

Supplementary webappendix

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Supplemental text to “Genomic epidemiology of *Neisseria gonorrhoeae* with reduced susceptibility to cefixime in the United States”

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METHODS

Sample preparation

Neisseria gonorrhoeae isolates for MIC determination and DNA isolation were cultivated on GC Base agar (Difco/BD, Franklin Lakes, NJ) supplemented with 1% IsoVitaleX (BBL/BD, Franklin Lakes, NJ) with incubation at 35°C in 5·0% CO₂. Whole chromosomal DNA was isolated using the ArchivePure DNA purification kit Protocol 18 (5PRIME GbmH, Gaithersburg, MD) as described by the manufacturer with the following modifications: 1/3 of a 10 µl loop of overnight growth was suspended in 300 µl cell lysis solution, incubated at 56°C for 30 minutes, with centrifugation time increased to 4 minutes in the protein precipitation step. In the DNA precipitation, the protocol was modified in that the tube was spun down briefly to facilitate removal of remaining ethanol. In the DNA hydration step, the rehydration time was changed to 1 h 15 minutes at 56°C.

MIC determination by agar dilution and Etest

Agar plate dilution MIC values were determined and interpreted as previously described (see <http://www.cdc.gov/std/gisp/GISP-Protocol07-15-2010.pdf>).

Genome comparison visualizations

Pairwise comparisons of gonococcal genomes, as shown in Supplemental Figure S12, were visualized using the Artemis Comparison Tool.

RESULTS

Comparison to MLST and NG-MAST

Comparison of the whole-genome phylogeny with multilocus typing schemes, MLST and NG-MAST, which are based on 7 housekeeping loci and two hypervariable genes (*porB* and *tbpB*), respectively, shows that the cef^{RS} isolates include many sequence types (Supplemental Figure S2). Among the cef^{RS}, MLST 1901 and NG-MAST 1407 are the most common sequence types. However, while MLST 1901 comprises 73% of cef^{RS}, it also comprises the plurality (47%) of cefixime susceptible isolates. NG-MAST1407 represents 38% of the cef^{RS} isolates and is, along with NG-MAST 2992, the largest single NG-MAST type among the cefixime susceptible isolates (both 9·3%). As expected, both typing schemes are incapable of capturing the resolution attainable from whole genome-based methods (Supplemental Figure S2 and

Supplemental Table S2). Genome sequencing also reveals the limits of NG-MAST, as *tbpB* is a null allele in one cluster of isolates due to presence of an *N. meningitidis* *tbpB* that lack the PCR primer target sequences at this locus (Supplemental Figure S11).

Interspecies mosaicism

Neisseria are known to undergo interspecies recombination. Evidence for this process in *N. gonorrhoeae* includes mosaic *penA* alleles and the novel *penA* alleles described below, as well as the suspected recombination with *N. meningitidis* *tbpB*. Of the predicted recombinant regions in this dataset at least 50 bp in length and with unique matches in the *de novo* assembled genomes, 6.0% match most closely to non-gonococcal *Neisseria* species based on comparison to the nt database. Additional loci that appear to have been shared among *Neisseria* include DhpS, involved in sulfonamide resistance, and regions of the *lgt* locus, involved in production of lipooligosaccharide.

penA genotype – phenotype correlation and MIC retesting

Seven isolates initially reported as susceptible carry mosaic *penA* XXXIV, one isolate (GCGS126) with mosaic *penA* XXXIV varying by a single amino acid substitution (E101D) was initially reported as susceptible, and two isolates initially reported as *cef^{RS}* were found to have the same *penA* amino acid sequences as susceptible isolates. On rechecking MICs for each of these isolates by agar dilution, isolates with *penA* XXXIV and the isolate with a single amino acid substitution were determined to have MICs of 0.125 mg/mL, with the exception of two isolates, GCGS046 and GCGS048, which remained were 0.06 mg/mL and 0.03 mg/mL, respectively, and that the two isolates initially reported as *cef^{RS}* that share *penA* alleles with susceptible isolates are in fact susceptible to cefixime (MICs decreased from 0.25 mg/mL to 0.03-0.06 mg/mL; Supplemental Table S2). The *penA* allele in GCGS126 (with MIC of 0.125 mg/mL) differs from mosaic *penA* XXXIV by only one amino acid, but there are a total of 8 nucleotide differences (7 synonymous substitutions) also mostly clustered in the C-terminal transpeptidase domain, suggesting a likely recombination event.

1. Carver TJ, Rutherford KM, Berriman M, Rajandream MA, Barrell BG, Parkhill J. ACT: the Artemis Comparison Tool. *Bioinformatics*. 2005; **21**(16): 3422-3.

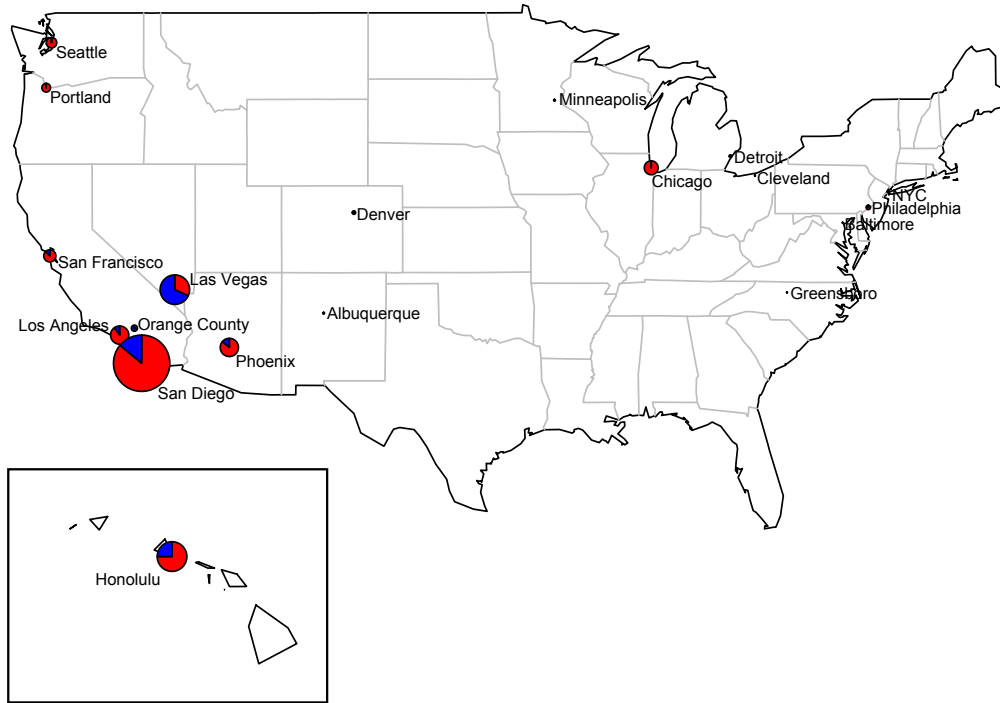


Figure S1. Map of clinic locations contributing isolates to this study. The size of the pie charts is proportional to the number of isolates from each location; blue represents the fraction of isolates from self-identified MSM and red from MSM.

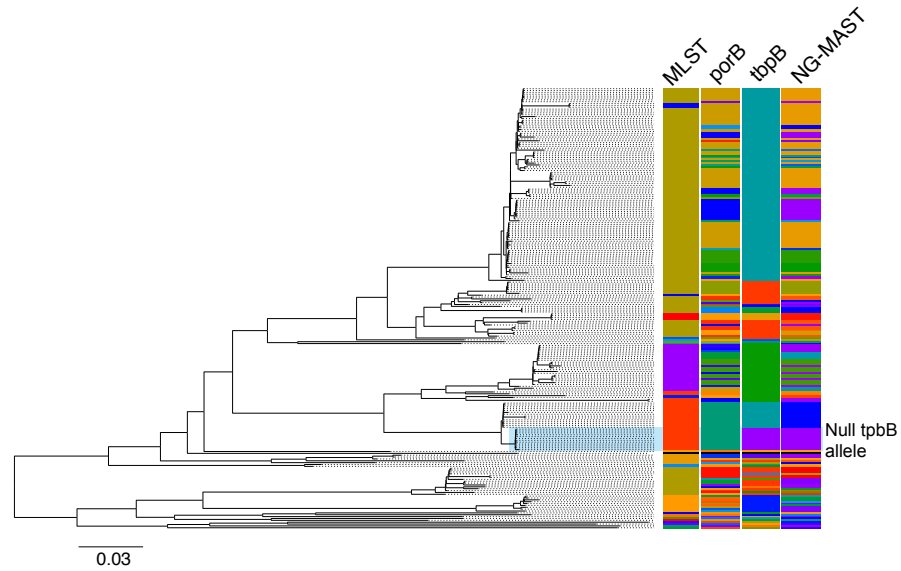


Figure S2. Maximum likelihood phylogeny from whole genome SNPs from the 236 isolates and the reference genome. The *in silico* determined MLST, *porB*, *tbpB*, and NG-MAST types are denoted in the columns as labeled. Highlighted in the blue rectangle is a branch of isolates with a null *tbpB* allele; the common ancestor of these isolates underwent recombination replacing the *N. gonorrhoeae* *tbpB* with the *tbpB* locus from *N. meningitidis* (see Supplemental Figure S11). Full listings of MLST and NG-MAST types are in Supplemental Table S1.

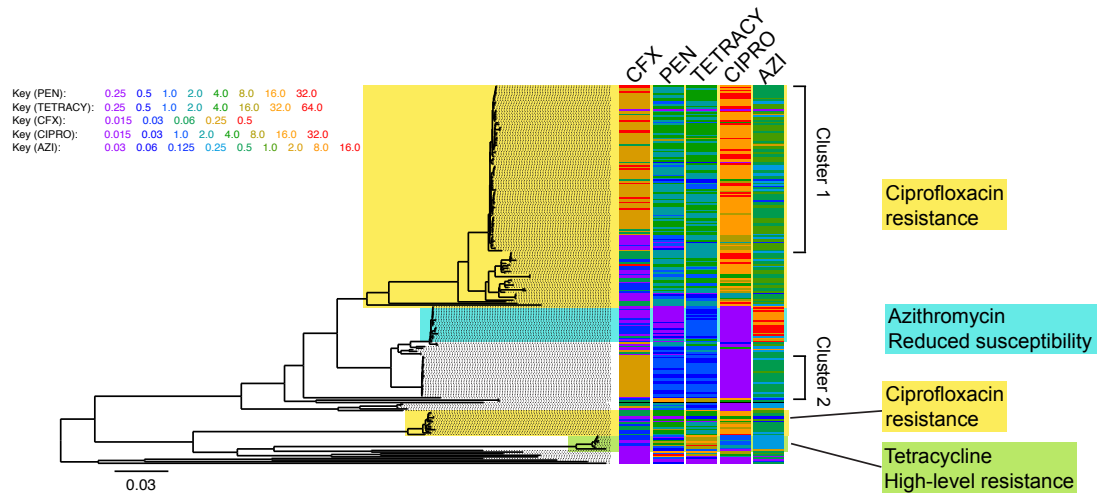


Figure S3. Maximum likelihood phylogeny from core genome SNPs (predicted recombinant regions removed) and MICs for penicillin, tetracycline, cefixime, ciprofloxacin, and azithromycin. Blue and green rectangles highlight branches of the tree that are notable for sharing elevated MICs, including clusters with resistance to ciprofloxacin (MIC \geq 1 mg/mL), high level resistance to tetracycline (MIC \geq 16 mg/mL), and reduced susceptibility to azithromycin (MIC \geq 2 mg/mL). Clusters of isolates with reduced susceptibility to cefixime are not highlighted on this figure.

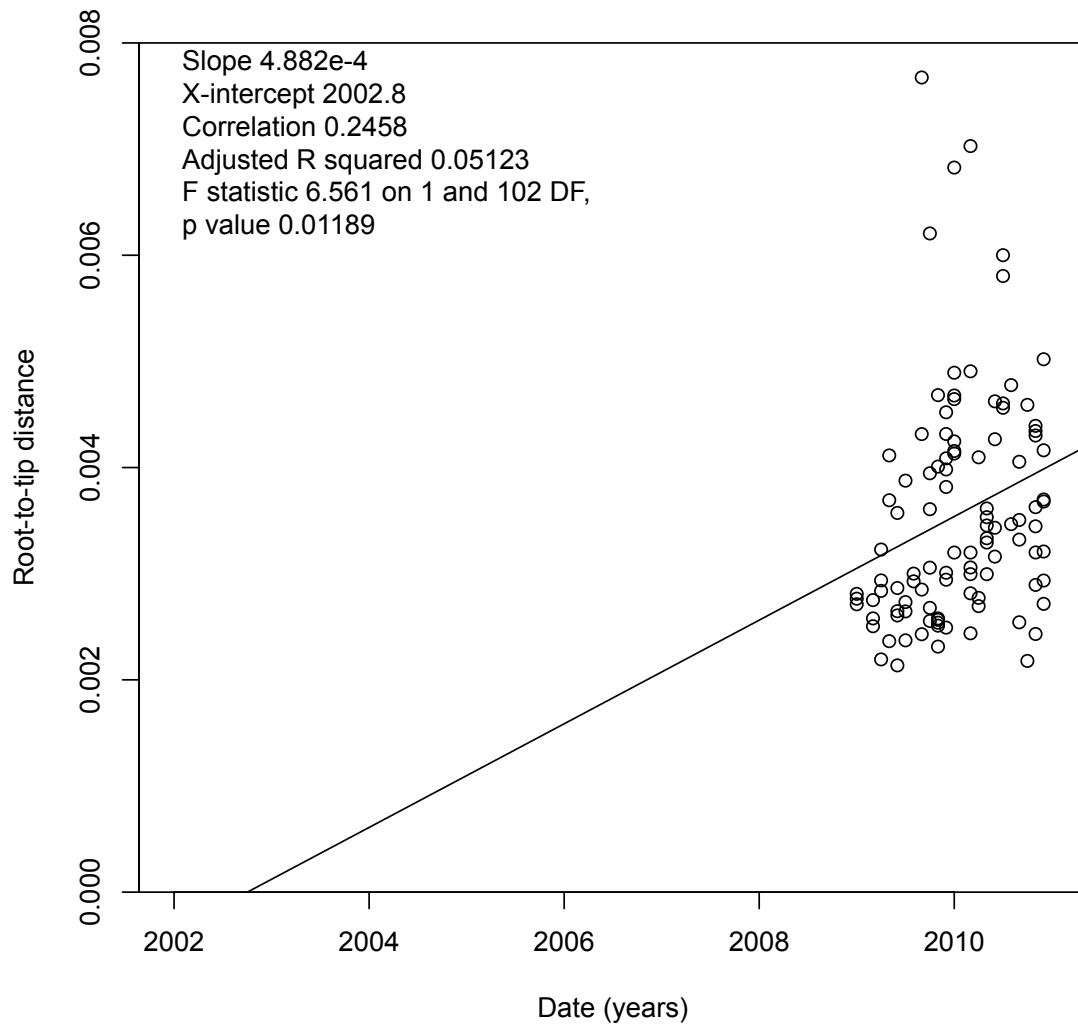


Figure S4. Root-to-tip distance by year for all isolates in cluster 1, with linear regression showing that the branching of the isolates in the maximum likelihood phylogeny based on the core-SNPs (Figure 1) demonstrates phylogenetic signal.

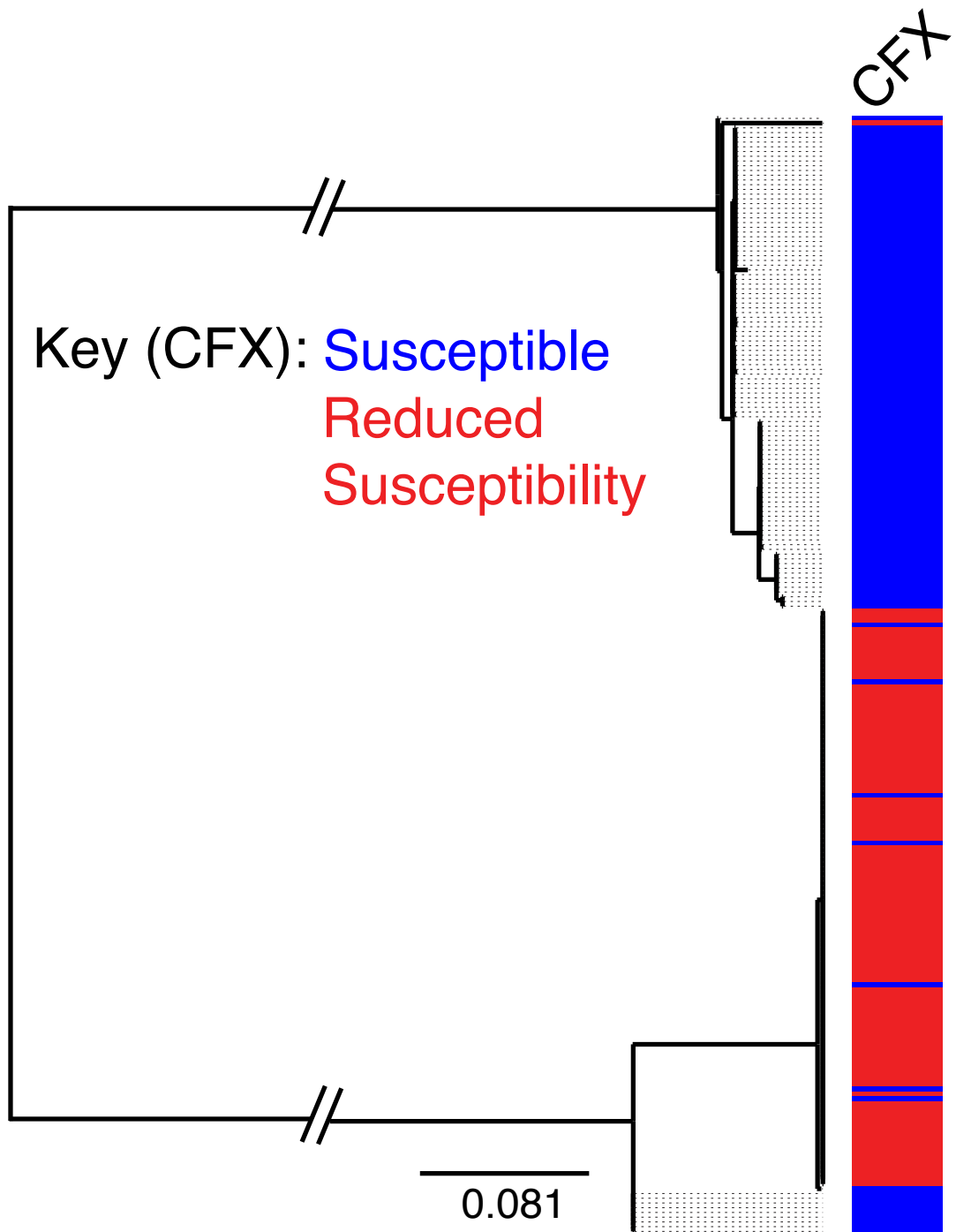


Figure S5. Maximum likelihood phylogenies of *penA* with accompanying report of cefixime susceptibility (red) or reduced susceptibility (blue) for each isolate in the column on the right.

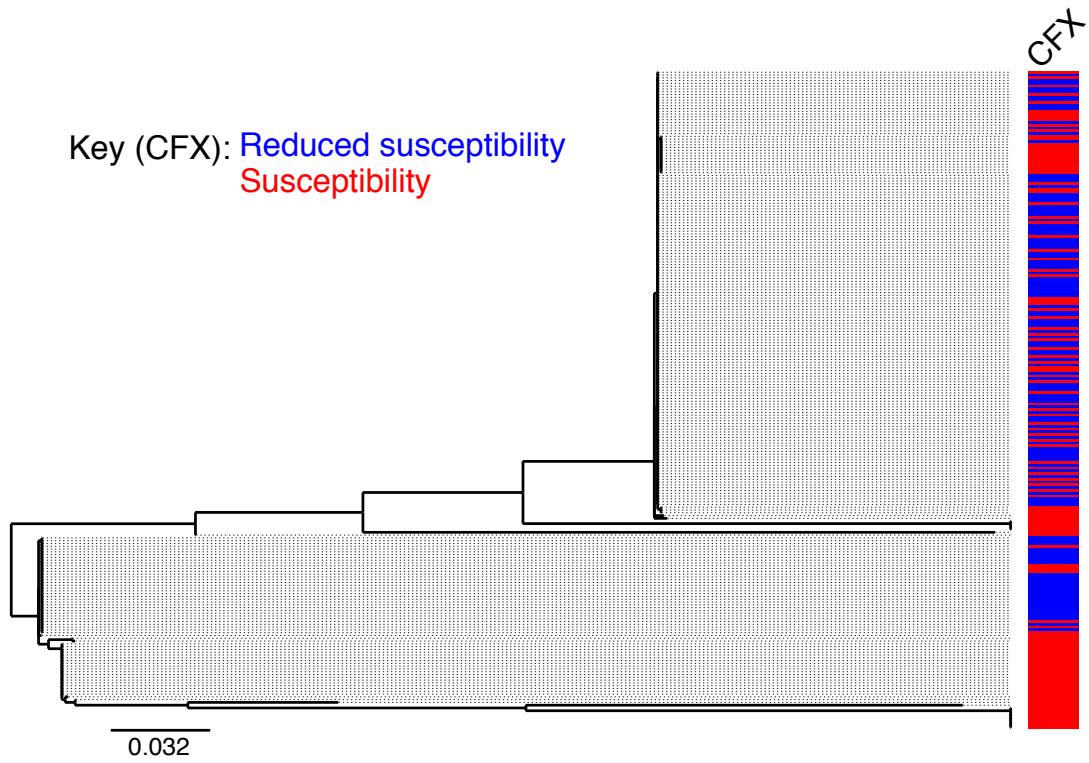


Figure S6. Maximum likelihood phylogenies of *mtrR* with accompanying report of cefixime susceptibility (red) or reduced susceptibility (blue) for each isolate in the column on the right.

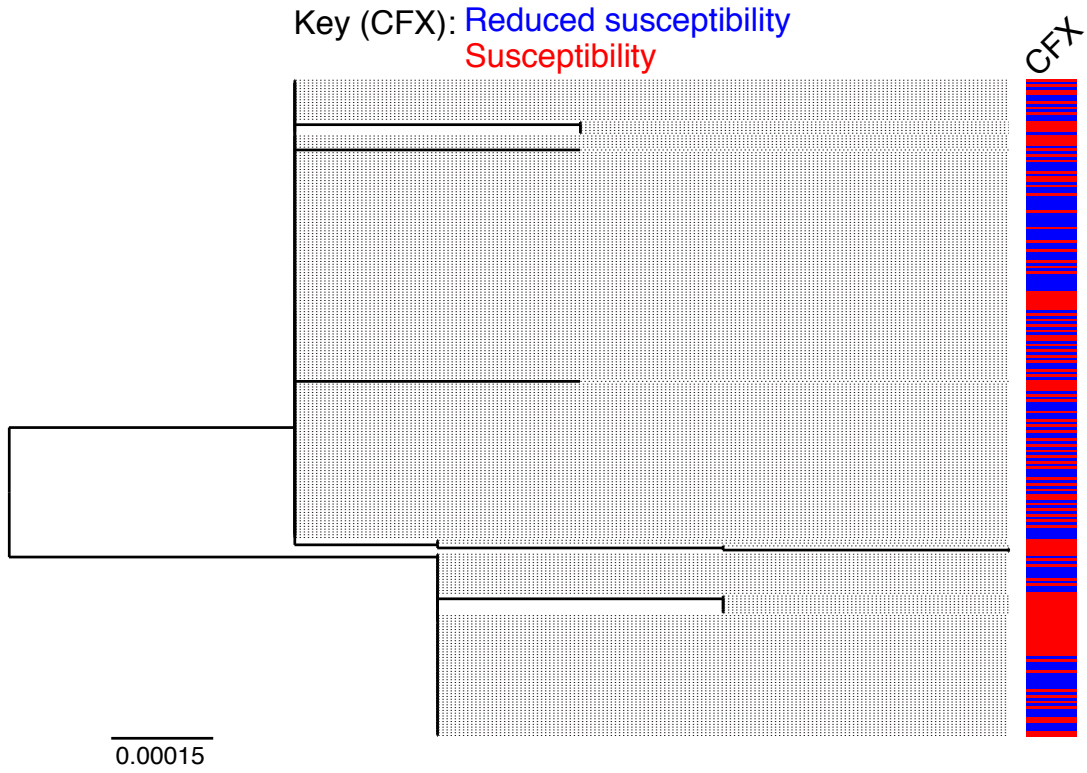


Figure S7. Maximum likelihood phylogenies of *ponA* with accompanying report of cefixime susceptibility (red) or reduced susceptibility (blue) for each isolate in the column on the right.

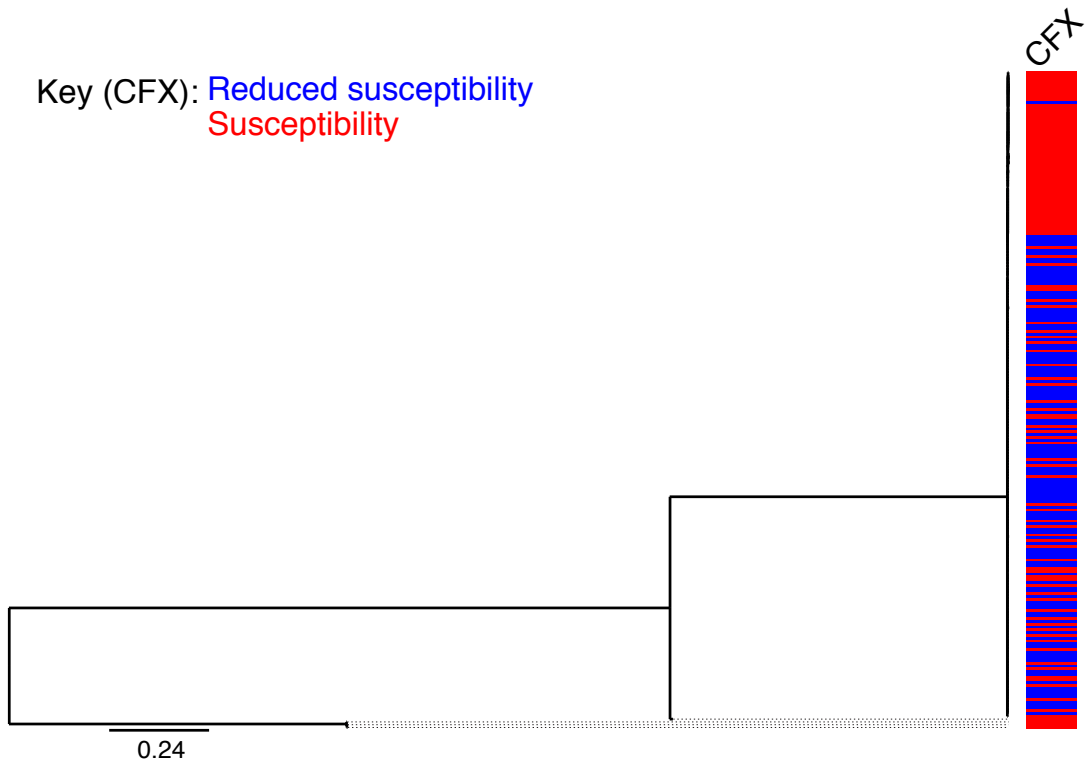


Figure S8. Maximum likelihood phylogenies of pilQ with accompanying report of cefixime susceptibility (red) or reduced susceptibility (blue) for each isolate in the column on the right.

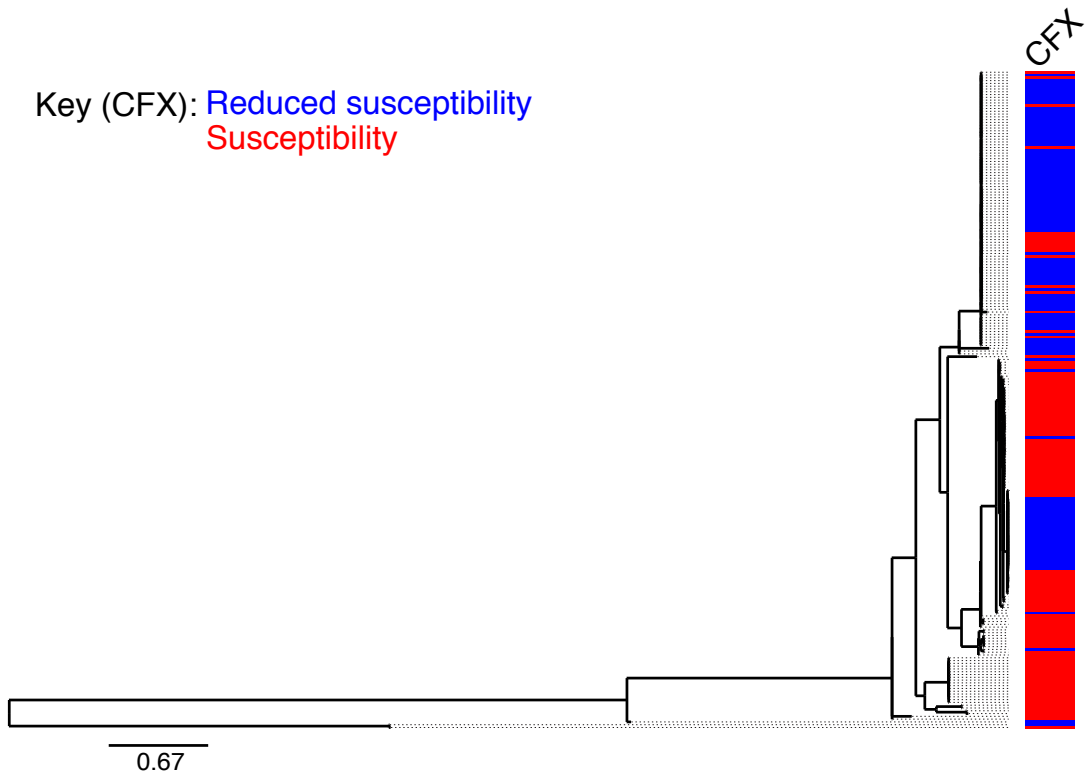


Figure S9. Maximum likelihood phylogenies of *porB* with accompanying report of cefixime susceptibility (red) or reduced susceptibility (blue) for each isolate in the column on the right.

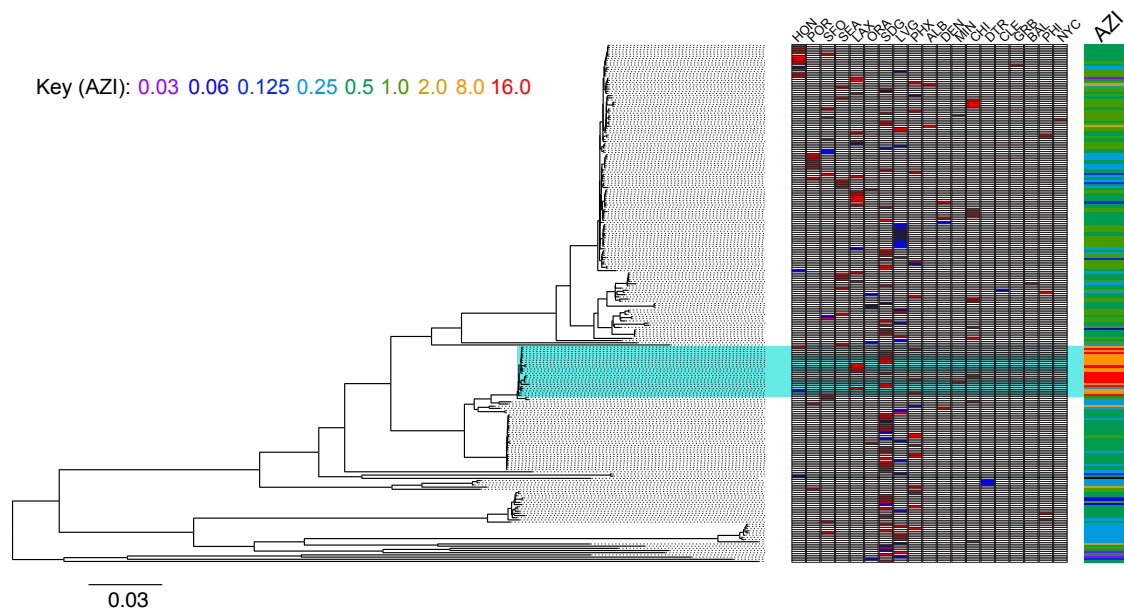


Figure S10. Maximum likelihood phylogeny from core genome SNPs (predicted recombinations removed). The geographic location of the clinic of origin for each isolate is denoted in the matrix to the right of the phylogenetic tree, colored according to sexual orientation of the infected individual (red=MSM, blue=MSW). Location abbreviations: HON – Honolulu; POR – Portland; SFO – San Francisco; SEA – Seattle; LAX – Los Angeles; ORA – Orange County; SDG – San Diego; LVG – Las Vegas; PHX – Phoenix; ALB – Albuquerque; DEN – Denver; MIN – Minneapolis; CHI – Chicago; DTR – Detroit; CLE – Cleveland; GRB – Greensborough; BAL – Baltimore; PHI – Philadelphia; NYC – New York City. (C) The azithromycin MIC ($\mu\text{g}/\text{mL}$) is presented in the column on the far right. The cluster of azi^{RS} isolates is indicated in the blue highlighted region.

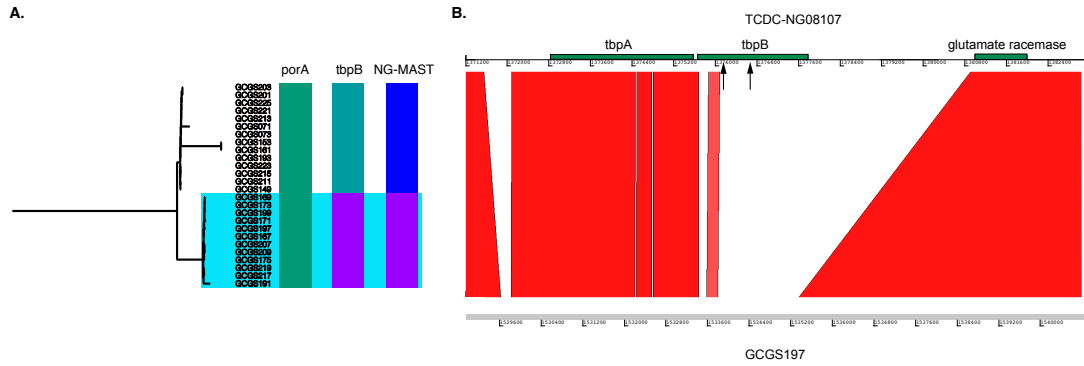


Figure S11. A. Phylogeny of *cef^{RS}* cluster 2 based on full SNP set. The clade highlighted in blue identifies those that share the null *tbpB* allele (see Supplemental Figure 2). B. Comparison of the genome sequence for the reference genome TCDC-NG08107 and GCGS197, a representative isolate from the branch with null *tbpB* allele as seen in (A), showing recombination at the *tbpB* locus. The red blocks indicate homologous sequence. The sequence for *tbpA*, *tbpB*, and glutamate racemase are noted in the TCDC-NG08107 track. The arrows indicate the locations of the primers (forward primer 5' end at 1376716 and reverse primer 5' end at 1376138 in TCDC-NG08107 numbering).

Supplemental Table 1

Sample	YEAR	clinic	SEXOR2	B_LAC	PEN	TETRACY	SPCTINO	CFX	CRO	CIPRO	CPD	AZI	MLST	porB	tbpB	NG-MAST	CFX	Cluster	Notes
GCGS001	2009	CHI	MSM	2	4	4	1	0.25	0.125	16	2	1	1901	908	110	1407	RS	1	
GCGS002	2009	CHI	MSM	2	1	4	1	0.015	0.03	16	0.06	1	1901	1582	4	6712	S		
GCGS003	2009	CHI	MSM	2	4	4	1	0.25	0.125	16	1	1	1901	908	110	1407	RS	1	
GCGS004	2009	CHI	MSM	2	2	4	1	0.015	0.03	16	0.06	1	1901	99%_Allele3745	4	Unknown	S		
GCGS005	2009	CHI	MSM	2	4	4	1	0.5	0.06	16	1	1	1901	99%_Allele3013	110	Unknown	RS	1	
GCGS006	2009	CHI	MSM	2	2	1	1	0.015	0.03	8	0.06	1	8110	1417	4	2265	S		
GCGS007	2009	CHI	MSM	2	2	2	1	0.25	0.06	16	1	0.5	1901	3025	110	4986	RS	1	
GCGS008	2009	CHI	MSM	2	0.5	16	1	0.015	0.008	1	0.03	0.25	1588	3403	743	New	S		
GCGS009	2009	DEN	MSM	2	1	2	1	0.25	0.03	0.015	0.25	0.25	8126	301	29	359	RS	-	Novel mosaic
GCGS010	2009	LVG	MSM	2	0.25	0.5	1	0.015	0.015	0.015	0.03	0.5	1580	99%_Allele1986	29	Unknown	S		
GCGS011	2009	DTR	MSW	2	2	0.5	1	0.25	0.06	0.015	1	0.25	1893	586	25	860	S		Rechecked CFX MIC as per supplemental table 2: 0.06
GCGS012	2009	DTR	MSW	2	1	0.5	1	0.06	0.06	0.015	0.25	0.25	1893	97%_Allele3680	96%_Allele1456	Unknown	S		
GCGS017	2009	HON	MSM	2	4	4	1	0.25	0.06	32	2	1	1901	908	110	1407	RS	1	
GCGS018	2009	HON	MSM	2	0.25	1	1	0.015	0.008	0.015	0.015	8	9363	1808	29	2992	S		
GCGS019	2009	HON	MSM	2	4	4	1	0.25	0.06	32	2	1	1901	908	110	1407	RS	1	
GCGS020	2009	SDG	MSM	2	1	0.5	1	0.015	0.008	2	0.03	0.5	1579	105	21	286	S		
GCGS021	2009	HON	MSM	2	4	2	1	0.25	0.06	32	2	1	1901	908	110	1407	RS	1	
GCGS022	2009	SDG	MSM	2	4	4	1	0.06	0.06	16	0.125	0.5	1901	4	4	225	S		
GCGS023	2009	HON	MSW	2	2	2	1	0.25	0.03	16	0.5	0.5	1901	543	110	1405	RS	1	
GCGS024	2009	HON	MSW	1	16	16	1	0.06	0.06	4	0.125	0.5	7823	543	110	1405	S		
GCGS025	2009	HON	MSM	2	2	2	1	0.25	0.06	16	1	0.5	1901	908	110	1407	RS	1	
GCGS026	2009	SDG	MSM	2	8	4	1	0.06	0.06	16	0.25	0.5	1901	99%_Allele223	5	Unknown	S		
GCGS027	2009	HON	MSM	2	2	4	1	0.25	0.03	32	1	0.5	1901	908	110	1407	RS	1	
GCGS028	2009	SDG	MSM	2	0.5	2	1	0.015	0.008	8	0.06	1	1901	908	110	1407	S		
GCGS029	2009	HON	MSM	2	2	4	1	0.25	0.06	32	1	0.5	1901	908	110	1407	RS	1	
GCGS030	2009	SDG	MSM	2	0.5	2	1	0.015	0.008	8	0.06	1	1901	908	110	1407	S		
GCGS031	2009	HON	MSM	2	2	4	1	0.5	0.03	32	2	0.5	1901	908	110	1407	RS	1	
GCGS032	2009	SDG	MSM	2	4	2	1	0.03	0.03	16	0.125	0.5	1901	2329	4	New	S		
GCGS033	2009	LAX	MSW	2	4	4	1	0.25	0.06	16	1	0.25	1901	1388	110	2212	RS	1	
GCGS034	2009	ORA	MSW	2	2	2	1	0.06	0.06	32	0.125	1	7371	3522	29	7155	S		
GCGS035	2009	LAX	MSW	2	4	4	1	0.25	0.125	32	2	1	1901	99%_Allele1914	110	Unknown	RS	1	
GCGS036	2009	ORA	MSW	2	2	1	1	0.03	0.015	16	0.06	0.5	1901	1678	1169	New	S		
GCGS037	2009	LAX	MSM	2	2	4	1	0.5	0.125	32	2	0.5	1901	908	110	1407	RS	1	
GCGS038	2009	LAX	MSM	2	0.5	1	1	0.015	0.008	0.015	0.015	8	9363	1808	29	2992	S		
GCGS039	2009	LVG	MSW	2	4	4	1	0.25	0.125	16	2	1	1901	99%_Allele2147	110	Unknown	RS	1	
GCGS040	2009	LVG	MSW	1	16	16	1	0.03	0.03	16	0.125	0.125	7823	543	29	1288	S		
GCGS041	2009	LVG	MSM	2	4	4	1	0.25	0.125	16	2	1	1901	908	110	1407	RS	1	

GCGS042	2009	LVG	MSM	2	4	4	1	0.06	0.06	8	0.125	1	1901	14	4	437	S	
GCGS043	2009	LVG	MSW	2	4	2	1	0.25	0.06	16	1	1	1901	99%_Allele2147	110	Unknown	RS	1
GCGS044	2009	LVG	MSW	2	4	4	1	0.06	0.06	16	0.125	0.5	1901	99%_Allele866	4	Unknown	S	
GCGS045	2009	LVG	MSW	2	2	2	1	0.25	0.06	16	0.5	1	1901	99%_Allele2147	110	Unknown	RS	1
GCGS046	2009	LVG	MSW	2	4	4	1	0.06	0.03	16	0.5	1	1901	99%_Allele2147	110	Unknown	S	
GCGS047	2009	LVG	MSW	2	4	4	1	0.25	0.06	16	1	1	1901	99%_Allele2147	110	Unknown	RS	1
GCGS048	2009	LVG	MSW	2	4	4	1	0.06	0.06	16	1	1	1901	99%_Allele2147	110	Unknown	S	
GCGS049	2009	LVG	MSW	2	2	4	1	0.25	0.06	16	1	1	1901	99%_Allele2147	110	Unknown	RS	1
GCGS050	2009	LVG	MSW	2	0.25	0.25	1	0.015	0.008	0.015	0.015	0.03	8154	4754	16	7949	S	
GCGS051	2009	LVG	MSW	2	2	2	1	0.25	0.06	16	1	0.5	1901	99%_Allele2147	110	Unknown	RS	1
GCGS052	2009	LVG	MSW	2	2	2	1	0.03	0.06	8	0.125	0.5	1901	14	4	437	S	
GCGS053	2009	LVG	MSW	2	2	4	1	0.25	0.125	16	1	1	1901	99%_Allele1914	110	Unknown	RS	1
GCGS054	2009	LVG	MSW	2	2	4	1	0.06	0.06	16	0.125	1	1901	14	4	437	S	
GCGS055	2009	PHI	MSM	2	2	2	1	0.25	0.06	16	1	1	1901	908	110	1407	RS	1
GCGS056	2009	PHI	MSM	2	2	2	1	0.06	0.06	8	0.06	0.5	1901	260	4	3169	S	
GCGS057	2009	PHX	MSM	2	2	2	1	0.25	0.06	16	1	0.5	1901	1914	110	3158	RS	1
GCGS058	2009	PHX	MSM	2	2	2	1	0.03	0.03	16	0.125	0.5	1901	14	1514	8258	S	
GCGS059	2009	PHX	MSM	2	4	4	1	0.25	0.125	16	2	1	1901	2237	110	3709	RS	1
GCGS060	2009	PHX	MSM	2	4	2	1	0.06	0.06	8	0.125	0.5	1901	2329	4	New	S	
GCGS061	2009	POR	MSM	2	4	2	1	0.5	0.06	32	1	0.5	1901	1914	110	3158	RS	1
GCGS062	2009	POR	MSM	2	0.5	1	1	0.015	0.015	4	0.03	0.25	1580	59	29	730	S	
GCGS063	2009	POR	MSM	2	2	4	1	0.25	0.06	16	1	0.5	1901	1900	110	3128	RS	1
GCGS064	2009	POR	MSM	2	4	32	1	0.06	0.03	0.015	0.25	1	1893	14	4	437	S	
GCGS065	2009	POR	MSM	2	2	4	1	0.25	0.06	16	1	0.5	1901	1900	110	3128	RS	1
GCGS066	2009	LVG	MSM	2	1	4	1	0.015	0.008	8	0.125	1	1901	908	110	1407	S	
GCGS067	2009	SDG	MSM	2	4	2	1	0.25	0.125	16	1	0.5	1901	1903	110	3149	RS	1
GCGS068	2009	SDG	MSM	2	1	2	1	0.015	0.008	8	0.06	0.5	1901	908	110	1407	S	
GCGS069	2009	SDG	MSW	2	4	4	1	0.25	0.125	16	1	1	1901	99%_Allele1914	110	Unknown	RS	1
GCGS070	2009	SDG	MSW	2	1	1	1	0.03	0.015	4	0.06	0.5	1901	4016	33	6734	S	
GCGS071	2009	SDG	MSM	2	0.5	1	1	0.25	0.03	0.015	0.5	0.5	1580	2700	110	5895	RS	2
GCGS072	2009	SDG	MSM	2	0.5	0.25	1	0.015	0.008	0.015	0.03	0.5	6712	19	27	7468	S	
GCGS073	2009	SDG	MSM	2	1	0.5	1	0.25	0.06	0.015	0.5	0.25	1580	2700	110	5895	RS	2
GCGS074	2009	SDG	MSM	2	0.5	0.5	1	0.015	0.008	0.015	0.03	0.25	1583	35	18	766	S	
GCGS075	2009	SDG	MSM	2	2	2	1	0.25	0.06	8	1	0.5	1901	908	110	1407	RS	1
GCGS076	2009	SDG	MSM	2	1	2	1	0.015	0.008	8	0.06	0.25	1901	908	110	1407	S	
GCGS077	2009	SDG	MSW	2	4	2	1	0.25	0.06	16	2	1	1901	2684	110	4378	RS	1
GCGS078	2009	SDG	MSW	2	0.25	0.25	1	0.015	0.008	4	0.015	0.06	1901	3022	28	New	S	
GCGS079	2009	SEA	MSM	2	2	1	1	0.25	0.06	16	2	0.5	1901	1914	110	3158	RS	1
GCGS080	2009	SEA	MSM	2	4	4	1	0.015	0.06	16	0.125	1	1901	430	4	690	S	

GCGS081	2009	SEA	MSM	2	2	1	1	0.25	0.06	16	1	0.125	1901	1914	110	3158	RS	1	
GCGS082	2009	SEA	MSM	2	2	2	1	0.06	0.06	16	0.125	0.25	1902	1053	107	3449	S		
GCGS083	2009	SFO	MSM	2	2	2	1	0.25	0.03	0.03	0.125	0.5	9363	908	29	3935	S		Rechecked CFX MIC as per supplemental table 2: 0.03
GCGS084	2009	SFO	MSM	2	2	4	1	0.015	0.015	0.015	0.25	1	9363	908	29	3935	S		
GCGS085	2009	SFO	MSM	2	2	2	1	0.25	0.03	32	1	0.5	1901	2763	110	4533	RS	1	
GCGS086	2009	SFO	MSM	2	2	1	1	0.03	0.015	4	0.03	1	1901	105	4	1440	S		
GCGS087	2009	SFO	MSW	2	2	4	1	0.25	0.03	32	1	0.5	1901	2078	110	3431	RS	1	
GCGS088	2009	SFO	MSW	2	0.5	0.5	1	0.06	0.008	0.015	0.015	0.5	1901	2078	110	3431	S		
GCGS089	2009	SFO	MSM	2	2	16	1	0.25	0.03	32	2	1	1901	908	110	1407	RS	1	
GCGS090	2009	SFO	MSM	1	8	32	1	0.015	0.008	1	0.03	0.25	1588	2583	743	4205	S		
GCGS091	2010	CHI	MSM	2	4	4	1	0.25	0.06	16	1	1	1901	99%_Allele3013	110	Unknown	RS	1	
GCGS092	2010	CHI	MSM	2	1	1	1	0.06	0.06	0.015	0.5	0.25	1580	59	29	730	S		
GCGS093	2010	CHI	MSM	2	4	4	1	0.25	0.125	16	1	1	1901	99%_Allele3013	110	Unknown	RS	1	
GCGS094	2009	CHI	MSM	2	2	2	1	0.03	0.03	8	0.125	1	1901	99%_Allele1582	4	Unknown	S		
GCGS095	2010	CHI	MSM	2	2	4	1	0.25	0.06	16	1	1	1901	908	110	1407	RS	1	
GCGS096	2010	CHI	MSM	2	0.5	1	1	0.03	0.008	0.015	0.03	16	9363	1808	29	2992	S		
GCGS097	2010	CHI	MSM	2	2	4	1	0.5	0.125	16	2	0.5	1901	908	110	1407	RS	1	
GCGS098	2010	CHI	MSM	2	0.25	1	1	0.03	0.008	0.015	0.03	16	9363	1808	29	2992	S		
GCGS099	2010	CLE	MSW	2	4	4	1	0.5	0.25	16	2	1	1901	14	4	437	RS	-	Individual isolate with penA mosaic XXXIV outside clusters 1 and 2
GCGS100	2010	DTR	MSW	2	2	1	1	0.06	0.06	0.015	0.25	0.25	1893	97%_Allele3680	96%_Allele1456	Unknown	S		
GCGS101	2010	DEN	MSM	2	2	2	1	0.25	0.06	16	2	0.125	1901	908	110	1407	RS	1	
GCGS102	2010	ALB	MSM	2	4	2	1	0.06	0.03	16	0.5	2	1901	3003	110	4951	S		
GCGS103	2010	DEN	MSM	2	2	4	1	0.25	0.03	16	1	0.5	1901	908	110	1407	RS	1	
GCGS104	2010	ALB	MSM	2	4	4	1	0.06	0.03	16	0.5	2	1901	908	110	1407	S		
GCGS105	2010	DEN	MSW	2	1	2	1	0.25	0.06	16	1	0.5	1901	2623	110	4269	RS	1	
GCGS106	2010	PHX	MSW	2	0.5	1	1	0.015	0.008	0.015	0.03	8	8126	301	29	359	S		
GCGS107	2010	GRB	MSM	2	4	4	1	0.25	0.125	16	0.5	0.5	1901	908	110	1407	RS	1	
GCGS108	2009	BAL	MSM	2	2	4	1	0.03	0.06	16	0.125	0.25	1901	1259	4	1978	S		
GCGS109	2010	HON	MSM	2	4	4	1	0.25	0.06	32	2	0.5	1901	908	110	1407	RS	1	
GCGS110	2010	SFO	MSM	2	0.25	1	1	0.03	0.008	0.015	0.015	16	9363	1808	29	2992	S		
GCGS113	2010	HON	MSM	2	2	4	1	0.25	0.03	32	2	0.5	1901	99%_Allele4576	110	Unknown	RS	1	
GCGS114	2009	SDG	MSM	2	0.25	0.5	1	0.015	0.008	2	0.015	0.125	1901	908	110	1407	S		
GCGS115	2010	HON	MSM	2	4	4	1	0.5	0.06	16	2	0.5	1901	908	110	1407	RS	1	
GCGS116	2009	SDG	MSM	2	0.25	0.5	1	0.015	0.008	4	0.015	0.06	1901	4	4	225	S		
GCGS117	2010	HON	MSW	2	1	2	1	0.25	0.06	16	1	0.25	8129	908	110	1407	RS	1	
GCGS118	2010	HON	MSW	2	0.25	1	1	0.03	0.03	0.015	0.03	8	9363	99%_Allele1808	29	Unknown	S		
GCGS119	2010	HON	MSM	2	1	2	1	0.25	0.06	16	1	0.25	8129	908	110	1407	RS	1	
GCGS120	2009	SDG	MSM	2	0.25	0.5	1	0.015	0.008	0.015	0.015	8	9363	2577	29	4198	S		
GCGS121	2010	LAX	MSM	2	2	2	1	0.5	0.06	16	4	0.5	1901	908	110	1407	RS	1	

GCGS122	2010	LAX	MSM	2	2	2	1	0.06	0.03	16	0.25	0.25	1901	1259	4	1978	S	
GCGS123	2010	LAX	MSM	2	2	2	1	0.25	0.125	16	2	0.5	1901	908	110	1407	RS	1
GCGS124	2010	LAX	MSM	2	0.25	0.25	1	0.015	0.008	0.015	0.015	0.03	1901	908	110	1407	S	
GCGS125	2010	LAX	MSM	2	4	2	1	0.25	0.06	16	2	0.5	1901	908	110	1407	RS	1
GCGS126	2010	LAX	MSM	2	0.5	0.5	1	0.06	0.03	0.015	0.125	0.25	9363	1808	29	2992	S	
GCGS127	2010	LAX	MSM	2	1	0.5	1	0.25	0.03	8	1	0.25	1901	98%_Allele875	110	Unknown	RS	1
GCGS128	2010	LAX	MSM	2	0.25	0.5	1	0.03	0.008	0.015	0.03	8	9363	1808	29	2992	S	
GCGS129	2010	LAX	MSM	2	2	4	1	0.25	0.03	32	1	0.5	1901	908	110	1407	RS	1
GCGS130	2009	SDG	MSM	2	2	2	1	0.03	0.03	16	0.125	0.5	1901	1259	4	1978	S	
GCGS131	2010	LAX	MSM	2	2	4	1	0.25	0.03	32	1	0.5	1901	908	110	1407	RS	1
GCGS132	2009	SDG	MSM	2	4	2	1	0.06	0.03	16	0.125	0.5	1901	14	4	437	S	
GCGS133	2010	LAX	MSM	2	2	2	1	0.25	0.03	32	1	0.5	1901	908	110	1407	RS	1
GCGS134	2009	SDG	MSM	2	4	2	1	0.03	0.03	16	0.125	0.5	1901	14	4	437	S	
GCGS135	2010	LAX	MSM	2	2	4	1	0.5	0.03	16	1	0.5	1901	908	110	1407	RS	1
GCGS136	2010	LAX	MSM	2	0.25	1	1	0.03	0.008	0.015	0.03	8	9363	99%_Allele3523	29	Unknown	S	
GCGS137	2010	LAX	MSM	2	2	2	1	0.25	0.06	32	1	0.5	1901	908	110	1407	RS	1
GCGS138	2010	LAX	MSM	2	0.25	1	1	0.015	0.008	0.015	0.015	16	9363	1808	29	2992	S	
GCGS139	2010	LAX	MSM	2	4	4	1	0.25	0.125	32	2	1	1901	908	110	1407	RS	1
GCGS140	2010	LAX	MSM	2	4	4	1	0.06	0.06	32	0.25	1	1901	1259	4	1978	S	
GCGS141	2010	LVG	MSW	2	4	4	1	0.25	0.06	16	1	0.5	1901	99%_Allele2147	110	Unknown	RS	1
GCGS142	2009	LVG	MSW	2	0.5	1	1	0.015	0.008	0.015	0.015	2	1579	760	33	3485	S	
GCGS143	2010	LVG	MSW	2	4	2	1	0.25	0.125	16	1	0.5	1901	99%_Allele2147	110	Unknown	RS	1
GCGS144	2009	LVG	MSW	2	0.5	1	1	0.06	0.03	0.015	0.25	0.5	1580	99%_Allele1986	29	Unknown	S	
GCGS145	2010	LVG	MSM	2	4	4	1	0.25	0.06	16	1	1	1901	908	110	1407	RS	1
GCGS146	2009	LVG	MSM	1	4	16	1	0.03	0.008	1	0.06	0.25	1588	30	743	3307	S	
GCGS147	2010	LVG	MSW	2	2	4	1	0.25	0.125	16	2	1	1901	99%_Allele2147	110	Unknown	RS	1
GCGS148	2009	SFO	MSW	2	2	1	1	0.03	0.015	4	0.03	0.5	1901	105	4	1440	S	
GCGS149	2010	LVG	MSW	2	1	1	1	0.25	0.03	0.015	0.25	0.5	1580	2700	110	5895	RS	2
GCGS150	2009	SDG	MSW	1	16	32	1	0.015	0.015	8	0.03	0.5	1903	4270	129	7132	S	
GCGS151	2010	LVG	MSW	2	2	2	1	0.25	0.06	16	1	1	8129	908	110	1407	RS	1
GCGS152	2010	LVG	MSW	2	2	2	1	0.06	0.06	16	0.25	0.5	7822	543	186	1791	S	
GCGS153	2010	LVG	MSW	2	0.5	0.5	1	0.25	0.03	0.015	0.25	0.5	1580	2700	110	5895	RS	2
GCGS154	2009	SDG	MSW	1	32	16	1	0.015	0.008	4	0.015	0.5	8130	90	29	758	S	
GCGS155	2010	MIN	MSM	2	4	4	1	0.25	0.125	16	2	1	1901	908	110	1407	RS	1
GCGS156	2010	MIN	MSM	2	0.25	1	1	0.03	0.008	0.015	0.03	16	9363	99%_Allele4354	29	Unknown	S	
GCGS157	2010	NYC	MSM	2	8	4	1	0.25	0.125	16	0.5	1	1901	908	110	1407	RS	1
GCGS158	2009	PHI	MSM	2	4	2	1	0.03	0.03	16	0.125	0.25	1901	14	4	437	S	
GCGS159	2010	ORA	MSM	2	2	2	1	0.25	0.06	8	1	0.5	1901	908	110	1407	RS	1
GCGS160	2009	SDG	MSM	2	0.25	2	1	0.015	0.008	8	0.06	1	1901	908	110	1407	S	

GCGS161	2010	ORA	MSM	2	0.5	0.5	1	0.25	0.03	0.015	0.5	0.5	1580	2700	110	5895	RS	2
GCGS162	2009	SDG	MSM	1	1	0.5	1	0.015	0.008	0.015	0.015	0.5	8152	19	25	5268	S	
GCGS163	2010	PHI	MSM	2	4	4	1	0.25	0.25	16	2	0.5	1901	2078	110	3431	RS	1
GCGS164	2009	PHI	MSM	2	4	2	1	0.03	0.03	16	0.125	0.25	1901	4	29	2493	S	
GCGS165	2010	PHX	MSM	2	2	4	1	0.25	0.06	16	1	1	1901	908	110	1407	RS	1
GCGS166	2009	PHX	MSM	2	1	2	1	0.015	0.008	8	0.06	1	1901	908	110	1407	S	
GCGS167	2010	PHX	MSM	2	1	1	1	0.25	0.03	0.015	0.5	0.5	1580	2700	alt_tpbB	Unknown	RS	2
GCGS168	2010	PHX	MSM	2	4	4	1	0.06	0.06	16	0.25	1	1901	4	4	225	S	
GCGS169	2010	PHX	MSM	2	1	1	1	0.25	0.03	0.015	0.5	0.5	1580	2700	alt_tpbB	Unknown	RS	2
GCGS170	2010	PHX	MSM	2	4	4	1	0.06	0.06	16	0.25	0.5	1901	4	4	225	S	
GCGS171	2010	PHX	MSM	2	1	1	1	0.25	0.06	0.015	0.5	0.5	1580	2700	alt_tpbB	Unknown	RS	2
GCGS172	2010	PHX	MSM	2	1	1	1	0.015	0.008	0.015	0.03	2	1893	3716	25	6306	S	
GCGS173	2010	PHX	MSM	2	1	1	1	0.25	0.06	0.015	0.5	1	1580	2700	alt_tpbB	Unknown	RS	2
GCGS174	2010	PHX	MSM	2	0.25	1	1	0.03	0.008	0.015	0.015	16	9363	1808	29	2992	S	
GCGS175	2010	PHX	MSM	2	1	1	1	0.25	0.06	0.015	0.5	0.5	1580	2700	alt_tpbB	Unknown	RS	2
GCGS176	2010	PHX	MSM	2	0.25	1	1	0.015	0.008	0.015	0.015	16	9363	99%_Allele3381	29	Unknown	S	
GCGS177	2010	PHX	MSW	2	1	1	1	0.25	0.03	4	0.25	1	1901	7	110	New	RS	1
GCGS178	2010	PHX	MSW	2	1	2	1	0.03	0.015	16	0.25	1	1901	908	110	1407	S	
GCGS179	2010	POR	MSM	2	2	2	1	0.5	0.06	16	1	0.25	1901	1900	110	3128	RS	1
GCGS180	2009	LVG	MSM	2	1	16	1	0.015	0.015	2	0.06	0.25	1588	5014	743	New	S	
GCGS181	2010	POR	MSM	2	1	2	1	0.5	0.125	16	1	0.25	1901	1900	110	3128	RS	1
GCGS182	2009	LVG	MSM	2	1	1	1	0.015	0.008	4	0.03	0.5	1579	105	21	286	S	
GCGS183	2010	POR	MSM	2	1	2	1	0.25	0.06	16	1	0.25	1901	1900	110	3128	RS	1
GCGS184	2009	LVG	MSM	1	16	0.25	1	0.015	0.008	2	0.015	0.03	1582	35	944	4424	S	
GCGS185	2010	POR	MSM	2	2	2	1	0.25	0.06	16	1	0.25	1901	1900	110	3128	RS	1
GCGS186	2009	LVG	MSM	1	8	64	1	0.03	0.03	2	0.06	0.25	1588	997	743	New	S	
GCGS187	2010	POR	MSM	2	2	2	1	0.25	0.125	16	1	0.25	1901	1900	110	3128	RS	1
GCGS188	2009	LVG	MSM	2	0.25	16	1	0.03	0.015	1	0.03	0.25	1588	1142	743	New	S	
GCGS189	2010	SDG	MSM	2	4	2	1	0.25	0.06	16	2	1	1901	2780	110	4556	RS	1
GCGS190	2010	SDG	MSM	2	4	4	1	0.03	0.03	16	0.25	1	1901	4	135	1861	S	
GCGS191	2010	SDG	MSM	2	1	1	1	0.25	0.03	0.015	0.5	0.5	1580	2700	alt_tpbB	Unknown	RS	2
GCGS192	2010	SDG	MSM	2	0.5	1	1	0.03	0.008	0.015	0.03	2	9363	1808	29	2992	S	
GCGS193	2010	SDG	MSW	2	0.5	1	1	0.25	0.03	0.015	0.5	0.5	1580	2700	110	5895	RS	2
GCGS194	2009	ORA	MSW	2	1	1	1	0.015	0.03	4	0.06	0.5	1901	4016	33	6734	S	
GCGS195	2010	SDG	MSM	2	4	2	1	0.25	0.06	16	1	0.5	1901	908	110	1407	RS	1
GCGS196	2010	SDG	MSM	2	4	4	1	0.03	0.03	16	0.25	1	1901	14	4	437	S	
GCGS197	2010	SDG	MSM	2	1	1	1	0.25	0.015	0.015	0.5	0.5	1580	2700	alt_tpbB	Unknown	RS	2
GCGS198	2009	SDG	MSM	2	0.25	1	1	0.015	0.008	0.015	0.015	16	9363	2577	29	4198	S	
GCGS199	2010	SDG	MSW	2	1	1	1	0.25	0.015	0.015	0.5	0.5	1580	2700	alt_tpbB	Unknown	RS	2

GCGS200	2009	ORA	MSW	2	0.25	0.25	1	0.015	0.015	0.015	0.015	0.125	6722	99%_Allele521	5	Unknown	S	
GCGS201	2010	SDG	MSM	2	1	1	1	0.25	0.015	0.015	0.5	0.5	1580	2700	110	5895	RS	2
GCGS202	2009	SDG	MSM	2	0.25	0.5	1	0.03	0.008	0.015	0.015	8	9363	2577	29	4198	S	
GCGS203	2010	SDG	MSM	2	1	1	1	0.25	0.06	0.015	0.5	0.5	1580	2700	110	5895	RS	2
GCGS204	2010	SDG	MSM	2	0.25	1	1	0.015	0.008	0.015	0.015	8	9363	95%_Allele5049	29	Unknown	S	
GCGS205	2010	SDG	MSM	2	4	2	1	0.25	0.125	16	2	1	1901	908	110	1407	RS	1
GCGS206	2009	SDG	MSM	2	0.25	0.5	1	0.015	0.008	4	0.03	0.06	1901	4	4	225	S	
GCGS207	2010	SDG	MSM	2	1	1	1	0.25	0.06	0.015	0.5	0.5	1580	2700	alt_tpbB	Unknown	RS	2
GCGS208	2009	SDG	MSM	2	1	2	1	0.015	0.015	8	0.03	1	1582	99%_Allele53	21	Unknown	S	
GCGS209	2010	SDG	MSM	2	0.5	0.5	1	0.25	0.03	0.015	0.5	0.5	1580	2700	alt_tpbB	Unknown	RS	2
GCGS210	2009	PHX	MSM	2	0.5	16	1	0.03	0.015	1	0.06	0.25	1588	30	743	3307	S	
GCGS211	2010	SDG	MSM	2	0.5	0.5	1	0.25	0.03	0.015	0.5	0.25	1580	2700	110	5895	RS	2
GCGS212	2009	SDG	MSM	2	1	32	1	0.03	0.015	1	0.06	0.25	1588	28	743	4405	S	
GCGS213	2010	SDG	MSM	2	1	0.5	1	0.25	0.03	0.015	0.5	0.5	1580	2700	110	5895	RS	2
GCGS214	2009	SDG	MSM	2	0.5	0.5	1	0.015	0.008	2	0.03	0.5	1579	105	21	286	S	
GCGS215	2010	SDG	MSM	2	0.5	0.5	1	0.25	0.03	0.015	0.5	0.25	1580	2700	110	5895	RS	2
GCGS216	2009	SDG	MSM	1	4	16	1	0.015	0.015	2	0.03	0.25	1588	30	743	3307	S	
GCGS217	2010	SDG	MSM	2	1	1	1	0.25	0.03	0.015	0.5	0.5	1580	2700	alt_tpbB	Unknown	RS	2
GCGS218	2009	SDG	MSM	2	0.25	1	1	0.015	0.008	0.015	0.015	16	9363	1808	29	2992	S	
GCGS219	2010	SDG	MSM	2	1	1	1	0.25	0.03	0.015	0.5	0.5	1580	2700	alt_tpbB	Unknown	RS	2
GCGS220	2009	SDG	MSM	2	0.25	0.25	1	0.015	0.008	2	0.03	0.06	1579	105	21	286	S	
GCGS221	2010	SDG	MSW	2	1	0.5	1	0.25	0.03	0.015	0.5	0.5	1580	2700	110	5895	RS	2
GCGS222	2009	ORA	MSW	2	2	1	1	0.015	0.03	4	0.06	0.5	1901	4016	33	6734	S	
GCGS223	2010	SDG	MSM	2	0.5	1	1	0.25	0.03	0.015	0.5	0.5	1580	2700	110	5895	RS	2
GCGS224	2009	SDG	MSM	2	0.25	1	1	0.015	0.008	0.015	0.015	8	9363	2577	29	4198	S	
GCGS225	2010	SDG	MSM	2	1	1	1	0.25	0.03	0.015	0.5	0.5	1580	2700	110	5895	RS	2
GCGS226	2010	SDG	MSM	2	0.25	0.5	1	0.015	0.008	0.015	0.015	16	9363	99%_Allele3281	29	Unknown	S	
GCGS227	2010	SDG	MSM	2	4	4	1	0.25	0.06	16	1	1	1901	3960	110	6632	RS	1
GCGS228	2009	PHX	MSM	2	2	4	1	0.015	0.015	0.015	0.06	2	9363	99%_Allele1259	29	Unknown	S	
GCGS229	2010	SDG	MSM	2	2	2	1	0.25	0.06	16	1	0.5	1901	3960	110	6632	RS	1
GCGS230	2010	SDG	MSM	2	0.25	0.5	1	0.015	0.008	0.015	0.015	8	9363	99%_Allele3381	29	Unknown	S	
GCGS231	2010	SEA	MSM	2	2	1	1	0.25	0.03	32	0.25	0.25	1901	1914	110	3158	RS	1
GCGS232	2010	SEA	MSM	2	2	2	1	0.06	0.03	32	0.25	0.5	1901	1259	4	1978	S	
GCGS233	2010	SEA	MSM	2	2	4	1	0.25	0.06	32	1	0.5	1901	908	110	1407	RS	1
GCGS234	2010	SEA	MSM	2	2	2	1	0.06	0.03	32	0.25	0.5	1901	1259	4	1978	S	
GCGS235	2010	SEA	MSM	2	2	4	1	0.25	0.06	16	1	0.25	1901	908	110	1407	RS	1
GCGS236	2010	SEA	MSM	2	2	2	1	0.06	0.03	32	0.25	0.5	1901	1259	4	1978	S	
GCGS237	2010	SEA	MSM	2	2	4	1	0.25	0.06	16	1	0.25	1901	908	110	1407	RS	1
GCGS238	2009	SEA	MSM	2	2	4	1	0.06	0.03	32	0.25	0.5	8129	195	4	1103	S	

GCGS239	2010	SFO	MSM	2	2	4	1	0.5	0.125	16	2	0.5	1901	908	110	1407	RS	1
GCGS240	2010	SFO	MSM	2	2	4	1	0.06	0.06	32	0.25	0.5	1901	250	4	323	S	
GCGS241	2010	SFO	MSM	2	4	4	1	0.5	0.125	16	2	0.5	1901	908	110	1407	RS	1
GCGS242	2010	SFO	MSM	2	0.5	0.5	1	0.06	0.015	4	0.25	0.125	1901	98%_Allele875	110	Unknown	S	

Supplemental Table S2

ID	Reported CFX MIC (mg/mL)	CDC lab retest CFX MIC (mg/mL)	penA allele
GCGS011	0.25	0.06	non-mosaic
GCGS083	0.25	0.03	non-mosaic
GCGS046	0.06	0.06	mosaic penA XXXIV
GCGS048	0.06	0.03	mosaic penA XXXIV
GCGS088	0.06	0.125	mosaic penA XXXIV
GCGS102	0.06	0.125	mosaic penA XXXIV
GCGS104	0.06	0.125	mosaic penA XXXIV
GCGS124	0.015	0.125	mosaic penA XXXIV
GCGS126	0.06	0.125	mosaic penA XXXIV E101D
GCGS242	0.06	0.125	mosaic penA XXXIV