Supplementary Material

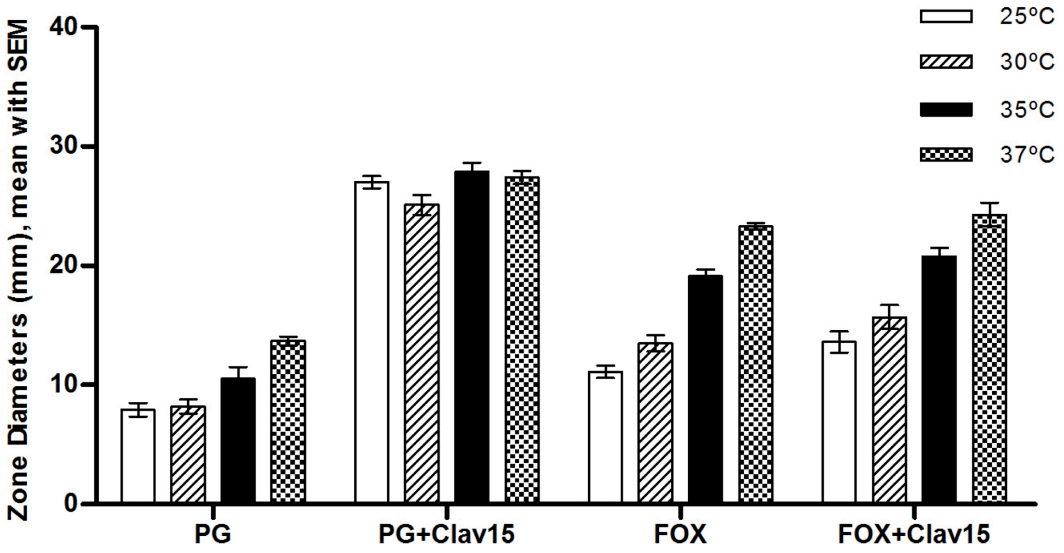

Figure S1: The effect of different incubation temperatures (ºC) on antimicrobial susceptibility testing for *mecC*-MRSA strains (n=10). Penicillin (PG) and cefoxitin (FOX) disc diffusion was performed on Iso-Sensitest agar (ISA) with or without 15 µg/ml clavulanic acid.

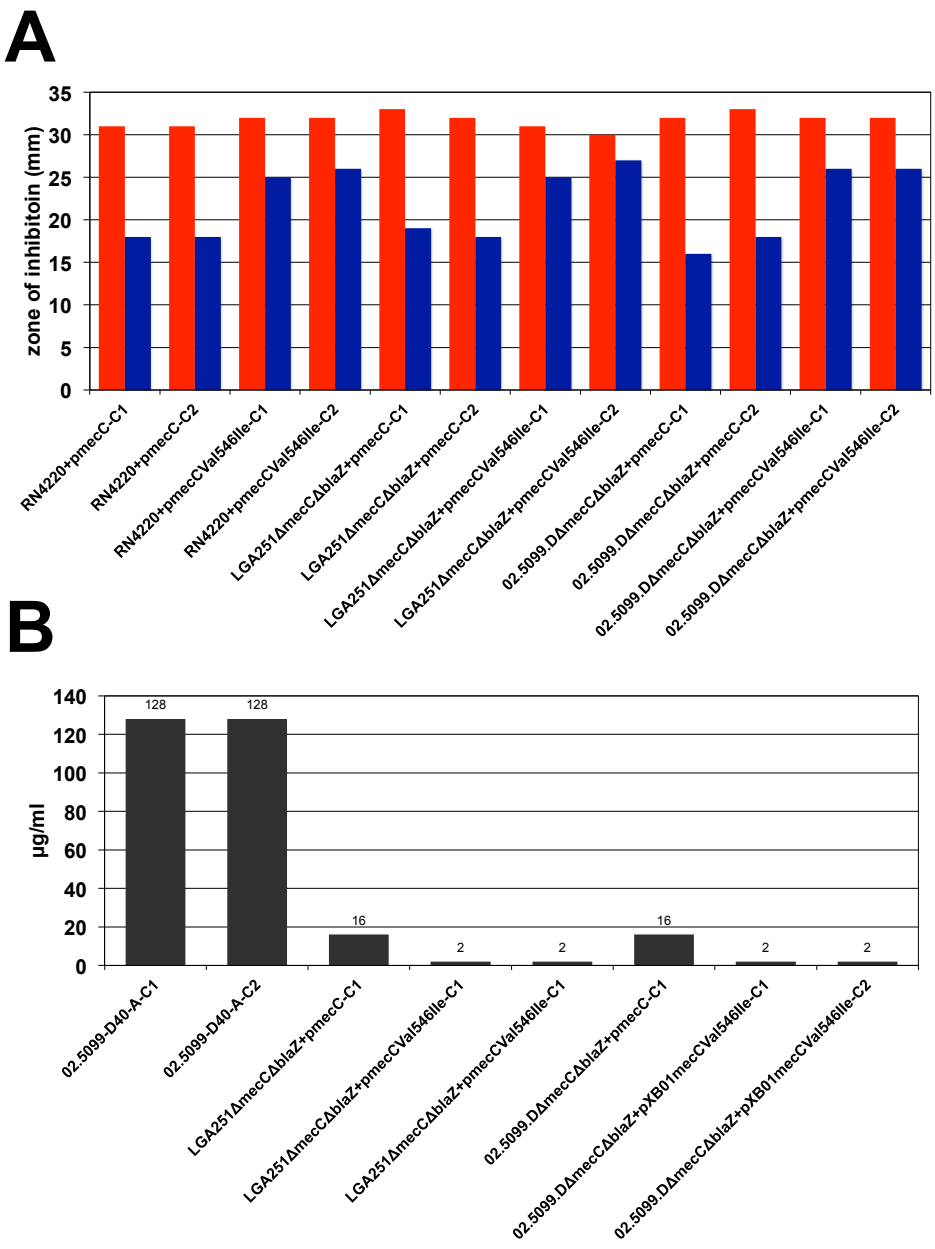

Figure S2: Effect of PBP2c Val546Ile substitution. (A) Comparison of disc diffusion results for penicillin (PG) (red bars) and cefoxitin (FOX) (blue bars) of strains RN4220, LGA251Δ*mecC*Δ*blaZ* and 02.5099.DΔ*mecC*Δ*blaZ* carrying plasmid borne copies of wildtype *mecC* and *mecC*^{Val546Ile}. C1 and C2 denote clone 1 and clone 2, respectively. (B) Comparison of minimum inhibitory concentrations for cefoxitin (FOX) of wildtype strain and strains RN4220, LGA251Δ*mecC*Δ*blaZ* and 02.5099.DΔ*mecC*Δ*blaZ* carrying plasmid borne copies of wildtype *mecC* and *mecC*^{Val546Iso}. C1 and C2 denote clone 1 and clone 2, respectively.

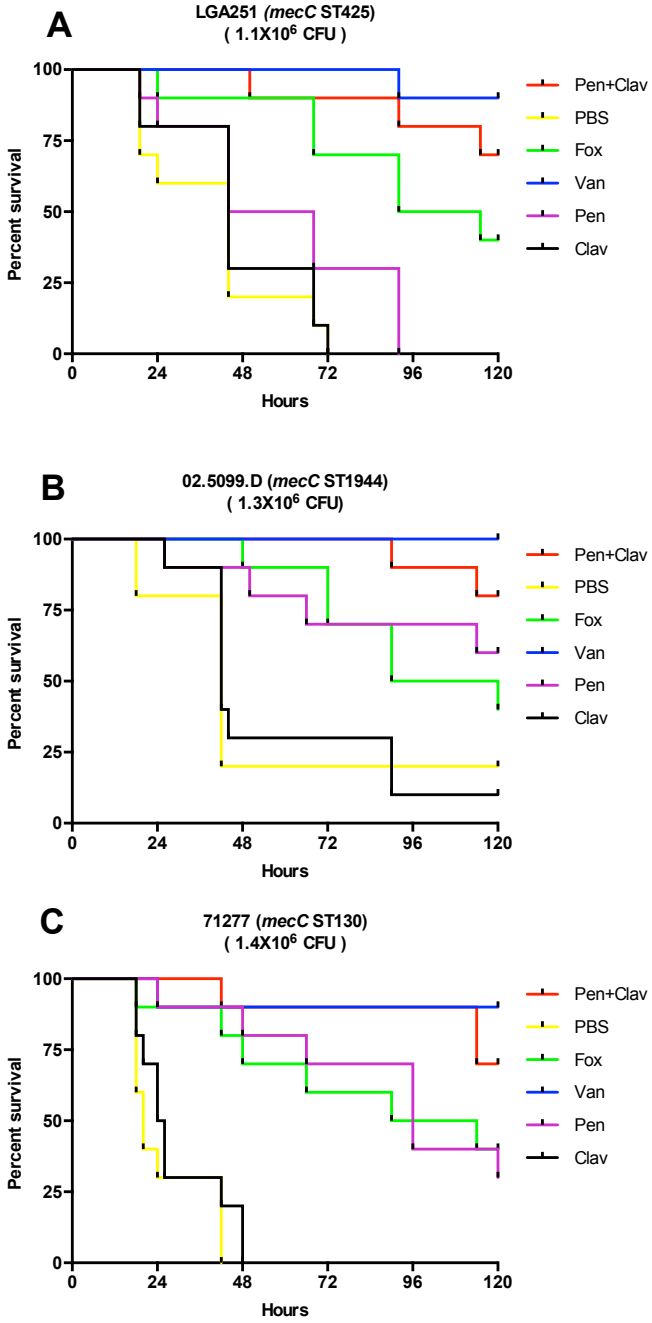

Figure S3: Biological repeats of experimental treatment of *Galleria mellonella* infected by *mecC*-MRSA strains: (A) LGA251 (B) 02.5099.D (C) 71277. Ten larvae in each group were experimentally infected and then treated at 2, 24 and 48 hours with Vancomycin (50 mg/kg / 6.73 x 10^{-9} mol), Penicillin (20 mg/kg / 1.12 x 10^{-8} mol), Clavulanic acid (20 mg/kg / 1.69 x 10^{-8} mol), Penicillin / Clavulanic acid, Cefoxitin (40 mg/kg / 1.78 x 10^{-8} mol), and PBS alone. Fig. shows a representative image of a single experiment.

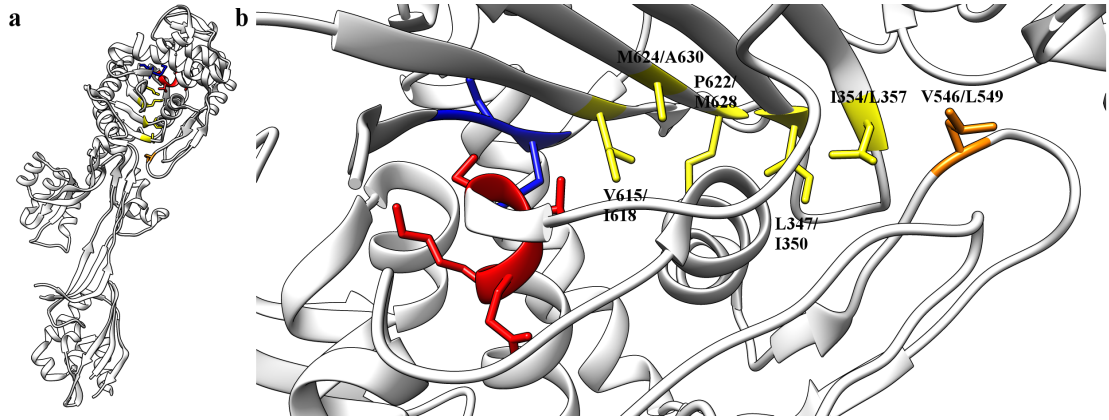

Figure S4: Structural view of PBP2a (A) in context of fold (B) close-up. Residues numbered first PBP2c and PBP2a second. SxxK motif in red and KT/SG motif in blue. Produced using UCSF Chimera (http://www.cgl.ucsf.edu/chimera/); from PDB code 1VQQ (PBP2a).

Table S3: Comparison of minimum inhibitory concentration of penicillin (PG) and cefoxitin (FOX) after *in vitro* selection for penicillin-resistance in strains lacking the SCC*mec* type XI encoded *blaZ*. C1, C2, C3 indicate multiple individual colonies tested.

Strains	**PG** μg/ml	**FOX** μg/ml
LGA251Δ*blaZ*	0.015	8
LGA251Δ*blaZ*-D9-C1	8	128
LGA251Δ*blaZ*-D9-C2	8	128
LGA251Δ*blaZ*-D9-C3	8	128
02.5099.DΔ*blaZ*	0.0625	8
02.5099.DΔ*blaZ*-D13-C1	16	128
02.5099.DΔ*blaZ*-D13-C2	16	>128
02.5099.DΔ*blaZ*-D13-C3	16	>128

Note for the table: 20 hours, at 35ºC. MIC breakpoint for PG is 0.12 μg/ml; for FOX is 4 μg/ml.
PG concentration range is 0.03125-128 μg/ml. FOX concentration range is 1-128 μg/ml. D9 = day 9 and D13 = day 13.

Table S5: Bacterial strains, plasmids and primers used in this study.

Bacterial strains, plasmids or primers	Description	Reference
***E. coli* strains**		
DC10B	Δ*dcm* in the DH10B background; Dam methylation only	(1)
***S. aureus* strains**		
RN4220	Derived from NCTC8325-4, selected for transformability with DNA from *E. coli*	(2)
RN4220(pXB01mecC)	RN4220 with wildtype *mecC*	This study
RN4220(pXB01*mecCVal546Iso*)	RN4220 with mutated *mecC*	This study
LGA251	ST425, *mecC* reference strain	(3)
LGA251Δ*mecC*	LGA251 with *mecC* deletion	This study
LGA251Δ*blaZ*	LGA251 with *blaZ* deletion	This study
LGA251Δ*mecC*Δ*blaZ*	LGA251 with *mecC* and *blaZ* deletion	This study
LGA251Δ*mecC* (pXB01)	LGA251Δ*mecC* with empty plasmid pXB01	This study
LGA251Δ*blaZ* (pXB01)	LGA251Δ*blaZ* with empty plasmid pXB01	This study
LGA251Δ*mecC*Δ*blaZ* (pXB01)	LGA251Δ*mecC*Δ*blaZ* with empty plasmid pXB01	This study
LGA251Δ*mecC* (pXB01-*mecC*)	LGA251Δ*mecC* complemented with pXB01-*mecC*	This study
LGA251Δ*blaZ* (pXB01-*blaZ*)	LGA251Δ*blaZ* complemented with pXB01-*blaZ*	This study
LGA251Δ*mecC*Δ*blaZ* (pXB01-*mecC*)	LGA251Δ*mecC*Δ*blaZ* complemented with pXB01-*mecC*	This study
LGA251Δ*mecC*Δ*blaZ* (pXB01-*blaZ*)	LGA251Δ*mecC*Δ*blaZ* complemented with pXB01-*blaZ*	This study
02.5099.D	CC130, *mecC* clinical isolate	(3)
02.5099.DΔ*mecC* (pXB01)	02.5099.D with *mecC* deletion	This study
02.5099.DΔ*blaZ* (pXB01)	02.5099.D with *blaZ* deletion	This study
02.5099.DΔ*mecC*Δ*blaZ* (pXB01)	02.5099.D with *mecC* and *blaZ* deletion	This study
02.5099.DΔ*mecC*	02.5099.DΔ*mecC* with empty plasmid pXB01	This study
02.5099.DΔ*blaZ*	02.5099.DΔ*blaZ* with empty plasmid pXB01	This study
02.5099.DΔ*mecC*Δ*blaZ*	02.5099.DΔ*mecC*Δ*blaZ* with empty plasmid pXB01	This study
02.5099.DΔ*mecC* (pXB01-*mecC*)	02.5099.DΔ*mecC* complemented with pXB01-*mecC*	This study
02.5099.DΔ*blaZ* (pXB01-*blaZ*)	02.5099.DΔ*blaZ* complemented with pXB01-*blaZ*	This study
02.5099.DΔ*mecC*Δ*blaZ* (pXB01-*mecC*)	02.5099.DΔ*mecC*Δ*blaZ* complemented with pXB01-*mecC*	This study
02.5099.DΔ*mecC*Δ*blaZ* (pXB01-*blaZ*)	02.5099.DΔ*mecC*Δ*blaZ* complemented with pXB01-*blaZ*	This study
Plasmids		
pRMC2	*E. coli* - *S. aureus* shuttle vector, Amp^r, Cm^r	(4)
pXB01	*E. coli* - *S. aureus* shuttle vector pRMC2 with *bla* deletion; Cm^r	This study
pXB01-*mecC*	pXB01 with *mecC* gene from LGA251	This study
pXB01-$mecC^{Val546Ile}$	pXB01 with *mecC* gene having a G to A mutation at position 1636 producing a Val546Ile substitution	This study
pXB01-*blaZ*	pXB01 with *blaZ* gene from LGA251	This study
pIMAY	pIMC5 with tetracycline inducible *secY* antisense from pKOR1; Cm^r	(1)
pIMAYΔ*mecC*	Modified pIMAY for deleting *mecC* gene, encompassing the entire gene, insert amplified from LGA251	This study
pIMAYΔ*blaZ*	Modified pIMAY for deleting *blaZ* gene, encompassing the entire gene, insert amplified from LGA251	This study
pIMAYΔ*mecC*Δ*blaZ*	Modified pIMAY for deleting *blaZ* gene, encompassing the entire gene, insert amplified from LGA251Δ*mecC*	This study
Primers	**Sequence (5'-3') restriction site underlined**	**Restriction site**
de-bla-F	ATATATATGCGGCCGCCTGTCAGACCAAGTTTACTC	NotI
de-bla-R	ATGCGCATGCGGCCGCACTCTTCCTTTTTCAATATTATT	NotI
Δ*mecC*_A	ATCGGGTACCTAATATGAATGAGTTCATGGC	KpnI
Δ*mecC*_B	CTCTTCCTCCTTTTGTACTAC	
Δ*mecC*_C	TAGTACAAAAGGAGGAAGAGTTCGAATACTTCTTTTGACTTGG	
Δ*mecC*_D	ATCGGAGCTCGTATCGCCACTTTTATTTTTG	SacI
Δ*mecC*_outF	GCTAAATGTAATGCTGGATTGAACC	
Δ*mecC*_outR	TCTTGTCCATCTGGATAAACAAACG	

Δ*blaZ*_A	ATATGGTACCAAATATCCCGAGTGATTATCC	KpnI
Δ*blaZ*_B	CCTACGTTAGCACCATATTTTTTTTCG	
Δ*blaZ*_C	AAATATGGTGCTAACGTAGGGTAAGTGAGGTTGCTG AAATTGTACT	
Δ*blaZ*_D	ATATGAGCTCCATCATAGAAGTTCCAGACG	SacI
Δ*blaZ*_outF	TGCCCGCATTGCATTAGCATTAGGA	
Δ*blaZ*_outR	AAATATTGGAAGCAAGCCATAGCAG	
Δ*blaZ*Δ*mecC*_A	ATATGGTACCGGAGATCTAATAGACAAGTC	KpnI
Δ*blaZ*Δ*mecC*_outF	GACCATTGGATTCTCTACTTCATCT	
*cat*up	GGATTTTTCGCTACGCTCAAA	
*cat*down	AAAGTACAGTCGGCATTATCTCA	
IM151	TACATGTCAAGAATAAACTGCCAAAGC	(1)
IM152	AATACCTGTGACGGAAGATCACTTCG	(1)
blaZ-F- KpnI	CGCGGGTACCTATATAGGAGGAATAAAATTG	KpnI
blaZ-R-SacI	GCGCGAGCTCTTACTCATTAATATTTTTTAG	SacI
mecC-F-KpnI	CGCGGGTACCCAAAAGGAGGAAGAGATG	KpnI
mecC-R-SacI	GCGCGAGCTCTTCGAATTACTGATCTAT	SacI
mecCf	GTTCACACCTCACTTCTTAAC	
mecCr	GTAATCTTTGTGGAAACGC	
mecCm	GCCGTGTTTATCCATTGAAC	

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4. **Corrigan, R. M., and T. J. Foster.** 2009. An improved tetracycline-inducible expression vector for Staphylococcus aureus. Plasmid **61:**126-9.