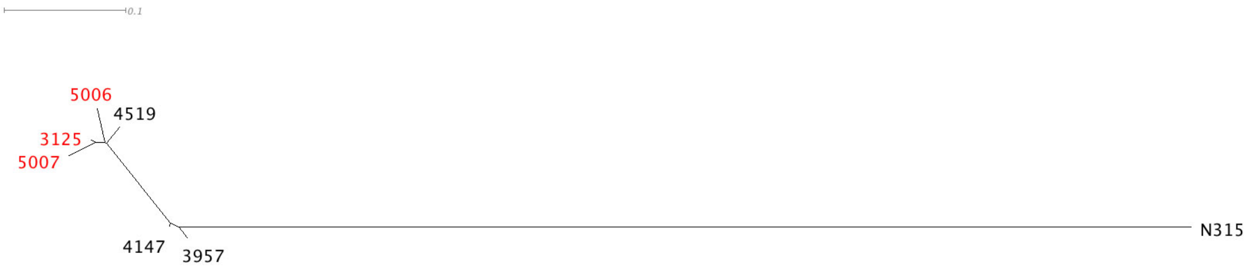
Supplemental Material



	N315	TMHS-SA-5007	TMHS-SA-5006	TMHS-SA-4519	TMHS-SA-4147	TMHS-SA-3957	TMHS-SA-3125
TMHS-SA-3125	523	16	22	16	55	66	
TMHS-SA-3957	467	72	74	68	11		
TMHS-SA-4147	468	67	65	57			
TMHS-SA-4519	521	30	26				
TMHS-SA-5006	529	34					
TMHS-SA-5007	525						
N315							

Figure S1. Neighbor-joining tree showing estimates of the phylogenetic relationship between the patient isolates. The tree shows the phylogenetic relationship between the genomes of the isolates studied. The genome of strain N315 was used as reference[1]. Ceftaroline-resistant isolates (MIC > 32 mg/L) are highlighted in red. The pairwise distances (i.e. the number of SNPs by which the strains differ) based on SNPs are shown in the bottom figure.

Table S1. Sequences of primers used for *mecA* mutagenesis.

Mutation	Primer
E239K	5’-CTAATGA AACAAAGAGTCGTAACTATCC
Y446N	5’-CGTTACAAGAAATGAAGTGGTAAATGG
E447K	5’-CGTTACAAGATATAAAGTGGTAAATGG
Y446N-E447K	5’-CGTTACAAGAAATAAAGTGGTAAATGG
E239K-Y446N-E447K*	5’-CGTTACAAGAAATAAAGTGGTAAATGG 5’-CTAATGA AACAAAGAGTCGTAACTATCC

*To create the E239K-Y446N-E447K triple mutant, a two-step mutagenesis procedure was performed and therefore two primers are listed.

Table S2. SNPs identified in ceftaroline-resistant isolates

Genome Position	**Gene**			**Base**		**Codon**		**Amino Acid**		**Change**
	ID	Name	Position	**Ref**	**Sam**	**Ref**	**Sam**	**Ref**	**Sam**	
3708	SA0003	--	39	C	T	GAC	GAT	D	D	S
45699	SA0038	*mecA*	1339	C	T	GAA	AAA	E	K	NS
45702	SA0038	*mecA*	1336	A	T	TAT	AAT	Y	N	NS
63952	SA0057	*ccrB*	1074	C	T	AAG	AAA	K	K	S
83412	SA0073	--	320	C	T	AGC	AAC	S	N	NS
88746	SA0078	--	1055	A	G	GAT	GGT	D	G	NS
98144	SA0088	--	274	A	G	ATT	GTT	I	V	NS
166783	SA0143	*adhE*	2327	C	T	GCA	GTA	A	V	NS
230901	SA0195	--	1384	G	A	GAA	AAA	E	K	NS
231999	SA0197	--	250	T	C	TTT	CTT	F	L	NS
254026	SA0214	*uhpT*	759	G	A	CTG	CTA	L	L	S
271741	SA0225	--	371	C	T	AGC	AAC	S	N	NS
296739	IG									
342882	SA0285	--	48	A	G	GTA	GTG	V	V	S
354593	IG									
543212	SA0470	*hslO*	8	A	G	CAC	CGC	H	R	NS
737892	SA0643	--	777	T	C	CAT	CAC	H	H	S
954599	IG									
994627	SA0877	*prfC*	412	A	G	ATT	GTT	I	V	NS
1167831	SA1030	--	618	T	C	CGT	CGC	R	R	S
1252611	SA1106	*proS*	701	A	G	GAA	GGA	E	G	NS
1309983	IG									
1363647	SA1193	*fmtC*	36	C	T	ATC	ATT	I	I	S
1433445	SA1260	*thyA*	97	A	G	TCG	CCG	S	P	NS

1433941	IG									
1487170	SA1283	*pbp2*	515	C	T	GCA	GTA	A	V	NS
1498423	SA1291	--	630	G	A	CGC	CGT	R	R	S
1521664	SA1313	--	123	T	C	GGA	GGG	G	G	S
1556732	SA1346	*bmfBB*	63	T	C	TTA	TTG	L	L	S
1585604	SAS047	*rpmG*	110	G	T	TCA	TAA	S	*	NS
1818069	SA1579	*leuS*	525	A	G	TCT	TCC	S	S	S
1823243	SA1584	--	368	C	T	GGA	GAA	G	E	NS
1977307	IG									
2052862	IG									
2075143	SA1838	--	652	G	A	GGA	AGA	G	R	NS
2117791	IG									
2210418	SA1959	*glmS*	1471	C	T	GCA	ACA	A	T	NS
2236392	SA1969	--	1004	T	A	GAT	GTT	D	V	NS
2282754	SA2010	--	984	G	A	GAC	GAT	D	D	S
2308472	SA2048	*rpsJ*	170	T	A	AAG	ATG	K	M	NS
2308476	SA2048	*rpsJ*	166	G	A	CAT	TAT	H	Y	NS
2469590	SA2200	--	515	A	C	CTT	CGT	L	R	NS
2484998	SA2214	*bioA*	1044	T	C	TCA	TCG	S	S	S
2533830	SA2256	--	668	T	C	GAC	GGC	D	G	NS
2565384	SA2287	*sarH2*	583	T	C	TCA	CCA	S	P	NS
2585465	IG									
2646228	SA2354	--	622	C	T	GGG	AGG	G	R	NS
2699250	SA2408	*cudT*	1097	A	G	TTA	TCA	L	S	NS
2743211	SA2439	--	1145	T	C	GAT	GGT	D	G	NS
2748178	SA2442	--	1686	A	G	AAT	AAC	N	N	S
2785787	IG									

This table shows the 51 SNPs identified in all ceftaroline resistant isolates (MIC > 32 mg/L) after filtering out the SNPs in common with susceptible isolate 3957. The genome of strain N315 was used as reference. Ref = Reference, Sam = Sample, NS = nonsynonymous, S = synonymous, IG = intergenic SNP, -- = name not annotated in reference genome. Nucleotide and amino acid codes are IUPAC standard.

Table S3. Nonsynonymous single nucleotide polymorphisms in *mecA* (PBP2a) active site causing high level resistance to ceftaroline in *S. aureus.*

Genome Position	**Gene**			**Base**		**Codon**		**Amino Acid**	
	ID	**Name**	**Position**	**Ref**	**Sam**	**Ref**	**Sam**	**Ref**	**Sam**
45699	SA0038	*mecA*	1339	C	T	GAA	AAA	E	K
45702	SA0038	*mecA*	1336	A	T	TAT	AAT	Y	N

The table shows the two SNPs identified in all ceftaroline-resistant isolates (MIC >32 mg/L) hypothesized to be responsible for the observed resistance phenotype. These two SNPs were not identified by VAAL simultaneously in the susceptible isolates. The genome of strain N315 was used as the reference. Ref = Reference, Sam = Sample. Nucleotide and amino acid codes are IUPAC standard.

Table S4. Ceftaroline IC_{50} values from competition binding assays

PBP2a Strain	**IC_{50} (μM)**
WT	1.5
E239K	0.6
Y446N	> 500[a]
E447K	1.4
Y446N-E447K	49
E239K-Y446N-E447K	40

The IC_{50} value of ceftaroline for PBP2a WT and mutants was determined using a competition assay with bocillin (20).

[a] The Y446N mutant did not display inhibition at the highest concentration of ceftaroline that could be used in this assay. Thus, we have provided an estimate of its IC_{50} value.

SUPPLEMENTAL REFERENCE

1. Kuroda M, Ohta, T, Uchiyama I, et al. Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. Lancet 2001;357:1225-40.