

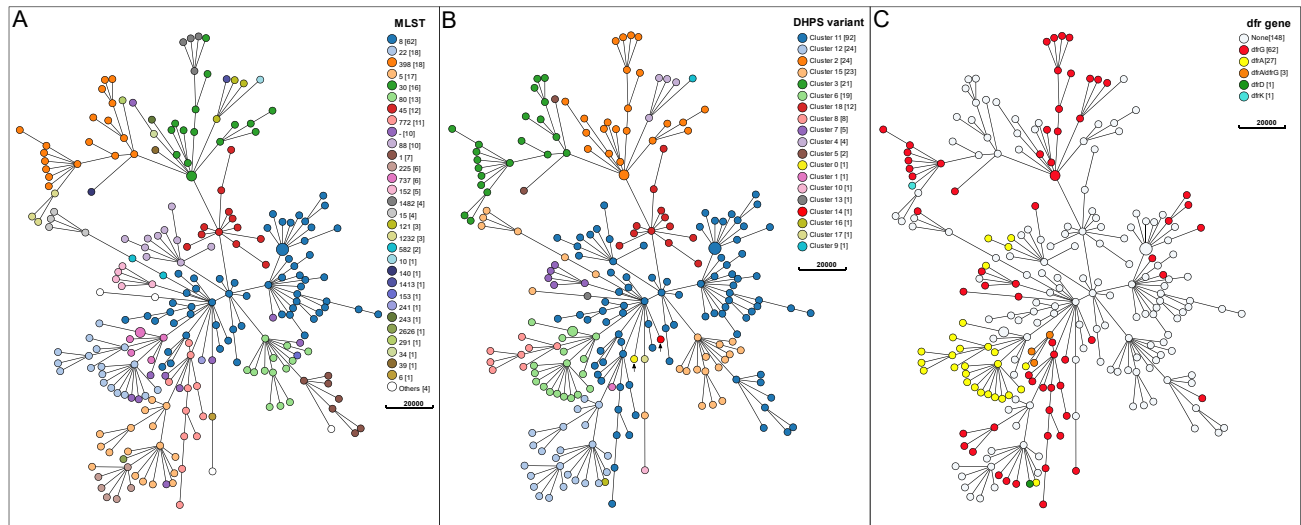
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dfrG reference MKVSLIAAMDKNRVIGKENDIPWRIPKDWYVKNNTTKGHPIILGRKNLESIGRALPDRRNIILTRDKGFTFNGCEIVHSIEDVFELCKNEBEIFIFGGEQIYNLFFPYVE 110
D1876 dfrG MKVSLIAAMDKNRVIGKENDIPWRIPKDWYVKNNTTKGHPIILGRKNLESIGRALPDRRNIILTRDKGFTFNGCEIVHSIEDVFELCKNEBEIFIFGGEQIYNLFFPYVE 110

dfrG reference KMYITKIHHEFEGDTFFPEVNYYEEWNEVFAQKGIKNDKNPYNYFFHVYERKNLLSX 166
D1876 dfrG KMYITKIHHEFEGDTFFQKX..... 130

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**Supplementary figure 1.** amino acid sequence of a truncated DHFR<sub>dfrG</sub> (D1876) due to a nucleotide deletion at position 381 of the *dfrG* resulting in a trimethoprim susceptible phenotype.



**Supplementary figure 2.** Minimum spanning tree (MST) of our study cohort. The MST is calculated using all 67571 polymorphic sites across the core-genome and with the MSTV” algorithm from the software grapetree. Strains are color-coded based either on A) their MLSTs, B) their DHPS variants, C) their *dfr* genes. The arrows in panel B indicate the two high-level SMZ resistant *S. aureus* isolates harboring the F17L mutation of the chromosomal DHPS.