Supporting Information

TITLE

Functional identification of serine hydroxymethyltransferase as a key gene involved in lysostaphin resistance and virulence potential of *Staphylococcus aureus* strains

RUNNING TITLE

Role of *shmT* in lysostaphin resistance and virulence

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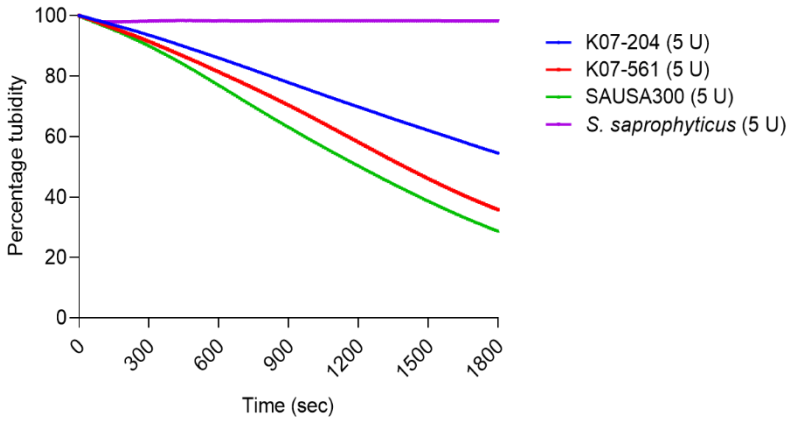
Content

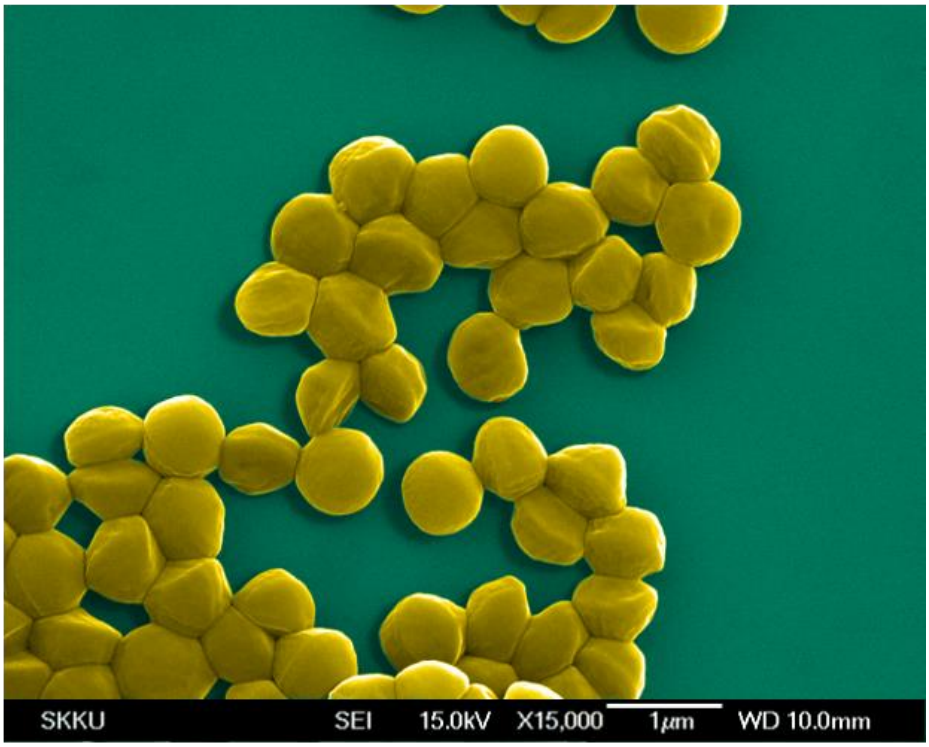
Supporting Fig. S1



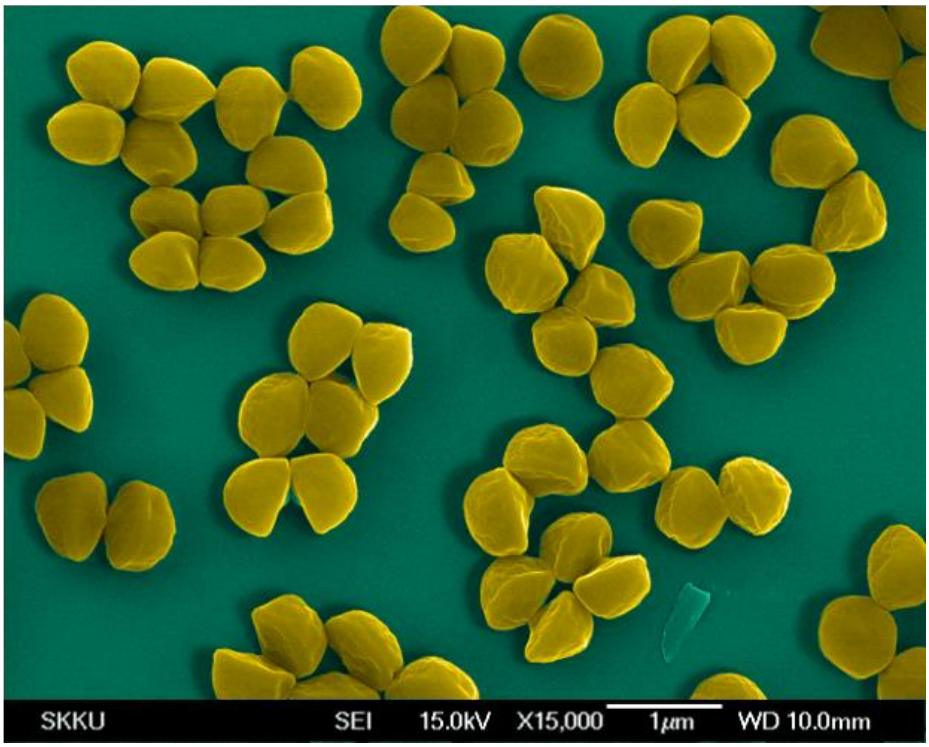
Supporting Fig. S1. Lysostaphin killing kinetics of human isolates of ST72. (A) Lysostaphin mediated killing efficiency using turbidity reduction among *lys*^{r} (K07-204) and *lys*^{s} (K07-561) in comparison to *S. aureus* USA300. Lysostaphin resistant *lys*^{r} K07-204 showed 37% percent turbidity reduction as compared to ≥60% turbidity reduction for K07-561 and *S. aureus* USA300 within 30 min of lysostaphin treatment. *S. saprophyticus* displayed resistance to lysostaphin treatment.

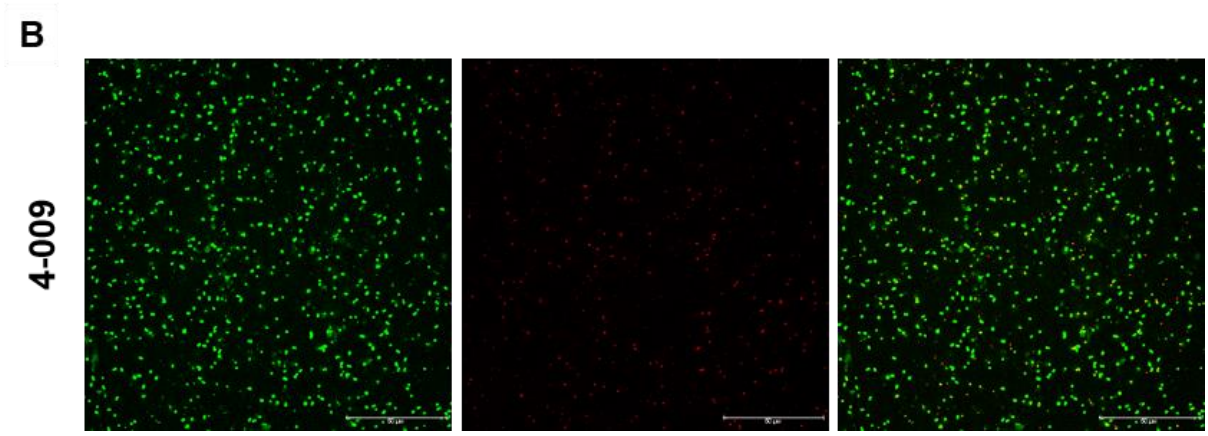
Supporting Fig. S2

A



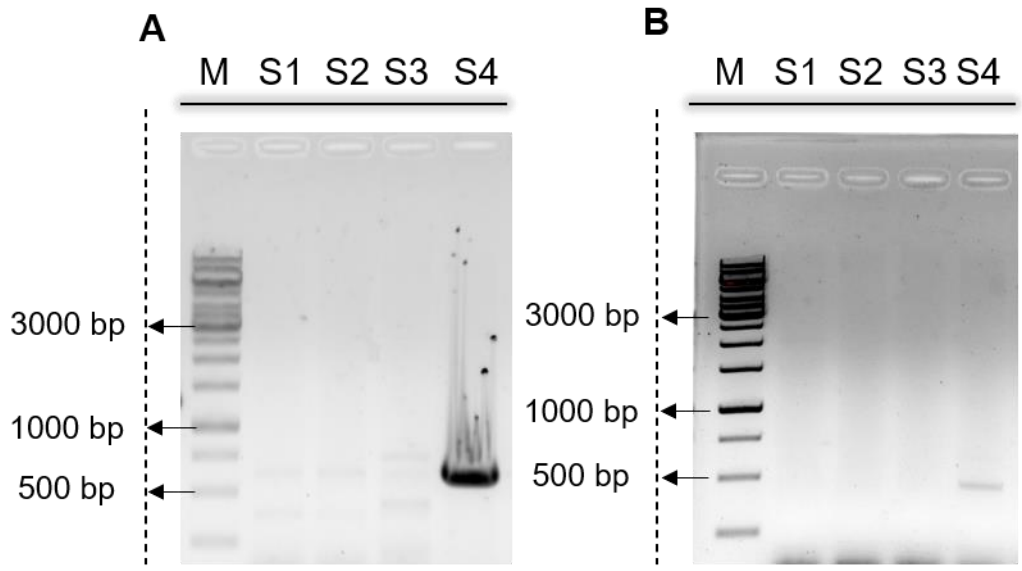
A'





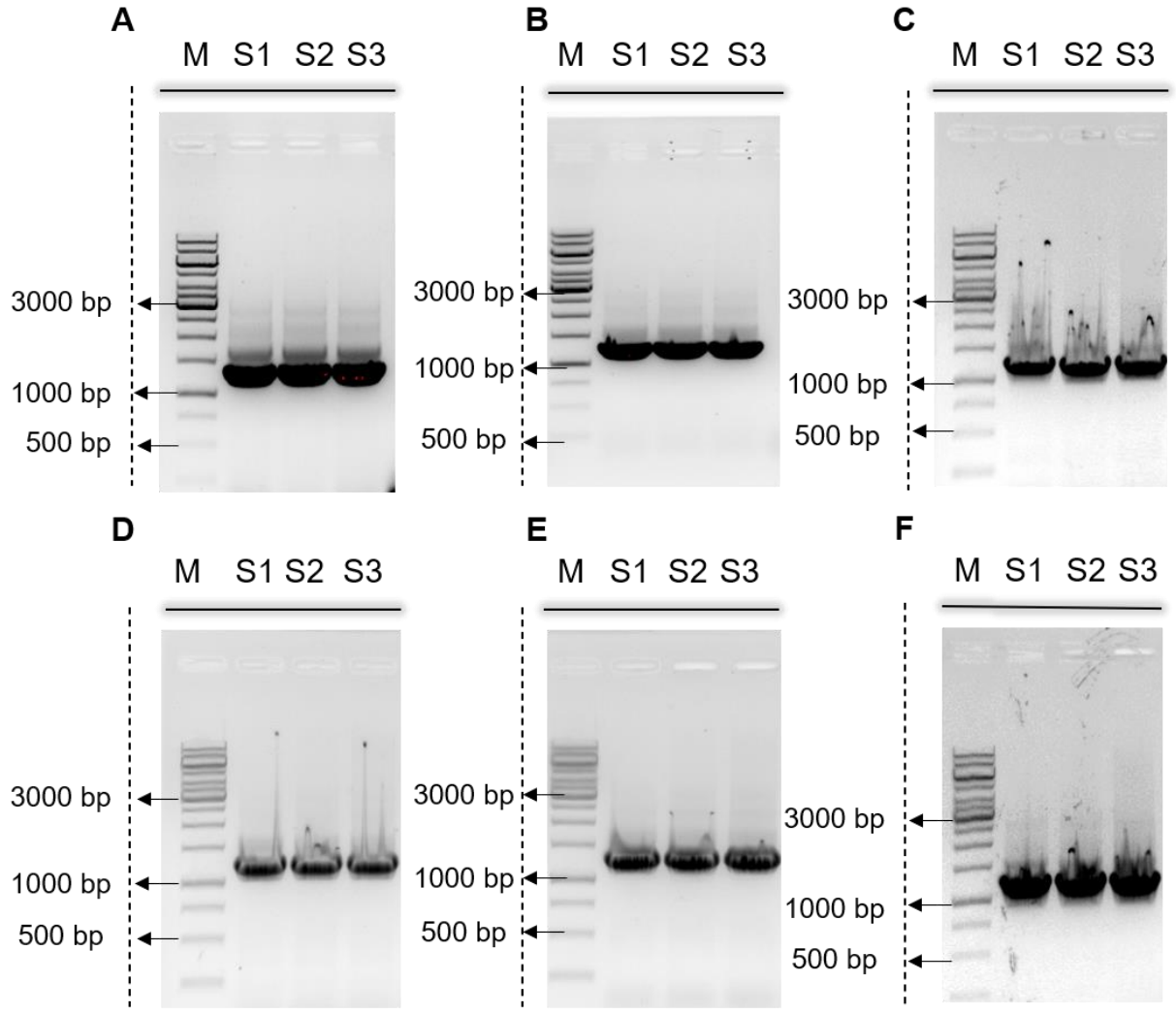
Supporting Fig. S2. **Scanning electron and confocal microscopy of ST72 resistant soil isolate, 4-009 to assess lysostaphin-mediated alteration in cell morphology and live/dead staining. (A-A')** SEM photomicrograph to assess the lysostaphin-mediated alteration in the cell morphology of lysostaphin resistant (*lys*^{r}) ST72 soil isolate 4-009 before (**A**) and after lysostaphin treatment (**A'**) and displayed no alterations post lysostaphin treatment (**A'**); **(B)** Live/dead images of *S. aureus* lysostaphin resistant (*lys*^{r}) ST72 isolate, 4-009 after lysostaphin treatment (4 U) using SYTO9/PI for 5 min. The total number of 4-009 cells were stained with SYTO9 stain (SYTO channel; green fluorescent cells) while a smaller proportion of cells were stained with PI (PI channel; red fluorescent cells) showing dead cells.

Supporting Fig. S3



Supporting Fig. S3. PCR based screening of presence/absence of *epr* and *lss* genes responsible for lysostaphin resistance in human isolates of ST72. (A) Agarose gel showing the presence/absence of endopeptidase gene (*epr*) screened by PCR amplification in ST72 isolates, K07-204 (S1), K07-561 (S2) as compared to *S. aureus* USA300 (negative control, S3) and *S. simulans* (positive control, S4) wherein the *epr* gene was amplified only in *S. simulans* on agarose gel conferring lysostaphin protection. **(B)** Agarose gel showing the PCR amplified lysostaphin gene (*lss*) in ST72 isolates, K07-204 (S1), K07-561 (S2) as compared to *S. aureus* USA300 (negative control, S3) and *S. simulans* (positive control, S4) wherein the *lss* gene was amplified only in *S. simulans* conferring lysostaphin production. (M denotes the 1kb DNA marker ranging from 250 bp to 10 kb). These results indicate that the ST72 isolates, K07-204 (lys^r) and K07-561 (lys^s) are not the lysostaphin producers.

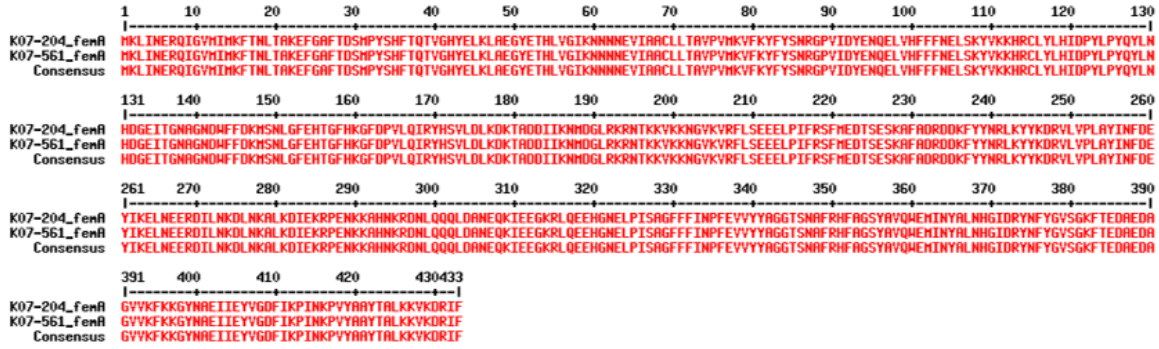
Supporting Fig. S4



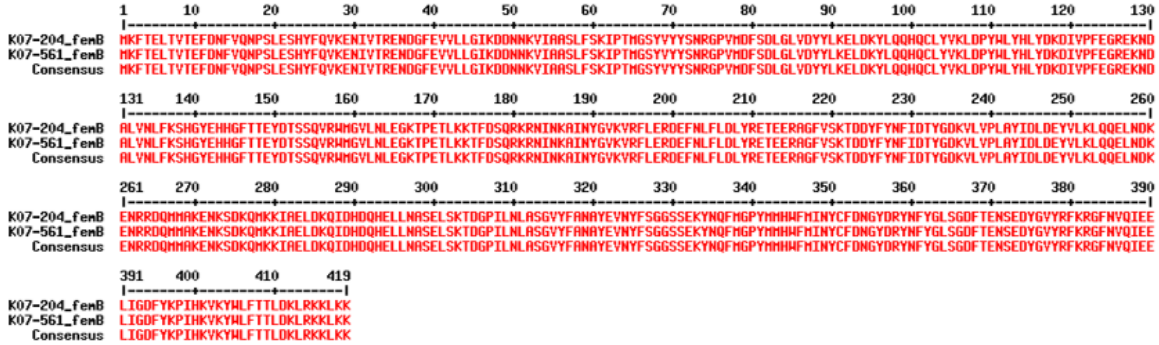
Supporting Fig. S4. PCR amplification, cloning, sequencing, and screening of associated mutation(s) in other key genes known for lysostaphin resistance. (A-F) Agarose gel showing the amplification of *femA* **(A)** *femB* **(B)** *femX* **(C)** *fmhC* **(D)** *lyrA* **(E)** and *shmT* **(F)** in ST72 isolates, K07-204 (S1), K07-561 (S2) and *S. aureus* USA300 (S3). These genes were amplified to clone in pCRTOPO2.1 cloning vector. Clones were sequenced to assess the mutation(s), if any, known to be responsible for lysostaphin resistance. M denotes the 1kb DNA marker ranging from 250 bp to 10 kb.

Supporting Fig. S5

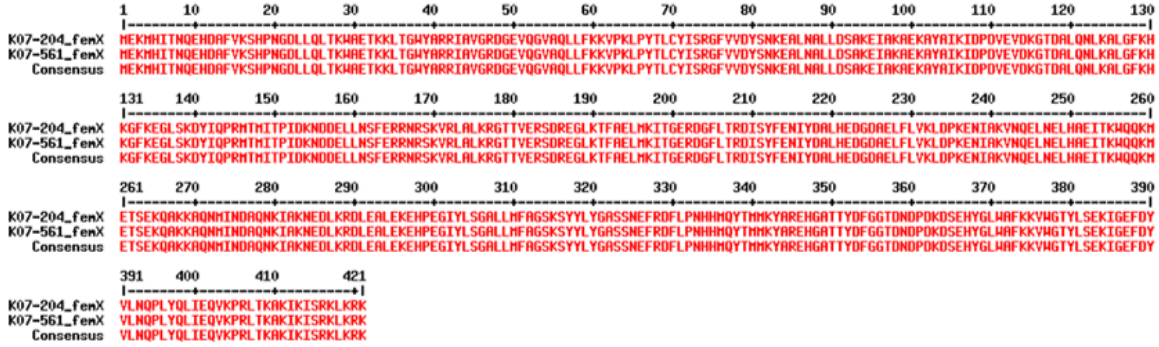
A

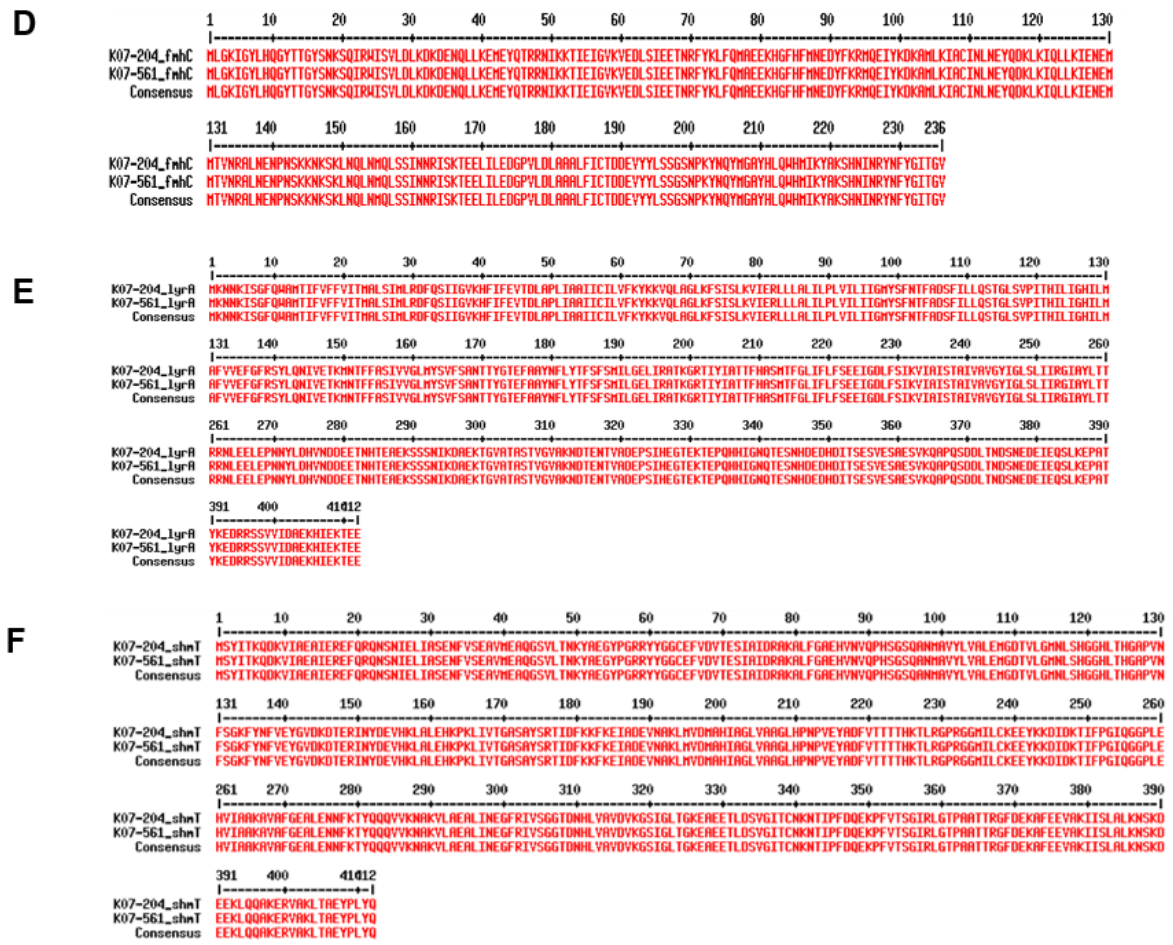


B



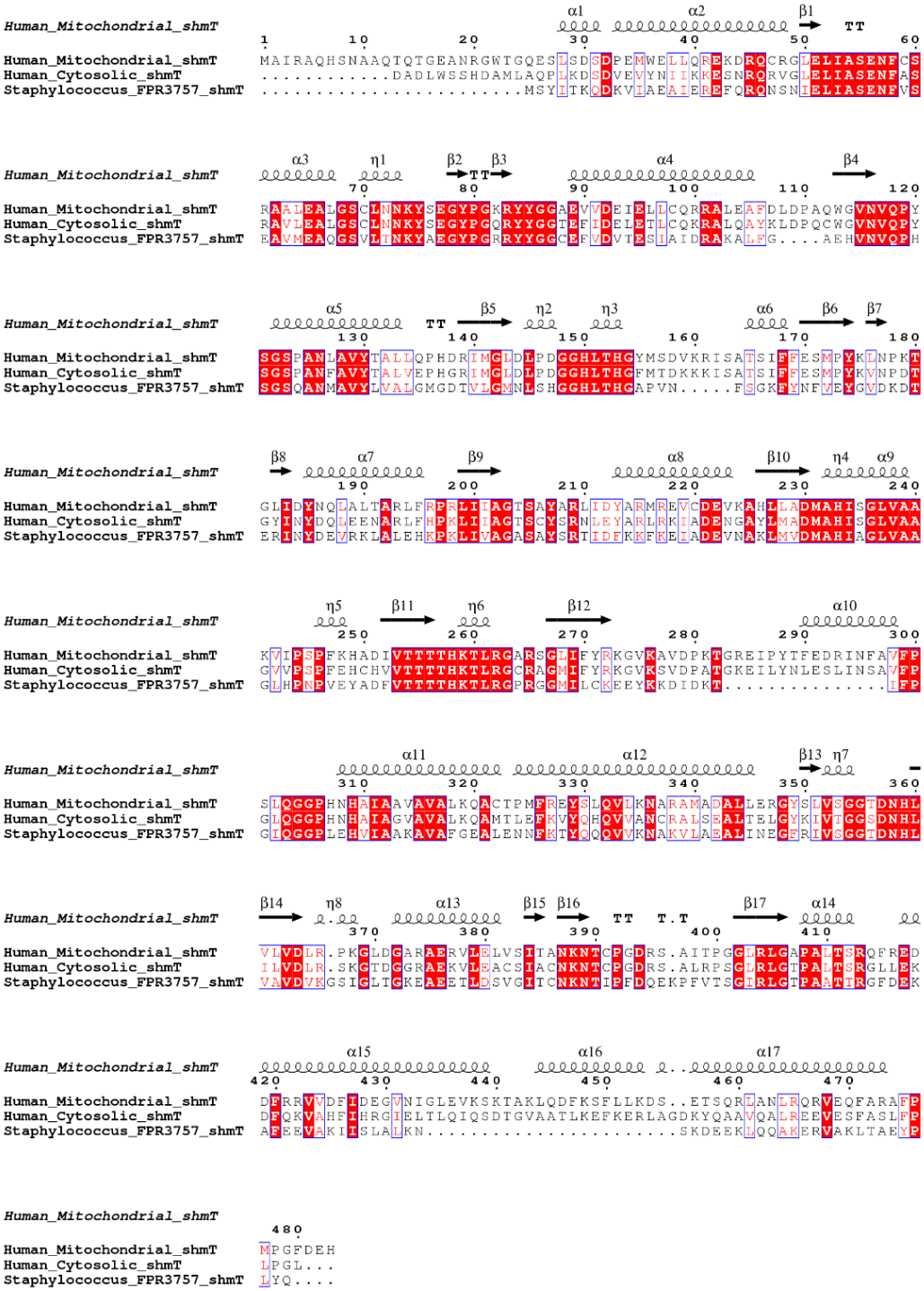
C





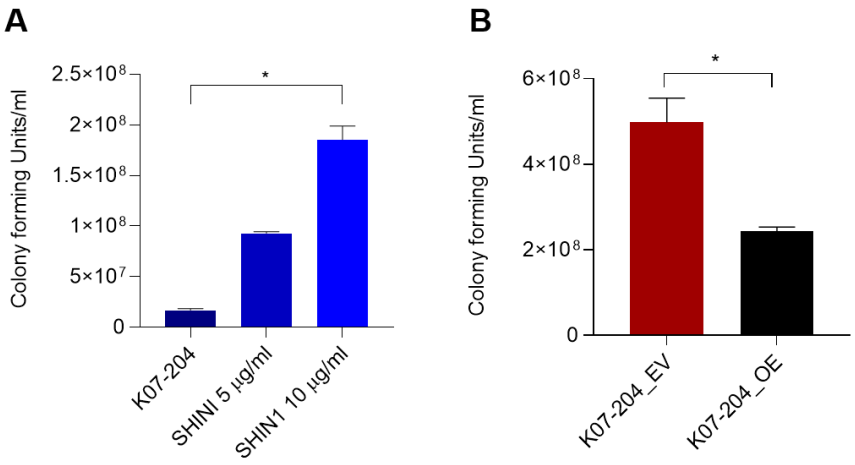
Supplementary Fig. S5. Cloning, sequencing, multiple sequence alignment to assess the mutation(s) upon translated DNA sequences. (A-F) Cloning of **(A)** *femA*, **(B)** *femB*, **(C)** *femX*, **(D)** *fmhC*, **(E)** *lyrA* **(F)** and *shmT* into pCR2.1TOPO cloning vector followed by DNA sequencing of multiple clones to assess mutation, if any, responsible for differential lysostaphin resistance between human isolates of ST72 K07-204 (*lys*^{r}) and K07-561 (*lys*^{s}). The sequenced DNA were translated *in-silico* to get amino acid sequences. The amino acid sequences of lysostaphin resistant (*lys*^{r}) K07-204 and lysostaphin susceptible (*lys*^{s}) K07-561 showed 100% identity. These results indicated that no known mechanism exists to explain the differential lysostaphin resistance between the lysostaphin resistant (*lys*^{r}) K07-204 and lysostaphin susceptible (*lys*^{s}) K07-561, human isolates of ST72.

Supporting Fig. S6



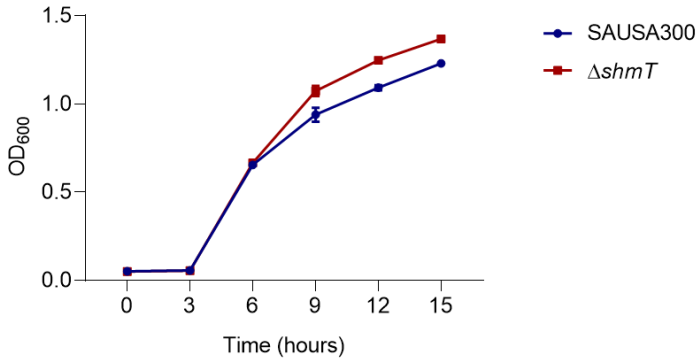
Supporting Fig. S6. Alignment of SHMT from *S. aureus* USA300 with human *SHMTs* to assess the overall similarity and identity. Alignment results showed a significantly high identity with two humans SHMTs, human cytosolic (UniProtKB - P34896) and mitochondrial SHMT (UniProtKB - P34897) with SHMT of *S. aureus* USA300 FPR3757 (CP000255.1). The human cytosolic and mitochondrial SHMT displayed 63.45% identity, while the human cytosolic and mitochondrial SHMT displayed 45.5%and 42% identity with SHMT of *S. aureus* USA300 FPR3757, respectively

Supporting Fig. S7



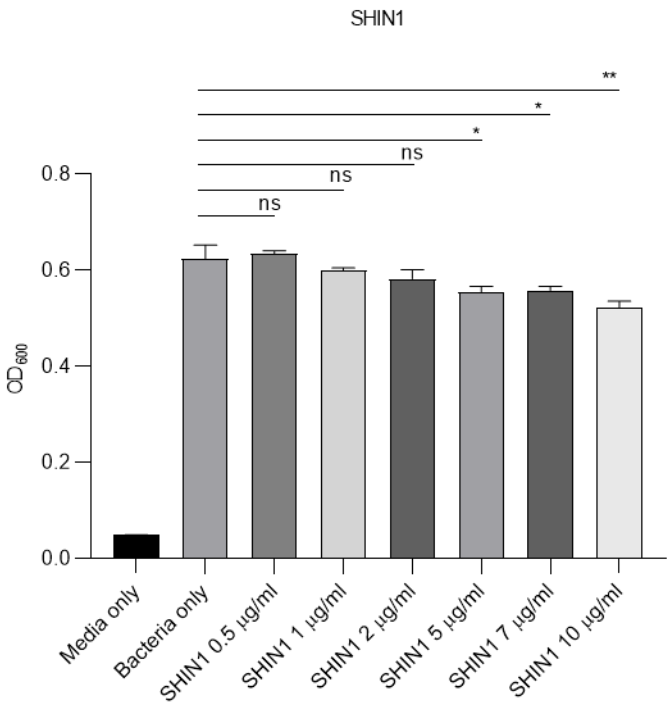
Supporting Fig S7. Role of SHMT in lysostaphin resistance of K07-204 human isolate of ST72. (**A**) The phenotypic assessment of lysostaphin resistance/susceptibility of K07-204 upon SHIN1-mediated inhibition of SHMT wherein the inhibition of SHMT marginally enhanced the resistance of K07-204, while (**B**) the overexpression of *shmT* (K07-204 with pRMC2_*shmT*) showed reduced lysostaphin resistance of K07-204. The lysostaphin killing assay was performed by using 5 units for 10 min incubation.

Supporting Fig. S8



Supporting Fig. S8. The role of *shmT* on the fitness of *S. aureus* USA300. The role of *shmT* on the fitness of SAUSA300 was assessed by comparing the growth of wild type SAUSA300 and Δ*shmT* knockout in TSB media for 16h. The growth of the Δ*shmT* knockout and wild type SAUSA300 was found to be comparable.

Supporting Fig. S9



Supporting Fig. S9. Serine hydroxymethyltransferase inhibitor 1 (SHIN1) toxicity to *S. aureus* USA300 cells at varying concentrations. The SHIN1 showed insignificant inhibition of bacterial growth up to 2 μg/mL while a mild inhibition in the cell division was observed beyond 2 to 10 μg/mL, measured by estimating the inhibition of cell density at $OD_{600\ nm}$.

Table S1. *S. aureus* ST72 isolates

Sequence type 72	MRSA/MSSA	Source of Isolation	Reference
K01-140	MRSA	Human	[1]
K07-204	MRSA	Human	
K07-322	MRSA	Human	
K01-799	MSSA	Human	
K07-006	MSSA	Human	
K07-561	MSSA	Human	
05-B-52	MRSA	Animal	
05-B-60	MRSA	Animal	
08-B-93	MRSA	Animal	
08-P-236	MRSA	Animal	
4-009	MRSA	Soil	

Table S2. Primers used in the study

Purpose	Name	Sequence (5'-3')	Reference/Source	Amplicon size (bp)
PCR	*lss*_fwd	GCTATTGGACTGAGTACATTTGCC	This study	Not amplified
	*lss*_rev	CTGCGGCATGCTTCTAAATGGACCAGTC		
	*epr*_fwd	CTAYWCACATMGMGGTCCWGTCATRRAC	This study	Not amplified
	*epr*_rev	TTAGAATTAGGGTTTTCTTTTAAT		
	*fmhC*_fwd_*KpnI*	AACATAGGTACCATGAAATTTTCAACTTTAAGTG	This study	1245
	*fmhC*_rev_*EcoRI*	AAGATAGAATTCTCAAACCTTATAAATAAGTTTTGC		
	*femA*_fwd_*KpnI*	AACATAGGTACCTTGCAGAGGGGAAATAGAAAAACTGC	This study	1338
	*femA*_rev_*EcoRI*	AAGATAGAATTCCTAAAAAATTCTGTCTTTAACTTTTT		
	*femB*_fwd_*KpnI*	AACATAGGTACCATGAAATTTACAGAGTTAACTG	This study	1260
	*femB*_rev_*EcoRI*	AAGATAGAATTCCTATTTCTTTAATTTTTTACGT		
	*femX*_fwd_*KpnI*	AACATAGGTACCATGGAAAAGATGCATATCACTAATC	This study	1266
	*femX*_rev_*EcoRI*	AAGATAGAATTCCTATTTTCGTTTTAATTTACGAG		
	*lyrA*_fwd_*KpnI*	AACATAGGTACCATGAAGAACAATAAAATTTCTG	This study	1260
	*lyrA*_rev_*EcoRI*	AAGATAGAATTCTTATTTGTTTTTATCTGAAGATTG		
	*shmT*_fwd_*KpnI*	AACATAGGTACCATGTCTTATATCACCAAGCAAG	This study	1239
	*shmT*_rev_*EcoRI*	AAGATAGAATTCTTATTGATATAGAGGATATTCAGC		
qRT-PCR	*gyrA*_fwd	CGTCAACGTATTGTTGTCAC	This study	180
	*gyrA*_rev	ACACTAGCATTTGCATCCTT		
	*shmT*_fwd	TCGGAAGCGGTTATGGAA	This study	196
	*shmT*_rev	CAGCCATGTTCGCTTGTG		

Table S3. Staphylococcal strains/isolates and plasmid used in the study

Strains	Organisms	Descriptions	Reference/Source
Strains	*Escherichia coli* DH5α	*F- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoRnupG Φ80dlacZΔM15 Δ(lacZYA-argF) U169, hsdR17(rK- mK+), λ–*	Invitrogen, USA
	E. coli DH5α_pRMC2	For amplification of pRMC2 vector, amp^r	This study
	WT USA300 FPR3757	JE2, wild-type epidemic community-associated methicillin-resistant *S. aureus* isolate USA300 LAC	NARSA
	RN4220	Restriction-deficient strain of NCTC8325	[2]
	WT *Staphylococcus simulans*	Lysostaphin synthesizing (*lss*) and resistance gene (*epr*)	Lab collection
	WT *Staphylococcus saprophyticus*	Lysostaphin resistant strain	KCTC3345
	RN4220_pRMC2	For amplification of pRMC2 vector in *S. aureus* RN4220 as cloning intermediate, Cm^r	This study
	RN4220_pRMC2_*shmT*	For amplification of pRMC2_*shmT* in *S. aureus* RN4220 as cloning intermediate, Cm^r	
	Δ*shmT*	Knock out of *shmT* gene, Em^r	Nebraska library
	SAUSA300_pRMC2	SAUSA300_EV, Cm^r	This study
	Δ*shmT*_pRMC2	Δ*shmT*_EV, Cm^r	This study
	Δ*shmT*_pRMC2_*shmT*	Δ*shmT*_Comp, Cm^r	This study
	SAUSA300_pRMC2_*shmT*	SAUSA300_OE, Cm^r	This study
	E. coli DH5α_pCR2.1 TOPO_ *K07-561_fmhC*	P_{lac}, *K07-561_fmhC*, Km^r, Amp^r	This study
	E. coli DH5α_pCR2.1 TOPO_*K07-561_femA*	P_{lac}, *K07-561_femA*, Km^r, Amp^r	This study
	E. coli DH5α_pCR2.1 TOPO_*K07-561_femB*	P_{lac}, *K07-561_femB*, Km^r, Amp^r	This study
	E. coli DH5α_pCR2.1 TOPO_*K07-561_femX*	P_{lac}, *K07-561_femX*, Km^r, Amp^r	This study
	E. coli DH5α_pCR2.1 TOPO_*K07-561_lyrA*	P_{lac}, *K07-561_lyrA*, Km^r, Amp^r	This study
	pCR2.1 TOPO_ *K07-561_shmT*	P_{lac}, *K07-561_shmT*, Km^r, Amp^r	This study

	E. coli DH5α_pCR2.1 TOPO_*K07-204_fmhC*	P_{lac}, *K07-204_fmhC*, Km^r, Amp^r	**[3]**
	E. coli DH5α_pCR2.1 TOPO_*K07-204_femA*	P_{lac}, *K07-204_ femA*, Km^r, Amp^r	Invitrogen
	E. coli DH5α_pCR2.1 TOPO_*K07-204_femB*	P_{lac}, *K07-204_femB*, Km^r, Amp^r	
	E. coli DH5α_pCR2.1 TOPO_*K07-204_femX*	P_{lac}, *K07-204_femX*, Km^r, Amp^r	
	E. coli DH5α_pCR2.1 TOPO_*K07-204_lyrA*	P_{lac}, *K07-204_lyrA*, Km^r, Amp^r	
	pCR2.1 TOPO_*K07-204_shmT*	P_{lac}, *K07-204_shmT*, Km^r, Amp^r	
Native Plasmids	pRMC2	Expression vector under control of tetracycline inducible P*xyl*/*tetO*, Amp^r, Cm^r	
	pCR2.1 TOPO	Expression vector under control of lactose inducible promoter P_{lac}, Km^r, Amp^r	

References

1. Ko, K. S.; Lim, S. K.; Jung, S. C.; Yoon, J. M.; Choi, J. Y.; Song, J. H., Sequence type 72 meticillin-resistant *Staphylococcus aureus* isolates from humans, raw meat and soil in South Korea. *J Med Microbiol* **2011,** 60, (Pt 4), 442-5.
2. Peng, H. L.; Novick, R. P.; Kreiswirth, B.; Kornblum, J.; Schlievert, P., Cloning, characterization, and sequencing of an accessory gene regulator (*agr*) in *Staphylococcus aureus*. *J Bacteriol* **1988,** 170, (9), 4365-72.
3. Corrigan, R. M.; Foster, T. J., An improved tetracycline-inducible expression vector for *Staphylococcus aureus*. *Plasmid* **2009,** 61, (2), 126-9.