Supplementary Figure 1 legend. Pairwise-SNP distances for *emm43.4/*ST3 isolates collected in King County, Washington, using kSNP3.0. The isolates from patients A and E exhibited 8-fold decreased susceptibility to ampicillin and shared the same deduced PBP2x substitution (T553K). Genome sequence accessions for these GAS isolates associated in SRA Bioproject PRJNA559889 from the indicated patients are SAMN12562623 (patient A), SAMN12562622 (patient B), SAMN12562624 (patient C), SAMN12562625 (patient D), and SAMN12562626 (patient E).

Supplementary Figure 2 legend. Lack of growth rate difference between *emm43.4/*ST3 strains containing PBP2x T553K substitution and near isogenic controls. Harvested colonies from fresh overnight culture were resuspended in 5 ml fresh Todd Hewitt broth with 0.5% yeast extract (THY) to make a suspension with OD600nm=0.5. 100ul of the suspension was inoculated into 5mL of fresh THY to make a starting culture of OD600nm. Mean optical density OD=600nm was measured every 15 minutes for 24 hours at 37°C. Each data point is a mean of ODs from the 16 wells for a same isolate at a given time point. Two isolates with point mutation (from Patient A and Patient E) didn’t show difference in growth characteristics compared to their near-isogenic controls (from Patients B, C, and D).