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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

Iclaprim is a diaminopyrimidine, which inhibits bacterial dihydrofolate reductase, and surveillance data prior to 2006 suggested that iclaprim was active against Gram-positive pathogens including emerging drug-resistant pathogens. In an era of increasing antimicrobial resistance, we undertook testing iclaprim and comparators against 931 Gram-positive clinical isolates from the United States and Europe collected between 2015-2016. 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} was 0.03/0.12 for all Staphylococcus aureus, 0.06/0.06 for methicillin susceptible S. aureus, 0.03/0.12 for methicillin resistant S. aureus, 0.12/0.5 for Streptococcus agalactiae, ⩽0.015 / ⩽ 0.015 for Streptococcus anginosus, 0.03 / 0.06 for Streptococcus dysgalactiae, and ⩽0.015 /0.03 µg/mL for Streptococcus pyogenes. Iclaprim was active against a contemporary collection (2015-2016) of Gram-positive bacteria isolated from the skin or soft tissue from patients with SSSI from the United States and Europe.

Keywords: iclaprim, surveillance, skin, soft tissue, in vitro

Key words: 159

Introduction

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

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

Iclaprim is a diaminopyrimidine, which inhibits bacterial dihydrofolate reductase (DHFR) and is active against emerging drug-resistant pathogens (Sader et al., 2009; Schneider et al., 2003). It is in the same class as trimethoprim, the only FDA approved dihydrofolate reductase inhibitor. Iclaprim was designed to be more active than trimethoprim and 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 renal toxicity, hepatotoxicity, blood dyscrasias, anaphylaxis, and hypersensitivity reactions. Iclaprim exhibits in vitro activity against Gram-positive pathogens such as Staphylococcus aureus and beta-hemolytic streptococci (BHS), including resistant phenotypes that cause SSSI (Sader et al., 2009; Morrissey et al., 2009). In a Phase 3 clinical trial, iclaprim has shown clinical cure rates comparable to vancomycin among patients treated for SSSI (Huang et al., 2017). Because of these findings, iclaprim is potentially well suited for treating patients with SSSI caused by or suspected Gram-positive bacteria, including multidrug resistant pathogens and is presently in Phase 3 clinical development for the treatment of acute bacterial skin and skin structure infections (ABSSSI). In an era of increasing antimicrobial resistance, we report contemporary surveillance data on 931 methicillin susceptible S. aureus (MSSA), methicillin resistant S. aureus (MRSA), S. agalactiae, S. anginosus, S. dysgalactiae, and S. pyogenes isolated from the skin or soft tissue from patients with SSSI in the United States and Europe.

Materials and Methods

Collection of bacterial isolates

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

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

Susceptibility testing

Antibacterial susceptibility testing was conducted by IHMA Laboratories (Monthey, Switzerland). Susceptibility testing was performed by broth microdilution in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines M07-A10 (2015) and the standard operating procedures at IHMA laboratories. Minimum Inhibitory Concentration (MIC) interpretations were based on CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (2015). There are no published breakpoints for iclaprim. S. aureus, both methicillin-susceptible and methicillin-resistant, were tested in cation-adjusted Mueller-Hinton broth (CA-MHB) and Streptococci were tested in CA-MHB supplemented with 5% lysed horse blood. Quality controls and interpretation of results were performed in accordance with CLSI M100 (2017). QC ranges for iclaprim were those approved by CLSI and published in M100. 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-sulfamethoxazole, erythromycin, clindamycin, gentamicin, cefoxitin, penicillin G, levofloxacin, tetracycline, vancomycin, linezolid, and daptomycin against S. aureus and ceftriaxone, meropenem, ampicillin, azithromycin, clindamycin, trimethoprim-sulfamethoxazole, levofloxacin, tetracycline, linezolid, and penicillin G against Streptococci).

Results

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

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

Iclaprim and comparator activity against S. aureus

Table 2 shows iclaprim exhibited activity against all 618 S. aureus isolates. The MIC_{50} and MIC_{90} values were 0.03 and 0.12 µg/mL, respectively. For trimethoprim-sulfamethoxazole, the MIC_{50} and MIC_{90} were ≤0.06 and ≤0.06 µg/mL, respectively. For isolates with a MIC for erythromycin of ≥1 µg/mL (n=319), the MIC_{50} and MIC_{90} for iclaprim were ≤0.25 and ≤0.25 µg/mL, respectively. For isolates with a MIC for clindamycin of ≥1 µg/mL (n=106), the MIC_{50} and MIC_{90} for iclaprim were ≤0.25 and ≤8 µg/mL, respectively. For isolates with a MIC for levofloxacin of ≥2 µg/mL (n=235),), the MIC_{50} and MIC_{90} for iclaprim were ≤0.25 and ≤0.5 µg/mL, respectively. All isolates with a MIC for TMS of ≥4 µg/mL had a MIC for iclaprim ≥ 8 µg/mL. For isolates with a MIC for TMS of 4, 8, 16 and 32 µg/mL, XXX (X.X%), X (X%) and X (X%) had a MIC for iclaprim of 8, 16 and ≥32 µg/mL.

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

For MSSA, the MIC_{50} and MIC_{90} were both 0.06 µg/mL. For MRSA, the MIC_{50} and MIC_{90} were 0.03 and 0.12 µg/mL, respectively. By comparison, trimethoprim-sulfamethoxazole MIC_{50} and MIC_{90} were both ≤0.06 µg/mL for MSSA, and ≤0.06 and 0.12 µg/mL for MRSA, respectively.

Iclaprim also maintained activity against S. aureus regardless of isolation from North America or Europe. For North America, the MIC_{50} and MIC_{90} were 0.03 and 0.12 µg/mL, respectively. For Europe, the MIC_{50} and MIC_{90} were 0.03 and 0.06 µg/mL, respectively.

Iclaprim and comparator activity against S. pyogenes

Iclaprim exhibited activity against all 159 S. pyogenes (Table 2). The MIC_{50}/MIC_{90} were ≤0.015 / 0.03 µg/mL, respectively. By comparison, MIC_{50}/MIC_{90} for trimethoprim-sulfamethoxazole were ≤0.06 / 0.12 µg/mL, respectively (Table 2). Iclaprim showed activity against S. pyogenes independent of the prevalence of macrolide resistance. The MIC_{90} of iclaprim was 0.03 µg/mL against isolates of S. pyogenes susceptible to azithromycin (MIC ≤ 0.5 µg/mL, n=133, 83.6 %) and also against isolates resistant to azithromycin (MIC ≥2 µg/mL, n=24, 15.1%).

Iclaprim and comparator activity against S. agalactiae

Iclaprim exhibited similar MICs against all 100 S. agalactiae (Table 2). The MIC_{50} and MIC_{90} were 0.12 and 0.5 µg/mL, respectively, for S. agalactiae. In comparison, MIC_{50} and MIC_{90} for trimethoprim-sulfamethoxazole were both 0.12 µg/mL (Table 2). Iclaprim showed activity against S. agalactiae independent of the prevalence of macrolide resistance. The MIC range (0.06 - 1 µg/mL) and MIC_{90} (0.5 µg/mL) for iclaprim were identical for isolates resistant

(MIC ≥2 µg/mL, 41%) or susceptible to azithromycin (59%).

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

Iclaprim exhibited activity against all 14 S. anginosus (Table 2). The MIC_{50} and MIC_{90} were both ⩽0.015 µg/mL for S. anginosus. In comparison, MIC_{50} and MIC_{90} for trimethoprim-sulfamethoxazole were both ⩽0.06 µg/mL (Table 2). Iclaprim showed activity against S. anginosus independent of the prevalence of macrolide resistance. The MIC for iclaprim against all S. anginosus isolates tested was constant at ≤0.015 µg/mL for an azithromycin MIC range ≤0.03 - >8 µg/mL.

Iclaprim exhibited activity against all 40 S. dysagalactiae (Table 2). The MIC_{50} and MIC_{90} were 0.03 and 0.06 µg/mL, respectively, for S. dysagalactiae. By comparison, MIC_{50}/MIC_{90} for trimethoprim-sulfamethoxazole were ⩽0.06 / 0.12 µg/mL, respectively (Table 2). Iclaprim showed activity against S. dysagalactiae independent of the prevalence of macrolide resistance. Of the nine isolates resistant to azithromycin (MIC ≥2 µg/mL, 22.5 %), only one had a MIC for iclaprim > 0.03 µg/mL. Based on EUCAST breakpoints (no breakpoints are available in CLSI for TMS against beta-hemolytic streptococci), only one S. dysagalactiae isolate was resistant to TMS (MIC ≥2 µg/mL), and the MIC of iclaprim was >32 µg/mL for this isolate.

Discussion

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

2015-2016 in the US and EU. Iclaprim activity (MIC_{50}, 0.03 µg/mL and MIC_{90}, 0.12 µg/mL) was similar to that of TMS (MIC_{50} ⩽0.06 µg/mL and MIC_{90}, 0.12 µg/mL). Although the in vitro activity of iclaprim alone was similar to TMS combination, not having to add a sulfonamide component to iclaprim may be clinically meaningful from a safety perspective. For example, sulfonamides are associated with the following severe toxicities: hypersensitivity reactions (Stevens Johnson syndrome), anaphylaxis, hepatotoxicity, and blood dyscrasias. The activity of iclaprim, the MIC_{50}/MIC_{90} of 0.03/0.12 µg/mL for S. aureus, 0.12/0.5 µg/mL for S. agalactiae, ≤0.015/0.03 µg/mL for S. pyogenes, ≤0.015 /≤0.015 µg/mL for S. anginosus and 0.03/0.06 µg/mL for S. dysgalactiae, documented in this analysis were consistent with those in a surveillance study performed a decade earlier, comprising 5,937 Gram-positive isolates from skin and soft tissue, blood stream and respiratory clinical specimens from patients in the US and EU (Sader et al., 2009) and with those in a surveillance study performed in 2012-2014, comprising 2,814 Gram-positive clinical isolates from skin and soft tissue from patients in the US and EU (Huang et al., in press).

Resistance in S. aureus to dihydrofolate reductase inhibitors is determined by a single amino acid change (F98Y) within the trimethoprim-binding site of DHFR. Iclaprim was rationally engineered, 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 trimethoprim resistance (TMP-R). Iclaprim retains sufficient binding affinity to F98Y DHFR due to additional hydrophobic interactions with surrounding amino acids (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. BHS are normally considered susceptible to TMP, although

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

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

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

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

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
S. aureus (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	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
S. pyogenes (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
S. agalactiae (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
S. anginosus (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
S. dysgalactiae (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 (μg/mL)**														
		⩽ 0.015	0.03	≤0.06	0.06	0.12	0.25	0.5	1	2	4	8	16	>16	32	>32
S. aureus (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
S. pyogenes (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
S. agalactiae (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
S. anginosus (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
S. dysgalactiae (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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