

Supplementary Appendix

Phenotypic detection of hemin-inducible trimethoprim-sulfamethoxazole hetero-resistance in *Staphylococcus aureus*

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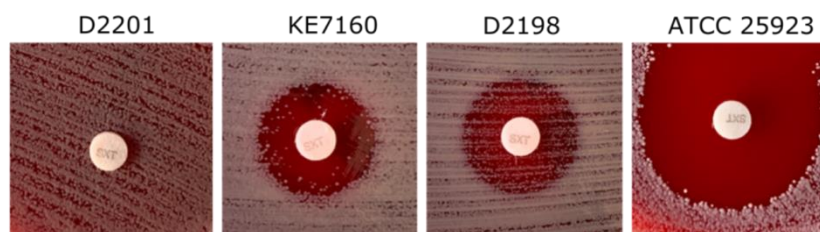
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Running title: monoclonal SXT heteroresistance *S. aureus*

Supplementary Figure S1. The different phenotypic resistance growth characteristics on Mueller-Hinton Agar supplemented with 5% horse blood.

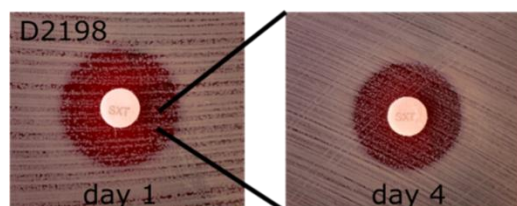
D2201=fully resistant phenotype; KE7160=low-density heteroresistance phenotype; D2198=high-density heteroresistance phenotype; ATCC25923=fully susceptible phenotype



Running title: monoclonal SXT heteroresistance *S. aureus*

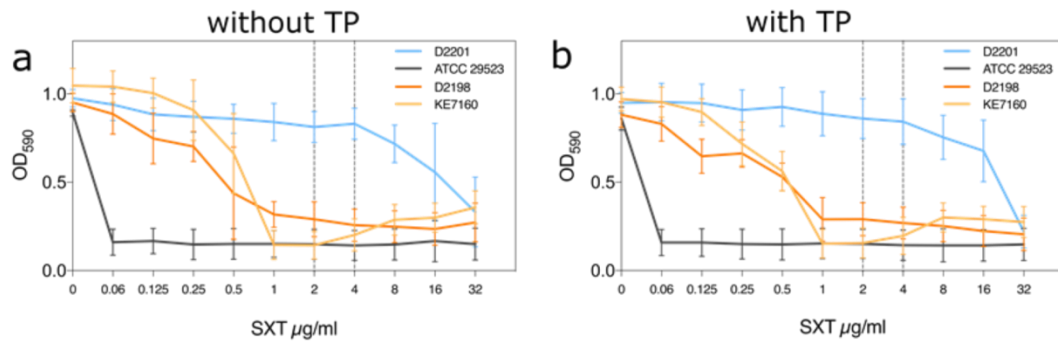
Supplementary Figure S2. Stability of heteroresistance phenotype.

Agar diffusion of a colony of trimethoprim-sulfamethoxazole resistant phenotype subcultured for 4 consecutive days exhibited similar heteroresistance phenotype as the original clone.



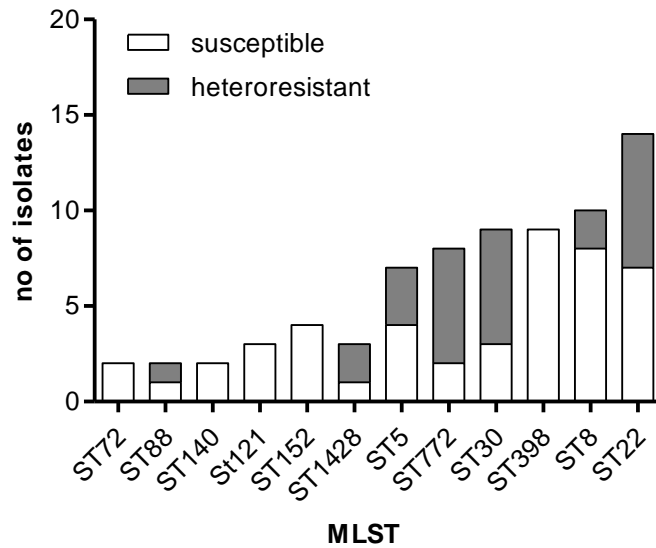
Supplementary Figure S3. Photometric population analysis profile.

Photometric growth analysis, measured at OD_{590nm} in various trimethoprim-sulfamethoxazole (1:19) concentrations in Mueller-Hinton broth without (a) and with 2.5 UI/ml thymidine phosphorylase (b). SXT concentration is expressed as concentration of trimethoprim component. Abbreviations: TP=thymidine phosphorylase, SXT=trimethoprim-sulfamethoxazole



Supplementary Figure S4. Association between MLST and heteroresistance phenotype in *Staphylococcus aureus*.

The heteroresistant phenotype towards trimethoprim-sulfamethoxazole is not confined to a particular ST-group. Only MLST with ≥ 2 isolates (n=81/95) were included in this analysis. Two isolates with fully resistant phenotype were not included in the figure. Abbreviations: MLST=multi-locus sequence typing; ST=sequence type



Running title: monoclonal SXT heteroresistance *S. aureus*

Supplementary Figure S5. Auxotrophy testing.

Staphylococcus aureus isolates D2198 and KE7160 did not exhibit thymidine, menadione or hemin auxotrophy. Both isolates exhibited normal growth on Mueller-Hinton agar and the presence of thymidine (T), menadione (M) or Hemin (X) did not change growth characteristics of the colony.

