E-910

Comparative evaluation of iclaprim potency and bactericidal activity tested against enterococci: Results from the International Study of Iclaprim Susceptibility (ISIS)

H.S. Sader[1], R.N. Jones[1], P. Rhomberg[1], K. Islam[2], S. Hawser[2], T.R. Fritsche[1]

[1]*JMI Laboratories, North Liberty, Iowa, USA* [2]*Arpida AG, Reinach, Switzerland*

H.S. Sader MD, PhD
Director, International Surveillance Programs, JMI Laboratories
345 Beaver Kreek Centre, Suite A, North Liberty, Iowa, 52317, USA
Phone: +1 (319) 665-3370 Fax: +1 (319) 665-3371 e-mail: helio-sader@jmilabs.com

ABSTRACT

Background: Iclaprim (ICL) is a novel diaminopyrimidine, which selectively inhibits bacterial dihydrofolate reductase. Vancomycin (VAN)-resistant (R) enterococci (VRE) is the 3rd leading cause of bloodstream infections in the USA and treatment options for VRE are limited. Potency and bactericidal activity (MBC) of ICL was evaluated against recent clinical strains of *enterococci*.

Methods: 613 strains (310 *E. faecalis* [EF] and 303 *E. faecium* [EFM]) from the USA (308) and Europe (EU; 305) were susceptibility (S) tested by CLSI broth microdilution method against ICL, trimethoprim (TMP), co-trimoxazole (TMP/SMX) and various comparators. MBC values for ICL, TMP and VAN were assessed for a subset of 20 strains..

Results: ICL (MIC_{50}, 0.03 µg/mL) was 16-fold more active than TMP (MIC_{50}, 0.5 µg/mL) and slightly more active than TMP/SMX (MIC_{50}, 0.06 µg/mL). USA isolates showed higher MIC values for ICL, TMP and TMP/SMX (MIC_{50} of 0.06, 2 and 0.12 µg/mL, respectively) compared to isolates from EU (MIC_{50} of 0.015, 0.25 and 0.06 µg/mL). R rates to VAN were also higher in the USA (36.6%) compared to EU (7.9%). Similar to TMP, TMP/SMX and VAN, ICL was more active against EF compared to EFM (MIC_{50}, 0.015 and 2 µg/mL, respectively). Although ICL MIC values were usually very low (95% at ≤0.03 µg/mL), the MBC results were higher and 13 strains (65%) had ICL MBC/MIC ratio ≥32. By contrast, all strains exhibited VAN MBC/MIC ratios consistent with bacteriostatic activity.

Antimicrobial	MIC_{50} (µg/mL) / MIC_{90} (µg/mL) / % S			
	USA (no. tested)		EU (no. tested)	
	E. faecalis (154)	*E. faecium* (154)	*E. faecalis* (156)	*E. faecium* (149)
ICL	0.015/4/-	4/>8/-	0.015/4/-	1/8/-
TMP	0.25/>64/-	>64/>64/-	0.25/>64/-	8/>64/-
TMP/SMX	0.03/4/-	>8/>8/-	0.03/>8/-	8/>64/-
VAN	1/2/97	>16/>16/29	1/2/98	≤0.5/>16/83
Teicoplanin	≤0.5/≤0.5/98	>16/>16/37	≤0.5/≤0.5/99	≤0.5/8/91

Conclusions: ICL was the most potent compound tested against *enterococci*. Further evaluations of ICL for treatment of enterococcal infections appear indicated in the ISIS program.

INTRODUCTION

Iclaprim is a novel dihydrofolate reductase (DFHR) inhibitor belonging to the diaminopyrimidine class of antibiotics for which trimethoprim (TMP) is the most well-known representative. TMP is frequently used in combination with sulfamethoxazole (SMX) and this combination has been used in clinical practice for almost five decades. SMX is highly synergistic with TMP as a result of inhibition of two sequential enzymes in the folate pathway, thereby enhancing the potency and bactericidal activity as well as reducing the potential for resistance development. However, SMX is often associated with allergic reactions. In contrast, iclaprim by itself shows a potent activity against a variety of pathogens and exhibits a potent bactericidal action against *Staphylococcus aureus* and *Streptococcus pneumoniae*. Iclaprim is being developed and administered as a stand-alone therapy for serious Gram-positive bacterial infections. The compound has been granted fast-track product designation and has recently completed two pivotal Phase III clinical studies in complicated skin and skin structures infections (cSSSI).

Most enterococcal infections occur in hospitalized patients or in patients undergoing long-term therapy such as peritoneal dialysis or hemodialysis. Currently, enterococci rank second or third as causes of nosocomial infections in the United States (USA) and treatment options remain limited.

The International Study of Iclaprim Susceptibility (ISIS) program evaluated the in-vitro activity of iclaprim, trimethoprim, trimethoprim/sulfamethoxazole and other comparator agents tested against contemporary clinical bacterial strains collected in USA and European medical centers. We report here the comparative potency and bactericidal activity of iclaprim tested against enterococcal strains.

METHODS

Bacterial isolates: A total of 613 clinical enterococcal strains, 310 *Enterococcus faecalis* (154 from USA and 156 from European medical centers) and 303 *Enterococcus faecium* (154 each from USA and 149 from European medical centers), were selected for this *in vitro* trial. All organisms were collected between 2004 and 2006. Sources of infection consisted of bloodstream, skin and soft tissue, respiratory and patients hospitalized from pneumonia. MBC values for iclaprim, trimethoprim and vancomycin were assessed for a subset of 20 randomly selected clinical strains (15 *E. faecalis* and 5 *E. faecium*).

Susceptibility testing: MIC values were determined by Clinical and Laboratory Standards Institute (CLSI) broth microdilution method per M7-A7 [2006]. Quality control (QC) ranges and interpretive criteria for comparator compounds were used as published in CLSI M10 0-S17 [2007]. QC ranges for iclaprim were those recently approved by CLSI and published in M100-S17 [2007]. Media quality and thymidine content of MIC panels was evaluated by QC testing with *E. faecalis* ATCC strains 29212 and 33186.

Bactericidal activity: MBC values were determined on 20 strains by plating the broth onto appropriate growth media from the microdilution tray well and those from at least five log_2 dilutions at and above the MIC for each organism. Quantitative colony counts were also performed on the starting inoculum. The lowest concentration of antimicrobial agent that killed ≥99.9% of the initial test inoculum was defined as the MBC endpoint. In addition, thymidine content of the blood agar media used in the MBC experiments was also assessed by the evaluation of the inhibition zone around a 1.25/23.75 µg trimethoprim/sulfamethoxazole disk when testing enterococcal strains *E. faecalis* ATCC 29212 and ATCC 33186.

RESULTS

- As expected, *E. faecalis* isolates were generally more susceptible than E. faecium to study drugs and resistance rates for *E. faecalis* did not differ significantly between the USA and Europe (Table 1).
- Resistance rates to study drugs were very high among *E. faecium* isolates and differed significantly between the USA and Europe with vancomycin resistance rates peaking to 71% for the USA isolates (Table 1).

Table 1. Activity of antimicrobial agents tested against enterococci (613 strains) from the USA and Europe.

Antimicrobial agent	MIC_{50}	MIC_{90}	Range	% susceptible/ resistant[a]
E. faecalis				
USA (154)				
Iclaprim	0.015	4	≤0.004–>8	- / -[b]
Trimethoprim	0.25	>64	0.06–>64	- / -
Trimethoprim-sulfamethoxazole	0.03	4	0.015–>8	- / -
Erythromycin	>4	>4	≤0.12–>4	13.0 / 56.5
Tetracycline	>16	>16	≤0.5–>16	26.0 / 73.4
Levofloxacin	1	>4	0.5–>4	68.8 / 31.2
Teicoplanin	≤0.5	≤0.5	≤0.5–>16	98.1 / 1.9
Vancomycin	1	2	≤0.5–>16	97.4 / 2.6
Ampicillin	≤4	≤4	≤4	100.0 / 0.0
Europe (156)				
Iclaprim	0.015	4	≤0.004–>8	- / -[b]
Trimethoprim	0.25	>64	≤0.03–>64	- / -
Trimethoprim-sulfamethoxazole	0.03	>8	0.008–>8	- / -
Erythromycin	2	>4	≤0.12–>4	14.1 / 49.4
Tetracycline	>16	>16	≤0.5–>16	30.8 / 69.2
Levofloxacin	1	>4	0.25–>4	61.5 / 37.2
Teicoplanin	≤0.5	≤0.5	≤0.5–16	98.7 / 0.0
Vancomycin	1	2	≤0.5–>16	98.1 / 1.3
Ampicillin	≤4	≤4	≤4	100.0 / 0.0
E. faecium				
USA (154)				
Iclaprim	4	>8	≤0.004–>8	- / -[b]
Trimethoprim	>64	>64	≤0.03–>64	- / -
Trimethoprim-sulfamethoxazole	>8	>8	≤0.004–>8	- / -
Erythromycin	>4	>4	≤0.12–>4	3.2 / 88.3
Tetracycline	≤0.5	>16	≤0.5–>16	64.9 / 34.4
Levofloxacin	>4	>4	0.5–>4	9.1 / 90.9
Teicoplanin	>16	>16	≤0.5–>16	37.0 / 59.1
Vancomycin	>16	>16	≤0.5–>16	29.2 / 70.8
Ampicillin	>16	>16	≤4–>16	8.4 / 91.6
Europe (149)				
Iclaprim	1	8	≤0.004–>8	- / -[b]
Trimethoprim	8	>64	≤0.03–>64	- / -
Trimethoprim-sulfamethoxazole	8	>8	≤0.004–>8	- / -
Erythromycin	>4	>4	≤0.12–>4	2.7 / 80.5
Tetracycline	≤0.5	>16	≤0.5–>16	69.8 / 29.5
Levofloxacin	>4	>4	1–>4	19.5 / 73.2
Teicoplanin	≤0.5	8	≤0.5–>16	90.6 / 8.1
Vancomycin	≤0.5	>16	≤0.5–>16	83.2 / 14.8
Ampicillin	>16	>16	≤4–>16	15.4 / 84.6
All enterococci (613)				
Iclaprim	0.03	>8	≤0.004–>8	- / -[b]
Trimethoprim	0.5	>64	≤0.03–>64	- / -
Trimethoprim-sulfamethoxazole	0.06	>8	≤0.004–>8	- / -
Erythromycin	>4	>4	≤0.12–>4	8.3 / 68.6
Tetracycline	16	>16	≤0.5–>16	47.7 / 51.8
Levofloxacin	>4	>4	0.25–>4	39.9 / 58.0
Teicoplanin	≤0.5	>16	≤0.5–>16	81.1 / 17.3
Vancomycin	1	>16	≤0.5–>16	77.0 / 22.3
Ampicillin	≤4	>16	≤4–>16	56.4 / 43.6

[a] Criteria as published by the CLSI [2007].
[b] - = No breakpoint has been established by the CLSI [2007].

- Against all enterococcal isolates tested, iclaprim (MIC_{50}, 0.03 µg/mL) was 16-fold more potent than TMP (MIC_{50}, 0.5 µg/mL) and slightly more active than the TMP/SMX combination (MIC_{50}, 0.06 µg/mL) (Table 1) and showed the bi/tri-modal MIC distribution typical of the diaminopyrimidine class (Figures 1 & 2).

Figure 1. *E. faecalis* (310 strains) from Europe and USA

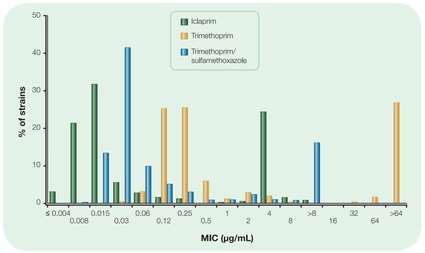
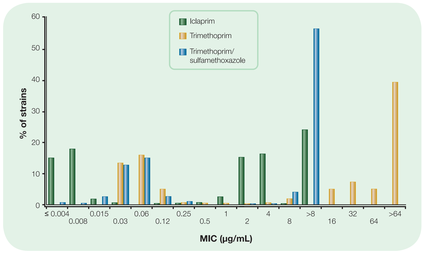


Figure 2. *E. faecium* (303 strains) from Europe and USA



- Iclaprim exhibited very good activity against *E. faecalis* strains from both Europe and the USA with MIC_{50} of 0.015 µg/mL and MIC_{90} of 4 µg/mL (Table 1).
- Against *E. faecium* isolates, iclaprim was more potent than TMP and of the TMP/SMX combination with MIC_{50} of 4, >64 and >8 µg/mL, respectively for the USA isolates and MIC_{50} of 1, 8 and 8 µg/mL, respectively against European isolates (Table 1).
- Against *E. faecium*, iclaprim exhibited MIC ≤4 µg/mL against more than 70% of the strains, in contrast to TMP and TMP/SMX for which MICs were ≤4 µg/mL for less than 40% of the isolates (Figure 2).
- Although iclaprim MIC values were very low (19 of 20 strains [95.0%] at ≤0.03 µg/mL), the MBC results were generally higher and 13 strains (65.0%) showed MBC/MIC ratios of ≥32 (Tables 2 & 3).

Table 2. Iclaprim MIC and MBC results for 20 enterococci.

Organism (no. of isolates)	No. of isolates at MIC or MBC value (µg/mL) of:									
	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	>*[a]
Iclaprim										
***E. faecalis* (15)**										
MIC	1	9	4	0	0	1	0	0	0	0
MBC	0	0	1	1	2	0	0	0	0	11
***E. faecium* (5)**										
MIC	2	3	0	0	0	0	0	0	0	0
MBC	0	0	2	0	1	0	0	0	0	2
Trimethoprim										
***E. faecalis* (15)**										
MIC	0	0	0	0	3	7	4	0	0	1
MBC	0	0	0	0	0	0	1	1	2	11
***E. faecium* (5)**										
MIC	0	0	0	2	2	1	0	0	0	0
MBC	0	0	0	0	0	1	1	1	0	2
Vancomycin										
***E. faecalis* (15)**										
MIC	0	0	0	0	0	0	0	7	8	0
MBC	0	0	0	0	0	0	0	0	0	15
***E. faecium* (5)**										
MIC	0	0	0	0	0	0	0	2	2	1
MBC	0	0	0	0	0	0	0	0	0	5

[a]MBC value was higher than the highest dilution tested indicating MBC/MIC >32.

Table 3. MBC/MIC ratio results for iclaprim, trimethoprim and vancomycin tested against a selected group of 20 enterococcal organisms.

Organism (no.) MBC/MIC ratio	No. of isolates at MBC/MIC		
	Iclaprim	Trimethoprim	Vancomycin
***E. faecalis* (15)**			
1	0	0	0
2	1	1	0
4	2	1	0
8	0	1	0
16	1	3	0
≥32	11	9	15
***E. faecium* (5)**			
1	0	0	0
2	1	1	0
4	1	1	0
8	1	1	0
16	0	0	0
≥32	2	2	5

- High MBC/MIC ratios were also obtained with trimethoprim (55.0% of strains with MBC/MIC of ≥32), while all enterococcal strains showed vancomycin MBC/MIC ratios consistent with bacteriostatic activity.

CONCLUSIONS

- **Iclaprim exhibited very good activity against *E. faecalis* strains from both Europe and the USA with MIC_{50} of 0.015 µg/mL and MIC_{90} of 4 µg/mL.**
- **Iclaprim exhibited good activity against multi-drug resistant *E. faecium* isolates with MICs ≤4 µg/mL for about 70% of the strains.**

SELECTED REFERENCES

Andrews J, Honeybourne D, Ashby J, Jevons G, Fraise A, Fry P, Warrington S, Hawser S, Wise R. Concentrations in plasma, epithelial lining fluid, alveolar macrophages and bronchial mucosa after a single intravenous dose of 1.5 mg/kg of iclaprim (AR-100) in healthy men. *Journal of Antimicrobial Chemotherapy* 2007; in press.

Bryskier A. Anti-MRSA agents: under investigation, in the exploratory phase and clinically available. *Expert Review of Anti-infective Therapy* 2005; 3: 505–53.

Clinical and Laboratory Standards Institute. (2006). *M7-A7, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard – seventh edition*. Wayne, PA. CLSI.

Clinical and Laboratory Standards Institute. (2007). M100-S17, *Performance standards for antimicrobial susceptibility testing; 17th informational supplement*. Wayne, PA. CLSI.

Hawser S, Lociuro S, Islam K. Dihydrofolate reductase inhibitors as antibacterial agents. *Biochemical Pharmacology* 2006; 71: 941–48.

Huovinen P. Resistance to trimethoprim-sulfamethoxazole. *Clinical Infectious Diseases* 2001; 32: 1608–14.

National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. *American Journal of Infection Control* 32: 2004; 470–85.

Rice LB. Antimicrobial resistance in Gram-positive bacteria. *American Journal of Infection Control* 2006; 34: S11–19.

Schmieder P, Hawser S, Islam K. Iclaprim, a novel diaminopyrimidine with potent activity on trimethoprim sensitive and resistant bacteria. *Bioorganic and Medicinal Chemistry Letters* 2003; 13: 4217–21.

Sorbera L A, Castaner J, Rabasseda X. Iclaprim. *Drugs of the Future* 2004; 29: 220–25.

Then RL. Antimicrobial dihydrofolate reductase inhibitors-Achievements and future options: Review. *Journal of Chemotherapy* 2004; 16: 3–12.

ICAAC 2007

PRINT NUMBER TO GO HERE XXXXX