**Supplementary File 1: Strains and plasmids used in this study**

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| **Strains** | **Relevant characteristics** | **Origin** |
| ***S. aureus*** |  |  |
| RN4220 | Restriction deficient derivative of *S. aureus* NCTC8325-4 strain capable of receiving foreign DNA | R. Novick |
| NCTC8325-4 | MSSA *S. aureus* strain | R. Novick |
| MW2 | CA-MRSA *S. aureus* strain | (Baba et al., 2008) |
| NCTCΔ*oatA* | NCTC8325-4 *oatA* null mutant; *oat* encodes a *O*-acetyltransferase that determines PGN resistance to  lysozyme (Bera et al., 2005) | This study |
| NCTCΔ*fmtA* | NCTC8325-4 *fmtA* null mutant; *fmtA* is involved in the modulation of the major autolysin and in the synthesis of PGN (Qamar and Golemi-Kotra, 2012) | This study |
| NCTCΔ*arlR* | NCTC8325-4 *arlR* null mutant; *arlR* encodes a regulator of the expression of autolysins and other proteins (Liang et al., 2005) | This study |
| NCTCΔ*atl* | NCTC8325-4 *atl* null mutant; *atl* encodes a major staphylococcal autolysin (Oshida et al., 1995) | This study |
| NCTCΔ*tagO* | NCTC8325-4 *tagO* null mutant ; *tagO* encodes to a glycosyltransferase required for the first step of WTA biosynthesis | (Atilano et al., 2010) |
| NCTCΔ*tarS* | NCTC8325-4 *tarS* null mutant *tarS* is required for the attachment of β-*O*-GlcNAc residues to WTA (Brown et al., 2012) | This study |
| NCTCΔ*dltA* | NCTC8325-4 *dltA* null mutant; *dltA* is required for the production of teichoic acids with attached D-alanyl esters (Peschel et al., 1999) | This study |
| NCTCΔ*atl*Δ*tagO* | NCTC8325-4 lacking *atl* and *tagO* genes | This study |
| MW2∆*atl* | MW2 *atl* null mutant | This study |
| AMH265A | NCTC8325-4 encoding Atl protein with mutation H265A in the amidase domain. | This study |
| GLE1128A | NCTC8325-4 encoding Atl protein with mutation E1128A in the glucosaminidase domain. | This study |
| AtlH265A/E1128A | NCTC8325-4 encoding Atl protein with mutations H265A and E1128A | This study |
| ***S. pneumoniae*** |  |  |
| R36A | Non-encapsulated laboratory strain | (Avery et al., 1944) |
| R36AΔ*lytA* | R36A *lytA* null mutant; *lytA* encodes a major pneumococcal autolysin (Mosser and Tomasz, 1970) | This study |
| R36AΔ*lytA*p*lytA* | R36A *lytA* null mutant complemented with replicative plasmid to express LytA | This study |
| ***L. lactis***  LL108 | RepA+ MG1363, Cmr | (Leenhouts et al., 1996) |
| ***E. coli*** |  |  |
| EC101 | *E. coli* cloning strain with  *repA* from pWVO1 integrated in the chromosome | (Law et al., 1995) |
| DH5α | *recA endA1 gyrA96 thi-1 hsdR17 supE44 relA1 ϕ80 ΔlacZ*ΔM15 | Lab stock |
| DC10B | Δ*dcm* in the DH10B background; Dam methylation only | (Monk et al., 2012) |
| BL21(DE3) | *F–ompT gal dcm lon hsdSB(rB-mB-) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])* | Lab stock |
| **Plasmids** |  |  |
| pMAD | *E. coli* – *S. aureus* shuttle vector with a thermosensitive origin of replication for Gram-positive bacteria; Ampr; Eryr; LacZ+ | (Arnaud et al., 2004) |
| pORI280 | Integrative vector which replicates in strains containing RepA; Eryr; LacZ+ | (Leenhouts et al., 1996) |
| pΔ*oatA* | pORI280 with *oatA* up and downstream regions | This study |
| pΔ*fmtA* | pORI280 with *fmtA* up and downstream regions | This study |
| pΔ*arlR* | pMAD with *arlR* up and downstream regions | This study |
| pΔ*atl* | pMAD with *atl* up and downstream regions | This study |
| pΔ*tarS* | pMAD with *tarS* up and downstream regions | This study |
| pΔ*dltA* | pMAD with *dltA* up and downstream regions | (Atilano et al., 2011) |
| pΔ*tagO* | pMAD with *tagO* up and downstream regions | (Atilano et al., 2010) |
| pAMH265A | pMAD encoding a truncated Atl protein with mutation H265Ain AM domain | This study |
| pGLE1128A | pMAD encoding a truncated Atl protein with mutation E1128Ain GL domain | This study |
| pBCSLF001 | High-copy-number vector, containing the −10 constitutive promoter of sigA from S. pneumoniae, Tetr | (Henriques et al., 2011) |
| p∆*lytA* | pORI280 with *lytA* up and downstream regions | This study |
| p*lytA* | pBCSLF01 expressing *S. pneumoniae* LytA | This study |
| pGL100 | *E. coli* replicative plasmid expressing *S. pneumoniae* LytA. Ampr | (Garcia et al., 1987) |
| pET28a(+) | *E. coli* expression vector. Kmr. | Novagen |
| pET-AMR1R2R3GL | pET28a(+) expressing His6-AMR1R2R3GL protein. | This study |
| pET-AMR1R2 | pET28a(+) expressing His6-AMR1R2 protein. | This study |
| pET-R3GL | pET28a(+) expressing His6-R3GL protein. | This study |
| pET-AMH265A | pET28a(+) expressing His6-AM(H265A)R1R2 protein. | This study |
| pET-GLE1228A | pET28a(+) expressing His6-R3GL(E1228A) protein. | This study |
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