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Supplemental Information

MRSA Isolates from United States Hospitals

Carry *dfrG* and *dfrK* Resistance Genes and Succumb to Propargyl-Linked Antifolates

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Table S1. Related to Table 1. Antibiotic Susceptibility Profiles of Clinical MRSA Isolates ($\mu\text{g/mL}$)

Strain	Erythromycin	Clindamycin	Tetracycline	Gentamicin	Levofloxacin	Ciprofloxacin	Gatifloxacin
UCH MRSA 1	>4 (R)	≤ 0.12 (S)	≤ 2 (S)	≤ 2 (S)	64 (R)	>64 (R)	>8(R)
UCH MRSA 115	<0.25 (S)	≤ 0.12 (S)	>16 (R)	>16 (R)	>64 (R)	>64 (R)	>8(R)
UCH MRSA 121	>4 (R)	0.25 (S)	≤ 2 (S)	≤ 2 (S)	>64 (R)	64 (R)	>8(R)
UCH MRSA 127	>4 (R)	≤ 0.12 (S)	≤ 2 (S)	≤ 2 (S)	8 (R)	32 (R)	4(R)
HH MRSA 714	2 (R)	≤ 0.12 (S)	≤ 2 (S)	≤ 2 (S)	>64 (R)	64 (R)	>8(R)
HH MRSA 1144	>4 (R)	>2 (R)	>16 (R)	>16 (R)	8 (R)	64 (R)	4 (R)
HH MRSA 1184	>4 (R)	0.25 (S)	≤ 2 (S)	≤ 2 (S)	0.25 (S)	1 (S)	<1 (S)
UCH MSSA 1	>4 (R)	>2 (R)	≤ 2 (S)	≤ 2 (S)	8 (R)	64 (R)	2 (S)

All strains are sensitive to synercid ($\text{MIC} \leq 0.5 \mu\text{g/mL}$), daptomycin ($\text{MIC} \leq 0.5 \mu\text{g/mL}$), rifampin ($\text{MIC} \leq 0.5 \mu\text{g/mL}$), vancomycin ($\text{MIC} \leq 2 \mu\text{g/mL}$), streptomycin ($\text{MIC} \leq 1000 \mu\text{g/mL}$), and linezolid ($\text{MIC} \leq 1 \mu\text{g/mL}$). All strains are resistant to ampicillin ($\text{MIC} \geq 16 \mu\text{g/mL}$), penicillin ($\text{MIC} > 8 \mu\text{g/mL}$). UCH MSSA-1 is sensitive to oxacillin.

Table S2. Related to Table 1 and Table S1. Molecular Mechanisms of Antibiotic Resistance for Clinical Isolates

	Resistance Mechanism	MIC Range ($\mu\text{g/mL}$)	Strains
Trimethoprim	<i>dfrA</i>	250	UCH115, HH1144
	<i>dfrG</i>	>1000	UCH MRSA1, UCH121, UCH127, HH714, UCH MSSA1
	<i>dfrK</i>	>1000	HH1184
Sulfamethoxazole	<i>folB</i> (F17L, V30I, T31N, M37I, I58V, T59S, V60L, L64M, I110M, V117I, V126I, E208K, F226L)	>500	UCH MRSA1
	<i>folB</i> (F17L, T28S, T59S, L64M, E205K)	≥ 500	UCH115, UCH 121, UCH127, HH714, HH1144, UCH MSSA1
	<i>folB</i> (V30I, I58V, T59S, V60L, L64M, I110M, V117I, V126I, F226L)	32	HH1184
Tetracycline	<i>TetM</i>	>16	UCH115, HH1144
Gentamicin	<i>aac(2')-apc(6'')</i>	>16	UCH115, HH1144
Erythromycin	<i>mphC</i>	8-32	UCH121, UCH127, HH1184
	<i>ermC</i>	>64	UCH MRSA1, HH1144, UCH MSSA 1
Clindamycin	<i>ermC</i>	>64	UCH MRSA1, HH1144, UCH MSSA 1
Levofloxacin/ Ciprofloxacin/ Gatifloxacin	<i>gyrA</i> (S84R ,S85P), <i>grlA</i> (S80F), <i>grlB</i> (E471K)	64/ >64/>8	UCH MRSA1
	<i>gyrA</i> (S84L, S85P), <i>gyrA</i> (S90K, E84K)	>64/>64/ >8	UCH115
	<i>gyrA</i> (S84R ,S85P), <i>grlA</i> (S80F), <i>grlB</i> (D432V)	>64/64/>8	UCH121, HH714
	<i>gyrA</i> (S84L) and <i>grlA</i> (S80F), <i>grlB</i> (D432V, E596D*)	8/32/4	UCH127
	<i>gyrA</i> (S84L) and <i>grlA</i> (S80F)	8/64/4	HH1144
Levofloxacin/ Ciprofloxacin	<i>gyrA</i> (S84L)	8/64	UCH MSSA1

Table S3. Related to Table 2. Fluoroquinolone Minimum Inhibitory Concentrations Supplemented with Reserpine (µg/mL)

Strain	Minimum Inhibitory Concentration		Minimum Inhibitory Concentration with 20 µg/mL Reserpine	
	Levofloxacin	Ciprofloxacin	Levofloxacin	Ciprofloxacin
UCH MRSA 1	64	>64	64 (1)	32 (≥2)
UCH MRSA 115	>64	>64	>64 (≥1)	32 (≥2)
UCH MRSA 121	>64	64	>64 (≥1)	32 (2)
UCH MRSA 127	8	32	4 (2)	8 (4)
HH MRSA 714	>64	64	>64 (≥1)	32 (2)
HH MRSA 1144	8	64	8 (1)	16 (4)
HH MRSA 1184	0.25	1	<0.125 (≥2)	<0.125 (>4)
UCH MSSA 1	8	64	8 (1)	32 (2)

Fold increases in MIC noted in parentheses

Table S4. Related to Table 2. Synergy Minimum Inhibitory Concentrations Supplemented with Sulfamethoxazole (µg/mL)

Strain		UCP1039	UCP1164	UCP1172	UCP1173	UCP1191	UCP1205	UCP1206
UCHC MRSA 115	<i>dfrA</i>	1.25	0.3125	1.25	0.1563	0.625	2.5	0.625
HH MRSA 714	<i>dfrG</i>	0.0391	0.3125	0.3125	0.3125	0.0391	0.0391	0.1563
HH MRSA 1184	<i>dfrK</i>	≤0.0098	≤0.0098	≤0.0098	≤0.0098	≤0.0098	≤0.0098	≤0.0098

*UCH MRSA115 and HH MRSA115 contained 100 µg/mL SMX and HH MRSA1184 contains 10 µg/mL

Table S5. Related to Figure 2. Crystallography Data Collection and Structure Refinement Statistics

	DfrB :NADPH:UCP1191
PDB ID	5JG0
Space group	<i>P6₁22</i>
No. monomers in asymmetric unit	1
Unit cell (<i>a, b, c</i> in Å)	78.86, 78.86, 106.43 90.0, 90.0, 120.0
Resolution (Å)	39.44-1.88 (1.91-1.88)
Completeness % (last shell, %)	99.75 (97.0)
Unique reflections	16, 498
Redundancy (last shell)	16.7 (17.4)
Rsym, (last shell)	0.107 (0.483)
$\langle I/\sigma \rangle$ (last shell)	41.2 (5.52)
R-factor/Rfree	0.1765/ 0.2172
No. of atoms (protein, ligands, solvent)	1,458
Rms deviation bond lengths (Å), angles (deg)	0.007, 1.238
Average B factor for protein (Å ²)	29.54
Average B factor for ligand (Å ²)	25.34 β-NADPH 34.66 Inhibitor
Average B factor for solvent molecules (Å ²)	35.74
Residues in most favored regions (%) ^a	98.12
Residues in additional allowed regions (%) ^a	1.88
Residues in disallowed regions (%) ^a	0
Collection Location	SSRL Beamline 7-1

^a According to an analysis of the Ramachandran plot

Table S6. Related to Figure 3. Structural analysis of residues involved in binding PLAs and NADPH

DfrB:PLA Interactions			
Residue	Binding Partner	Bond Distance (Å)	Comments
Leu5	Backbone to C ₂ -NH ₃	2.9	Leu5Ile Mutation in all TMP ^R enzymes
Asp27	Side chain to C ₄ -NH ₃	3.2	Conserved in all <i>dfr</i> enzymes
	Side chain to N ₅	2.6	
Leu28	Hydrophobic interactions with B-C ring system and C ₆ ethyl		Leu28Tyr mutation in <i>dfrG/K</i> no mutation in <i>dfrA</i>
His30	Coordinates H ₂ O with pyrimidine C ₄ -NH ₃	3.1, 3.2	His30Tyr mutations in <i>dfrK/G</i> , mutations known to be relevant ¹
Val31	Hydrophobic interaction with pyrimidine C ₆ -ethyl		Val31Ile in <i>dfrA</i> , mutations known to be relevant ²
Ile50	Side chains make hydrophobic interactions with B-C ring system		Conserved in all <i>dfr</i> enzymes
Leu54			Conserved in all <i>dfr</i> enzymes
Arg57	Side chain to C-ring COOH	2.8	Conserved in all <i>dfr</i> enzymes
Phe92	Backbone to pyrimidine C ₂ -NH ₃	3.1	Conserved in all <i>dfr</i> enzymes
Phe98	Mutations known to be relevant ³		Phe98Tyr mutation in all <i>dfr</i> enzymes.
DfrB:NADPH Interactions			
Residue	Binding Partners	Bond Distances (Å)	Comments
Ala7	BB Carbonyl to Nicotinamide amide (NH ₂)	2.8	Conserved in all <i>dfr</i> enzymes
	BB amine to Nicotinamide amide (OH)	2.7	
Gln19	Nicotinamide ribose	3.3	Gln19Asp in <i>dfrG/K</i>
Arg44	Guanidine to ribose phosphate	3.2	Conserved in all <i>dfr</i> enzymes
	γ -NH to ribose phosphate	2.9	
Thr46	Side chain to phosphate	2.5	Thr46His in <i>dfrG/K</i>
Thr63	Side chain to ribose phosphate	2.8	Conserved in all <i>dfr</i> enzymes
Glu100	Side chain to adenine	2.9/2.7	Glu100Leu in <i>dfrG/K</i> Glu100Ala in <i>dfrA</i>

(BB) Backbone (SC) side chain

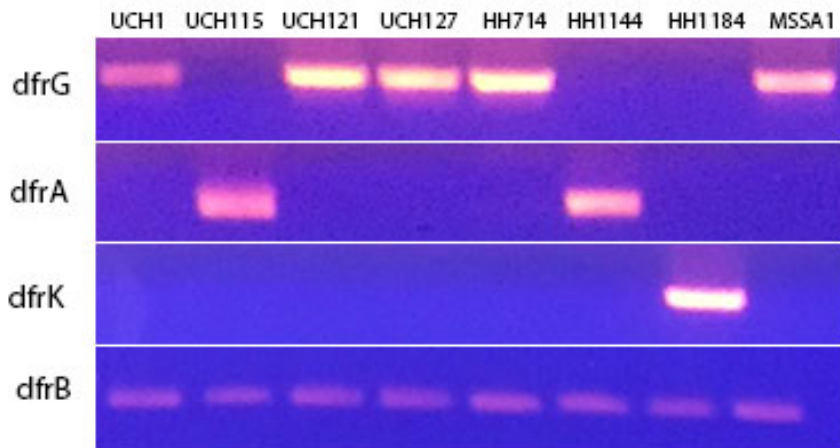


Figure S1. Related to Table 1. Composite PCR Gel for Gene Identification

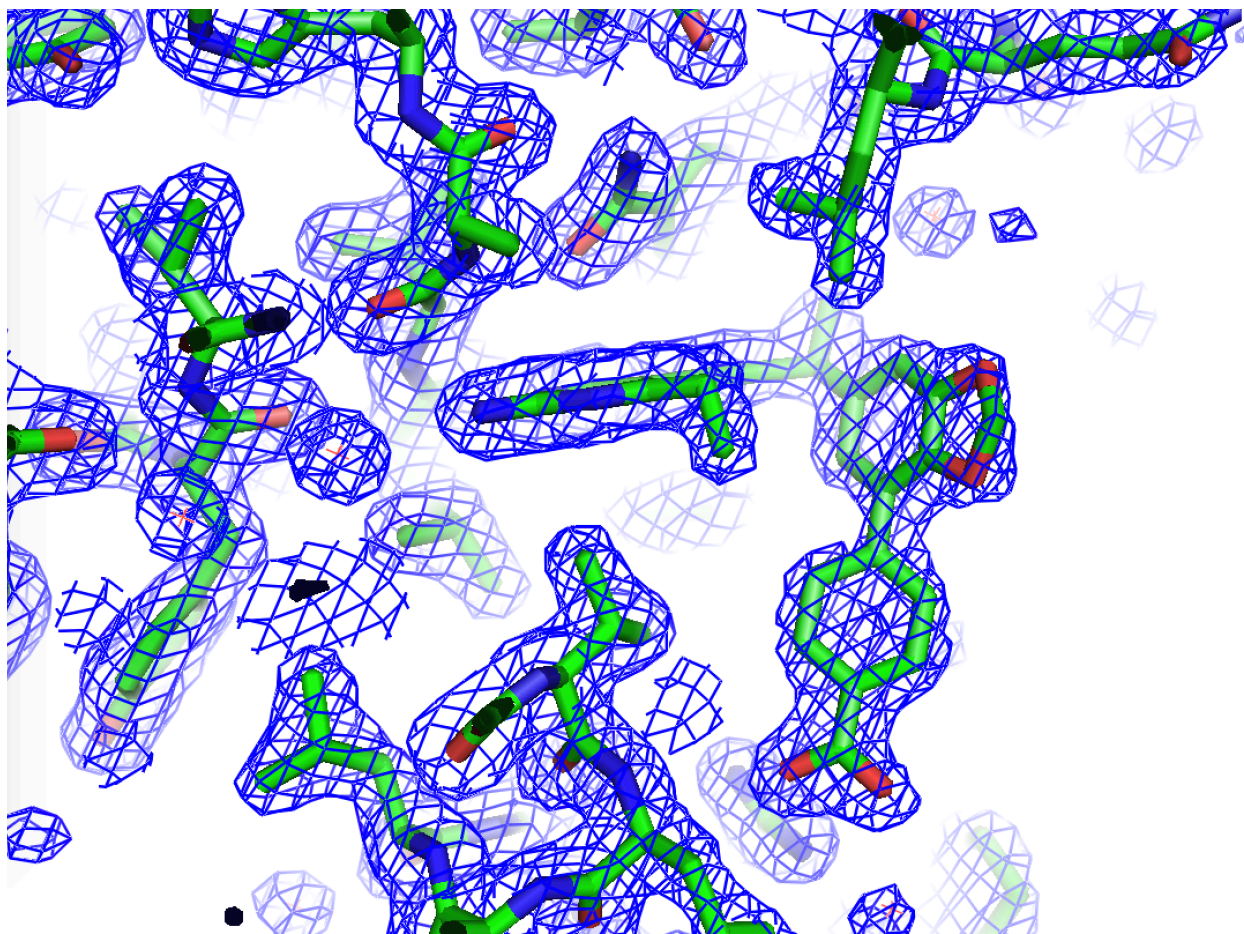


Figure S2. Related to Figure 2. OMIT Map from crystal structure of SaDHFR bound to NADPH and UCP1191.

Electron density ($2F_o - F_c$) of the active site residues for the Sa(WT)DHFR:NADPH:UCP1191, shown at 1.0σ

Fig. S3. Related to Experimentnal. ^1H and ^{13}C NMR of UCP1205

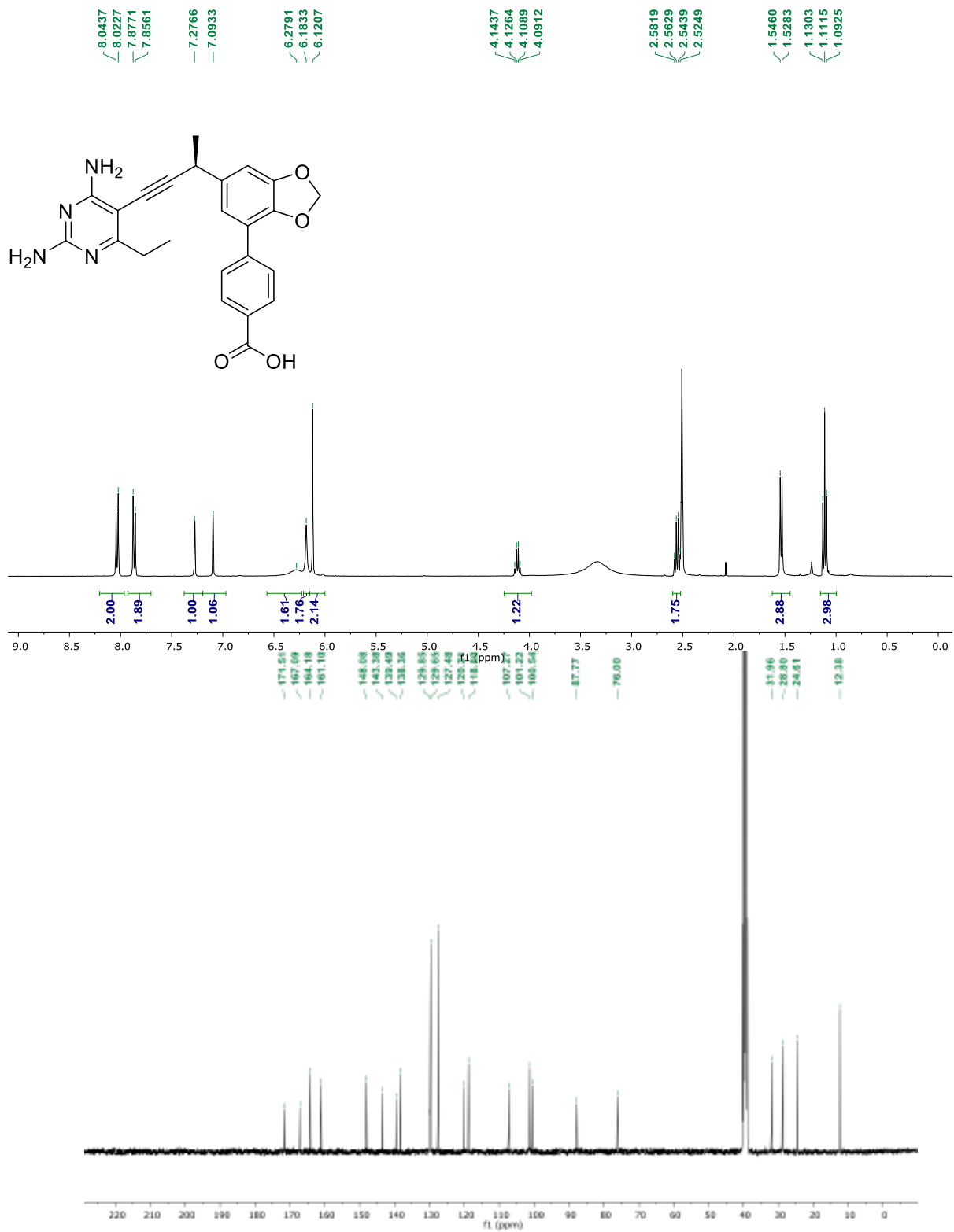


Fig. S4. Related to Experimental. ^1H and ^{13}C NMR of UCP1206

