

Table S1. Minimum inhibitory concentration (MIC) values of the strains from the exposure to sub-inhibitory sodium bituminosulfonate concentrations over the course of ten passages on the active agent and after five recovery passages on plain plates (labeled with R). All MIC values in g/L.

Strain	MIC values determined after incubation of the strains on														
	Agar plates containing sub-inhibitory sodium bituminosulfonate concentrations										Plain agar plates				
	Baseline	P1 ^a	P2	P3	P4	P5	P6	P7	P8	P9	P10	R1 ^b	R2	R3	R4
ATCC 29213	0.125	0.125	0.25	0.5	0.5	0.5	0.5	0.5	1	1	1	1	1	0.5	0.5
ATCC 25923	0.25	0.25	0.25	0.25	0.5	0.5	0.5	0.5	1	1	1	0.5	0.5	0.5	0.5
ATCC 43400	0.125	0.125	0.25	0.25	0.25	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.25
MOP4	0.25	1	1	1	1	2	1	2	2	1	2	1	1	1	0.5
MOP2	0.125	0.25	0.25	0.5	0.5	1	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5
MOP12	0.125	0.25	0.5	0.5	1	1	1	1	1	0.5	1	1	0.5	0.25	0.25
MOP14	0.06	0.125	0.25	0.25	0.5	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	0.5	0.5
MOP16	0.25	0.5	0.5	1	0.5	1	1	1	1	1	1	1	1	1	1
MOP18	0.06	0.125	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.25	0.25	0.25	0.25	0.25
MOP20	0.125	0.25	0.5	1	0.5	1	0.5	1	1	1	1	1	1	1	0.5
MOP22	0.06	0.125	0.25	0.5	0.5	0.5	0.25	0.5	0.5	0.5	0.5	0.5	0.25	0.25	0.5
MOP24	0.06	0.125	0.25	0.5	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
MOP26	0.125	0.125	0.125	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.5	0.25	0.25	0.25
11-03	0.25	0.25	0.25	0.5	0.5	0.25	0.5	0.5	0.5	1	1	1	0.5	0.5	0.5
11-15	0.25	0.25	0.25	0.5	0.25	0.25	0.25	0.5	0.5	0.5	1	0.5	0.5	0.25	0.25
11-12	0.06	0.06	0.06	0.125	0.125	0.06	0.125	0.125	0.125	0.25	0.5	0.5	0.5	0.25	0.25
10-50	0.25	0.25	0.25	0.5	0.25	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.25
09-32	0.25	0.25	0.25	0.25	0.25	0.5	0.25	0.5	0.5	0.5	0.5	0.25	0.25	0.25	0.25
11-04	0.125	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.5	0.25	0.25	0.25
17-48	0.25	0.25	0.25	0.25	0.5	0.5	0.5	0.5	1	1	1	1	1	1	0.5
10-17	0.25	0.25	0.25	0.5	0.25	0.25	0.25	0.5	0.5	0.5	1	0.5	0.5	0.5	0.25
09-26	0.125	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.25
13-02	0.125	0.25	0.25	0.25	0.25	0.5	0.25	0.5	0.5	1	0.5	0.25	0.5	0.25	0.25
11-31	0.125	0.25	0.25	0.25	0.25	0.5	0.5	0.5	1	1	1	0.5	0.5	0.25	0.25
30-38	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.5	1	1	1	1	0.5	1	0.5
20-35	0.125	0.125	0.25	0.25	0.5	0.25	0.5	0.25	0.5	0.5	0.25	0.25	0.25	0.25	0.125
LM380270	0.125	0.5	0.5	1	0.5	1	1	1	1	1	1	1	0.5	0.5	0.5
LM379438	0.125	0.25	0.5	0.25	0.25	0.5	0.5	0.5	1	1	1	0.5	0.5	0.5	0.25
LM297503	0.125	0.25	0.5	0.5	0.5	0.5	0.5	0.5	1	1	1	1	0.5	0.5	0.5
023-7049	0.125	0.25	0.5	1	1	1	1	1	1	1	1	0.5	0.5	0.5	0.5

^aPassages on plates containing a linear gradient of the microbial agent. ^bPassages on plain agar plates.

Table S2. MIC values of the strains from the exposure to sub-inhibitory fusidic acid concentrations over the course of ten passages on the active agent and after five recovery passages on plain plates (labeled with R). All MIC values in mg/L.

Strain	MIC values determined after incubation of the strains on															
	Agar plates containing sub-inhibitory fusidic acid concentrations										Plain agar plates					
	Baseline	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	R1	R2	R3	R4	R5
ATCC 29213	0.125	2	8	16	8	8	8	16	16	16	16	16	16	16	16	16
ATCC 25923	0.125	2	2	2	8	8	8	8	8	16	16	8	8	16	8	8
ATCC 43300	0.06	8	8	16	16	8	16	16	16	16	16	16	16	16	16	16
MOP4	0.06	0.125	0.25	2	4	4	8	8	8	8	8	8	4	4	8	8
MOP2	0.06	0.125	0.25	0.5	2	1	1	2	1	1	1	2	2	2	2	2
11-03	0.125	0.25	2	4	4	4	4	2	4	8	8	16	8	8	8	8

^aPassages on plates containing a linear gradient of the microbial agent. ^bPassages on plain agar plates.

Table S3. Clinical strain collection used in the study.

Strain	<i>spa</i> -type	Methicillin resistance phenotype (<i>mec</i> gene type)	Source
MOP4	t150	PSSA-MSSA (n.d.)	[33]
MOP2	(11–17–12–17)	PRSA-MSSA (n.d.)	[33]
MOP12	t002	PRSA-MSSA (n.d.)	[33]
MOP14	t1909	PRSA-MSSA (n.d.)	[33]
MOP16	t084	PRSA-MSSA (n.d.)	[33]
MOP18	t021	PRSA-MSSA (n.d.)	[33]
MOP20	t548	PRSA-MSSA (n.d.)	[33]
MOP22	t949	PRSA-MSSA (n.d.)	[33]
MOP24	t298	PRSA-MSSA (n.d.)	[33]
MOP26	t015	PRSA-MSSA (n.d.)	[33]
11–03	t003	HA-MRSA (<i>mecA</i>)	[34]
11–15	t032	HA-MRSA (<i>mecA</i>)	[34]
11–12	t008	HA-MRSA (<i>mecA</i>)	[34]
10–50	t002	HA-MRSA (<i>mecA</i>)	[34]
09–32	t045	HA-MRSA (<i>mecA</i>)	[34]
11–04	t004	HA-MRSA (<i>mecA</i>)	[34]
17–48	t014	HA-MRSA (<i>mecA</i>)	[34]
10–17	t041	HA-MRSA (<i>mecA</i>)	[34]
09–26	t022	HA-MRSA (<i>mecA</i>)	[34]
13–02	t038	HA-MRSA (<i>mecA</i>)	[34]
11–31	t044	PVL-positive CA-MRSA (<i>mecA</i>)	[34]
30–38	t008	PVL-positive CA-MRSA (<i>mecA</i>)	[34]
20–35	t693	PVL-positive CA-MRSA (<i>mecA</i>)	[34]
LM380270	t011	LA-MRSA (<i>mecA</i>)	[35]
LM379438	t034	LA-MRSA (<i>mecA</i>)	[35]
LM297503	t108	LA-MRSA (<i>mecA</i>)	[35]
023–7049	t843	LA-MRSA (<i>mecC</i>)	[36]

MSSA, methicillin-susceptible *S. aureus*; PSSA, penicillin-susceptible *S. aureus*; PRSA, penicillin-resistant *S. aureus*; MRSA, methicillin-resistant *S. aureus*; HA-MRSA, healthcare-associated *S. aureus*; CA-MRSA, community acquired *S. aureus*; PVL-pos, Panton Valentine leucocidin positive; LA-MRSA, livestock associated *S. aureus*; n.d., not detected.

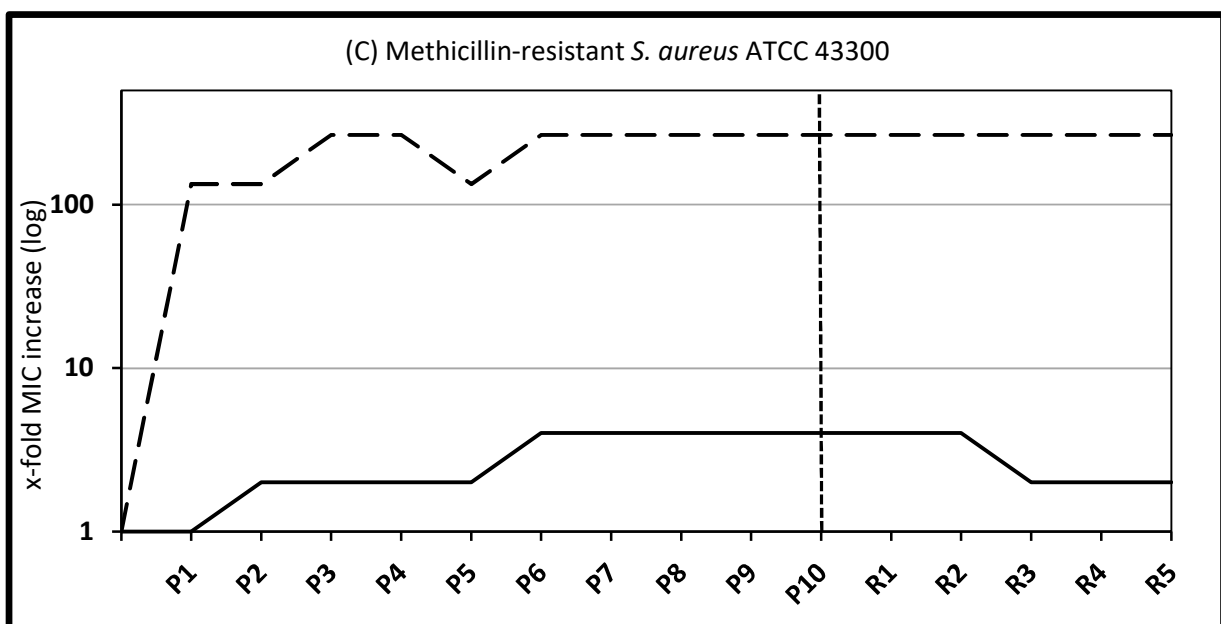
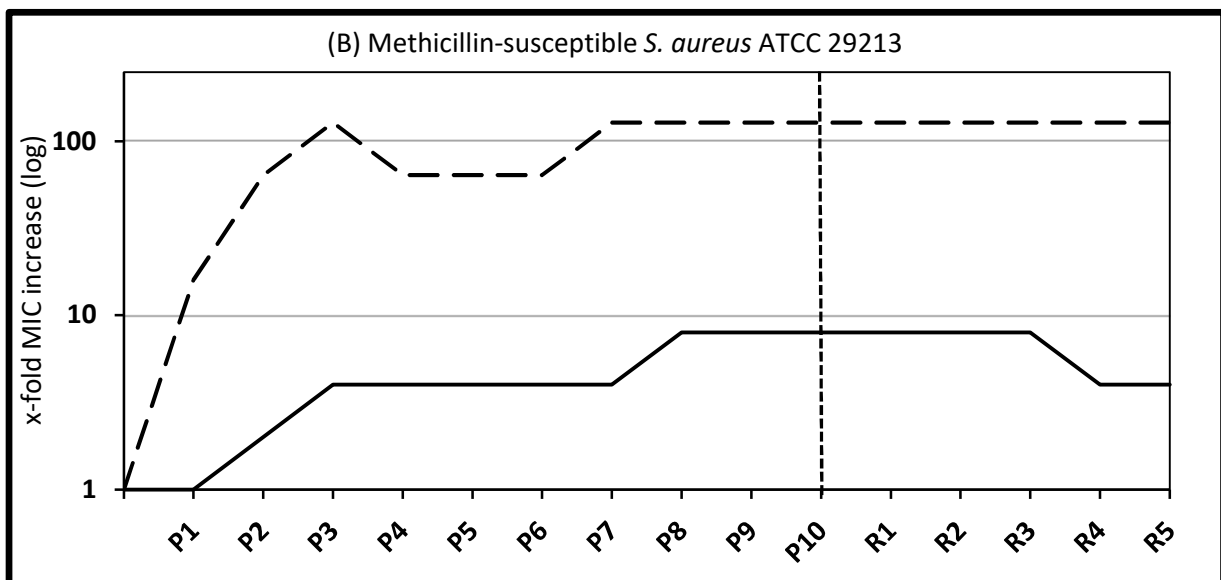
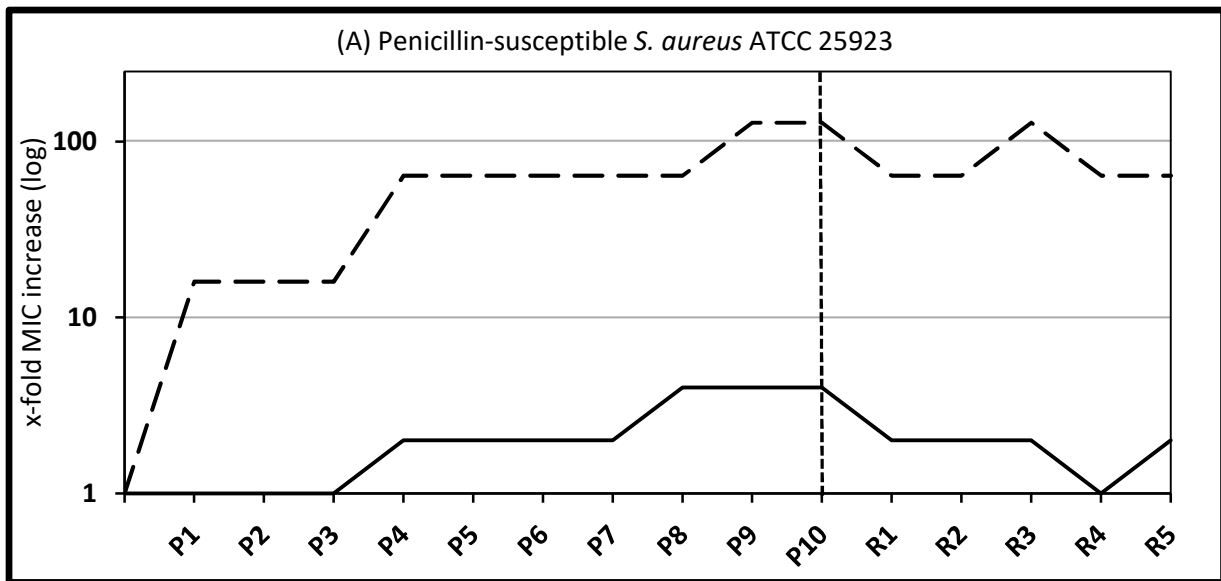


Figure S1. Data from the penicillin-susceptible (**A**), methicillin-susceptible (**B**) and methicillin-resistant (**C**) reference strains exposed to ten passages of sodium bituminosulfonate (solid line) and fusidic acid (dashed line) on linear gradient plates. The dotted vertical line indicates the endpoint after ten passages on linear gradient plates containing the active agents (labeled with P) and five recovery passages on plain agar plates (labeled with R).



Figure S2. Pairwise alignment of obtained polymorphisms within the *fusA* gene from the screening for resistance mechanism.



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