

Supplementary Information

Exposure of Methicillin-Resistant *Staphylococcus aureus* to Low Levels of the Antibacterial THAM-3ΦG Generates a Small Colony Drug-Resistant Phenotype

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SUPPORTING INFORMATION

Table S1. Passage number, Passage Concentration of THAM-3ΦG, and Minimum Inhibitory Concentrations of Cell Isolates.

Passage No.	Passage [T3PG]	MIC (mg/L)	n
P-0	0 mg/L	2	48
P-1L	1 mg/L	4	8
P-1S		8	10
P-2L	2 mg/L	4	6
P-2S		8	12
P-3L	2.5 mg/L	4	6
P-3S		8	12
P-4L	3 mg/L	4	6
P-4S		8	8
P-5L	4 mg/L	4	6
P-5S		8	6
P6	5 mg/L	8	6
P7	6 mg/L	8	9
P8	8 mg/L	32	11
P9	16 mg/L	32	8
P10	32 mg/L	32	9
P11	64 mg/L	64	7

With each passage, the concentration of THAM-3ΦG was increased, which is reflected as the passage concentration. MICs were recorded for each cell type (denoted by L or S for large and small) isolated in the resistance assay. MIC is greater than or equal to the concentration of THAM-3ΦG at each passage. The number of technical replicates for each MIC test is represented by n. A minimum of 3 biological replicates were conducted for each isolate.

Table S2. Loading Factors from the PCA of WT and SCV, Treated and Untreated with THAM-3ΦG

Metabolite	PC1	PC2
2-Hydroxyisobutyrate	-0.21931	0.066952
AMP	0.20476	0.10556
Acetate	-0.09675	0.14288
Alanine	-0.21148	0.041895
Asparagine	-0.04029	0.30841
Aspartate	0.19171	0.19284
Betaine	0.078021	0.30806
Choline	-0.04716	0.16751
Cystathionine	-0.05587	0.082503
Dimethylamine	-0.15678	0.18598
Formate	-0.21685	0.10983
Glucose-1-phosphate	-0.24133	-0.0313
Glutamate	0.085426	0.27294
Glutamine	-0.12531	0.048285
Glycine	-0.20279	0.10716
Isoleucine	-0.16718	0.23029
Isovalerate	-0.19481	0.003794
Lactate	0.16269	0.23403
Leucine	-0.18304	0.20341
Lysine	-0.24527	-0.01398
Methionine	-0.07726	0.23719
NAD	0.20178	0.16067
NADP	0.20042	0.18237
Niacinamide	0.15796	0.11186
Phenylalanine	-0.22942	0.10733
Proline	0.18245	0.16791
Succinate	-0.23823	0.015148
Trimethylamine	0.16618	0.23992
Trimethylamine N-oxide	0.11707	0.28054
UDP-galactose	-0.24	0.023353
Valine	-0.16669	0.21628
sn-Glycero-3-phosphocholine	-0.13242	0.25066
pi-Methylhistidine	-0.16544	0.024453

Table S3. Loading Factors from the PCA of WT and SCV at Mid-log Phase.

Metabolite	PC1	PC2
2-Hydroxyisobutyrate	-0.21931	0.066952
AMP	0.20476	0.10556
Acetate	-0.09675	0.14288
Alanine	-0.21148	0.041895
Asparagine	-0.04029	0.30841
Aspartate	0.19171	0.19284
Betaine	0.078021	0.30806
Choline	-0.04716	0.16751
Cystathionine	-0.05587	0.082503
Dimethylamine	-0.15678	0.18598
Formate	-0.21685	0.10983
Glucose-1-phosphate	-0.24133	-0.0313
Glutamate	0.085426	0.27294
Glutamine	-0.12531	0.048285
Glycine	-0.20279	0.10716
Isoleucine	-0.16718	0.23029
Isovalerate	-0.19481	0.003794
Lactate	0.16269	0.23403
Leucine	-0.18304	0.20341
Lysine	-0.24527	-0.01398
Methionine	-0.07726	0.23719
NAD	0.20178	0.16067
NADP	0.20042	0.18237
Niacinamide	0.15796	0.11186
Phenylalanine	-0.22942	0.10733
Proline	0.18245	0.16791
Succinate	-0.23823	0.015148
Trimethylamine	0.16618	0.23992
Trimethylamine N-oxide	0.11707	0.28054
UDP-galactose	-0.24000	0.023353
Valine	-0.16669	0.21628
sn-Glycero-3-phosphocholine	-0.13242	0.25066
pi-Methylhistidine	-0.16544	0.024453

Table S4. Primers Used for PCR Amplification and Gene Sequencing.

Primer Name	Gene Target	Primer sequence: 5' - 3'
TP-menFF	menF	CGAAGAAAGAAGTGAAATGGATGGCTACGGGCG
TP-menFR	menF	GCTTCAAATGCAAGTGCAAGTGGCGTTGAGCG
TP-menDF	menD	GTACGGCGTAAGGGAAGTAGTTATCAGTCCG
TP-menDR	menD	CGGTCTAACAACGTCGTAATATAATCAAAATTCCACG
TP-menHF	menH	GCCACATTGTTATCTGAAACTTCGACG
TP-menHR	menH	GCGAGGGATTTGGTCTTCACCTACATAACC
TP-menBF	menB	GGGAAAAGTTGCCATTATTTCAATCGC
TP-menBR	menB	GGTTGGCGAGAATTCAATTTCTCACCAGCTCG
TP-menEF	menE	CATAACCACTGCTAGAATGTCATAAAATCG
TP-menER	menE	CGGTGTTACAATTTGTGACTTAAATGGCTC
TP-menCF	menC	GCGAAGCAATTTCCAGGTATCAGTGACGC
TP-menCR	menC	GGCGGCTTTGACCCTGTTCCG