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Short Communication

In vitro activity of dihydrofolate reductase inhibitors and other antibiotics against Gram-positive pathogens collected globally between 2004 and 2016

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ABSTRACT

Objectives: The objective of this study was to determine the *in vitro* activity of iclaprim and comparator agents against 7618 Gram-positive clinical isolates collected in the periods 2004–2006, 2012–2014 and 2015–2016.
Methods: Antimicrobial susceptibility testing was performed by the broth microdilution method and the minimum inhibitory concentrations (MICs) were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines.
Results: Iclaprim MIC_{50}/MIC_{90} values were 0.06/0.12 μg/mL for *Staphylococcus aureus*, including methicillin-susceptible and methicillin-resistant strains, and 0.015/0.03, 0.12/0.5 and 0.03/0.06 μg/mL, respectively, for *Streptococcus pyogenes*, *Streptococcus agalactiae* and *Streptococcus dysgalactiae* over 8 years within the period from 2004 to 2016. Iclaprim was 8–32-fold more potent than trimethoprim. Against *S. aureus*, including methicillin-resistant strains, iclaprim was more active than standard-of-care intravenous antibiotics used to treat Gram-positive skin infections. Iclaprim was up to 16-fold more potent than vancomycin and linezolid and was 4–8-fold more potent than daptomycin.
Conclusions: Iclaprim demonstrated potent and consistent activity among Gram-positive clinical isolates collected globally between 2004 and 2016.

1. Introduction

Iclaprim is a selective bacterial dihydrofolate reductase (DHFR) inhibitor [1]. Iclaprim was designed using X-ray crystallography of isolated DHFR to enhance potency against Gram-positive pathogens, and specifically against strains with mutational changes in DHFR that determine trimethoprim resistance. Iclaprim has demonstrated *in vitro* and *in vivo* activity against Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), linezolid-resistant *S. aureus*, daptomycin-non-susceptible *S. aureus* and vancomycin-resistant *S. aureus* [2].

Currently, trimethoprim is the only DHFR inhibitor approved by the US Food and Drug Administration (FDA) for bacterial infections and has been widely used for more than three decades since its approval in 1981, largely in the formulation of trimethoprim with sulfamethoxazole. Due to its relatively low potency, trimethoprim is typically combined with sulfamethoxazole to synergistically treat skin and soft-tissue infections, pneumonia and urinary tract infections due to Gram-positive and Gram-negative pathogens. However, with its widespread use, pathogens have developed resistance to this antibiotic [3–5].

In this study, surveillance data on the *in vitro* activity of iclaprim and comparator antibiotics, including trimethoprim with or without sulfamethoxazole, against Gram-positive clinical isolates collected from 2004–2006, 2012–2014 and 2015–2016 are summarised.

2. Methods

2.1. Collection of bacterial isolates

Antimicrobial susceptibility testing was conducted by JMI Laboratories (North Liberty, IA) and IHMA Europe Sàrl (Monthey, Switzerland). A total of 7618 non-duplicate, non-consecutive

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clinical isolates of *S. aureus* [*n* = 6312; comprising 2413 methicillin-sensitive *S. aureus* (MSSA) and 3899 MRSA] and β-haemolytic streptococci (*n* = 1306), including *Streptococcus pyogenes* (*n* = 861), *Streptococcus agalactiae* (*n* = 405) and *Streptococcus dysgalactiae* (*n* = 40) from 2004–2006 (*n* = 5324), 2012–2014 (*n* = 1377) and 2015–2016 (*n* = 917) were collected from North America (49.5%), Europe (46.2%), Asia-Pacific (2.3%) and South America (2.0%). The isolates were from skin and skin-structure infections, pneumonia, and bloodstream infections.

2.2. Antimicrobial susceptibility testing

Clinical isolates were identified by the submitting laboratories and were confirmed by either JMI Laboratories for the 2004–2006 and 2012–2014 isolates or by IHMA Europe Sàrl for the 2015–2016 isolates using standard bacteriological methods.

Briefly, antimicrobial susceptibility testing was performed by the broth microdilution method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [M07-A7 (2006) and M07-A10 (2015)] [6,7]. Quality control and minimum inhibitory concentration (MIC) values were calculated using CLSI [M100-S18 (2008) for the 2004–2006 isolates, M100-S25 (2015) for the 2012–2014 isolates and M100, 27th ed (2017) for the 2015–2016 isolates] [8–10]. Trimethoprim alone was not tested in the 2015–2016 surveillance study. *Staphylococcus aureus* (both MSSA and MRSA) were tested in cation-adjusted Mueller–Hinton broth (CA-MHB), and β-haemolytic streptococci were tested in CA-MHB supplemented with lysed horse blood. Iclaprim and comparator antibiotic MIC results were within the CLSI published ranges against *S. aureus* ATCC 29213. Isolates were tested with commercially manufactured MIC panels (Thermo Fisher Scientific, Cleveland, OH).

3. Results

Overall, across 8 years within the period from 2004 to 2016, iclaprim had potent and consistent activity against *S. aureus*, including MSSA and MRSA, and β-haemolytic streptococci, including *S. pyogenes*, *S. agalactiae* and *S. dysgalactiae*, based on MIC_{50}/MIC_{90} values (Table 1). Based on the MIC_{50}/MIC_{90} values, the activity of iclaprim was 8–32-fold more potent than trimethoprim alone and was similar to that of trimethoprim/sulfamethoxazole against *S. aureus*, including MRSA, and β-haemolytic streptococci between 2004 and 2016.

3.1. Staphylococcus aureus

The MIC_{50}/MIC_{90} values of *S. aureus* for iclaprim were 0.06/0.12, 0.06/0.12 and 0.03/0.06 μg/mL for 2004–2006, 2012–2014 and 2015–2016, respectively. In comparison, MIC_{50}/MIC_{90} values for trimethoprim were 1/2 μg/mL, respectively, for both 2004–2006 and 2012–2014. For trimethoprim/sulfamethoxazole (trimethoprim MIC value reported herein), the MIC_{50}/MIC_{90} values were 0.06/0.12, 0.06/0.12 and ≤0.06/≤0.06 μg/mL during the same respective time periods. Iclaprim was active against *S. aureus* resistant to erythromycin, clindamycin and levofloxacin. For isolates with an erythromycin MIC of ≥1 μg/mL (*n* = 3805), the MIC_{50} and MIC_{90} values for iclaprim were 0.06 μg/mL and 0.12 μg/mL, and 3506 (92.1%), 165 (4.3%) and 134 (3.5%) isolates had an iclaprim MIC of ≤0.25, 0.5–4 and ≥8 μg/mL, respectively. For isolates with a clindamycin MIC of ≥1 μg/mL (*n* = 1579), the MIC_{50} and MIC_{90} values for iclaprim were 0.06 μg/mL and 1 μg/mL, and 1401 (88.7%), 113 (7.2%) and 65 (4.1%) isolates had an iclaprim MIC of ≤0.25, 0.5–4 and ≥8 μg/mL, respectively. For isolates with a levofloxacin MIC of ≥2 μg/mL (*n* = 3298), the MIC_{50} and MIC_{90} values for iclaprim were 0.06 μg/mL and 0.25 μg/mL, and 2994 (90.8%), 175 (5.3%) and 129 (3.9%) isolates had an iclaprim MIC of ≤0.25, 0.5–4 and ≥8 μg/mL, respectively.

Iclaprim maintained activity both against MSSA and MRSA isolates. For MSSA, the MIC_{50}/MIC_{90} values were 0.06/0.12, 0.06/0.12 and 0.06/0.06 μg/mL for 2004–2006, 2012–2014 and 2015–2016, respectively. For MRSA, the MIC_{50}/MIC_{90} values were 0.06/0.12, 0.06/0.5 and 0.03/0.12 μg/mL for the same periods. The MIC_{50}/MIC_{90} values were higher for trimethoprim: for MSSA, the MIC_{50}/MIC_{90} values were 1/1 μg/mL and 1/2 μg/mL for 2004–2006 and 2012–2014; and for MRSA, they were 1/2 μg/mL and 1/8 μg/mL, respectively. The MIC_{50}/MIC_{90} values for trimethoprim/sulfamethoxazole were similar to iclaprim: 0.06/0.06, 0.06/0.06 and ≤0.06/≤0.06 μg/mL for MSSA and 0.06/0.25, 0.06/0.25 and ≤0.06/0.12 μg/mL for MRSA for 2004–2006, 2012–2014 and 2015–2016, respectively.

Overall during the 8-year evaluation period between 2004 and 2016, resistance of *S. aureus* to trimethoprim/sulfamethoxazole

Table 1
In vitro activity of iclaprim and comparator agents against Gram-positive clinical isolates collected worldwide from 2004–2006, 2012–2014 and 2015–2016.

Organism	Year	*N*	Iclaprim		TMP		SXT[a]		VAN		LNZ		DAP	
			MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Staphylococcus aureus	2004–2006	4516	0.06	0.12	1	2	0.06	0.12	1	1	2	2	–	–
	2012–2014	1178	0.06	0.12	1	2	0.06	0.12	1	1	1	1	0.25	0.5
	2015–2016	618	0.03	0.06	–	–	≤0.06	≤0.06	1	1	1	2	0.25	0.5
MSSA	2004–2006	1513	0.06	0.12	1	1	0.06	0.06	1	1	2	2	–	–
	2012–2014	596	0.06	0.12	1	2	0.06	0.06	1	1	1	1	0.25	0.5
	2015–2016	304	0.06	0.06	–	–	≤0.06	≤0.06	1	1	1	2	0.25	0.5
MRSA	2004–2006	3003	0.06	0.12	1	2	0.06	0.25	1	1	2	2	–	–
	2012–2014	582	0.06	0.5	1	8	0.06	0.25	1	1	1	1	0.25	0.5
	2015–2016	314	0.03	0.12	–	–	≤0.06	0.12	1	1	1	2	0.25	0.5
β-Haemolytic streptococci	2004–2006	808	0.015	0.25	0.25	2	0.06	0.12	≤0.5	≤0.5	1	1	–	–
	2012–2014	199	0.06	0.25	1	2	0.12	0.25	0.25	0.5	1	1	0.12	0.25
	2015–2016	299	0.03	0.25	–	–	≤0.06	0.12	–	–	1	1	–	–
S. pyogenes	2004–2006	604	0.015	0.03	0.25	0.5	0.06	0.12	≤0.5	≤0.5	1	1	–	–
	2012–2014	98	0.015	0.06	0.25	1	0.12	0.25	0.25	0.5	1	1	0.12	0.25
	2015–2016	159	≤0.015	0.03	–	–	≤0.06	0.12	–	–	1	1	–	–
S. agalactiae	2004–2006	204	0.12	0.25	1	4	0.06	0.12	≤0.5	≤0.5	1	1	–	–
	2012–2014	101	0.12	0.25	2	4	0.12	0.12	0.25	0.5	1	1	0.12	0.25
	2015–2016	100	0.12	0.5	–	–	0.12	0.12	–	–	1	1	–	–
S. dysgalactiae	2015–2016	40	0.03	0.06	–	–	0.03	0.06	–	–	1	1	–	–

TMP, trimethoprim; SXT, trimethoprim/sulfamethoxazole; VAN, vancomycin; LNZ, linezolid; DAP, daptomycin; $MIC_{50/90}$, minimum inhibitory concentrations (in μg/mL) against 50% and 90% of the isolates, respectively; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*.
[a] Tested at a fixed trimethoprim/sulfamethoxazole ratio of 1:19 (trimethoprim $MIC_{50/90}$ values shown in table).

was low and ranged from 0.3–0.7% for MSSA to 2.6–3.9% for MRSA; resistance was comparatively higher to trimethoprim at 1.3–4.5% for MSSA and 7.1–9.6% for MRSA.

3.2. Streptococcus pyogenes

The MIC_{50}/MIC_{90} values for iclaprim were 0.015/0.03, 0.015/0.06 and ≤0.015/0.03 μg/mL for 2004–2006, 2012–2014 and 2015–2016, respectively, for a combined total of 861 *S. pyogenes* isolates (Table 1). For trimethoprim, the MIC_{50}/MIC_{90} values were 0.25/0.5 μg/mL for 2004–2006 and 0.25/1 μg/mL for 2012–2014. For trimethoprim/sulfamethoxazole, the MIC_{50}/MIC_{90} values were 0.06/0.12, 0.12/0.25 and ≤0.06/0.12 μg/mL for 2004–2006, 2012–2014 and 2015–2016, respectively.

3.3. Streptococcus agalactiae

The MIC_{50}/MIC_{90} values for iclaprim were 0.12/0.25, 0.12/0.25 and 0.12/0.5 μg/mL for 2004–2006, 2012–2014 and 2015–2016, respectively, for a combined total of 405 *S. agalactiae* isolates (Table 1). For trimethoprim, the MIC_{50}/MIC_{90} values were 1/4 μg/mL for 2004–2006 and 2/4 μg/mL for 2012–2014. For trimethoprim/sulfamethoxazole, the MIC_{50}/MIC_{90} values were 0.06/0.12, 0.12/0.12 and 0.12/0.12 μg/mL for 2004–2006, 2012–2014 and 2015–2016, respectively. For *S. agalactiae*, resistance to trimethoprim was between 10.8–12.9%, whereas resistance to trimethoprim/sulfamethoxazole ranged from 0–1.0%.

3.4. Streptococcus dysgalactiae

The MIC_{50}/MIC_{90} values for iclaprim were 0.03/0.06 μg/mL for 2015–2016 against a total of 40 *S. dysgalactiae* isolates (Table 1). For trimethoprim/sulfamethoxazole, the MIC_{50}/MIC_{90} values were the same as for iclaprim (0.03/0.06 μg/mL).

4. Discussion

Iclaprim is a potent bactericidal DHFR inhibitor and is in development as a stand-alone agent for the treatment of acute bacterial skin and skin-structure infections (ABSSSIs) and hospital-acquired bacterial pneumonia. This report highlights that iclaprim has remained highly active against a collection of >7500 Gram-positive bacterial isolates during an 8-year period spanning 2004 through 2016. Despite selective pressure with trimethoprim, the only currently approved bacterial DHFR inhibitor, iclaprim maintains low MICs for *S. aureus* (MIC_{50}/MIC_{90} values of 0.06/0.12 μg/mL) and β-haemolytic streptococci (MIC_{50}/MIC_{90} values of 0.015/0.25 μg/mL), common causes of ABSSSI. Iclaprim was 8–32-fold more potent than trimethoprim alone and had similar activity to trimethoprim/sulfamethoxazole against Gram-positive clinical isolates collected from around the world.

Iclaprim has been shown to be bactericidal against Gram-positive bacteria and to possess a low potential for resistance development when it is used on its own, without the synergistic combination of a sulfonamide agent, which has contributed negatively to the safety profile of trimethoprim/sulfamethoxazole (e.g. hypersensitivity reactions, blood dyscrasias and others) [11,12]. Importantly, the activity of iclaprim against *S. aureus* was not impacted by the methicillin resistance phenotype, and iclaprim maintained consistent activity against target Gram-positive cocci across the evaluated geographic regions.

In conclusion, the results from this surveillance of susceptibility from 2004–2006, 2012–2014 and 2015–2016 confirm the potent and consistent activity of iclaprim over a significant time frame during which trimethoprim and trimethoprim/sulfamethoxazole resistance has been reported. Iclaprim was up to 16-fold more potent than vancomycin and linezolid and 4–8-fold more potent than daptomycin against *S. aureus*, including MRSA. Future surveillance is warranted to track the continued potency of antibiotics, including DHFR inhibitors.

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Competing interests

SN and DBH are employees of Motif BioSciences.

Ethical approval

Not required.

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