=11) and 0% of isolates, respectively, from Brazil had a MIC >2 µg/mL.

A total of 134 (5.6%) isolates had reduced susceptibility to iclaprim with MICs >8 µg/mL (Table 3). With the exception of Brazil, these isolates were not clustered in time, infection type and/or place.

3.3 Iclaprim and comparator activity against beta-hemolytic streptococci

The MIC_{50} and MIC_{90} for iclaprim were 0.06 and 0.25 µg/mL, respectively, against all 199 BHS, including *S. pyogenes* and *Streptococcus agalactiae* isolates (Tables 2 and 3). In comparison, MIC_{50}/MIC_{90} for trimethoprim and trimethoprim-sulfamethoxazole were 1 / 2 µg/mL and 0.12/0.25 µg/mL, respectively (Table 2). For isolates with a MIC for erythromycin of ≥4 µg/mL (n=60), 52 (86.7%), 2 (3.3%), and 6 (10.0%) isolates had a MIC for iclaprim of ≤0.25, 4 and ≥8 µg/mL, respectively.

3.4 Iclaprim and comparator activity against S. pneumoniae

The MIC_{50} and MIC_{90} for iclaprim were 0.06 and 2 µg/mL, respectively, against 259 *S. pneumoniae* isolates (Tables 2 and 3). In comparison, MIC_{50}/MIC_{90} for trimethoprim and trimethoprim-sulfamethoxazole were 2/64 µg/mL and 0.25/8 µg/mL, respectively. Trimethoprim-sulfamethoxazole susceptibility was only 69.1% (CLSI criteria) against 259 respiratory isolates of *S. pneumoniae*. Iclaprim activity against these strains are shown in Table 2. One isolate with a MIC value of 4 µg/mL for penicillin (intermediate susceptibility) had a MIC value of 0.03 µg/mL for iclaprim. Another isolate with a MIC value of >8 µg/mL for penicillin (resistance) had a MIC value of 1 µg/mL for iclaprim. Iclaprim showed good activity against *S. pneumoniae* independent of the prevalence of macrolide and tetracycline resistance. For isolates with a MIC for erythromycin of ≥1 µg/mL (resistance; n=65), 56 (86.2%), 2 (3.1%),

1 (1.5%), 1 (1.5%) and 5 (7.7%) isolates had a MIC for iclaprim of ≤0.12, 1, 2, 4 and ≥8 µg/mL, respectively. For isolates with a MIC for tetracycline of ≥8 µg/mL (resistance; n=49), 42 (85.7%), 3 (6.1%), 1 (2.0%) and 3 (6.1%) isolates had a MIC for iclaprim of ≤0.25, 1, 2 and ≥8 µg/mL, respectively.

4. Discussion

This report shows iclaprim on its own, without the synergistic combination of a sulfonamide, is highly active against a collection of 2,814 Gram-positive clinical isolates, including those with resistant phenotypes, collected between 2012-2014 from the US, AP, LA, and EU. Iclaprim (MIC_{50}, 0.06 µg/mL and MIC_{90}, 0.12 µg/mL) was more potent than trimethoprim (MIC_{50} and MIC_{90} were 1 and 2 µg/mL) and identical to trimethoprim-sulfamethoxazole (MIC_{50}, 0.06 µg/mL and MIC_{90}, 0.12 µg/mL). The MIC_{50}/MIC_{90} 0.06/0.12 µg/mL for *S. aureus*, the MIC_{50}/MIC_{90} 0.06/0.25 µg/mL for BHS, and the MIC_{50}/MIC_{90} 0.06/2 µg/mL for *S. pneumoniae* documented in this analysis were consistent with those in a previous surveillance of 5,937 Gram-positive isolates (Sader et al., 2009).

Resistance in *S. aureus* to trimethoprim is determined by a single amino acid change (F98Y) within the TMP-binding site of DHFR. Iclaprim was rationally designed, using information from X-ray crystal data of isolated DHFR, for enhanced activity against Gram-positive bacteria including strains with mutational changes in DHFR that determine TMP resistance. Iclaprim retains sufficient binding affinity to F98Y DHFR due to additional hydrophobic interactions with surrounding amino acids to overcome TMP resistance (Oefner et al., 2009). Its activity against TMP-R clinical isolates of *S. aureus* and BHS has been demonstrated in a number of studies and is driven by the greatly increased affinity of iclaprim to

the DHFR target site including mutant DHFR.

In conclusion, the results from this surveillance report confirm potent and widespread iclaprim susceptibility rates among contemporary (2012-2014) clinical pathogens from the US, AP, LA and EU. The number of nonsusceptible isolates of *S. aureus*, both methicillin susceptible and methicillin resistant, BHS, and *S. pneumoniae* to iclaprim were limited. Continued surveillance is warranted to track the continued potentcy of iclaprim in the future and to detect any potential emergence of resistance.

Table 1 Distribution of organisms collected from the United States, Asia Pacific, Latin America and Europe, 2012-2014

Organism	**US**	**EU**	**AP**	**LA**	**Total**
Staphylococcus aureus	385	403	191	199	1,178
MRSA	192	202	90	98	582
MSSA	193	201	101	101	596
BHS	75	75	23	26	199
S. pyogenes	37	36	12	13	98
S. agalactiae	38	39	11	13	101
Streptococcus pneumoniae	98	100	27	34	259
Total	943 (33.5%)	981 (34.9%)	432 (15.4%)	458 (16.3%)	2,814

Abbrevaitions: US, United States; EU, Europe; AP, Asia Pacific; LA, Latin America; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; BHS, beta-hemolytic streptococci

Table 2 In vitro activity of iclaprim and comparators against isolates collected from the United States, Asia Pacific, Latin America and Europe, 2012-2014

Organism	Drug	MIC_{50}	MIC_{90}	Range	CLSI			EUCAST		
					%S	%I	%R	%S	%I	%R
S. aureus (n=1,178)	Iclaprim	0.06	0.12	0.015-->8	-	-	-	-	-	-
	Trimethoprim	1	2	0.25-- >64	93.0	-	7.0	92.4	0.3	7.3
	TMS	0.06	0.12	0.03 -->6	98.1	-	1.9	98.1	0.2	1.7
	Erythromycin	0.5	>16	≤0.12-->16	50.2	5.2	44.7	50.7	1.2	48.1
	Levofloxacin	0.25	>4	≤0.12-->4	59.3	59.3	0.6	40.1	59.3	40.1
	Oxacillin	1	>2	≤ 0.25-->2	50.6	-	49.4	50.6	-	49.4
	Ceftriaxone	8	>16	1—>16	50.6	-	49.4	-	-	-
	Meropenem	0.12	>8	≤0.06-->8	50.6	-	49.4	-	-	-
	Tetracycline	≤0.5	8	≤ 0.5-->8	89.8	0.5	9.7	88.8	0.8	10.4
	Tigecycline	0.06	0.12	≤ 0.015—0.5	100.0	-	-	100.0	-	0.0
	Vancomycin	1	1	0.25—2	100.0	0.0	0.0	100.0	-	0.0
	Linezolid	1	1	≤ 0.12—2	100.0	-	0.0	100.0	-	0.0
	Daptomycin	0.25	0.5	≤ 0.06—2	99.9	-	-	99.9	0	0.1
MRSA (n=582)	Iclaprim	0.06	0.5	0.015-->8	-	-	-	-	-	-
	Trimethoprim	2	8	0.25 -->64	90.4	-	9.6	89.9	0.0	10.1
	TMS	0.06	0.25	0.03-->8	96.9	-	3.1	96.9	0.3	2.7
	Erythromycin	>16	>16	≤ 0.12-->16	23.2	4.0	72.9	23.5	0.5	75.9
	Levofloxacin	>4	>4	≤ 0.12-->4	22.3	1.0	76.6	22.3	1.0	76.6
	Oxacillin	>2	>2	2-->2	0.0	-	100.0	0.0	-	100.0
	Ceftriaxone	>16	>16	4-->16	0.0	-	100.0	-	-	-
	Meropenem	8	>8	≤ 0.06-->8	0.0	-	100.0	-	-	-
	Tetracycline	≤ 0.5	>8	≤ 0.5-->8	85.9	0.3	13.7	84.0	1.4	14.6
	Tigecycline	0.06	0.12	≤ 0.015-->0.5	100.0	-	-	100.0	-	0.0
	Vancomycin	1	1	0.25—2	100.0	0.0	0.0	100.0	-	0.0
	Linezolid	1	1	≤ 0.12—2	100.0	-	0.0	100.0	-	0.0
	Daptomycin	0.25	0.5	≤ 0.06--1	100.0	-	-	-	-	0.0

MSSA (n=596)	Iclaprim	0.06	0.12	0.015-->8	-	-	-	-	-	-
	Trimethoprim	1	2	0.25—>64	95.5	-	4.5	95.0	0.5	4.5
	TMS	0.06	0.06	0.03—>8	99.3	-	0.7	99.3	0.0	0.7
	Erythromycin	0.25	>16	≤0.12--->16	76.5	6.4	17.1	77.2	1.8	21.0
	Levofloxacin	0.25	0.25	≤0.12-->4	95.5	0.2	4.4	95.5	0.2	4.4
	Oxacillin	0.5	0.5	≤0.25--1	100.0	-	0.0	100.0	-	0.0
	Ceftriaxone	4	4	1—>16	100.0	-	0.0	-	-	-
	Meropenem	≤0.06	0.12	≤0.06--2	100.0	-	0.0	-	-	-
	Tetracycline	≤0.05	≤0.5	≤0.5-->8	93.6	0.7	5.7	93.5	0.2	6.4
	Tigecycline	0.06	0.06	≤0.015--0.5	100.0	-	-	100.0	-	0.0
	Vancomycin	1	1	0.5--2	100.0	0.0	0.0	100.0	-	0.0
	Linezolid	1	1	0.25--2	100.0	-	0.0	100.0	-	0.0
	Daptomycin	0.25	0.5	0.12¯02	99.8	-	-	99.8	-	0.2
BHS (n=199)	Iclaprim	0.06	0.25	0.008-- >8	-	-	-	-	-	-
	Trimethoprim	1	2	0.12 --- >64	-	-	-	93.0	-	7
	TMS	0.12	0.25	0.03-- >8	-	-	-	98.0	1.5	0.5
	Erythromycin	≤0.12	>16	≤0.12—16	72.9	1.5	25.6	72.9	1.5	25.6
	Levofloxacin	0.5	1	≤0.12-- >4	99.0	0.0	1.0	94.0	5.0	1.0
	Oxacillin	≤0.06	≤0.06	≤0.06 – >2	100.0	-	-	100.0	-	0.0
	Ceftriaxone	0.06	0.12	≤ 0.015 –>16	99.5	-	-	100.0	-	0
	Meropenem	≤0.06	≤0.06	≤0.015 –32	100.0	-	-	100.0	-	0.0
	Tetracycline	>8	>8	≤0.5 -- >8	43.7	0.5	55.8	42.7	1.0	56.3
	Tigecycline	0.03	0.03	≤0.015—0.06	100.0	-	-	100.0	0.0	0.0
	Vancomycin	0.25	0.5	0.25-1	100.0	-	-	100.0	-	0.0
	Linezolid	1	1	0.5 –1	100.0	-	-	100.0	0.0	0.0
	Daptomycin	0.12	0.25	≤0.06 – 0.5	100.0	-	-	100.0	-	0.0
S. pyogenes (N=98)	Iclaprim	0.06	0.25	0.008-- >8	-	-	-	-	-	-
	Trimethoprim	1	2	0.12 --- >64	-	-	-	93.0	-	7
	TMS	0.12	0.25	0.03-- >8	-	-	-	98.0	1.5	0.5
	Erythromycin	≤0.12	>16	≤0.12—16	72.9	1.5	25.6	72.9	1.5	25.6
	Levofloxacin	0.5	1	≤0.12-- >4	99.0	0.0	1.0	94.0	5.0	1.0
	Oxacillin	≤0.06	≤0.06	≤0.06 – >2	100.0	-	-	100.0	-	0.0

	Ceftriaxone	0.06	0.12	≤ 0.015 –>16	99.5	-	-	100.0	-	0
	Meropenem	≤0.06	≤0.06	≤0.06 –32	100.0	-	-	100.0	-	0.0
	Tetracycline	>8	>8	≤0.5 -- >8	43.7	0.5	55.8	42.7	1.0	56.3
	Tigecycline	0.03	0.03	≤0.015—0.06	100.0	-	-	100.0	0.0	0.0
	Vancomycin	0.25	0.5	0.25-1	100.0	-	-	100.0	-	0.0
	Linezolid	1	1	0.5 –1	100.0	-	-	100.0	0.0	0.0
	Daptomycin	0.12	0.25	≤0.06 – 0.5	100.0	-	-	100.0	-	0.0
S. agalactiae (N=101)	Iclaprim	0.06	0.25	0.008-- >8	-	-	-	-	-	-
	Trimethoprim	1	2	0.12 --- >64	-	-	-	93.0	-	7
	TMS	0.12	0.25	0.03-- >8	-	-	-	98.0	1.5	0.5
	Erythromycin	≤0.12	>16	≤0.12—16	72.9	1.5	25.6	72.9	1.5	25.6
	Levofloxacin	0.5	1	≤0.12-- >4	99.0	0.0	1.0	94.0	5.0	1.0
	Oxacillin	≤0.06	≤0.06	≤0.06 – >2	100.0	-	-	100.0	-	0.0
	Ceftriaxone	0.06	0.12	≤ 0.015 –>16	99.5	-	-	100.0	-	0
	Meropenem	≤0.06	≤0.06	≤0.015 –32	100.0	-	-	100.0	-	0.0
	Tetracycline	>8	>8	≤0.5 -- >8	43.7	0.5	55.8	42.7	1.0	56.3
	Tigecycline	0.03	0.03	≤0.015—0.06	100.0	-	-	100.0	0.0	0.0
	Vancomycin	0.25	0.5	0.25-1	100.0	-	-	100.0	-	0.0
	Linezolid	1	1	0.5 –1	100.0	-	-	100.0	0.0	0.0
	Daptomycin	0.12	0.25	≤0.06 – 0.5	100.0	-	-	100.0	-	0.0
S. pneumoniae (n=259)	Iclaprim	0.06	2	0.015-->8	-	-	-	-	-	-
	Trimethoprim	2	64	0.25-- >64	-	-	-	-	-	-
	TMS	0.25	8	0.12-- >8	69.1	12.0	18.9	78.0	3.1	18.9
	Erythromycin	≤0.12	>16	≤0.12-- >16	58.7	0.8	40.5	58.7	0.8	40.5
	Levofloxacin	1	1	0.5-- >4	97.7	0.4	1.9	97.7	-	2.3
	Penicillin	≤0.06	2	≤0.06-->8	55.2	24.7	20.1	55.2	-	44.8
	Ceftriaxone	0.03	1	≤0.015—16	75.3	15.8	8.9	75.3	24.3	0.4
	Meropenem	≤0.06	0.5	≤0.06—2	76.4	18.9	4.6	76.4	22.8	0.8
	Tetracycline	≤0.5	>8	≤0.5—0.8	67.6	0.4	32.0	67.6	0.4	32.0
	Tigecycline	0.03	0.03	≤0.015—0	99.6	-	.	-	-	-
	Vancomycin	0.25	0.5	≤0.12—0.5	100.0	-	-	100.0	-	0.0

	Linezolid	1	1	≤0.12--2	100.0	-	-	100.0	0.0	0.0
	Daptomycin	0.12	0.25	≤0.06—0.5	-	-	-	-	-	-

Abbreviations: MIC, minimal inhibitory concentration; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; S, susceptible; I, intermediate; R, resistant; TMS, trimethoprim-sulfamethoxazole; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; BHS, beta-hemolytic streptococci

Table 3 Cumulative inhibition by iclaprim, trimethorprim and trimethoprim-sulfamethoxazole at MIC values by pathogen group, 2012-2014

Organism	**Drug**	**Number (cumulative percentage) inhibited by drug MIC (µg/mL)**												
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>*
S. aureus (n=1,178)	Iclaprim	143 (12.5)	746 (75.5)	196 (92.1)	7 (92.7)	1 (92.8)	6 (93.3)	0 (93.3)	4 (93.6)	8 (94.3)	--	--	--	67 (100)
	Trimethoprim	--	--	--	18 (1.5)	268 (24.3)	707 (84.3)	96 (92.4)	3 (92.7)	3 (93.2)	3 (93.2)	4 (93.5)	6 (94.1)	70 (100.0)
	TMS	55 (4.7)	963 (86.4)	67 (92.1)	26 (94.3)	19 (95.9)	18 (97.5)	8 (98.1)	2 (98.3)	5 (98.7)	--	--	--	15 (100.0)
MSSA (n=596)	Iclaprim	67 (11.7)	370 (73.3)	127 (94.6)	5 (95.5)	0 (95.5)	0 (95.5)	0 (95.5)	1 (95.6)	0 (95.6)	--	--	--	26 (100.0)
	Trimethoprim	--	--	--	8 (1.3)	120 (21.5)	376 (84.6)	62 (95.0)	3 (95.5)	0 (95.5)	0 (95.5)	1 (95.6)	0 (95.6)	26 (100.0)
	TMS	23 (3.9)	529 (92.6)	18 (95.6)	4 (96.3)	8 (97.70)	7 (98.8)	3 (99.3)	0 (99.3)	1 (99.5)	--	--	--	3 (100.0)
MRSA (n=582)	Iclaprim	76 (13.4)	376 (77.7)	69 (89.5)	2 (89.9)	1 (90.0)	6 (91.1)	0 (91.1)	3 (91.6)	8 (93.0)	--	--	--	41 (100.0)
	Trimethoprim	--	--	--	--	10 (1.7)	148 (27.1)	331 (84.0)	34 (89.9)	3 (90.4)	3 (90.9)	3 (91.4)	6 (92.4)	44 (100.0)
	TMS	32 (5.5)	434 (80.1)	49 (88.5)	22 (92.3)	11 (94.2)	11 (96.0)	5 (96.9)	2 (97.3)	4 (97.9)	--	--	--	12 (100.0)
BHS (n=199)	Iclaprim	84 (42.2)	20 (52.3)	53 (78.9)	32 (95.0)	4 (97.0)	1 (97.5)	0 (97.5)	0 (97.5)	0 (97.5)	--	--	--	5 (100.0)
	Trimethoprim	--	--	11 (5.5)	48 (29.6)	32 (45.7)	51 (71.4)	43 (93.0)	8 (97.0)	0 (97.5)	1 (97.5)	0 (97.5)	0 (97.5)	5 (100.0)
	TMS	2 (1.0)	61 (31.7)	111 (87.4)	19 (97.0)	1 (97.5)	1 (98.0)	3 (99.5)	0 (99.5)	0 (99.5)	--	--	--	1 (100.0)
S. pyogenes (N=98)	Iclaprim	42 (42.9)	11 (54.1)	24 (78.6)	15 (93.9)	2 (95.9)	1 (96.9)	0 (96.9)	0 (96.9)	0 (96.9)	--	--	--	3 (100.0)
	Trimethoprim	--	--	6 (6.1)	23 (29.6)	16 (45.9)	26 (72.4)	21 (93.9)	3 (96.9)	0 (96.9)	0 (96.9)	0 (96.9)	0 (96.9)	3 (100.0)
	TMS	1	31	52	9	1	1	2	0	0	--	--	--	1

		(1.0)	(32.7)	(85.7)	(94.9)	(95.9)	(96.9)	(99.0)	(99.0)	(99.0)				(100.0)
S. agalactiae (N=101)	Iclaprim	42 (41.6)	9 (50.5)	29 (79.2)	17 (96.0)	2 (98.0)	0 (98.0)	0 (98.0)	0 (98.0)	0 (98.0)	--	--	--	2 (100.0)
	Trimethoprim	--	--	5 (5.0)	25 (29.7)	16 (45.5)	25 (70.3)	22 (92.1)	5 (97.0)	0 (97.0)	1 (98.0)	0 (98.0)	0 (98.0)	2 (100.0)
	TMS	1 (1.0)	30 (30.7)	57 (87.1)	12 (99.0)	0 (99.0)	0 (99.0)	1 (99.0)	0 (99.0)	0 (99.0)	--	--	--	0 (100.0)
S. pneumoniae (n=259)	Iclaprim	73 (28.2)	109 (70.3)	23 (79.2)	0 (79.2)	3 (80.3)	8 (83.4)	24 (92.7)	3 (93.8)	12 (98.5)	--	--	--	4 (100.0)
	Trimethoprim	--	--	--	1 (0.4)	6 (2.7)	54 (23.6)	123 (71.0)	21 (79.2)	1 (79.5)	1 (79.9)	7 (82.6)	28 (93.4)	17 (100.0)
	TMS	--	--	17 (6.2)	143 (61.4)	20 (69.1)	23 (78.0)	8 (81.1)	7 (83.8)	36 (97.7)	--	--	--	6 (100.0)

*Greater than the highest MIC dilution tested

Abbreviations: TMS, trimethoprim-sulfamethoxazole; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; BHS, beta-hemolytic streptococci

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Highlights

- Iclaprim is active *in vitro* against Gram-positive pathogens.
- Iclaprim was 8 to 32-fold more potent than trimethoprim.
- The MIC_{90} of iclaprim was also lower than most of the antibiotics tested.
- Iclaprim may be a treatment for skin and skin structure and pneumonia infections.