Chrom	Position	Reference	Altrenate	Region	Effect
CP000255	417	G	T	NON-CODING	-
CP000255	16135	G	A	CODING	START LOST
CP000255	35817	A	T	NON-CODING	-
CP000255	76849	A	G	NON-CODING	-
CP000255	87551	C	A	CODING	SYNONYMOUS
CP000255	115262	A	G	CODING	NON-SYNONYMOUS
CP000255	238924	G	A	CODING	NON SYNONYMOUS
CP000255	334170	T	A	CODING	NON-SYNONYMOUS
CP000255	334171	T	A	CODING	STOP GAINED
CP000255	350947	A	G	CODING	SYNONYMOUS
CP000255	414724	C	T	NON-CODING	-
CP000255	507017	A	G	CODING	NON-SYNONYMOUS
CP000255	534272	G	A	CODING	NON-SYNONYMOUS
CP000255	691057	T	C	NON-CODING	-
CP000255	802202	T	C	NON-CODING	-
CP000255	816938	A	G	CODING	SYNONYMOUS
CP000255	848576	G	A	CODING	NON SYNONYMOUS
CP000255	855559	C	A	NON-CODING	-
CP000255	865624	C	T	CODING	NON SYNONYMOUS
CP000255	891071	C	T	CODING	STOP GAINED
CP000255	906463	T	A	NON-CODING	-
CP000255	941455	G	A	CODING	STOP GAINED
CP000255	950418	A	G	CODING	SYNONYMOUS
CP000255	975709	T	A	CODING	NON SYNONYMOUS
CP000255	975798	A	T	CODING	STOP GAINED
CP000255	1046867	T	A	CODING	STOP GAINED
CP000255	1074284	T	G	NON-CODING	-
CP000255	1111775	T	C	CODING	SYNONYMOUS
CP000255	1181644	C	A	CODING	NON-SYNONYMOUS
CP000255	1246149	T	A	CODING	NON-SYNONYMOUS
CP000255	1263354	A	T	CODING	NON SYNONYMOUS
CP000255	1302306	T	C	CODING	NON-SYNONYMOUS
CP000255	1324043	A	G	CODING	SYNONYMOUS
CP000255	1405837	C	T	CODING	NON-SYNONYMOUS
CP000255	1420581	T	A	CODING	NON-SYNONYMOUS
CP000255	1449721	G	A	CODING	NON-SYNONYMOUS
CP000255	1622717	A	T	CODING	SYNONYMOUS
CP000255	1770310	C	T	CODING	SYNONYMOUS
CP000255	1885211	G	A	CODING	SYNONYMOUS

CP000255	1926344	G	T	CODING	NON-SYNONYMOUS
CP000255	1955851	G	C	CODING	NON-SYNONYMOUS
CP000255	2019634	T	C	CODING	SYNONYMOUS
CP000255	2019637	T	C	CODING	SYNONYMOUS
CP000255	2130440	T	C	CODING	NON-SYNONYMOUS
CP000255	2149492	G	A	CODING	NON-SYNONYMOUS
CP000255	2165813	T	C	CODING	NON-SYNONYMOUS
CP000255	2476503	T	G	CODING	NON-SYNONYMOUS
CP000255	2488715	A	G	CODING	NON-SYNONYMOUS
CP000255	2499313	A	G	NON-CODING	-
CP000255	2504807	G	C	NON-CODING	-
CP000255	2554758	C	T	CODING	STOP-GAINED
CP000255	2624155	A	C	NON-CODING	-
CP000255	2678946	A	G	CODING	SYNONYMOUS
CP000255	2705337	G	A	CODING	NON-SYNONYMOUS
CP000255	2734666	C	T	CODING	NON-SYNONYMOUS
CP000255	2746919	C	T	CODING	NON-SYNONYMOUS
CP000255	2766651	G	A	CODING	NON-SYNONYMOUS
CP000255	2807973	A	C	CODING	NON-SYNONYMOUS
CP000255	2808635	G	T	CODING	NON-SYNONYMOUS
CP000255	2848174	A	G	CODING	SYNONYMOUS
CP000255	2849850	A	G	CODING	SYNONYMOUS
CP000255	2857261	G	T	CODING	NON-SYNONYMOUS

Codon	AA Change	Gene
-	-	-
atG/atA	M -> I	SAUSA300_0012
-	-	-
-	-	-
gtC/gtA	V -> V	*copA*
tAt/tGt	Y -> C	SAUSA300_0104
Gaa/Aaa	E -> K	SAUSA300_0202
Ttg/Atg	L -> M	SAUSA300_0280
tTg/tAg	L -> STOP	SAUSA300_0280
tcA/tcG	S -> S	SAUSA300_0299
-	-	-
gAa/gGa	E -> G	*treC*
gGc/gAc	G -> D	*glmU*
-	-	-
-	-	-
ggA/ggG	G -> G	yfiA
Gta/Ata	V -> I	*eno*
-	-	-
aCa/aTa	T -> I	SAUSA300_0775
Caa/Taa	Q -> STOP	SAUSA300_0811
-	-	-
Cag/Tag	Q -> STOP	*glpQ*
gcA/gcG	A -> A	*rexB*
aTt/aAt	I -> N	*oppC*
Aaa/Taa	K -> STOP	*oppC*
Aaa/Taa	K -> STOP	*atl*
-	-	-
ggT/ggC	G -> G	*pyc*
gaC/gaA	D -> E	*ftsZ*
aTa/aAa	L -> K	*rnhB*
Atg/Ttg	M -> L	*pyrH*
tTg/tCg	L -> S	SAUSA300_1182
tcA/tcG	S -> S	*glnA*
cCt/cTt	P -> L	*pepF*
Tca/Aca	S -> T	*dapB*
Cca/Tca	P -> S	*msrA*
gcT/gcA	A -> A	*lpdA*
gcG/gcA	A -> A	*hemD*
gaC/gaT	D -> D	*leuS*

atG/atT	M -> I	SAUSA300_1744
tCt/tGt	S -> C	*lukD*
aaT/aaC	N -> N	SAUSA300_1858
gaT/gaC	D -> D	SAUSA300_1858
gAt/gGt	D -> G	SAUSA300_1975
aGa/aAa	R -> K	*agrA*
aTg/aCg	M -> T	*ilvD*
Act/Cct	T -> P	*tcaR*
atA/atG	I -> M	SAUSA300_2314
-	-	-
-	-	-
tGg/tAg	W -> STOP	SAUSA300_2375
-	-	-
gcA/gcG	A -> A	SAUSA300_2481
gCa/gTa	A -> V	SAUSA300_2500
Gaa/Aaa	E -> K	*panC*
aGt/aAt	S -> N	SAUSA300_2542
gCg/gTg	A -> V	SAUSA300_2556
aTc/aGc	I -> S	SAUSA300_2586
Cgt/Agt	R -> S	SAUSA300_2587
taT/taC	Y -> Y	SAUSA300_2621
ttA/ttG	L -> L	SAUSA300_2622
Gca/Tca	A -> S	*nixA*

Gene Name	Patient
-	D2
Putative homoserine O-acetyltransferase	B1
-	B2
-	B2
ATPase copper transport	D1
Transcriptional regulator, AraC	B1
peptide ABC transporter permease	C
hypothetical protein	D1
hypothetical protein	D1
hypothetical protein	B2
-	A
Alpha-phosphotrehalase	B1
bifunctional N-acetylglucosamine-1-phosphate uridyltransferase/glucosamine-1-phosphate acetyltransferase	A
-	D2
-	A
ribosomal subunit interface protein	B2
phosphopyruvate hydratase	D1
-	D1
Hypothetical protein	C
hypothetical protein	A
-	D2
glycerophosphoryl diester phosphodiesterase	A
ATP-dependent helicase/deoxyribonuclease subunit B	C
Peptide ABC transporter permease	C
Peptide ABC transporter permease	C
Autolysin	B1
-	A
pyruvate carboxylase	B2
cell division protein FtsZ	D1
ribonuclease HII	A
Uridylate kinase	C
pyruvate ferredoxin oxidoreductase, alpha subunit	A
glutamine synthetase, type I	D2
oligoendopeptidase F	D2
dihydrodipicolinate reductase	D1
methionine sulfoxide reductase A	A
dihydrolipoamide dehydrogenase	A
uroporphyrinogen III synthase	A
leucyl-tRNA synthetase	D2

hypothetical protein	D2
leukotoxin LukD	D1
hypothetical protein	B2
hypothetical protein	B2
Aerolysin/leukocidin family protein	A
accessory gene regulator protein A	B2
dihydroxy-acid dehydratase	D2
transcriptional regulator TcaR	A
hypothetical protein	D1
-	D2
-	A
ABC transporter ATP-binding/permease	D2
-	D1
hypothetical protein	D2
Glycosyl transferase	B1
pantoate--beta-alanine ligase	D2
putative AMP-binding enzyme	B2
ABC transporter protein	B2
hypothetical protein	D1
accessory secretory protein Asp1	D1
Hypothetical protein	B1
hypothetical protein	B2
high-affinity nickel-transporter	A

Supplemental Table 1. Average between-patient/group single nucleotide polymorphism differences. The number of base differences per sequence from averaging over all sequence pairs between patient groups are shown. The analysis involved 27 nucleotide sequences.

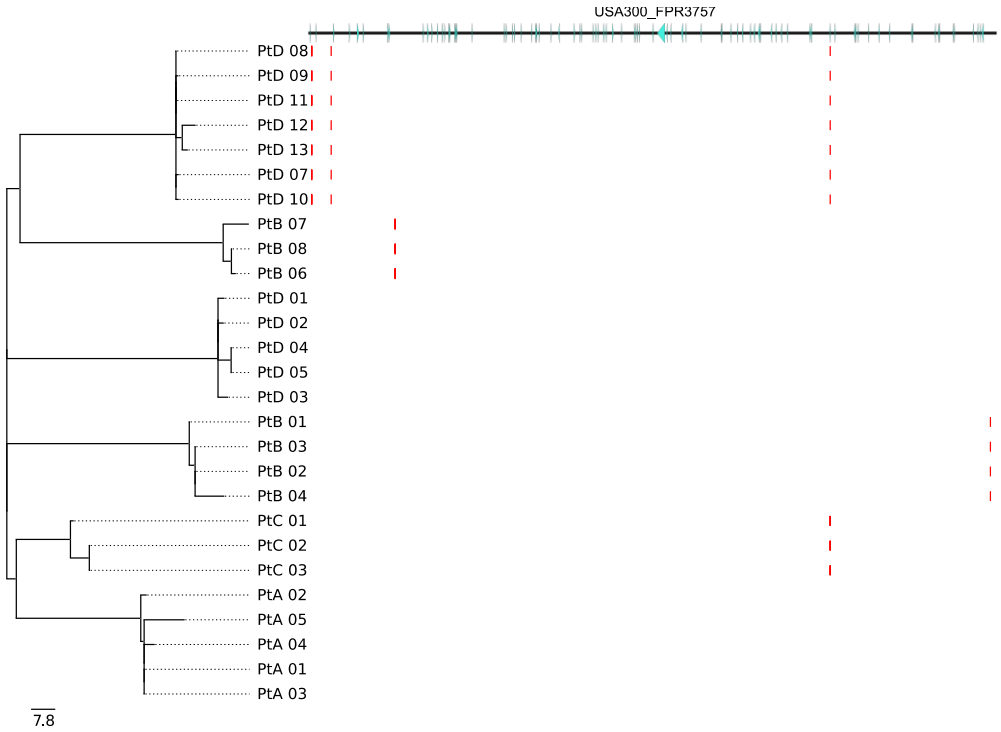
	PtA	PtB1	PtB2	PtC	PtD1
PtA	-				
PtB1	49.20	-			
PtB2	46.87	69.67	-		
PtC	22.87	47.67	45.33	-	
PtD1	52.80	73.60	73.27	51.27	-
PtD2	44.91	67.71	57.38	43.38	71.31

Supplemental Table 2. Frequency and characteristics of single nucleotide polymorphisms by patient and MRSA USA300 population.

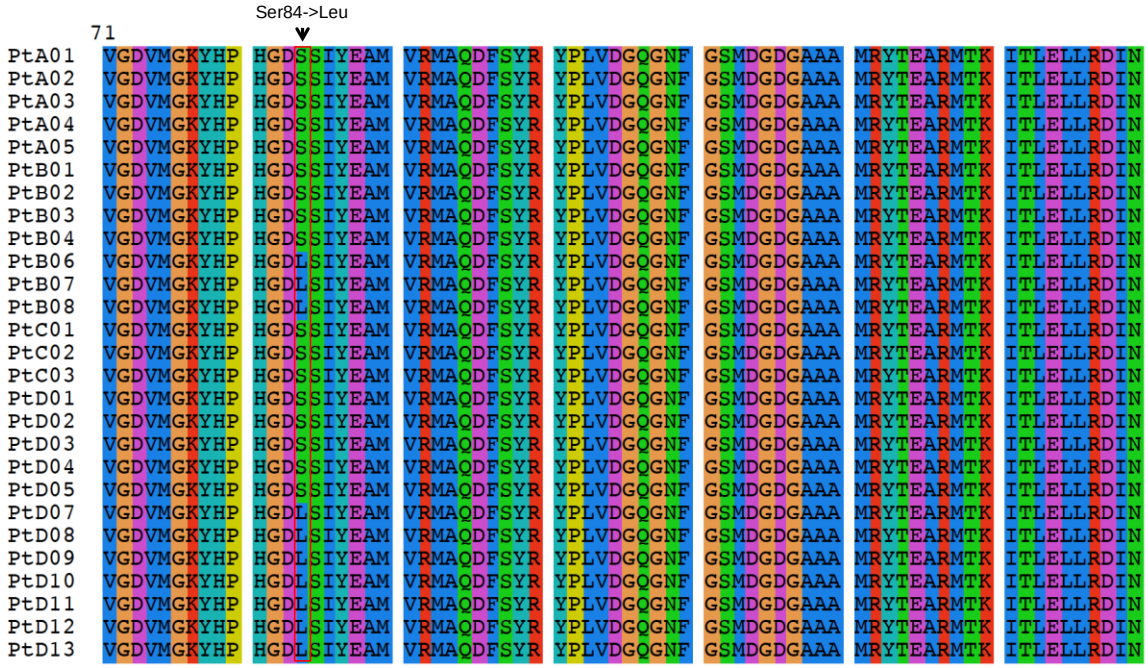
	Patient A	Patient B	Patient B-2	Patient C	Patient D	Patient D-2
Isolates	5	4	3	3	5	7
Days	744	424	46	222	112	227
Synonymous	2 (14.3%)	1 (16.7%)	3 (42.9%)	1 (16.7%)	2 (33.3%)	0 (0.0%)
Non-synonymous	7 (50.0%)	3 (50.0%)	3 (42.9%)	4 (66.7%)	2 (33.3%)	5 (83.3%)
Stop lost	0 (0.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Stop gained	2 (14.4%)	1 (16.7%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)
Non-coding	3 (21.4%)	0 (0.0%)	1 (14.3%)	0 (0.0%)	2 (33.3%)	1 (16.7%)
Total SNPs	14	6	7	6	6	6
Mean pairwise distance (SNP)	6	2.6	7.3	4	6	4
Rate of Diversification (SNPs/year)	2.9	2.2	57.9	6.6	19.6	6.4

Supplementary Table 3. SNP rate by USA300 subsystem. Subsystems were annotated using RAST (http://rast.nmpdr.org/). SNPs for all 27 USA300 isolates were stratified by subsystem. The sum of nucleotides among genes annotated as a specific subsystem were used to calculate a SNP rate (per 10,000 nucleotides) by subsystem, which was then compared to the rate of SNPs occurring in other subsystems. A rate ratio and 95% confidence interval was calculated. Asterisks are used to denote statistically significant p-values for rate ratios.

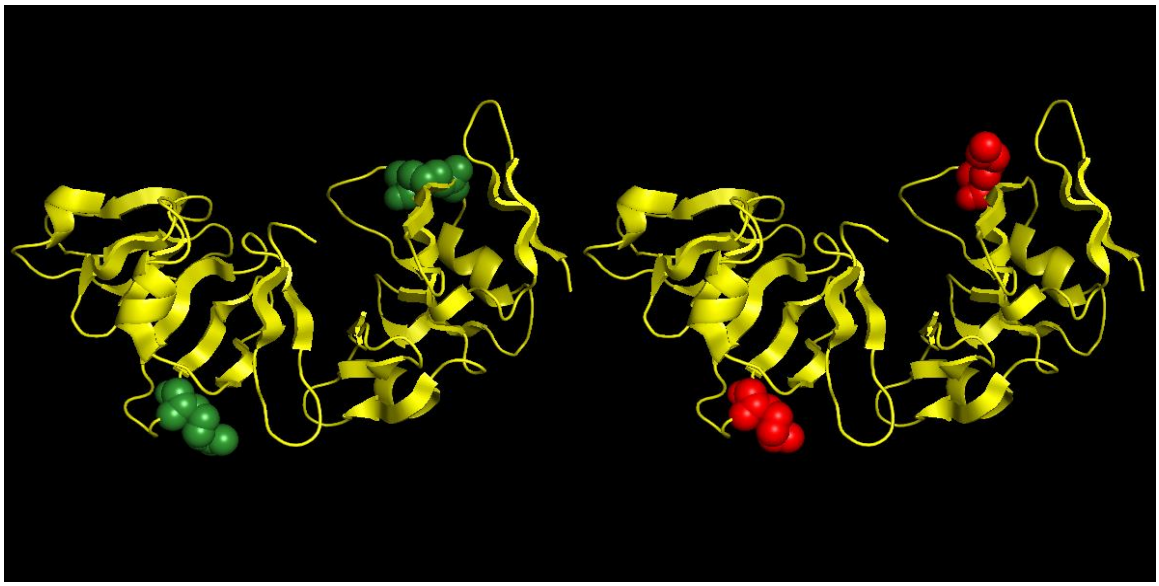
USA300 Subsystem Category	SNPs (% of total)	Nucleotides in subsystem (% of total)	Rate (per 10k) in subsystem	Rate (per 10k) among other subsystems	Rate Ratio (95% CI)
Membrane Transport	8 (27.6)	48,639 (3.8)	1.64	0.25	6.69 (2.97-15.1)*
Regulation and Cell signaling	3 (10.3)	45,701 (3.5)	0.66	0.30	2.17 (0.65-7.16)
Cell Division and Cell Cycle	1 (3.4)	18,397 (1.4)	0.54	0.32	1.72 (0.23-12.6)
Virulence, Disease and Defense	5 (17.2)	130,574 (10.1)	0.38	0.31	1.23 (0.47-3.23)
Amino Acids and Derivatives	4 (13.8)	114,296 (8.8)	0.35	0.32	1.11 (0.38-3.17)
Respiration	1 (3.4)	31,176 (2.4)	0.32	0.32	0.99 (0.14-7.34)
Protein Metabolism	2 (6.9)	106,703 (8.2)	0.19	0.34	0.55 (0.13-2.33)
Cofactors, Vitamins, Prosthetic Groups, Pigments	2 (6.9)	127,096 (9.8)	0.16	0.35	0.45 (0.11-1.90)
RNA Metabolism	1 (3.4)	71,479 (5.5)	0.14	0.34	0.42 (0.06-3.06)
Cell Wall and Capsule	1 (3.4)	88,193 (6.8)	0.11	0.34	0.33 (0.05-2.43)
Carbohydrates	1 (3.4)	121,218 (9.4)	0.08	0.36	0.23 (0.03-1.69)



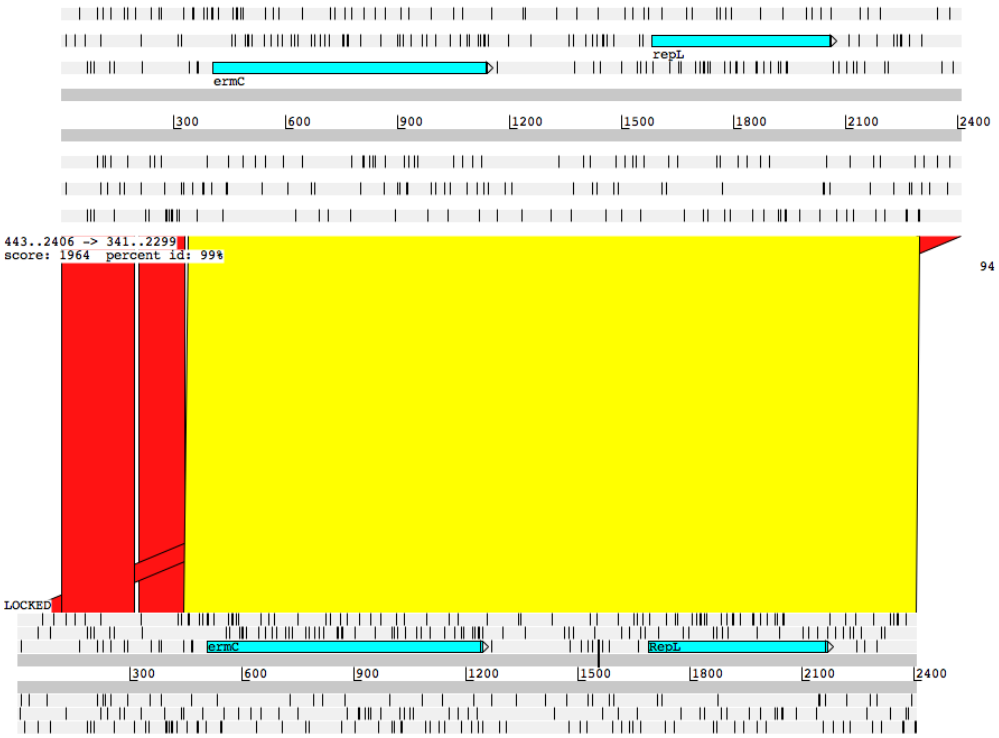
Supplemental Figure 1. Gubbins recombination analysis of 27 USA300 MRSA isolates. A recombination-free maximum-likelihood phylogeny is displayed on the left of the figure. The red bars display the location of recombination events against the USA300_FPR3757 reference genome. All detected recombination events were shared among all intra-host isolates. SNPs introduced through recombination events were removed from the analysis.



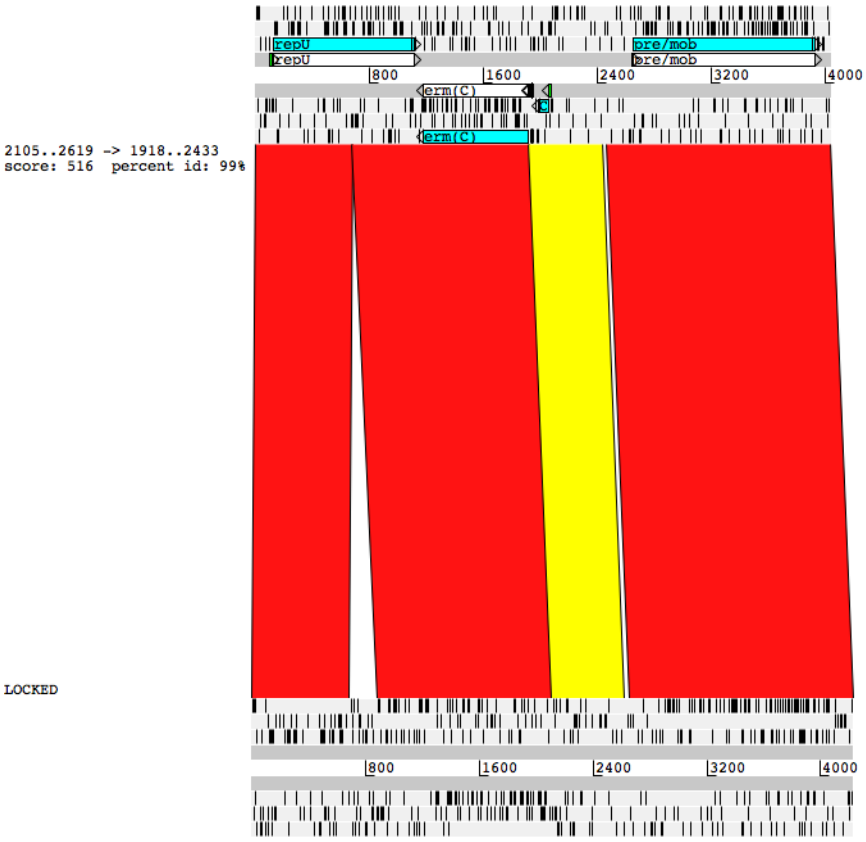
Supplemental Figure 2. Alignment of DNA Gyrase A (gyrA) among 27 USA300 isolates demonstrating Ser84->Leu mutation conferring fluoroquinolone resistance.



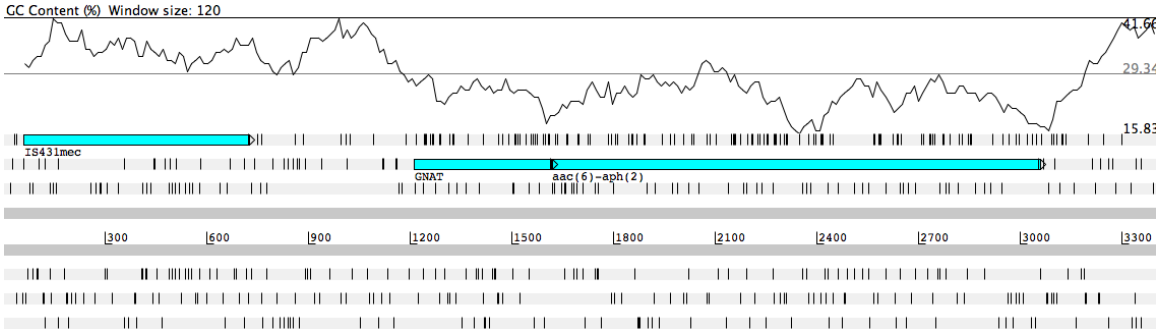
Supplemental Figure 3. Visualization of R170K mutation in *S. aureus* Accessory gene regulator protein A (T1YBU1). *agrA* is responsible for regulating the expression of several virulence genes and may have a role in biofilm production. The arginine to lysine mutation retained the same amino acid charge but varied in size.



Supplementary Figure 4. Alignment of small, 2,407 bp plasmid present in isolates PtC-01 and PtC-03 with homology to 2.3-kb erythromycin resistance plasmid pPV141 from *Staphylococcus chromogenes* (GenBank: U82607). Plasmid pPV141 has a 58 bp deletion in the translational attenuator that results in the constitutive expression of *ermC* and clindamycin resistance (1).



Supplementary Figure 5. Alignment of 4,295 bp plasmid present in isolates PtD-03 and PtD-04 to *Staphylococcus saprophyticus* pSES22 plasmid (GenBank: AM159501). Plasmid pSES22 possesses a constitutively expressed *ermC* gene that confers resistance to erythromycin and clindamycin, which is linked to a 22-bp tandem duplication in the *ermC* regulatory region (2). This regulatory region is highlighted in yellow and displays 99% identity. Plasmids present in PtD-03 and PtD-04 are identical to each other and possess a truncated version of the 22-bp duplication. Both isolates PtD-03 and PtD-04 were constitutively resistant to clindamycin. Visualization was constructed with Artemis Comparison Tool (ACT v13.0.0).



Supplementary Figure 6. Artemis review of 3,465 bp transposon containing IS431mec transposase and *aac(6')-aph(2'').*

References

1. Werckenthin C, Schwarz S, Westh H. Structural Alterations in the Translational Attenuator of Constitutively Expressed ermC Genes. Antimicrob Agents Chemother. **1999**; 43(7):1681–1685.

2. Somkuti GA, Solaiman DK, Steinberg DH. Molecular properties of the erythromycin resistance plasmid pPV141 from Staphylococcus chromogenes. **1997**; 37(2):119–27.