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Surveillance of Iclaprim Activity: *In Vitro* Susceptibility of Gram-positive Skin Infection Pathogens Collected from 2015-2016 From North America and Europe

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Abstract

25
26
27 Iclaprim is a diaminopyrimidine, which inhibits bacterial dihydrofolate reductase, and
28 surveillance data prior to 2006 suggested that iclaprim was active against Gram-positive
29 pathogens including emerging drug-resistant pathogens. In an era of increasing antimicrobial
30 resistance, we undertook testing iclaprim and comparators against 931 Gram-positive clinical
31 isolates from the United States and Europe collected between 2015-2016. Susceptibility testing
32 was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.
33 Minimum inhibitory concentration (MIC) interpretations were based on CLSI and European
34 Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. MIC₅₀/MIC₉₀ was
35 0.03/0.12 for all *Staphylococcus aureus*, 0.06/0.06 for methicillin susceptible *S. aureus*,
36 0.03/0.12 for methicillin resistant *S. aureus*, 0.12/0.5 for *Streptococcus agalactiae*, ≤ 0.015 / \leq
37 0.015 for *Streptococcus anginosus*, 0.03 / 0.06 for *Streptococcus dysgalactiae*, and ≤ 0.015 / 0.03
38 $\mu\text{g/mL}$ for *Streptococcus pyogenes*. Iclaprim was active against a contemporary collection
39 (2015-2016) of Gram-positive bacteria isolated from the skin or soft tissue from patients with
40 SSSI from the United States and Europe.

41
42 Keywords: iclaprim, surveillance, skin, soft tissue, *in vitro*

43 Key words: 159

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48 **Introduction**

49 Bacterial skin and soft tissue infections (SSTIs) are one of the most common causes of
50 infection in patients of all ages (Stevens et al., 2014). In particular, these infections represent the
51 most common infection type presenting in patients visiting emergency rooms and account for a
52 substantial portion of hospital admissions (Stevens et al., 2014; Tognetti et al., 2012). Gram-
53 positive bacteria are the most frequently isolated etiology of skin infections, occurring in more
54 than 80% of ABSSSI cases, with *S. aureus* the most common pathogen as the cause of wound
55 infections, abscesses, and cellulitis (Tognetti et al., 2012).

56 In 2014, the Infectious Diseases Society of America (IDSA) issued a practice guideline to
57 provide recommendations for the diagnosis and management of SSTIs (Stevens et al., 2014). The
58 recommendations were issued in response to the dramatic increase in the frequency and severity
59 of these types of infections and the emergence of pathogens that are resistant to many of the
60 antimicrobial agents commonly used to treat these infections. There are many antibiotics
61 approved for the treatment of SSTIs, but all have safety concerns or reported resistant pathogens
62 (Steinkraus et al., 2007; Sanchez Garcia et al., 2010; Mishra et al., 2012; Steenbergen et al., 2005;
63 Long et al., 2014). Therefore, there is a medical need for a well-tolerated antimicrobial agent
64 with rapid bactericidal action with activity against MRSA and other Gram-positive pathogens,
65 with an alternative mode of action, which is not cross-resistant to available antibiotics.

66 Iclaprim is a diaminopyrimidine, which inhibits bacterial dihydrofolate reductase (DHFR)
67 and is active against emerging drug-resistant pathogens (Sader et al., 2009; Schneider et al.,
68 2003). It is in the same class as trimethoprim, the only FDA approved dihydrofolate reductase
69 inhibitor. Iclaprim was designed to be more active than trimethoprim and overcome
70 trimethoprim resistance among Gram-positive pathogens (Oefner et al., 2009). In addition,

71 iclaprim does not need to be combined with a sulfonamide, which is commonly associated with
72 adverse events including renal toxicity, hepatotoxicity, blood dyscrasias, anaphylaxis, and
73 hypersensitivity reactions. Iclaprim exhibits *in vitro* activity against Gram-positive pathogens
74 such as *Staphylococcus aureus* and beta-hemolytic streptococci (BHS), including resistant
75 phenotypes that cause SSSI (Sader et al., 2009; Morrissey et al., 2009). In a Phase 3 clinical trial,
76 iclaprim has shown clinical cure rates comparable to vancomycin among patients treated for
77 SSSI (Huang et al., 2017). Because of these findings, iclaprim is potentially well suited for
78 treating patients with SSSI caused by or suspected Gram-positive bacteria, including multidrug
79 resistant pathogens and is presently in Phase 3 clinical development for the treatment of acute
80 bacterial skin and skin structure infections (ABSSSI). In an era of increasing antimicrobial
81 resistance, we report contemporary surveillance data on 931 methicillin susceptible *S. aureus*
82 (MSSA), methicillin resistant *S. aureus* (MRSA), *S. agalactiae*, *S. anginosus*, *S. dysgalactiae*,
83 and *S. pyogenes* isolated from the skin or soft tissue from patients with SSSI in the United States
84 and Europe.

85

86 **Materials and Methods**

87 *Collection of bacterial isolates*

88 A total of 931 non-duplicative, non-consecutive isolates of methicillin susceptible *S.*
89 *aureus* (n=314), methicillin resistant *S. aureus* (n=304), *S. pyogenes* (n=159), *S. agalactiae*
90 (n=100), *S. dysgalactiae* (n=40), and *S. anginosus* (n=14) were collected from skin or soft tissue
91 from patients with SSSI in multiple locations in the US and EU between 2016-2016. Clinical
92 isolates were identified by the submitting laboratories and confirmed by IHMA Laboratories
93 using the Bruker Matrix-Assisted Laser Desorption Ionization-Time Of Flight Mass

94 Spectrometry (MALDI-TOF) biotyper for all isolates. The distribution of pathogens by country
95 are shown in Table 1. Of the 931 isolates, 467 (50.2%) were collected from North America and
96 464 (49.8%) from Europe.

97

98 *Susceptibility testing*

99 Antibacterial susceptibility testing was conducted by IHMA Laboratories (Monthey,
100 Switzerland). Susceptibility testing was performed by broth microdilution in accordance with
101 the Clinical and Laboratory Standards Institute (CLSI) guidelines M07-A10 (2015) and the
102 standard operating procedures at IHMA laboratories. Minimum Inhibitory Concentration (MIC)
103 interpretations were based on CLSI and European Committee on Antimicrobial Susceptibility
104 Testing (EUCAST) criteria (2015). There are no published breakpoints for iclaprim. *S. aureus*,
105 both methicillin-susceptible and methicillin-resistant, were tested in cation-adjusted Mueller-
106 Hinton broth (CA-MHB) and *Streptococci* were tested in CA-MHB supplemented with 5% lysed
107 horse blood. Quality controls and interpretation of results were performed in accordance with
108 CLSI M100 (2017). QC ranges for iclaprim were those approved by CLSI and published in
109 M100. Iclaprim and comparator antibiotic MIC results were within the CLSI published ranges
110 against *S. aureus* ATCC 29213 and *S. pneumoniae* ATCC 49619. Isolates were tested with MIC
111 panels (ThermoFisher Scientific, Cleveland, OH, USA) of comparator antibiotics (trimethoprim-
112 sulfamethoxazole, erythromycin, clindamycin, gentamicin, ceftioxin, penicillin G, levofloxacin,
113 tetracycline, vancomycin, linezolid, and daptomycin against *S. aureus* and ceftriaxone,
114 meropenem, ampicillin, azithromycin, clindamycin, trimethoprim-sulfamethoxazole,
115 levofloxacin, tetracycline, linezolid, and penicillin G against *Streptococci*).

116

117 **Results**

118 *Iclaprim and comparator activity against Gram-positive pathogens from 2015-2016*

119 Iclaprim demonstrated antimicrobial activity against key Gram-positive pathogens,
120 including strains with resistant phenotypes, isolated from the skin or soft tissue from patients
121 with SSSI. Table 2 shows the *in vitro* activity of iclaprim and comparators against *S. aureus*,
122 methicillin susceptible *S. aureus*, methicillin resistant *S. aureus*, *S. agalactiae*, *S. anginosus*, *S.*
123 *dysgalactiae*, and *S. pyogenes*. Table 3 shows the cumulative percentage of isolates inhibited at
124 each iclaprim MIC value. Iclaprim MIC values ranged from ≤ 0.015 to >32 $\mu\text{g/mL}$. MIC values
125 were similar to trimethoprim-sulfamethoxazole (TMS).

126

127 *Iclaprim and comparator activity against S. aureus*

128 Table 2 shows iclaprim exhibited activity against all 618 *S. aureus* isolates. The MIC₅₀
129 and MIC₉₀ values were 0.03 and 0.12 $\mu\text{g/mL}$, respectively. For trimethoprim-sulfamethoxazole,
130 the MIC₅₀ and MIC₉₀ were ≤ 0.06 and ≤ 0.06 $\mu\text{g/mL}$, respectively. For isolates with a MIC for
131 erythromycin of ≥ 1 $\mu\text{g/mL}$ (n=319), the MIC₅₀ and MIC₉₀ for iclaprim were ≤ 0.25 and ≤ 0.25
132 $\mu\text{g/mL}$, respectively. For isolates with a MIC for clindamycin of ≥ 1 $\mu\text{g/mL}$ (n=106), the MIC₅₀
133 and MIC₉₀ for iclaprim were ≤ 0.25 and ≤ 8 $\mu\text{g/mL}$, respectively. For isolates with a MIC for
134 levofloxacin of ≥ 2 $\mu\text{g/mL}$ (n=235), the MIC₅₀ and MIC₉₀ for iclaprim were ≤ 0.25 and ≤ 0.5
135 $\mu\text{g/mL}$, respectively. All isolates with a MIC for TMS of ≥ 4 $\mu\text{g/mL}$ had a MIC for iclaprim \geq
136 8 $\mu\text{g/mL}$. For isolates with a MIC for TMS of 4, 8, 16 and 32 $\mu\text{g/mL}$, XXX (X.X%), X (X%) and
137 X (X%) had a MIC for iclaprim of 8, 16 and ≥ 32 $\mu\text{g/mL}$.

138 Iclaprim maintained activity against *S. aureus* regardless of methicillin susceptibility.

139 For MSSA, the MIC₅₀ and MIC₉₀ were both 0.06 µg/mL. For MRSA, the MIC₅₀ and MIC₉₀
140 were 0.03 and 0.12 µg/mL, respectively. By comparison, trimethoprim-sulfamethoxazole MIC₅₀
141 and MIC₉₀ were both ≤0.06 µg/mL for MSSA, and ≤0.06 and 0.12 µg/mL for MRSA,
142 respectively.

143 Iclaprim also maintained activity against *S. aureus* regardless of isolation from North
144 America or Europe. For North America, the MIC₅₀ and MIC₉₀ were 0.03 and 0.12 µg/mL,
145 respectively. For Europe, the MIC₅₀ and MIC₉₀ were 0.03 and 0.06 µg/mL, respectively.

146

147 *Iclaprim and comparator activity against S. pyogenes*

148 Iclaprim exhibited activity against all 159 *S. pyogenes* (Table 2). The MIC₅₀/MIC₉₀ were
149 ≤0.015 / 0.03 µg/mL, respectively. By comparison, MIC₅₀/MIC₉₀ for trimethoprim-
150 sulfamethoxazole were ≤0.06 / 0.12 µg/mL, respectively (Table 2). Iclaprim showed activity
151 against *S. pyogenes* independent of the prevalence of macrolide resistance. The MIC₉₀ of
152 iclaprim was 0.03 µg/mL against isolates of *S. pyogenes* susceptible to azithromycin (MIC ≤ 0.5
153 µg/mL, n=133, 83.6 %) and also against isolates resistant to azithromycin (MIC ≥2 µg/mL, n=24,
154 15.1%).

155

156 *Iclaprim and comparator activity against S. agalactiae*

157 Iclaprim exhibited similar MICs against all 100 *S. agalactiae* (Table 2). The MIC₅₀ and
158 MIC₉₀ were 0.12 and 0.5 µg/mL, respectively, for *S. agalactiae*. In comparison, MIC₅₀ and
159 MIC₉₀ for trimethoprim-sulfamethoxazole were both 0.12 µg/mL (Table 2). Iclaprim showed
160 activity against *S. agalactiae* independent of the prevalence of macrolide resistance. The MIC
161 range (0.06 - 1 µg/mL) and MIC₉₀ (0.5 µg/mL) for iclaprim were identical for isolates resistant

162 (MIC ≥ 2 $\mu\text{g/mL}$, 41%) or susceptible to azithromycin (59%).

163

164 *Iclaprim and comparator activity against S. anginosus and S. dysgalactiae*

165 Iclaprim exhibited activity against all 14 *S. anginosus* (Table 2). The MIC₅₀ and MIC₉₀
166 were both ≤ 0.015 $\mu\text{g/mL}$ for *S. anginosus*. In comparison, MIC₅₀ and MIC₉₀ for trimethoprim-
167 sulfamethoxazole were both ≤ 0.06 $\mu\text{g/mL}$ (Table 2). Iclaprim showed activity against *S.*
168 *anginosus* independent of the prevalence of macrolide resistance. The MIC for iclaprim against
169 all *S. anginosus* isolates tested was constant at ≤ 0.015 $\mu\text{g/mL}$ for an azithromycin MIC range
170 ≤ 0.03 - > 8 $\mu\text{g/mL}$.

171 Iclaprim exhibited activity against all 40 *S. dysgalactiae* (Table 2). The MIC₅₀ and
172 MIC₉₀ were 0.03 and 0.06 $\mu\text{g/mL}$, respectively, for *S. dysgalactiae*. By comparison,
173 MIC₅₀/MIC₉₀ for trimethoprim-sulfamethoxazole were $\leq 0.06 / 0.12$ $\mu\text{g/mL}$, respectively (Table
174 2). Iclaprim showed activity against *S. dysgalactiae* independent of the prevalence of
175 macrolide resistance. Of the nine isolates resistant to azithromycin (MIC ≥ 2 $\mu\text{g/mL}$, 22.5 %),
176 only one had a MIC for iclaprim > 0.03 $\mu\text{g/mL}$. Based on EUCAST breakpoints (no breakpoints
177 are available in CLSI for TMS against beta-hemolytic streptococci), only one *S. dysgalactiae*
178 isolate was resistant to TMS (MIC ≥ 2 $\mu\text{g/mL}$), and the MIC of iclaprim was > 32 $\mu\text{g/mL}$ for this
179 isolate.

180

181 **Discussion**

182 This study shows that iclaprim alone, without the synergistic combination of a
183 sulfonamide, is active against a collection of 931 Gram-positive clinical isolates, including those
184 with resistant phenotypes, collected from skin or soft tissue from patients with SSSI between

185 2015-2016 in the US and EU. Iclaprim activity (MIC₅₀, 0.03 µg/mL and MIC₉₀, 0.12 µg/mL)
186 was similar to that of TMS (MIC₅₀ ≤0.06 µg/mL and MIC₉₀, 0.12 µg/mL). Although the *in*
187 *vitro* activity of iclaprim alone was similar to TMS combination, not having to add a sulfonamide
188 component to iclaprim may be clinically meaningful from a safety perspective. For example,
189 sulfonamides are associated with the following severe toxicities: hypersensitivity reactions
190 (Stevens Johnson syndrome), anaphylaxis, hepatotoxicity, and blood dyscrasias. The activity of
191 iclaprim, the MIC₅₀/MIC₉₀ of 0.03/0.12 µg/mL for *S. aureus*, 0.12/0.5 µg/mL for *S. agalactiae*,
192 ≤0.015/0.03 µg/mL for *S. pyogenes*, ≤0.015 /≤0.015 µg/mL for *S. anginosus* and 0.03/0.06
193 µg/mL for *S. dysgalactiae*, documented in this analysis were consistent with those in a
194 surveillance study performed a decade earlier, comprising 5,937 Gram-positive isolates from
195 skin and soft tissue, blood stream and respiratory clinical specimens from patients in the US and
196 EU (Sader et al., 2009) and with those in a surveillance study performed in 2012-2014,
197 comprising 2,814 Gram-positive clinical isolates from skin and soft tissue from patients in the
198 US and EU (Huang et al., in press).

199 Resistance in *S. aureus* to dihydrofolate reductase inhibitors is determined by a single
200 amino acid change (F98Y) within the trimethoprim-binding site of DHFR. Iclaprim was
201 rationally engineered, using information from X-ray crystal data of isolated DHFR, for enhanced
202 activity against Gram-positive bacteria including strains with mutational changes in DHFR that
203 determine trimethoprim resistance (TMP-R). Iclaprim retains sufficient binding affinity to F98Y
204 DHFR due to additional hydrophobic interactions with surrounding amino acids (Oefner et al.,
205 2009). Its activity against TMP-R clinical isolates of *S. aureus* and BHS has been demonstrated
206 in a number of studies and is driven by the greatly increased affinity of iclaprim to the DHFR
207 target site including mutant DHFR. BHS are normally considered susceptible to TMP, although

208 no clinical breakpoints for TMP exist and consequently no information concerning mechanisms
209 of TMP resistance in such organisms are available.

210 In conclusion, the results from this surveillance study confirm widespread iclaprim
211 susceptibility rates among contemporary (2015-2016) pathogens from the skin or soft tissue from
212 patients with SSSI from the US and EU. The rates are unchanged a decade after another large
213 surveillance study (Sader et al., 2009). The proportions of nonsusceptible isolates of methicillin
214 susceptible *S. aureus*, methicillin resistant *S. aureus*, *S. agalactiae*, *S. anginosus*, *S. dysgalactiae*,
215 and *S. pyogenes* to iclaprim were limited. Continued surveillance is warranted to track the
216 activity of iclaprim and to detect any potential emergence of resistance to iclaprim in the future.

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230 Table 1 Distribution of organisms collected from North America and Europe, 2015-2016

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Organism	North America	Europe	Total
<i>S. aureus</i>	307	311	618
MRSA	154	160	314
MSSA	153	151	304
<i>S. agalactiae</i>	50	50	100
<i>S. anginosus</i>	11	3	14
<i>S. dysgalactiae</i>	20	20	40
<i>S. pyogenes</i>	79	80	159
Total	467 (50.2%)	464 (49.8%)	931

232

233 Abbreviations: MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*

Table 2 *In vitro* activity of iclaprim and comparators against isolates collected from North America and Europe, 2015-2016

Organism	Drug	MIC ₅₀	MIC ₉₀	Range	CLSI			EUCAST		
					%S	%I	%R	%S	%I	%R
<i>S. aureus</i> (n=618)	Iclaprim	0.03	0.12	≤0.015->32	NB	NB	NB	NB	NB	NB
	Cefoxitin	>4	>4	1->4	49.2	0	50.8	NB	NB	NB
	Clindamycin	0.12	>4	≤0.03->4	82.9	0	17.2	82.9	0	17.2
	Daptomycin	0.25	0.5	0.12-1	100.0	0	0	100.0	0	0
	Erythromycin	16	>16	≤0.12->16	48.4	0.7	51.0	48.4	0	51.6
	Gentamicin	0.25	0.5	≤0.06->16	95.3	0.2	4.5	95.3	0	4.7
	Levofloxacin	0.25	>4	0.06->4	62.0	0.5	37.5	62.0	0	38.0
	Linezolid	1	2	0.5-4	100.0	0	0	100.0	0	0
	Penicillin	>4	>4	≤0.06->4	15.4	0	84.6	15.4	0	84.6
	Tetracycline	0.25	1	≤0.06->16	92.6	0.3	7.1	91.10	1.13	7.8
	Trimethoprim-Sulfamethoxazole	≤0.06	≤0.06	≤0.06-32	98.5	0	1.5	98.5	0.3	1.13
	Vancomycin	1	1	≤0.25-2	100.0	0	0	100.0	0	0
	MRSA (n=314)	Iclaprim	0.03	0.12	≤0.015->32	NB	NB	NB	NB	NB
Cefoxitin		> 4	> 4	>4->4	0.0	0.0	100.0	NB	NB	NB
Clindamycin		0.12	> 4	≤0.03->4	70.1	0.0	29.9	70.1	0.0	29.9
Daptomycin		0.25	0.5	0.12-1	100.0	0.0	0.0	100.0	0.0	0.0
Erythromycin		> 16	> 16	≤0.12->16	25.2	0.3	74.5	25.2	0.0	74.8
Gentamicin		0.25	0.5	0.12->16	92.7	0.0	7.3	92.7	0.0	7.3
Levofloxacin		4	>4	0.06->4	33.1	0.6	66.2	33.1	0.0	66.9
Linezolid		1	2	0.5-4	100.0	0.0	0.0	100.0	0.0	0.0
Penicillin		>4	>4	0.25->4	0.0	0.0	100.0	0.0	0.0	100.0
Tetracycline		0.25	2	≤0.06->16	90.1	0.3	9.6	87.9	2.2	9.9
Trimethoprim-Sulfamethoxazole		≤0.06	0.12	≤0.06-32	97.5	0.0	2.6	97.5	0.6	1.9

	Vancomycin	1	1	≤0.25-2	100.0	0.0	0.0	100.0	0.0	0.0
MSSA (n=304)	Iclaprim	0.06	0.06	≤0.015->32	NB	NB	NB	NB	NB	NB
	Cefoxitin	4	4	1-4	100.0	0.0	0.0	NB	NB	NB
	Clindamycin	0.06	0.12	≤0.03->4	96.1	0.0	3.9	96.1	0.0	3.9
	Daptomycin	0.25	0.5	0.12-1	100.0	0.0	0.0	100.0	0.0	0.0
	Erythromycin	0.25	>16	≤0.12->16	72.4	1.0	26.6	72.4	0.0	27.6
	Gentamicin	0.25	0.5	≤0.06->16	98.0	0.3	1.6	98.0	0.0	2.0
	Levofloxacin	0.12	0.5	0.06-> 4	91.8	0.3	7.9	91.8	0.0	8.2
	Linezolid	1	2	0.5-2	100.0	0.0	0.0	100.0	0.0	0.0
	Penicillin	1	>4	≤ 0.06-> 4	31.3	0.0	68.8	31.3	0.0	68.8
	Tetracycline	0.25	0.25	≤ 0.06-> 16	95.1	0.3	4.6	94.4	0.0	5.6
	Trimethoprim- Sulfamethoxazole	≤0.06	≤0.06	≤ 0.06-16	99.7	0.0	0.3	99.7	0.0	0.3
	Vancomycin	1	1	≤ 0.25-2	100.0	0.0	0.0	100.0	0.0	0.0
<i>S. pyogenes</i> (n=159)	Iclaprim	≤0.015	0.03	≤ 0.015-0.5	NB	NB	NB	NB	NB	NB
	Ampicillin	≤0.03	≤0.03	≤ 0.03-0.12	100.0	0.0	0.0	NB	NB	NB
	Azithromycin	0.12	>8	≤ 0.03-> 8	83.7	1.3	15.1	83.7	0.0	16.4
	Ceftriaxone	0.03	0.03	≤ 0.015-0.06	100.0	0.0	0.0	NB	NB	NB
	Clindamycin	0.06	0.06	0.03-> 2	97.5	0.6	1.9	98.1	0.0	1.9
	Levofloxacin	0.5	1	0.25-2	100.0	0.0	0.0	100.0	0.0	0.0
	Linezolid	1	1	0.5-1	100.0	0.0	0.0	100.0	0.0	0.0
	Meropenem	≤0.015	≤0.015	≤ 0.015-0.06	100.0	0.0	0.0	NB	NB	NB
	Penicillin	≤0.06	≤0.06	≤ 0.06-≤ 0.06	100.0	0.0	0.0	100.0	0.0	0.0
	Tetracycline	0.12	>8	0.06-> 8	85.5	0.6	13.8	85.5	0.0	14.5
	Trimethoprim- sulfamethoxazole	≤0.06	0.12	≤ 0.06-0.5	NB	NB	NB	100.0	0.0	0.0
<i>S. agalactiae</i> (n=100)	Iclaprim	0.12	0.5	0.06-1	NB	NB	NB	NB	NB	NB
	Ampicillin	0.12	0.12	0.06-0.25	100.0	0.0	0.0	NB	NB	NB
	Azithromycin	0.12	>8	0.06-> 8	59.0	0.0	41.0	59.0	0.0	41.0

	Ceftriaxone	0.06	0.06	0.06-0.12	100.0	0.0	0.0	NB	NB	NB
	Clindamycin	0.06	>2	0.03-> 2	72.0	2.0	26.0	74.0	0.0	26.0
	Levofloxacin	1	1	0.5-2	100.0	0.0	0.0	100.0	0.0	0.0
	Linezolid	1	1	0.5-1	100.0	0.0	0.0	100.0	0.0	0.0
	Meropenem	0.03	0.06	0.03-0.06	100.0	0.0	0.0	NB	NB	NB
	Penicillin	≤0.06	≤0.06	≤ 0.06-0.5	99.0	0.0	1.0	99.00	0.0	1.0
	Tetracycline	>8	>8	0.06-> 8	23.0	1.0	76.0	22.0	1.0	77.0
	Trimethoprim-sulfamethoxazole	0.12	0.12	≤ 0.06-0.5	NB	NB	NB	100.0	0.0	0.0
<i>S. anginosus</i> (n=14)	Iclaprim	≤0.015	≤0.015	≤ 0.015-≤ 0.015	NB	NB	NB	NB	NB	NB
	Ampicillin	0.06	0.12	≤ 0.03-0.12	100.0	0.0	0.0	100.0	0.0	0.0
	Azithromycin	0.06	>8	≤ 0.03-> 8	57.1	7.1	35.7	NB	NB	NB
	Ceftriaxone	0.12	0.25	0.03-0.5	100.0	0.0	0.0	100.0	0.0	0.0
	Clindamycin	0.03	>2	≤ 0.015-> 2	78.6	0.0	21.4	78.6	0.0	21.4
	Levofloxacin	0.5	0.5	≤ 0.12-1	100.0	0.0	0.0	NB	NB	NB
	Linezolid	1	1	0.5-1	100.0	0.0	0.0	NB	NB	NB
	Meropenem	0.03	0.12	≤ 0.015-0.12	100.0	0.0	0.0	100.0	0.0	0.0
	Penicillin	≤0.06	≤0.06	≤ 0.06-≤ 0.06	100.0	0.0	0.0	100.0	0.0	0.0
	Tetracycline	4	>8	≤ 0.03-> 8	42.9	7.1	50.0	NB	NB	NB
	Trimethoprim-sulfamethoxazole	≤0.06	≤0.06	≤ 0.06-≤ 0.06	NB	NB	NB	NB	NB	NB
<i>S. dysgalactiae</i> (n=40)	Iclaprim	0.03	0.06	≤ 0.015-> 32	NB	NB	NB	NB	NB	NB
	Ampicillin	≤0.03	≤0.03	≤ 0.03-0.12	100.0	0.0	0.0	NB	NB	NB
	Azithromycin	0.12	>8	0.12-> 8	77.5	0.0	22.5	77.5	0.0	22.5
	Ceftriaxone	0.03	0.06	≤ 0.015-0.06	100.0	0.0	0.0	NB	NB	NB
	Clindamycin	0.06	0.06	0.03-> 2	97.5	0.0	2.5	97.5	0.0	2.5
	Levofloxacin	0.5	1	0.25-> 8	97.5	0.0	2.5	97.5	0.0	2.5
	Linezolid	1	1	1-1	100.0	0.0	0.0	100.0	0.0	0.0
	Meropenem	≤0.015	≤0.015	≤ 0.015-≤ 0.015	100.0	0.0	0.0	NB	NB	NB
	Penicillin	≤0.06	≤0.06	≤ 0.06-≤ 0.06	100.0	0.0	0.0	100.0	0.0	0.0

	Tetracycline	0.25	>8	0.12-> 8	67.5	10.0	22.5	67.5	0.0	32.5
	Trimethoprim-sulfamethoxazole	≤0.06	0.12	≤ 0.06-> 16	NB	NB	NB	97.5	0.0	2.5

Abbreviations: MIC, minimal inhibitory concentration; NB, no breakpoint; 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*

Table 3 MIC values and Cumulative MIC distributions for iclaprim and TMS by pathogen group, 2015-2016

Organism	Drug	Number (cumulative percentage) inhibited by drug MIC ($\mu\text{g/mL}$)														
		≤ 0.015	0.03	≤ 0.06	0.06	0.12	0.25	0.5	1	2	4	8	16	>16	32	>32
<i>S. aureus</i> (n=618)	Iclaprim	4.2	52.1	NA	89.97	93.0	93.9	94.3	94.8	94.8	95.1	95.5	96.4	NA	96.8	100
	TMS	NA	NA	91.9	NA	91.9	96.9	98.1	98.4	98.5	98.9	98.9	99.5	NA	100	100
MSSA (n=304)	Iclaprim	5.6	45.7	NA	91.4	96.1	97.4	97.4	97.7	97.7	97.7	97.7	98.4	NA	98.4	100
	TMS	NA	NA	97	NA	97.4	98.7	99.3	99.7	99.7	99.7	99.7	100	NA	100	100
MRSA (n=314)	Iclaprim	2.9	58.3	NA	88.5	90.1	90.4	91.4	92	92	92.7	93.3	94.6	NA	95.2	100
	TMS	NA	NA	86.9	NA	91.7	95.2	96.8	97.1	97.5	98.1	98.1	99	NA	100	100
<i>S. pyogenes</i> (n=159)	Iclaprim	84.9	95	NA	99.4	99.4	99.4	100	100	100	100	100	100	NA	100	100
	TMS	NA	NA	65.4	NA	96.2	99.4	100	100	100	100	100	100	100	NA	NA
<i>S. agalactiae</i> (n=100)	Iclaprim	0	0	NA	49	53	80	98	100	100	100	100	100	NA	100	100
	TMS	NA	NA	21	NA	93	99	100	100	100	100	100	100	100	NA	NA
<i>S. anginosus</i> (N=14)	Iclaprim	100	100	NA	100	100	100	100	100	100	100	100	100	NA	100	100
	TMS	NA	NA	100	NA	100	100	100	100	100	100	100	100	100	NA	NA
<i>S. dysgalactiae</i> (N=40)	Iclaprim	2.5	50	NA	95	95	95	97.5	97.5	97.5	97.5	97.5	97.5	NA	97.5	100
	TMS	NA	NA	65	NA	95	97.5	97.5	97.5	97.5	97.5	97.5	97.5	100	NA	NA

Abbreviations: NA, not applicable; TMS, trimethoprim-sulfamethoxazole; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*

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