

Resistance development to PLG0206, a novel engineered antimicrobial peptide

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Background

- PLG0206 is an engineered antimicrobial peptide (1, 2) with rapid bactericidal, broad-spectrum, and anti-biofilm properties being developed for the treatment of periprosthetic joint infections (PJI). The aim of this study was to determine the spontaneous mutation frequency (SMF) of PLG0206 with a variety of Gram-positive and Gram-negative pathogens that can cause PJIs.

Methods

- Isolates (*S. aureus*, *S. epidermidis*, enterococci, *S. pyogenes*, and *P. aeruginosa*) were sourced from the ATCC and Micromyx strain repository.
- Bacteria were plated onto RPMI-1640 agarose containing PLG0206 at 4, 8, or 16-fold the agar dilution MIC value.
- After a 48 hour incubation, the SMF was calculated and putative spontaneous mutant colonies were picked and patched to fresh agar plates containing the same PLG0206 concentration.
- Putative spontaneous mutant broth microdilution MIC testing utilized RPMI for PLG0206 while comparators were tested in CLSI standard media (3, 4).

Results

Table 1. Agar dilution MIC of isolates

Organism	Isolate	Type	MIC (µg/ml)				
			PLG0206 ¹	GM	IPM	VAN	
<i>S. aureus</i>	ATCC 29213	MSSA, QC	1	0.25 (0.12-1) ²	0.016 (0.016-0.06) ²	0.25 (0.06-0.5) ²	1 (0.5-2) ²
	NRS130	MSSA; ERY ^R	2	0.25	0.008	0.25	1
	NRS169	MSSA; ERY ^R , CLI ^R	1	0.25	0.016	0.12	1
	NRS1/Mu50	MRSA; VISA	2	>4	>8	8	4
	NRS384 (USA300)	MRSA	2	0.25	0.25	0.5	1
	NRS382 (USA100)	MRSA	1	0.25	1	4	1
<i>S. epidermidis</i>	MMX 8740	MRSE	1	8	2	16	2
	MMX 8655	MRSE	1	0.25	0.016	4	2
	ATCC 49134	MSSE	0.5	0.06	0.008	0.25	2
<i>E. faecalis</i>	ATCC 29212	VSE	1	8 (4-16) ²	1 (0.5-2) ²	1 (0.25-2) ²	4 (1-4) ²
	ATCC 700802	V583 VRE	1	>16	1	0.5	32
<i>E. faecium</i>	MMX 0485	VanA VRE	1	>16	>16	2	>32
<i>S. pyogenes</i>	ATCC 700294	-	32	>16	0.004	0.5	0.5
<i>P. aeruginosa</i>	ATCC 27853	QC	2	1 (0.5-2) ²	2 (1-4) ²	1 (0.5-4) ²	>16
	CDC 0241	IMP-1	2	>4	>8	>16	>16
	MMX 3025	CIP ^R	4	>4	2	>16	>16

¹ MIC as determined in RPMI-1640 Agar; ² CLSI QC ranges shown in parentheses where applicable; GM: gentamicin, IPM: imipenem, LVX: levofloxacin, VAN: vancomycin, MSSA: methicillin-susceptible *S. aureus*, MRSA: methicillin-resistant *S. aureus*, VISA: vancomycin-intermediate *S. aureus*, QC: quality control CIP^R: ciprofloxacin-resistant, ERY^R: erythromycin-resistant, CLI^R: clindamycin-resistant

Results

Table 2. Spontaneous mutation frequency values

Organism	Isolate	Type	Spontaneous Mutation Frequency at X-fold the MIC			MPC (µg/ml)
			4x	8x	16x	
<i>S. aureus</i>	ATCC 29213	MSSA; QC	2.01E-07	4.54E-08	1.03E-09	>16
	NRS130	MSSA; ERY ^R	1.76E-08	1.18E-09	≤1.18E-09	32
	NRS169	MSSA; ERY ^R , CLI ^R	3.14E-08	4.24E-09	≤8.47E-10	16
	NRS1	MRSA; VISA	≤1.98E-09	≤1.98E-09	≤1.98E-09	≤8
	NRS384	MRSA (USA300)	3.48E-08	7.09E-10	≤7.09E-10	32
<i>S. epidermidis</i>	MMX 8740	MRSE	NA	≤1.22E-09	≤1.22E-09	8
	MMX 8655	MRSE	NA	≤1.19E-09	≤1.19E-09	8
	ATCC 49134	MSSE	NA	NA	≤1.19E-09	8
<i>E. faecalis</i>	ATCC 700802	V583 VRE	≤2.94E-10	≤2.94E-10	≤2.94E-10	≤4
<i>E. faecium</i>	MMX 0485	VanA VRE	≤8.93E-10	≤8.93E-10	≤8.93E-10	≤4
<i>S. pyogenes</i>	ATCC 700294	-	≤4.55E-10	≤4.55E-10	≤4.55E-10	≤128
<i>P. aeruginosa</i>	ATCC 27853	QC	8.00E-08	6.81E-08	1.27E-08	>32
	CDC 0241	IMP-1	8.67E-08	1.71E-07	1.13E-07	>32
	MMX 3025	CIP ^R	6.80E-08	5.33E-09	≤8.20E-10	64

Table 3. Broth dilution MIC values for putative *S. aureus* spontaneous mutants

Isolate	Selection Concentration at X-fold the MIC	Isolate Name	PLG0206 ¹	VAN	RIF	ERY	CLI	LZD	LVX	TET
ATCC 29213	N/A	parent	0.5	1	0.008	0.5	0.25	4	0.25	1
	4X	P-4-100-A	1	1	0.008	0.25	0.25	4	0.12	0.5
		P-4-100-2	1	0.5	0.008	0.25	0.25	4	0.12	0.5
	8X	P-8-100-A	1	0.5	0.008	0.25	0.25	4	0.12	0.5
		P-8-100-B	1	0.5	0.008	0.25	0.25	4	0.12	0.5
	16X	P-16-100-1	1	0.5	0.008	0.25	0.12	4	0.12	0.5
P-16-100-2		1	0.5	0.008	0.25	0.25	4	0.12	0.5	
NRS130	N/A	parent	0.5	0.5	0.008	>32	0.12	2	0.12	0.25
	4X	P-8-2131-A	1	0.5	0.008	>32	0.25	2	0.12	0.25
		P-8-2131-B	1	1	0.008	>32	0.25	2	0.25	0.25
8X	P-16-2131-1	1	1	0.008	>32	0.25	2	0.25	0.25	
	P-16-2131-2	1	1	0.008	>32	0.5	2	0.25	0.25	
	N/A	parent	0.5	0.5	0.016	0.25	0.12	4	0.12	0.25
8X	P-8-2134-A	0.5	1	0.016	0.5	0.25	4	0.12	0.25	
	P-8-2134-B	0.5	1	0.016	0.5	0.25	4	0.12	0.25	
NRS384/MMX 2011	N/A	parent	0.5	0.5	0.008	>32	0.12	2	0.5	32
	4X	P-8-2011-A	0.5	0.5	0.008	>32	0.12	2	0.5	32
		P-8-2011-B	0.5	0.5	0.008	>32	0.12	2	0.5	32
		P-16-2011-1	0.5	1	0.008	>32	0.12	2	1	32
8X	P-16-2011-2	0.5	0.5	0.008	>32	0.12	2	0.5	32	
	N/A	parent	0.5	1	0.016	>32	>4	2	4	0.5
NRS382/MMX 2119	4X	P-4-2119-A	0.5	1	0.008	>32	>4	2	4	0.25
		P-4-2119-B	0.5	1	0.016	>32	>4	2	4	0.25
	8X	P-8-2119-A	1	1	0.016	>32	>4	2	4	0.25
		P-8-2119-B	0.5	1	0.016	>32	>4	2	4	0.25

¹ MIC as determined in RPMI-1640, comparators per CLSI. VAN: vancomycin, RIF: rifampin, ERY: erythromycin, CLI: clindamycin, LZD: linezolid, LVX: levofloxacin, TET: tetracycline, N/A: not applicable

Results

Table 4. Broth dilution MIC values for *P. aeruginosa* spontaneous mutants

Isolate	Selection Concentration at X-fold the MIC	Isolate Name	PLG0206 ¹	IMP	COL	AZT	LVX	GM	CAZ	TZP ²	
ATCC 27853	N/A	parent	0.5	1	0.25	4	1	1	2	4	
	8-4X	P-8-103-A	1	2	0.25	4	0.5	1	2	4	
		P-8-103-B	1	1	0.25	4	0.5	1	2	4	
	16-8X	P-16-103-A	2	1	0.25	4	1	1	2	4	
		P-16-103-B	2	2	0.25	8	1	2	2	8	
		P-32-103-A	8	1	0.25	4	1	1	2	4	
	CDC 0241	32-16X	P-32-103-B	4	1	0.25	8	1	1	2	4
parent			0.5	>32	0.25	16	16	32	>32	128	
8-4X		P-8-10166-A	4	>32	0.5	16	32	64	>32	128	
		P-8-10166-B	8	>32	1	16	32	64	>32	128	
		P-8-10166-1	1	>32	0.5	16	32	64	>32	128	
		P-8-10166-2	2	>32	0.5	16	32	64	>32	128	
		P-16-10166-A	16	>32	1	16	32	64	>32	128	
16-8X		P-16-10166-B	16	>32	2	16	32	64	>32	128	
		P-16-10166-1	1	>32	0.5	16	32	64	>32	128	
		P-16-10166-2	2	>32	0.5	16	32	32	>32	128	
		P-32-10166-A	16	>32	4	32	32	64	>32	128	
		P-32-10166-B	32	>32	4	32	32	64	>32	128	
		P-32-10166-1	2	>32	0.5	16	32	64	>32	128	
		P-32-10166-2	4	>32	2	16	32	64	>32	128	
MMX 3025		N/A	parent	0.5	1	0.25	16	16	32	8	>256
			P-16-3025-A	8	1	0.25	16	16	32	4	>256
		16-4X	P-16-3025-B	4	1	0.25	16	16	32	8	>256
	P-16-3025-1		1	1	0.25	16	16	32	4	>256	
	P-32-3025-A		8	1	0.25	16	32	32	8	>256	
	32-8X	P-32-3025-1	4	1	0.5	16	32	32	8	>256	
		P-32-3025-2	2	1	0.25	16	32	32	8	>256	
		P-32-3025-3	8	1	0.25	16	32	32	8	>256	

¹ MIC as determined in RPMI-1640, comparators per CLSI. IMP: imipenem, COL: colistin, AZT: aztreonam, LVX: levofloxacin, GM: gentamicin, CAZ: ceftazidime, TZP: piperacillin/tazobactam, N/A: not applicable

Summary

- No true spontaneous mutants to PLG0206 were selected for *S. aureus*, *S. epidermidis*, enterococci, or *S. pyogenes*.
- Smeared growth and false spontaneous mutant colonies suggest either the presence of heteroresistance or an inoculum effect.
- PLG0206 spontaneous *P. aeruginosa* mutants were selected.
- Whole genome sequencing of these mutants failed to confirm the presence of new mutations.
- The underlying mechanism behind the increase in PLG0206 MIC for *P. aeruginosa* will be further investigated.
- Topical application of PLG0206 for PJI treatment will utilize 1-3 mg/mL PLG0206, which is far above the MIC of the *P. aeruginosa* spontaneous mutants.

References

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