

**Anti-virulence potential of iclaprim, a novel folic acid synthesis inhibitor, against
*Staphylococcus aureus***

Applied Microbiology and Biotechnology

Lingyun Hao^{1,†}, Jingwen Zhou^{2,†}, Han yang¹, Chunyan He¹, Wen Shu¹, Haoyue Song¹, Qingzhong Liu^{2,*}

¹ Department of Clinical Laboratory, Shanghai General Hospital, Shanghai Jiaotong University School of Medicine; Shanghai, China

² Department of Clinical Laboratory, Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine; Shanghai 200071, China.

Lingyun Hao: haolingyun_sjtu@163.com

Jingwen Zhou: zhoujingwenstudy@163.com

Han yang: yanghansjtu@163.com

Chunyan He: hechunyan_hcy@163.com

Wen Shu: shuwen654321@163.com

Haoyue Song: songhaoyuex@sina.com

Qingzhong Liu: jiaodamedicine@foxmail.com

[†]Lingyun Hao and Jingwen Zhou contributed equally to this work.

*Corresponding author's mailing address: Department of Clinical Laboratory, Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, 274 Zhijiang Middle Rd., Shanghai, People's Republic of China; Zip Code: 200071. E-mail: jiaodamedicine@foxmail.com

Running title: Anti-virulence capacity of iclaprim on *S. aureus*

Supplementary Table 1 Virulence genes and primers used for qRT-PCR

Gene	Sequence of primer (5'-3')	References
<i>hla-F</i>	AATGAATCCTGTCGCTAATGCCGC	(Xiong et al. 2006)
<i>hla-R</i>	CTGAAGGCCAGGCTAAACCACTTT	
<i>coa-F</i>	AGGTCTTGAAGGTAGCTCAT	(Blickwede et al. 2005)
<i>coa-R</i>	GTTGTATTACGGATACCTGTA	
<i>clfA-F</i>	TGCTGCACCTAAAACAGACG	(Kim et al. 2017)
<i>clfA-R</i>	TGTGTCGTTTCCTGTTGTGC	
<i>clfB-F</i>	TTGCCGCCATAAAAGTATTA	(Blickwede et al. 2005)
<i>clfB-R</i>	TCACCACAAACAATTTCCAA	
<i>fnbA-F</i>	ACAAGTTGAAGTGGCACAGCC	(Blickwede et al. 2005)
<i>fnbA-R</i>	CCGCTACATCTGCTGATCTTGTC	
<i>fnbB-F</i>	CGTTATTTGTAGTTGTTTGTGTT	(Blickwede et al. 2005)
<i>fnbB-R</i>	TGGAATGGGACAAGAAAAAGAA	
<i>spa-F</i>	GCGCAACACGATGAAGCTCAACAA	(Blickwede et al. 2005)
<i>spa-R</i>	ACGTTAGCACTTTGGCTTGGATCA	
<i>sasG-F</i>	GGTTTTTCAGGTCCTTTTGGAT	(Blickwede et al. 2005)
<i>sasG-R</i>	CTGGTGAAGAGCGAGTGAAA	
<i>sdrD-F</i>	AGTACACAGTGGGAACAGCATC	(Blickwede et al. 2005)
<i>sdrD-R</i>	TCTGCAGCCTTTGCTTCTTGGTTC	
<i>atIA-F</i>	TGTCGAAGTATTTGCCGACTTCGC	(Bose et al. 2012)
<i>atIA-R</i>	TGGAATCCTGCACATCCAGGAAC	

<i>icaA-F</i>	AACAGAGGTAAAGCCAACGCACTC	(Wang et al. 2010)
<i>icaA-R</i>	CGATAGTATCTGCATCCAAGCAC	
<i>icaD-F</i>	ATGGTCAAGCCCAGACAGAG	(Wang et al. 2010)
<i>icaD-R</i>	AGTATTTTCAATGTTTAAAGCAA	
<i>RNAIII-F</i>	TAATTAAGGAAGGAGTGATTTC AATG	(Boisset et al. 2007)
<i>RNAIII-R</i>	TTTTTAGTGAATTTGTTCACTGTGTC	
<i>agrA-F</i>	TGATAATCCTTATGAGGTGCTT	(Boisset et al. 2007)
<i>agrA-R</i>	CACTGTGACTCGTAACGAAAA	
<i>saeS-F</i>	ATCCGAACAACAAGAAAAAACAG	(Shang et al. 2009)
<i>saeS-R</i>	TGATTATACCATCACGTAGTCCTTCA	
<i>sarA-F</i>	CCTCGCAACTGATAATCCTTATG	(Andrey et al. 2010)
<i>sarA-R</i>	ACGAATTTCACTGCCTAATTTGA	
<i>sigB-F</i>	TCAGCGGTTAGTTCATCGCTCACT	(Tran and Bonilla 2021)
<i>sigB-R</i>	GTCCTTTGAACGGAAGTTTGAAGCC	
<i>rot-F</i>	AAGAGCGTCCTGTTGACGAT	(Jefferson et al. 2004)
<i>rot-R</i>	TTTGCATTGCTGTTGCTCTA	
<i>16S</i>	CGTGCTACAATGGACAATACAAA	(Zhou et al. 2022)
<i>rRNA-F</i>		
<i>16S</i>	ATCTACGATTACTAGCGATTCCA	
<i>rRNA-R</i>		

qRT-PCR, quantitative real-time polymerase chain reaction

Reference

- Andrey DO, Renzoni A, Monod A, Lew DP, Cheung AL, Kelley WL (2010) Control of the *Staphylococcus aureus* toxic shock tst promoter by the global regulator SarA. J Bacteriol 192(22):6077-85. <https://doi.org/10.1128/JB.00146-10>
- Blickwede M, Wolz C, Valentin-Weigand P, Schwarz S (2005) Influence of clindamycin on the stability of coa and fnbB transcripts and adherence properties of *Staphylococcus aureus* Newman. FEMS Microbiology Letters 252(1):73-78. <https://doi.org/10.1016/j.femsle.2005.08.022>
- Boisset S, Geissmann T, Huntzinger E, Fechter P, Bendridi N, Possedko M, Chevalier C, Helfer AC, Benito Y, Jacquier A, Gaspin C, Vandenesch F, Romby P (2007) *Staphylococcus aureus* RNAIII coordinately represses the synthesis of virulence factors and the transcription regulator Rot by an antisense mechanism. Genes Dev 21(11):1353-66. <https://doi.org/10.1101/gad.423507>
- Bose JL, Lehman MK, Fey PD, Bayles KW (2012) Contribution of the *Staphylococcus aureus* Atl AM and GL murein hydrolase activities in cell division, autolysis, and biofilm formation. PLoS One 7(7):e42244. <https://doi.org/10.1371/journal.pone.0042244>
- Jefferson KK, Pier DB, Goldmann DA, Pier GB (2004) The teicoplanin-associated locus regulator (TcaR) and the intercellular adhesin locus regulator (IcaR) are transcriptional inhibitors of the ica locus in *Staphylococcus aureus*. J Bacteriol 186(8):2449-56. <https://doi.org/10.1128/JB.186.8.2449-2456.2004>
- Kim MK, Zhao A, Wang A, Brown ZZ, Muir TW, Stone HA, Bassler BL (2017)

- Surface-attached molecules control *Staphylococcus aureus* quorum sensing and biofilm development. Nat Microbiol 2:17080. <https://doi.org/10.1038/nmicrobiol.2017.80>
- Shang F, Xue T, Sun H, Xing L, Zhang S, Yang Z, Zhang L, Sun B (2009) The *Staphylococcus aureus* GGDEF domain-containing protein, GdpS, influences protein A gene expression in a cyclic diguanylic acid-independent manner. Infect Immun 77(7):2849-56. <https://doi.org/10.1128/IAI.01405-08>
- Tran HT, Bonilla CY (2021) SigB-regulated antioxidant functions in gram-positive bacteria. World J Microbiol Biotechnol 37(3):38. <https://doi.org/10.1007/s11274-021-03004-7>
- Wang Q, Sun FJ, Liu Y, Xiong LR, Xie LL, Xia PY (2010) Enhancement of biofilm formation by subinhibitory concentrations of macrolides in icaADBC-positive and -negative clinical isolates of *Staphylococcus epidermidis*. Antimicrob Agents Chemother 54(6):2707-11. <https://doi.org/10.1128/AAC.01565-09>
- Xiong YQ, Willard J, Yeaman MR, Cheung AL, Bayer AS (2006) Regulation of *Staphylococcus aureus* alpha-toxin gene (hla) expression by agr, sarA, and sae in vitro and in experimental infective endocarditis. The Journal of Infectious Diseases 194(9):1267-1275. <https://doi.org/10.1128/10.1086/508210>
- Zhou J, Zhao H, Yang H, He C, Shu W, Cui Z, Liu Q (2022) Insights Into the Impact of Small RNA SprC on the Metabolism and Virulence of *Staphylococcus aureus*. Front Cell Infect Microbiol 12:746746. <https://doi.org/10.3389/fcimb.2022.746746>

Supplementary Table 2. The differential folds and P values of the expression of various genes influenced by grade concentrations of iclaprim in strains of MW2, N315 and ATCC 25923

Gene		MW2+no antibiotic	
		Mean	Std Deviation
Exoenzyme genes	<i>hla</i>	1.000	0.17
	<i>coa</i>	1.000	0.11
Adhesion genes	<i>clfA</i>	1.000	0.2
	<i>clfB</i>	1.000	0.15
	<i>fnbA</i>	1.000	0.22
	<i>fnbB</i>	1.000	0.09
	<i>spa</i>	1.000	0.11
	<i>sasG</i>	1.000	0.21
	<i>sdrD</i>	1.000	0.15
	<i>altA</i>	1.000	0.22
	<i>icaA</i>	1.000	0.08
	<i>icaD</i>	1.000	0.07
Regulator genes	<i>RNAIII</i>	1.000	0.21
	<i>agrA</i>	1.000	0.2
	<i>saeS</i>	1.000	0.22
	<i>sarA</i>	1.000	0.14
	<i>sigB</i>	1.000	0.13
	<i>rot</i>	1.000	0.22

MW2+1/16 MIC iclaprim			
Mean	Std Deviation	Fold Change	p value
-1.79	0.07	0.56	0.001
0.91	0.18	0.91	0.43
2.58	0.39	2.58	0.007
2.94	0.24	2.94	0.001
2.82	0.57	2.82	0.003
2.54	0.71	2.54	0.014
1.04	0.08	1.04	0.37
1.49	0.08	1.49	0.03
1.86	0.22	1.86	0.005
1.72	0.31	1.72	0.024
1.79	0.18	1.79	0.01
1.63	0.2	1.63	0.006
-1.52	0.05	0.66	0.021
4.19	0.72	4.19	0.002
3.33	0.46	3.33	0.001
1.74	0.27	1.74	0.015
4.47	0.37	4.47	<0.001
3.54	0.72	3.54	0.003

MW2+1/32 MIC iclaprim			
Mean	Std Deviation	Fold Change	p value
8.56	1.39	8.56	0.001
1.2	0.21	1.2	0.56
5.53	1.18	5.53	0.004
1.94	0.48	1.94	0.016
3.53	1.12	3.53	0.006
3.82	1.21	3.82	0.004
1.16	0.24	1.16	0.28
2.48	0.36	2.48	0.003
3.59	0.88	3.59	0.002
3.48	0.86	3.48	0.003
6.62	0.93	6.62	0.011
3.86	0.41	3.86	0.001
-2.06	0.06	0.49	0.003
5.93	0.37	5.93	<0.001
8.48	1.43	8.48	<0.001
7.51	0.96	7.51	<0.001
3.3	0.19	3.3	<0.001
2.3	0.21	2.3	0.02

Gene		N315+no antibiotic	
		Mean	Std Deviation
Exoenzyme genes	<i>hla</i>	1.000	0.23
	<i>coa</i>	1.000	0.2
Adhesion genes	<i>clfA</i>	1.000	0.31
	<i>clfB</i>	1.000	0.19
	<i>fnbA</i>	1.000	0.22
	<i>fnbB</i>	1.000	0.16
	<i>spa</i>	1.000	0.19
	<i>sasG</i>	1.000	0.15
	<i>sdrD</i>	1.000	0.22
	<i>altA</i>	1.000	0.21
	<i>icaA</i>	1.000	0.22
	<i>icaD</i>	1.000	0.17
Regulator genes	<i>RNAIII</i>	1.000	0.08
	<i>agrA</i>	1.000	0.15
	<i>saeS</i>	1.000	0.09
	<i>sarA</i>	1.000	0.13
	<i>sigB</i>	1.000	0.1
	<i>rot</i>	1.000	0.11

N315+1/16 MIC iclaprim			
Mean	Std Deviation	Fold Change	p value
3	0.83	3	0.009
1.89	0.18	1.89	0.002
-1.52	0.11	0.66	0.02
2.17	0.33	2.17	0.003
-1.58	0.16	0.63	0.011
-1.52	0.14	0.66	0.017
3.15	0.67	3.15	0.003
3.76	0.52	3.76	<0.001
1.67	0.21	1.67	0.012
2.67	0.51	2.67	0.003
1.79	0.14	1.79	0.157
2.57	0.22	2.57	<0.001
2.73	0.5	2.73	0.004
3.83	0.44	3.83	0.01
3.89	0.61	3.89	0.001
2.04	0.22	2.04	0.014
-1.81	0.13	0.55	0.002
3.91	0.46	3.91	0.008

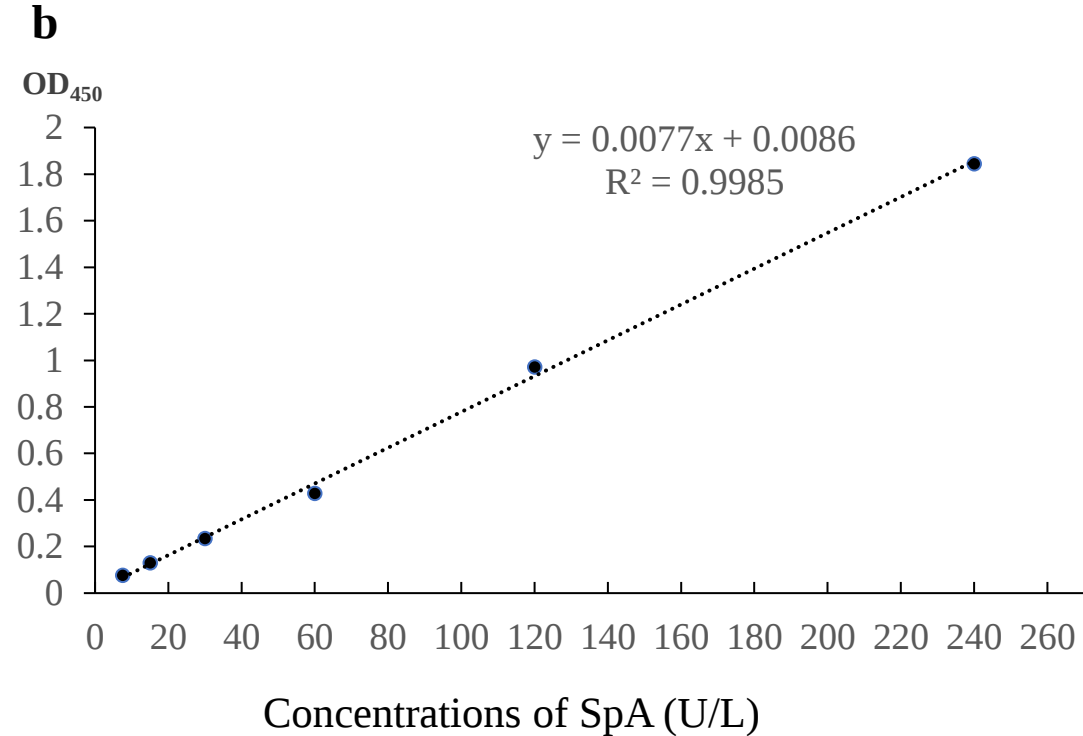
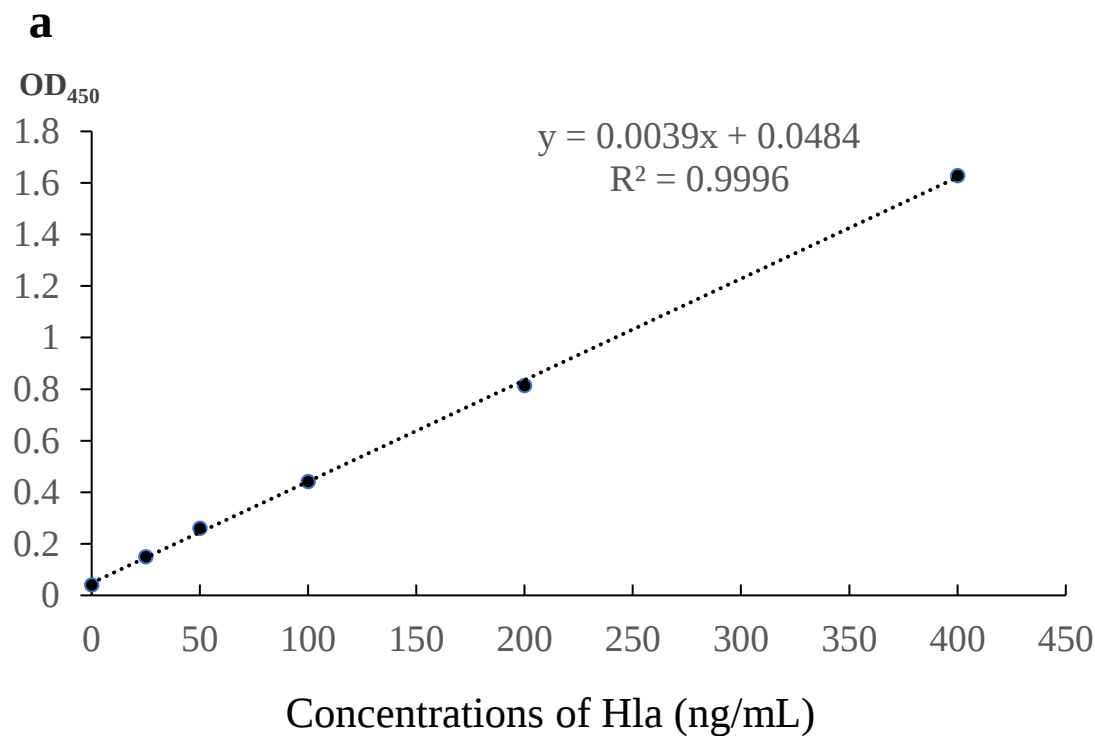
N315+1/32 MIC iclaprim			
Mean	Std Deviation	Fold Change	p value
6.62	0.71	6.62	0.01
1.86	0.21	1.86	0.004
0.9	0.13	0.9	0.314
2.07	0.17	2.07	0.01
6.26	1.81	6.26	0.002
6.26	1.92	6.26	0.006
2.41	0.49	2.41	0.005
2.7	0.47	2.7	0.002
2.35	0.52	2.35	0.008
6.51	1.05	6.51	<0.001
6.26	0.82	6.26	0.004
3.46	0.84	3.46	0.004
-1.64	0.22	0.61	0.012
5.54	0.75	5.54	<0.001
9.62	1.37	9.62	<0.001
6.28	0.98	6.28	0.012
6.34	1.29	6.34	0.021
7.48	1.04	7.48	<0.001

Gene		ATCC25923+no antibiotic	
		Mean	Std Deviation
Exoenzyme genes	<i>hla</i>	1.000	0.2
	<i>coa</i>	1.000	0.18
Adhesion genes	<i>clfA</i>	1.000	0.31
	<i>clfB</i>	1.000	0.21
	<i>fnbA</i>	1.000	0.2
	<i>fnbB</i>	1.000	0.24
	<i>spa</i>	1.000	0.2
	<i>sasG</i>	1.000	0.11
	<i>sdrD</i>	1.000	0.14
	<i>altA</i>	1.000	0.21
	<i>icaA</i>	1.000	0.18
	<i>icaD</i>	1.000	0.15
Regulator genes	<i>RNAIII</i>	1.000	0.15
	<i>agrA</i>	1.000	0.24
	<i>saeS</i>	1.000	0.2
	<i>sarA</i>	1.000	0.11
	<i>sigB</i>	1.000	0.14
	<i>rot</i>	1.000	0.17

ATCC25923+1/16 MIC iclaprim			
Mean	Std Deviation	Fold Change	p value
-1.52	0.18	0.66	0.04
2.53	0.27	2.53	0.001
4.04	0.63	4.04	0.001
1.86	0.28	1.86	0.04
-2.22	0.11	0.45	0.004
1.85	0.09	1.85	0.003
1.1	0.21	1.1	0.623
2.62	0.61	2.62	0.03
1.76	0.13	1.76	0.005
-1.37	0.08	0.73	0.043
1.11	0.2	1.11	0.241
1.13	0.19	1.13	0.133
2.86	0.77	2.86	0.008
5.51	0.82	5.51	0.001
2.92	0.31	2.92	0.003
2.77	0.82	2.77	0.013
-1.62	0.17	0.62	0.008
-2.94	0.18	0.34	0.03

ATCC25923+1/32 MIC iclaprim			
Mean	Std Deviation	Fold Change	p value
3.23	0.51	3.23	0.01
2.78	0.22	2.78	0.009
9.42	1.16	9.42	0.001
4.18	0.51	4.18	0.001
-2.03	0.1	0.49	0.005
1.56	0.11	1.56	0.008
0.92	0.2	0.92	0.144
4.23	0.54	4.23	0.001
2.04	0.48	2.04	0.023
-1.56	0.14	0.64	0.021
1.22	0.18	1.22	0.082
1.06	0.21	1.06	0.18
3.65	0.52	3.65	<0.001
6.32	0.84	6.32	<0.001
2.91	0.94	2.91	0.21
3.13	0.32	3.13	<0.001
-1.35	0.16	0.74	0.014
-2.17	0.18	0.46	0.02

Figure S1 Calibration Curve-Hla and SPA



a Hla; b SpA. Hla, α -hemolysin; SpA, staphylococcal protein A