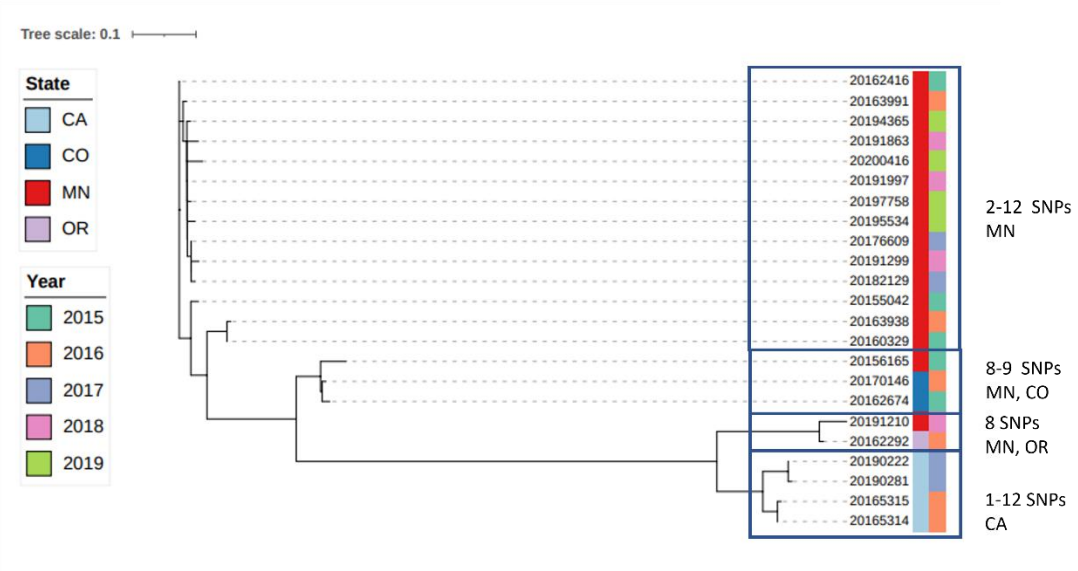
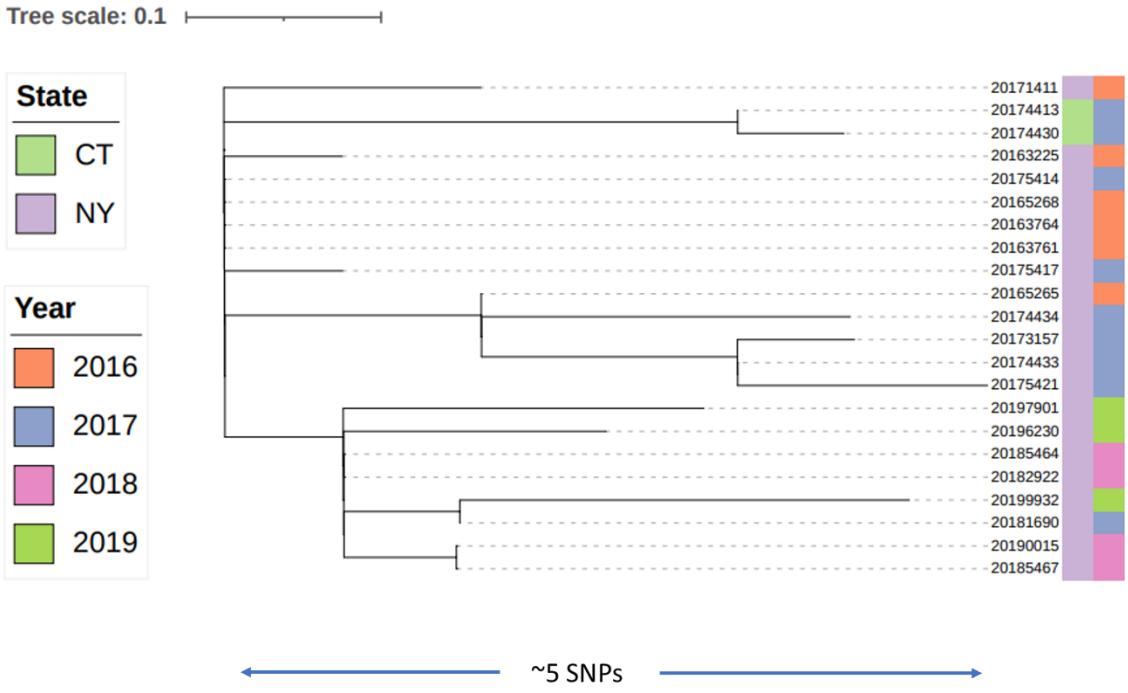
sFig1.
*emm*101 ST182
PBP2x14 (I502V,
A397V)



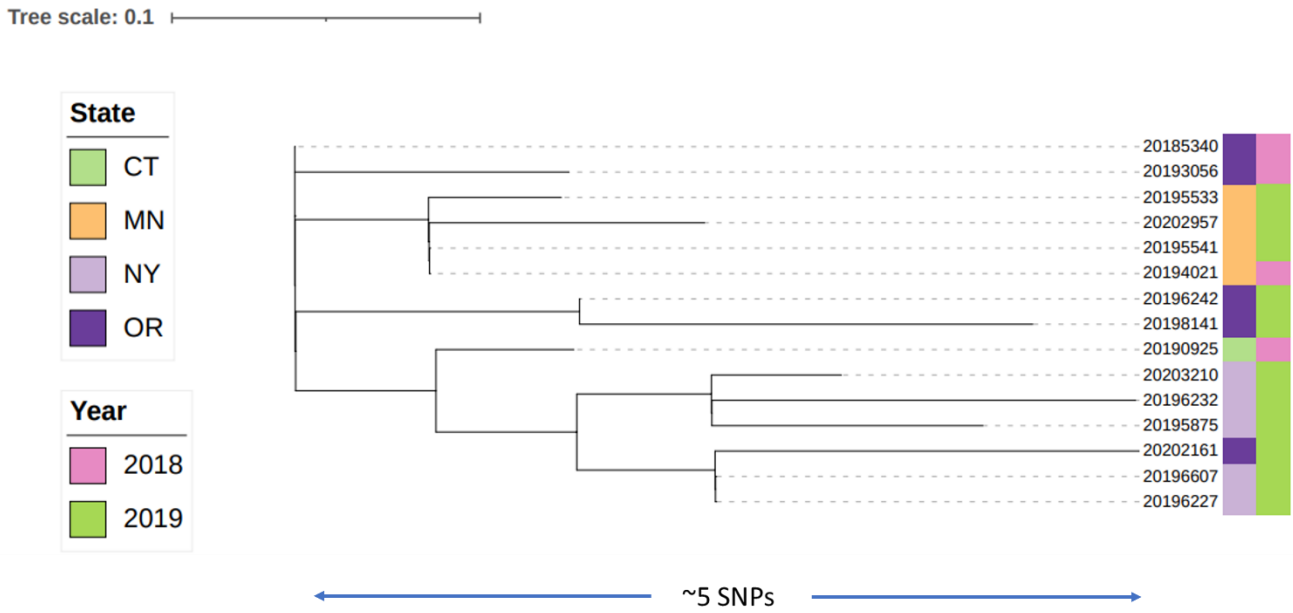
sFigure 1. Core Phylogeny of *emm101*/ST182 lineage sharing type PBP2x14 (Ile502Val, Ala397Val) recovered in 5 states during 2015-2019. Figure represents a mixture of temporally related and unrelated strains, suggestive of potentially long-standing origin of these mutants. The inside color band codes for state and outside color band for the isolation year.

sFig. 2
*emm*1 ST28
PBP2x18



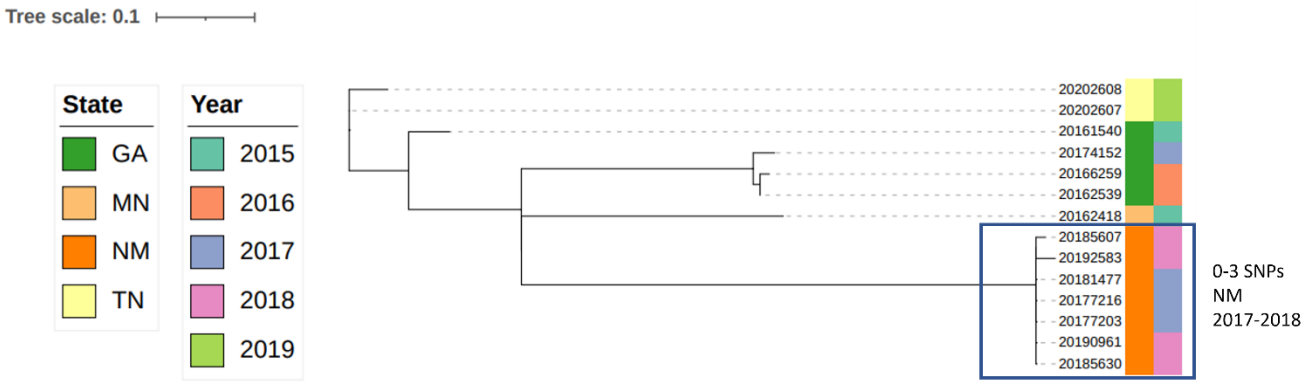
sFigure 2. Core phylogeny of the 22 nearly genetically indistinguishable *emm1* isolates sharing PBP2x18 (Ala397Val), with an average of 5 SNPs over the entire shared core genome. Except for 2 isolates from CT, all were recovered in NY during 2016-2019 which was also consistent with recent transmission. The inside color band codes for state and outside color band for the isolation year.

sFig. 3
*emm*1 ST28
PBP2x47



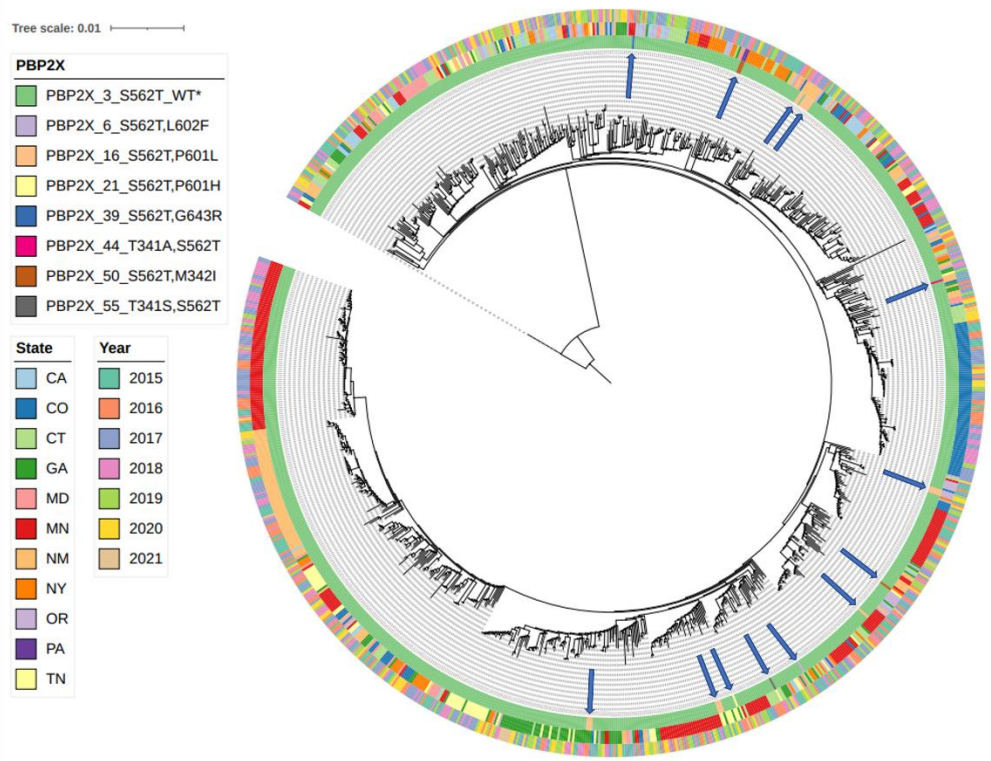
sFigure 3. Core phylogeny of PBP2x47 (K410E) variants detected in 23 isolates primarily among *emm1*/ST28 lineage. These 23 isolates shared nearly identical genomes (average SNP distance = 5) and were found in four states (NY, CT, MN, and OR) during 2018-2019. The inside color band codes for state and outside color band for the isolation year.

sFig 4.
*emm*89 ST101
PBP2x16



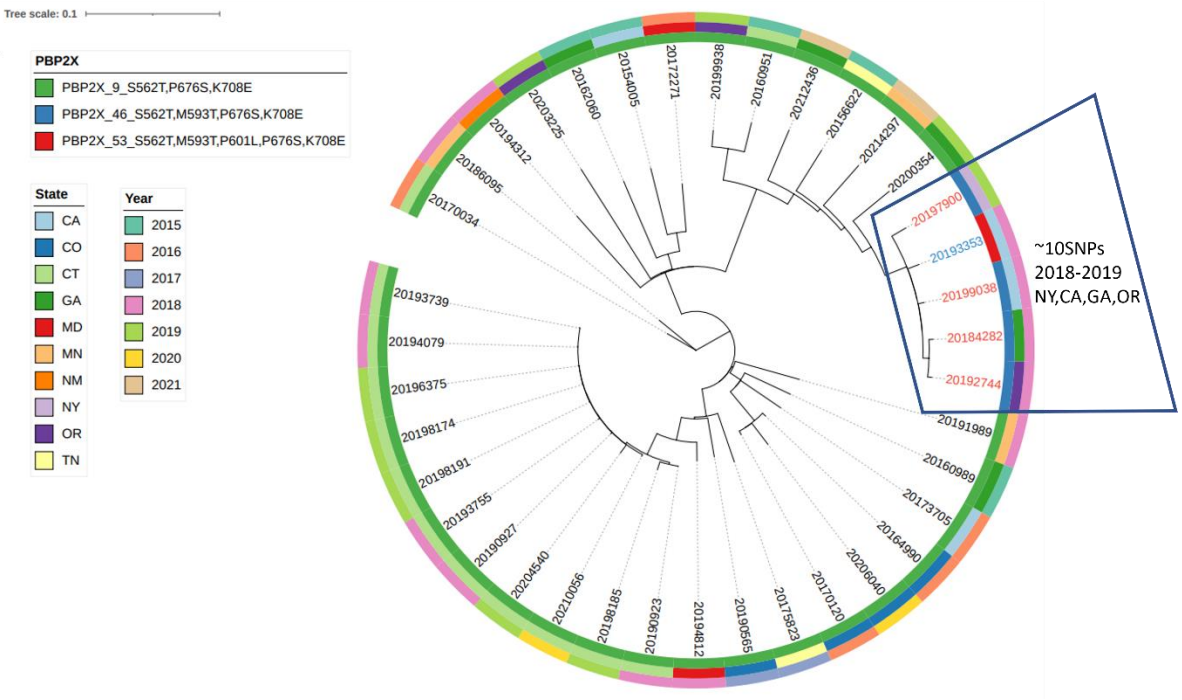
sFigure 4. Core phylogeny of PBP2x16 (Ser562Thr, Pro601Leu) was associated with increased MICs to four of the five beta-lactams tested and was found only in *emm89*/ST101 strains recovered from multiple states during 2015-2019. Seven of these isolates were recovered from NM in 2017-2018 and shared near identity (differed by 0 to 3 SNPs). The remaining eight isolates from GA, TN, MN, and OR were more distantly related.

sFig. 5
*emm*89 ST101



sFigure 5. Core phylogeny of *emm89*/ST101 lineage, average distance 41 SNPs (all 1014 recovered 2015-2021 are characterized). In contrast to core phylogeny of *emm1*/ST28 lineage, PBP2x types of this lineage are represented by fewer isolates spread among PBP2x3 type (that has beta-lactam susceptibility of wild type*), potentially indicative of more recent emergence.

sFig 6.
*emm*73 ST331



sFigure 6. Core phylogeny of *emm73* ST331 lineage depicting the single PBP2x53 variant likely to have arisen from a point mutation of the *pbp2x46* allele within an *emm73* precursor strain. Legends for this and all other phylogenies depicted employ same scheme as follows: innermost circle is color coded for PBP2x substitution, the second circle from inside is depicting state, and the third circle from inside is depicting year of isolation.

sTable 1. Compiled dataset of the combined PBP2x transpeptidase types/flanking substitutions with genomic accession numbers and selected bioinformatics pipeline information.

Abbreviations and explanations for selected columns. For more information regarding our WGS-based characterization of GAS please see reference [21]:

PBP2x Type: Penicillin binding protein 2X transpeptidase type for detection of the first-step mutations leading to β-lactam nonsusceptibility.

PBP2x Substitution: Based upon 1167 bp query (bases 808-1974) corresponding to amino acids 270 – 688.

PBP2x Flanking Substitution: All substitutions outside of our query region (residues 270 – 688) for potential reduced susceptibility to β-lactam antibiotics.

emm Type: *emm* subtype from WGS, from *emm* subtype database at ftp://ftp.cdc.gov/pub/infectious_diseases/biotech/tsemm/

ST: Multilocus sequence type, from database at https://pubmlst.org/spyogenes/

T Type: Important virulence factor that function in epithelial adhesion. 60 bp sequencing query for major pilus protein T antigen gene (tee).

Group A: Presence/absence of Group A antigen.

EMM Family: Presence/absence of emm family genes *mrp* and *enn*.

Other surface proteins: Presence or absence of additional surface protein genes. SOF - Serum opacity factor is an important hypervariable virulence factor expressed by a large percentage of GAS strains; FBAA, PRTF2, SFB1 - Fibronectin binding proteins; R28 - Adhesin.

Capsule: Hyaluronate capsule, presence/absence of hasA hyaluronic acid synthetase operon determinant.

SDA1: Virulence associated DNAse.

SIC: Streptococcal inhibitor of complement.

ROCA: RocA null mutations.

PNGA3: Pnga 3 - Clade 3 up-regulated promoter of the nga operon.

NADase D330G: NADase D330G substitution associated with increased NADase activity.

Exotoxins: Exotoxin gene profile (speA to speC, speG to speM, ssa, smeZ).

ERY, CLI: Macrolide and/or clindamycin resistance determinants (ermB, ermT, ermTR).

TET: Presence/absence of tet genes, conferring resistance to tetracyclines. tet refers to determinant other than tetM and tetO.

ParC, GyrA: Certain substitutions within ParC and/or GyrA confer resistance to fluoroquinolones.

Other Resistance: Other resistance features that include accessory resistance features not already listed (cat, Agly, Ant6-Ia, Aph3-III, Sat4A). The aph3 and sat4a determinants encode resistance to certain aminoglycosides. The cat gene confers chloramphenicol-resistance.

Contig No: Total number of DNA sequence segments that comprise the genomic data.

N50: Average length (base pairs) of contig.

Longest contig: Longest contig described as number of base pairs.

Total bases: Sum of the base pairs comprising the genomic data.

Neg: Stands for negative and denotes absence of the genomic determinant.

sTable 2. PBP2x types and their corresponding sequences based upon 1167 bp query (bases 808-1974) corresponding to amino acids 270 – 688.