**SUPPLEMENTARY DATA**

**Supplementary Table 2 – Phenotypic antibiotic susceptibilities of *S. aureus* wild-type, mutant and complemented strains to fusidic acid (FA) and mupirocin as determined by broth micro-dilution assays in this study.**

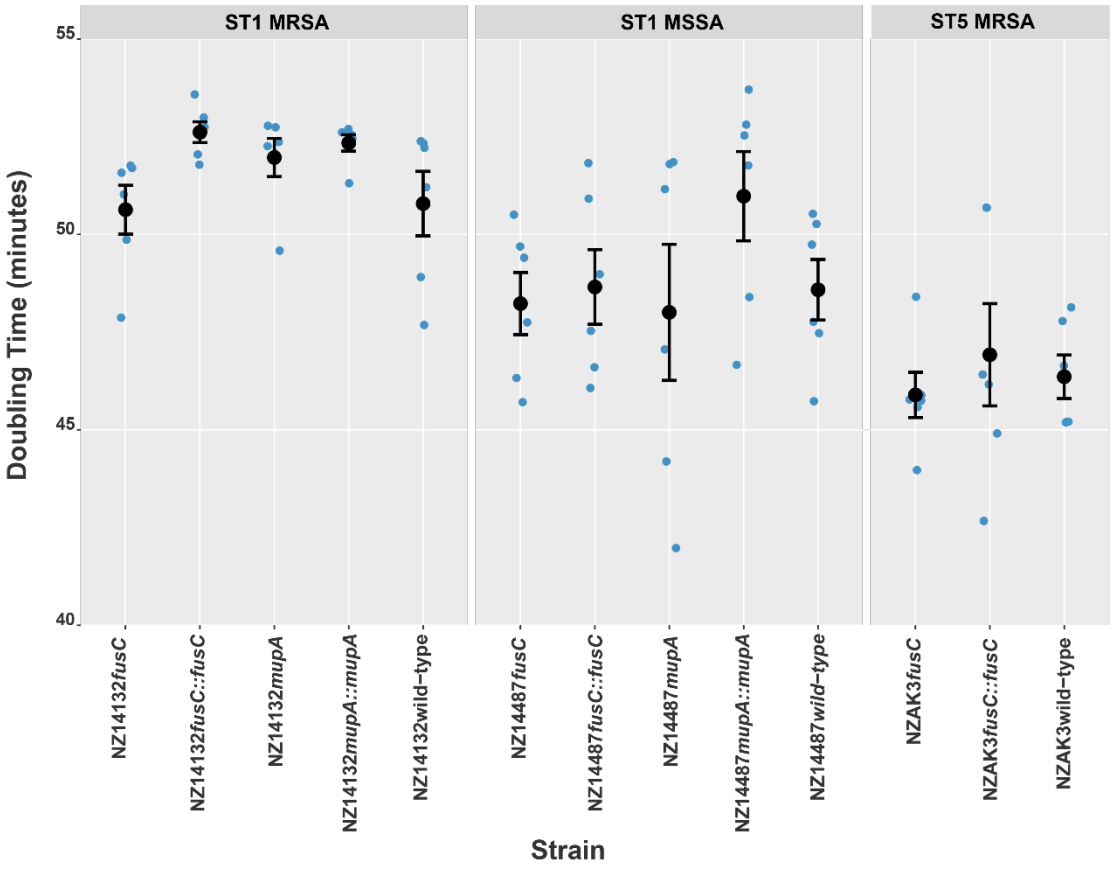
| Strain | WGS ID | FA MIC (mg/L) / $Log_2$ fold-change relative to wild-type MIC | Mupirocin MIC (mg/L) / $Log_2$ fold-change relative to wild-type MIC |
|---|---|---|---|
| NZ14132 | DW-38-S034-SA | 4 / 0 | >1024 / 0 |
| NZ14132*fusC* | DW-38-S035-SA | 0.0625 / -6 | ND* |
| NZ14132*fusC*::*fusC* | DW-38-S001-SA | 4 / 0 | ND |
| NZ14132*mupA* | DW-38-S036-SA | ND | 0.25 / -12 |
| NZ14132*mupA*::*mupA* | DW-38-S005-SA | ND | >1024 / 0 |
| NZ14487 | DW-38-S047-SA | 4 / 0 | >1024 / 0 |
| NZ14487*fusC* | DW-38-S048-SA | 0.0625 / -6 | ND |
| NZ14487*fusC*::*fusC* | DW-38-S060-SA | 4 / 0 | ND |
| NZ14487*mupA* | DW-38-S049-SA | ND | 0.25 / -12 |
| NZ14487*mupA*::*mupA* | DW-38-S007-SA | ND | >1024 / 0 |
| NZAK3 | DW-38-S061-SA | 4 / 0 | ND |
| NZAK3*fusC* | DW-38-S062-SA | 0.0625 / -6 | ND |
| NZAK3*fusC*::*fusC* | DW-38-S003-SA | 4 / 0 | ND |

*Abbreviations: ND, not determined.

**Supplementary Table 3 – Primer sequences used in the study**

| Primer | Sequence (5' to 3' direction) | Description |
|---|---|---|
| FUSC-Fp | CCTCACTAAAGGGAACAAAAGC TGGGTACCAATAAAATAATGGTG CTTGGGAAAGAAAAG | Flanking primers used for amplification of gene cassettes for *fusC* deletion and complementation |
| FUSC-Rp | CGACTCACTATAGGGCGAATTGG AGCTCAAAACAATAATAGCTATC TGTCAGTCTACC | |
| FUSC-KO-Fp | GTACTTCAACAAAAATGGAGGA ATATGAAATCCAAACAGCCCTGA TCTTTAGAACTAATG | Construction of gene cassettes for *fusC* deletion by SOE PCR |
| FUSC-KO-Rp | CATTAGTTCTAAAGATCAGGGCT GTTTGGATTTCATATTCCTCCATT TTTGTTGAAGTAC | |
| FUSC-COMP-Fp | ATGAATTAAAAGTCTACATCCAA GATTTTG | Construction of gene cassettes for *fusC* complementation by SOE PCR |
| FUSC-COMP-Rp | CAAAATCTTGGATGTAGACTTTT AATTCAT | |
| FUSC-OUT-Fp | CATTATCCTTGAAGACAGTTTATC CTGTAG | Confirmation on chromosomal integration of gene cassettes for *fusC* deletion and complementation |
| FUSC-OUT-Rp | CAGCAATATGATAACCACAATTA AACGTAC | |
| MUPA-Fp | CCTCACTAAAGGGAACAAAAGC TGGGTACCGAAAGGATGATTAAC TGATGAATAGAGCAG | Flanking primers used for amplification of gene cassettes for *mupA* deletion and complementation |
| MUPA-Rp | CGACTCACTATAGGGCGAATTGG AGCTCCAATATCAAATTCTCTATC TCCATATAAAC | |
| MUPA-KO-Fp | GAAATAAGTGATACTCTAGGAGG CTGAAAAGTTACAAACATGGCCA CTCTATTTTAGTAGAGTG | Construction of gene cassettes for *mupA* deletion by SOE PCR |
| MUPA-KO-Rp | CACTCTACTAAAATAGAGTGGCC ATGTTTGTAACTTTTCAGCCTCCT AGAGTATCACTTATTTC | |
| MUPA-COMP- | CTTAGTTGCCCTAAGTGTAATGG | Construction of gene cassettes |

| | | |
|---|---|---|
| Fp | GAAAATGTCGCGAGTAGAAGAAGTAATCGATGTTTG | for *mupA* complementation by SOE PCR |
| MUPA-COMP-Rp | CAAACATCGATTACTTCTTCTACTCGCGACATTTTCCCATTACACTTAGGGCAACTAAG | |
| MUPA-OUT-Fp | AGTTAAAAAGTAGAACCATTAATTTTAAATGG | Confirmation on chromosomal integration of gene cassettes for *mupA* deletion and complementation |
| MUPA-OUT-Rp | AATCTAATGGAAATTTTTCTAATGCTAGAG | |
| pIMAY-Z-Fp | GGTACCCAGCTTTTGTTCCCTTTAGTGAGG | Amplification of pIMAY-Z vector |
| pIMAY-Z-Rp | GAGCTCCAATTCGCCCTATAGTGAGTCG | |




**Supplementary Figure 1 – Doubling times for *S. aureus* wild-type, complemented, and mutant strains grown in BHI broth.** For each strain tested, six biological replicates (blue dots) were included for determining mean doubling times (black dots) and SEM (black error bars). No significant difference ($P > 0.05$, unpaired t test) in doubling time was observed when comparing the complemented and mutant strains to their respective wild-type.