

Table S1: Oligonucleotide primers used for PCR amplification of *Staphylococcus aureus* fluoroquinolone target genes

Primer Name	Product (bp)	Tm (°C)	Target gene ^a	Sequence (5'->3')
oCSSau01F	848	61	5' of <i>gyrB</i>	CGCGTCAAACGCATTTATTAAG
oCSSau01R	848	60	in <i>gyrB</i>	AATACCGCCCTCATAGTGATAG
oCSSau01F2	854	55	5' of <i>gyrB</i>	TTGTCACTACGACATCTG
oCSSau01R2	854	55	in <i>gyrB</i>	TGGCTCATCATGAATAGG
oCSSau01F3	511	62	START of	ATGGTGACTGCATTGTCAGATG
oCSSau01R3	511	60	in <i>gyrB</i>	TCTTATCAGTTGTGCCAACTTC
oCSSau02F	586	60	in <i>gyrB</i>	AAGTTGGCACAACCTGATAAGAC
oCSSau02R	586	60	in <i>gyrB</i>	ACTTGACGCACTTCAGAATTAC
oCSSau02F2	829	60	in <i>gyrB</i>	ACGCATTGTCACAAGACTTAG
oCSSau02R2	829	61	in <i>gyrB</i>	GCTGATTTACGACGTGTTACTTC
oCSSau03F	834	61	in <i>gyrB</i>	TCTGGTGAAGATACACGTGAAG
oCSSau03R	834	61	in <i>gyrB</i>	CTCAGGGTTCATTGTTGTTTCC
oCSSau03F2	867	62	in <i>gyrB</i>	GACGGATTCAAACGTGCATTAAC
oCSSau03R2	867	62	in <i>gyrB</i>	ACCTTTGTATCGTGCAATAGACC
oCSSau04F	885	61	in <i>gyrB</i>	GCCACCGTTGTATAAACTGAC
oCSSau04R	885	63	in <i>gyrA</i>	ATACCTACCGCGATACCTGATG
oCSSau05F	748	62	in <i>gyrA</i>	CAGCAATGCGTTATACTGAAGC
oCSSau05R	748	62	in <i>gyrA</i>	TGCGTACGTCTTCTAACAACCTG
oCSSau05F2	875	61	in <i>gyrA</i>	CAGTTATCGTTATCCGCTTGTTG
oCSSau05R2	875	61	in <i>gyrA</i>	CAAGTGCGATACGTAATCCTTC
oCSSau06F	657	61	in <i>gyrA</i>	TGTGCGTAAGGATGCAAATG
oCSSau06R	657	60	in <i>gyrA</i>	CGATATGTAGATACCGGCAAAC
oCSSau07F	632	60	in <i>gyrA</i>	GATCGTTTTCGGTGATGATCG
oCSSau07R	632	60	in <i>gyrA</i>	GTTGCTGTACGGCCTAAAG
oCSSau07F2	889	61	in <i>gyrA</i>	GCAACAACGCTTCAAACCTTTC
oCSSau07R2	889	61	in <i>gyrA</i>	AGCTACATCAAGCCCTACAAC
oCSSau08F	949	61	in <i>gyrA</i>	GTGGTGTGCTTAAACGTTTCAG
oCSSau08R	949	60	3' of <i>gyrA</i>	GTGCCACCATCAAGACTTATC
oCSSau08F2	1072	61	in <i>gyrA</i>	GGTATTCTGTAGTGAATGCTATTG
oCSSau08R2	1072	62	3' of <i>gyrA</i>	ATATGTATGTGGTGCCACCATC
oCSSau09F	982	63	5' of <i>parE</i>	TAGCGCCAGTATTACCACTACC
oCSSau09R	982	62	in <i>parE</i>	TGCTCTTGACGCTCTTTACC
oCSSau09F2	821	60	in <i>parE</i>	TGCAGGACGACCTAATACTC
oCSSau09R2	821	60	in <i>parE</i>	CAGAAGATGGCGAACCAC
oCSSau10F	728	60	in <i>parE</i>	CGCCATCTTCTGGTTTAGTG
oCSSau10R	728	60	in <i>parE</i>	AGCTTTACGTGCAGCTTC
oCSSau10F2	788	61	in <i>parE</i>	GCAGGAGGTAAATTTGGACAAG
oCSSau10R2	788	60	in <i>parE</i>	GGCAATTTGTCTGCAACAAC
oCSSau10F3	820	62	in <i>parE</i>	GTGAATGGCTTGAAGTTGAAATCC
oCSSau10R3	820	62	in <i>parE</i>	TTACCTGAACGAGCATCTTCAC
oCSSau11F	707	61	in <i>parE</i>	GCTGTTGTGTCTGTTTCGTATTC

oCSSau11R	707	62	in <i>parE</i>	GTCTGTCCAAGCGTATTCAACTC
oCSSau12F	683	60	in <i>parE</i>	AACCGCTTGTTCAAGCAG
oCSSau12R	683	60	in <i>parC</i>	GGACCATTGCTTCGTACAC
oCSSau12F2	773	62	in <i>parE</i>	TGATACTGATGGTGCGCATATTC
oCSSau12R2	773	62	in <i>parC</i>	ACATGTCGTAACCTCCAGTCTTG
oCSSau13F	841	62	in <i>parE</i>	TTGGTATGCAAGAGGACCAAAG
oCSSau13R	841	61	in <i>parC</i>	ACGTCCATTGCGTAAAGTTTC
oCSSau14F	791	63	in <i>parC</i>	TACGCGACAGATATAACCACCAC
oCSSau14R	791	63	in <i>parC</i>	CCTTCAAGCGCAACTATGTCAG
oCSSau15F	801	61	in <i>parC</i>	ACAGGCTGAAGCAATTGTAATG
oCSSau15R	801	62	in <i>parC</i>	TTAACACCAGCTGCCCTTAATC
oCSSau15F2	949	60	in <i>parC</i>	ACAAGCGTGACGCTAAAG
oCSSau15R2	949	63	in <i>parC</i>	TTTAACGAGCCGCGTTGTG
oCSSau16F	891	60	in <i>parC</i>	AGAATTGGGACAACATGTATCAC
oCSSau16R	891	60	3' of <i>parC</i>	ATTAGACCGAATTGAACCTTCAG
oCSSau16F2	768	61	in <i>parC</i>	GATTTGATTAGTGTTATGCGCTTTG
oCSSau16R2	768	60	3' of <i>parC</i>	AAACCATCCAGGTATTAACTATCG

^aThe sequence used for primer design was the published complete genome of *S. aureus* strain Newman (GenBank accession no. NC 009641)

Table S2a: Summary of all DNA gyrase amino acid variations among fluoroquinolone-susceptible *S. aureus* and MIC values for ATCC 29213 and 13 ocular isolates assigned to Group 1

	GyrA																	GyrB						GenBank accession no.								
	84 #	85 #	88 #	248	255	275	325	402	457	483	495	594	598	668	712	815	818	826	827	837	856	859	860		886	28	66	169	317	491	568	
GenBank strains																																
Newman	S	S	E	V	G	E	T	D	A	D	N	E	V	S	V	E	T	S	T	R	D	E	N	E	V	Q	D	E	G	E	NC_009641	
N315	S	S	E	V	G	E	T	D	T	D	D	E	V	S	V	E	T	S	T	R	D	E	N	E	V	K	D	E	G	E	NC_002745	
NCTC8325	S	S	E	V	G	E	T	D	A	D	D	E	V	S	V	E	T	S	T	R	D	E	N	E	V	Q	D	E	G	E	NC_007795	
RF122	S	S	E	V	R	E	T	D	A	E	D	E	V	S	V	--	V	--	--	R	D	E	N	E	V	Q	D	E	D	K	NC_007622	
MSSA476	S	S	E	V	G	E	T	D	A	D	D	E	V	S	V	E	T	S	T	R	D	E	N	E	V	Q	D	E	G	E	NC_002953	
COL	S	S	E	V	G	E	T	D	A	D	N	E	V	S	V	E	T	S	T	R	D	E	N	E	A	Q	D	E	G	E	NC_002951	
MW2	S	S	E	V	G	E	T	D	A	D	D	E	V	S	V	E	T	S	T	R	D	E	N	E	V	Q	D	E	G	E	NC_003923	

indicates amino acid positions located within the QRDR

^a Consensus residues reported for a total of 21 fluoroquinolone-susceptible isolates, bolded residues indicate the most prevalent amino acid

-- indicates amino acid deletions at GyrA positions 815, 826, and 827

Table S2b: Summary of all topoisomerase IV amino acid variations among fluoroquinolone-susceptible *S. aureus*, including ATCC 29213 and 13 ocular isolates assigned to Group 1

	ParC																			ParE																		
	45	80 #	84 #	144	223	267	320	372	400	402	404	410	437	521	594	641	650	656	688	694	70	107	139	230	327	330	422	432 #	478	530	585	596	609					
GenBank strains																																						
Newman	I	S	E	P	I	S	S	R	R	A	E	Y	E	F	F	D	K	V	A	M	I	G	N	S	V	P	E	D	H	G	P	E	V					
N315	I	S	E	P	I	S	S	R	R	A	E	F	E	F	F	D	K	V	A	M	I	G	N	S	V	P	E	D	H	G	P	E	V					
NCTC8325	I	S	E	P	I	S	S	R	R	A	E	Y	E	F	F	D	K	V	A	M	I	G	N	S	V	P	E	D	H	G	P	E	V					
RF122	I	S	E	P	I	S	S	H	R	A	E	Y	E	F	F	N	K	I	A	M	I	S	N	G	I	P	D	D	H	G	P	E	L					
MSSA476	I	S	E	P	I	S	S	R	R	A	E	Y	E	F	F	D	K	V	A	V	I	G	N	S	V	P	E	D	H	D	P	E	V					
COL	I	S	E	P	I	S	S	R	R	A	E	Y	E	F	F	D	K	V	A	M	I	G	N	S	V	P	E	D	H	G	P	E	V					
MW2	I	S	E	P	I	S	S	R	C	A	E	Y	E	F	F	D	K	V	A	M	I	G	N	S	V	P	E	D	H	G	P	E	V					
Study strains																																						
ATCC29213	I	S	E	P	I	S	S	R	R	A	E	F	E	F	F	D	K	V	A	M	I	G	S	S	V	P	E	D	H	G	P	E	V					
Sau31	I	S	E	P	I	S	S	R	R	A	G	F	E	F	F	D	R	V	A	M	I	G	N	S	V	P	E	D	H	G	P	E	V					
Sau38	I	S	E	S	I	S	S	R	R	A	E	Y	E	Y	F	D	K	I	A	M	I	G	N	S	V	P	D	D	H	G	P	E	V					
Sau12	I	S	E	P	I	S	S	R	R	A	E	Y	E	F	F	D	K	V	A	M	I	G	N	S	V	P	E	D	H	G	P	E	V					
Sau13	I	S	E	P	I	S	S	R	R	A	E	Y	E	F	F	D	K	V	A	M	I	G	N	S	V	P	E	D	H	G	P	E	V					
Sau32	M	S	E	P	I	G	S	R	R	A	E	Y	E	F	Y	D	K	V	V	M	I	G	N	S	V	P	D	D	H	G	P	D	V					
Sau33	I	S	E	S	I	S	S	R	R	A	E	Y	E	Y	F	D	K	I	A	M	I	G	N	S	V	P	D	D	H	G	P	E	V					
Sau34	I	S	E	P	V	S	S	R	R	A	E	F	E	F	F	D	K	V	A	M	I	G	N	S	V	P	E	D	Y	G	P	E	V					
Sau35	I	S	E	P	I	S	S	R	R	A	E	Y	E	F	F	D	K	V	A	M	I	G	N	S	V	P	E	D	H	G	P	E	V					
Sau36	I	S	E	P	V	S	S	R	R	A	E	F	E	F	F	D	K	V	A	M	I	G	N	S	V	P	E	D	Y	G	P	E	V					
Sau37	I	S	E	P	V	S	S	R	R	A	E	F	E	F	F	D	K	V	A	M	I	G	N	S	V	P	E	D	Y	G	P	E	V					
Sau39	I	S	E	P	V	S	S	R	R	A	E	F	E	F	F	D	K	V	A	M	I	G	N	S	V	P	E	D	H	G	P	E	V					
Sau7	I	S	E	P	I	S	S	R	R	A	E	Y	E	F	F	D	K	V	A	M	I	G	N	S	V	P	E	D	H	G	P	E	V					
Sau8	I	S	E	P	I	S	S	R	R	A	E	Y	E	F	F	D	K	V	A	M	I	G	N	S	V	P	E	D	H	G	P	E	V					
FQ-S Consensus ^a	I M	S	E	P S	I V	S G	S	R H	R C	A	E G	Y F	E	F Y	F Y	D N	K R	V I	A V	M V	I	G S	N S	S G	V I	P	E D	D	H Y	G D	P	E D	V L					

indicates amino acid positions located within the QRDR

^a Consensus residues reported for a total of 21 fluoroquinolone-susceptible isolates, bolded residues indicate the most prevalent amino acid

Table S3: Summary of all DNA gyrase and topoisomerase IV amino acid variations and MIC values among 12 fluoroquinolone-resistant ocular isolates assigned to Group 2

	GyrA											GyrB	ParC					ParE				MIC (µg/ml)					
	84 #	85 #	88 #	275	325	402	815	818	826	827	859	169	80 #	84 #	320	402	437	70	330	432 #	585	BES	MXF	GAT	CIP	LVX	
FQ-S Consensus ^a	S	S	E	E	T	D	E	T	S	T	E	D	S	E	S	A	E	I	P	D	P	0.03	0.06	0.12	0.25	0.25	
Study strains																											
Sau21	L	Y	0.25	2	2	16	4	
Sau22	L	F	0.25	2	2	16	8	
Sau30	L	F	0.25	1	1	16	4	
Sau42	L	Y	0.25	2	2	8	4	
Sau43	L	Y	0.25	2	2	16	8	
Sau11	L	Y	0.5	2	2	16	4	
Sau19	L	Y	0.5	2	2	16	4	
Sau23	L	Y	0.5	2	2	16	8	
Sau41	L	Y	0.5	2	2	16	4	
Sau45	L	Y	0.5	2	2	16	8	
Sau5	L	F	0.5	2	2	32	8	
Sau48	L	F	S	.	1	4	8	256	32	
MIC ₅₀																						0.5	2	2	16	8	

Periods indicate no change from the most prevalent amino acid in the fluoroquinolone-susceptible consensus sequence

indicates amino acid positions located within the QRDR

^a Consensus residues reported for a total of 21 fluoroquinolone-susceptible isolates, bolded residues indicate the most prevalent amino acid (see Tables S2a and S2b)

-- indicates amino acid deletions at GyrA positions 815, 826, and 827

Table S4: Summary of all DNA gyrase and topoisomerase IV amino acid variations and MIC values among 6 fluoroquinolone-resistant ocular isolates assigned to Group 3

	GyrA											GyrB	ParC					ParE				MIC (µg/ml)					
	84 #	85 #	88 #	275	325	402	815	818	826	827	859	169	80 #	84 #	320	402	437	70	330	432 #	585	BES	MXF	GAT	CIP	LVX	
FQ-S Consensus ^a	S	S	E	E	T	D	E	T	S	T	E	D	S	E	S	A	E	I	P	D	P	0.03	0.06	0.12	0.25	0.25	
Study strains																											
Sau1	L	Y	G	1	8	8	256	64	
Sau2	L	Y	G	.	V	1	8	4	256	32	
Sau28	L	Y	G	1	8	8	256	64	
Sau29	L	Y	G	1	8	8	256	64	
Sau44	L	Y	G	1	8	8	256	64	
Sau16	L	Y	G	2	16	16	256	128	
MIC ₅₀																						1	8	8	256	64	

Periods indicate no change from the most prevalent amino acid in the fluoroquinolone-susceptible consensus sequence

indicates amino acid positions located within the QRDR

^a Consensus residues reported for a total of 21 fluoroquinolone-susceptible isolates, bolded residues indicate the most prevalent amino acid (see Tables S2a and S2b)

-- indicates amino acid deletions at GyrA positions 815, 826, and 827

Table S5: Summary of all DNA gyrase and topoisomerase IV amino acid variations and MIC values among 3 fluoroquinolone-resistant ocular isolates assigned to Group 4

	GyrA											GyrB	ParC					ParE				MIC (µg/ml)					
	84 #	85 #	88 #	275	325	402	815	818	826	827	859	169	80 #	84 #	320	402	437	70	330	432 #	585	BES	MXF	GAT	CIP	LVX	
FQ-S Consensus ^a	S	S	E	E	T	D	E	T	S	T	E	D	S	E	S	A	E	I	P	D	P	0.03	0.06	0.12	0.25	0.25	
Study strains																											
Sau18	L	P	.	.	.	E	--	E	--	--	V	.	F	N	.	4	8	16	128	128	
Sau51	L	P	F	H	.	4	32	32	128	512	
Sau52	L	P	Y	H	.	4	64	32	128	512	
Median ^b																						4	32	32	128	512	

Periods indicate no change from the most prevalent amino acid in the fluoroquinolone-susceptible consensus sequence

indicates amino acid positions located within the QRDR

^a Consensus residues reported for a total of 21 fluoroquinolone-susceptible isolates, bolded residues indicate the most prevalent amino acid (see Tables S2a and S2b)

-- indicates amino acid deletions at GyrA positions 815, 826, and 827

^b Median value was used because the MIC₅₀ value could not be calculated for n<5 isolates

Table S6: Summary of all DNA gyrase and topoisomerase IV amino acid variations and MIC values among 10 fluoroquinolone-resistant ocular isolates assigned to Group 5

	GyrA											GyrB	ParC					ParE				MIC (µg/ml)					
	84 #	85 #	88 #	275	325	402	815	818	826	827	859	169	80 #	84 #	320	402	437	70	330	432 #	585	BES	MXF	GAT	CIP	LVX	
FQ-S Consensus ^a	S	S	E	E	T	D	E	T	S	T	E	D	S	E	S	A	E	I	P	D	P	0.03	0.06	0.12	0.25	0.25	
Study strains																											
Sau49	L	P	Y	G	.	.	D	4	32	32	256	512	
Sau15	L	P	Y	G	4	32	64	256	512	
Sau26	L	P	Y	G	4	64	64	256	512	
Sau3	L	P	Y	G	S	.	.	4	32	64	256	512	
Sau40	L	P	Y	G	4	64	64	256	512	
Sau47	L	P	Y	G	S	.	.	4	32	64	256	512	
Sau50	L	P	Y	G	S	.	.	4	64	64	256	512	
Sau54	L	P	Y	G	4	64	64	256	1024	
Sau55	L	P	Y	G	4	64	64	256	512	
Sau6	L	P	Y	G	4	32	64	256	512	
MIC ₅₀																						4	64	64	256	512	

Periods indicate no change from the most prevalent amino acid in the fluoroquinolone-susceptible consensus sequence

indicates amino acid positions located within the QRDR

^a Consensus residues reported for a total of 21 fluoroquinolone-susceptible isolates, bolded residues indicate the most prevalent amino acid (see Tables S2a and S2b)

-- indicates amino acid deletions at GyrA positions 815, 826, and 827

Table S7: Summary of all DNA gyrase and topoisomerase IV amino acid variations and MIC values among 8 fluoroquinolone-resistant ocular isolates assigned to Group 6

		GyrA										GyrB	ParC					ParE				MIC (µg/ml)					
		84 #	85 #	88 #	275	325	402	815	818	826	827	859	169	80 #	84 #	320	402	437	70	330	432 #	585	BES	MXF	GAT	CIP	LVX
FQ-S Consensus ^a		S	S	E	E	T	D	E	T	S	T	E	D	S	E	S	A	E	I	P	D	P	0.03	0.06	0.12	0.25	0.25
Study strains																											
	Sau25	L	.	A	D	Y	G	2	32	32	256	256
	Sau46	L	.	A	Y	G	F	2	32	32	256	256
	Sau27	L	.	A	Y	G	4	32	32	256	256
	Sau17	L	.	K	Y	G	4	64	64	256	512
	Sau20	L	.	K	Y	G	4	64	128	256	512
	Sau53	L	.	K	.	I	Y	G	4	64	128	256	512
	Sau14	L	.	K	H	.	Y	G	.	.	.	T	.	.	.	8	64	128	256	512
	Sau24	L	.	K	Y	G	8	64	128	256	512
MIC ₅₀																						4	64	128	256	512	

Periods indicate no change from the most prevalent amino acid in the fluoroquinolone-susceptible consensus sequence

indicates amino acid positions located within the QRDR

^a Consensus residues reported for a total of 21 fluoroquinolone-susceptible isolates, bolded residues indicate the most prevalent amino acid (see Tables S2a and S2b)

-- indicates amino acid deletions at GyrA positions 815, 826, and 827