Supplemental Material

Data S1. SUPPLEMENTAL METHODS

Bacterial strains, growth conditions, and antibiotic susceptibility testing.

The MSSA strain Laus102, which was isolated from a healthy carrier [16], and a panel of 62 *S. aureus* strains that had been previously isolated from humans and cows were used in this study (Supplemental Table 1). All *S. aureus* strains were stored in TSB (BD Difco™, Becton Dickinson, Sparks, MD) containing 10% (v/v) glycerol at -80 °C and sub-cultured on TSA plates to ensure purity before testing. For liquid cultures, TSB was inoculated with at least five single colonies and incubated for 24 h with agitation (200 rpm) at 37 °C.

The *P. aeruginosa* strain ATCC® 15442™ (LGC Standards, Molsheim, France) was stored in Lysogeny Broth (LB, BD Difco™, Becton Dickinson, Sparks, MD) containing 10% (v/v) glycerol at -80 °C and sub-cultured on LB agar plates to ensure purity before testing. For liquid cultures, LB was inoculated with at least five single colonies and incubated for 24 h with agitation (200 rpm) at 37 °C.

Flucloxacillin was purchased from OrPha Swiss (Küsnacht, Switzerland). The MICs of flucloxacillin were determined in Muller Hilton Broth (Becton Dickinson, Sparks, MD) using a standard micro-dilution procedure [51].

Bacteriophages.

The *Podoviridae* phage 66 and *Herelleviridae* phage vB_SauH_2002 genomes are publicly available (Genbank accession no. NC_007046 and MW528836, respectively) [17]. To produce large quantities of phages, amplification was performed using Laus102 as propagation strain. For each phage preparation, 2 L of TSB was inoculated 1:100 with 20 mL of an overnight culture of Laus102 and incubated at 37 °C under 200rpm until an OD_{595nm} of 0.1 was reached, and then 1 mL of phage stock (10^{10} PFU/mL) was added. The culture was further incubated at 37 °C under 200 rpm for 6 h, and then centrifuged twice at 8000 ×*g* for 15 min to remove bacterial debris. The supernatant containing the phages was passed through 0.22-µm filters (vacuum filtration 1000 rapid-filtermax, Techno Plastic Products AG, Trasadingen, Switzerland). The filtrate was further concentrated to 100 mL and buffer exchanged against 3 L of 1× phosphate buffer saline (PBS), pH 7.4 using tangential flow filtration through an mPES/500 KD column (Repligen, Waltham, MA). Phage concentrations in the

purified batches were determined in classical double agar overlay assays (DLAs) [52]. Briefly, 200 µL of an overnight culture of Laus102 was mixed with 100 µL of serial dilution of the phage preparations and 5 mL of TSB soft agar at 45 °C. This mixture was poured on TSA plates and incubated at 37 °C overnight after the TSB soft-agar layer solidified at room temperature. Concentration of phages was determined by counting PFUs. The equimolar phage cocktail at 10^{10} PFU/mL was assembled after adjusting the concentration of each phage to 10^{10} PFU/mL and by mixing equal volumes of the phages.

Phage vB_PaeM_4002 is a *Myoviridae* previously isolated from a sewage water sample collected at the Vidy wastewater treatment plant in Lausanne, Switzerland (unpublished) using *P. aeruginosa* PAO1 as a host strain. It is similar to the lytic phage vB_Pae_Ps44 (Genbank accession no. NC_028939). vB_PaeM_4002 was purified following the procedure described above, except that the propagation host used was *P. aeruginosa* strain ATCC® 15442™.

Electron microscopy.

Four-microliter phage suspension samples were deposited on a lacey carbon copper grid (EMS, Hatfield, PA) previously glow discharged for 30 s at 15 mA. The deposition was conducted in a Vitrobot Mark IV chamber (Thermo Fisher Scientific, Waltham, MA) in 100% humidity. A blotting time of 5 s with a force of -16 was used just before plunge freezing in liquid ethane. The grid was then transferred in an Elsa cryo-transfer holder (Gatan, Pleasanton, CA) and inserted in a 2100 Plus electron microscope (Jeol, Tokyo, Japan). Images (magnification, 120k; pixel size, 0.097 nm; 1-s exposure time) were collected by an XF416 camera (TVIPS GmbH, Gauting, Germany) with SerialEM software at 200 kV (electron dose of $25e^-/A^2/s$) [53].

Determination of phage host range and efficiency of plating.

Phage host range was determined on various *S. aureus* strains (Supplemental Table S1) using DLA (see above). Efficiency of plating scores were determined by dividing the phage titer in PFU/mL obtained on the tested strain by the phage titer obtained on the amplification strain Laus102 [54]. All experiments were done in triplicate.

***In vitro* turbidity assays.**

One hundred µL of an overnight culture of Laus102 were re-suspended in 10 mL of TSB and incubated at 37 °C under 200 rpm until the OD_{595nm} reached 0.6, corresponding to ~10^8 CFU/mL. Then, 10-µL samples of this bacterial suspension (10^6 CFU) were mixed in 96-well plates (Thermo Scientific, USA) with 280 µL of TSB and 10 µL of various dilutions of the phage solutions to achieve final MOIs of 0.01, 0.1, 1, 10, and 100. The microtiter plates were incubated at 37 °C in an Elx808IU absorbance microplate reader (BioTek®, Sursee, Switzerland) and the OD_{595nm} was recorded every 10 min for 24 h. The microplates were shaken for 3 s before each measurement. All experiments were performed in triplicate.

Phage time-kill curve assays.

One hundred-µL samples of an overnight culture of Laus102 were re-suspended in 10 mL of TSB and incubated at 37 °C under 200 rpm until the OD_{595nm} reached 0.6, corresponding to ~10^8 CFU/mL. The culture was diluted 1:100 in 10 mL of fresh TSB supplemented with either the equimolar phage cocktail at a final MOI of 1, flucloxacillin at 1× the MIC, or a combination of both at the same final concentrations and then incubated at 37 °C and 200 rpm. Cell viability was determined 0 h, 2 h, 4 h, and 24 h after inoculation (limit of detection 10^2 CFU/mL). Before plating, samples were diluted in 1× PBS (pH 3) to neutralize the phages. All experiments were performed in triplicate. A similar procedure was used to test vB_Pae_4002 on *P. aeruginosa* strain ATCC® 15442™.

For the experiments in the presence of plasma, 100-µL samples of an overnight culture of Laus102 or *P. aeruginosa* strain ATCC® 15442™ were re-suspended in 10 mL of TSB or LB, respectively, and incubated at 37 °C under 200 rpm until the OD_{595nm} reached 0.6, corresponding to ~10^8 CFU/mL. The culture was diluted 1:100 in 10 mL fresh TSB or LB supplemented 10% rat plasma (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland). After a 30-min pre-incubation at room temperature, *S. aureus* phage cocktail or phage vB_Pae_4002 (each at MOI = 100) was added accordingly, and test tubes were placed at 37 °C and 200rpm. Cell viability was determined 0 h, 2 h, and 4 h after initiation of the phage challenge (limit of detection 10^2 CFU/mL). Before plating, samples were diluted in 1× PBS (pH 3) to neutralize the phages. All experiments were performed in triplicate.

***In vitro S. aureus* mono-species biofilm assay.**

Maturation of the biofilm. MSSA Laus102 biofilms were produced in 96-well plates as previously described [18]. Briefly, overnight cultures were diluted 1:100 in TSB, and 100-µL samples of the subsequent solution containing ca. 10^7 CFU/mL were used to inoculate 96-well polystyrene plates (Greiner Bio-One, Kremsmünster, Austria) (final concentration of bacteria ~10^6 CFU per well). After a 24-h incubation at 37 °C without shaking, the supernatant was removed from each well, and the remaining adherent biofilm was carefully steam-washed for 45 min using the BiofilmCare™ technology procedure [18].

Treatment of biofilm. Mature biofilms were treated for 24 h at 37 °C with 10^8 PFU/mL, 10^9 PFU/mL, or 10^{10} PFU/mL (final MOIs = 1, 10, and 100, respectively) of phage vB_SauH_2002 alone, phage 66 alone, the phage cocktail, or flucloxacillin (1× or 10× MIC). In addition, the phage cocktail at all MOIs was evaluated in combination with both flucloxacillin concentrations.

Evaluation of treatment efficacy. The treated biofilms were rinsed two times with PBS and re-suspended in 100 µl of PBS by scraping the wells with sterile pipette tips. The 96-well microplate was sealed with a plastic film (Dutscher, Brumath, France), put in an ultrasound bath (Bactosonic, Bandelin electronic GmbH & Co.KG, Berlin, Germany) for 10 min at 40 Hz to detach attached bacteria and to remove clusters before determination of viable counts on TSA.

Additional information related to the EE model.

Randomization. Randomization of animals in groups was done using the online tool Research Randomizer (https://www.randomizer.org/).

Flucloxacillin dosing regimen. Rats received a suboptimal IV dose of flucloxacillin mimicking human kinetic treatment (2 g every 12 h for 24 h instead of 2 g every 6 h for 24 h for an optimal treatment). The administration protocol consisted in the infusion of a solution of flucloxacillin (0.3 g/10 mL in saline) according to the following cycle: 2.0mL/h for 15 min., followed by 0.4 mL/h for 1 h 45 min., 0.2 mL/h for 2 h, and 0.005 mL/h for 2 h. After this first 6 h infusion cycle, no treatment was given for 6 h and a second infusion cycle was performed thereafter followed by no treatment for 6 h before euthanasia.

Criteria for euthanasia. Animal welfare was assessed at least two times per day with an in-house welfare score sheet for rodents (see below). Animals were excluded from randomization if we

suspected that the catheter placed into the heart through the carotid artery had potentially damaged the aortic valve or was not properly inserted. Animals were euthanized humanely according to the score and status of the animal as indicated below (termination criteria). The mortality rate after surgery was 10%, and six rats were excluded before infection. Moreover, six rats were further excluded at the end of the experiment because the catheter was not properly inserted.

Welfare score sheet used in the *in vivo* experiment of EE rats.

	Score			
	0	1	2	3
Haircoat	Normal Well groomed	Fur ruffling	General lack of grooming	Hunched up with matted fur
Posture	Normal	Sporadic hunchback posture	Frequent hunchback posture	Head on cage floor
Activity	Normal	Decreased activity after slight stimulation	Significant decreased activity after moderate stimulation	Lethargy after moderate stimulation
Breath	Normal	Shallow	Labored breathing	Breathing noises
Behavior	Normal	Isolated from cage mates*		Convulsion

Termination criteria:
Score of 0: no action.
Score of 1: animal is observed twice daily. If animal does not return to normal within 48 hours it will be euthanized.
Score of 2: animal is observed three times daily. If animal does not return to normal within 12 hours it will be euthanized.
When an animal reaches a score of 3, either cumulative or in one observable criteria, it will be immediately euthanized.
*this score is not applicable for animals that are isolated in a cage, for instance animals equipped with a "swivel" system.

Blinding procedure. The rats receiving saline and phages or saline and antibiotics were connected to the same pumps, rendering the masking of group/treatment assignment challenging and unnecessary since blinding was performed during outcome assignment. Indeed, the technician who performed the experiments to evaluate the bacterial and phage loads in vegetations, and organs was blinded, i.e. she didn't know from which animal the samples we provided her originated from.

Bacterial loads in cardiac vegetations. The presence of macroscopic cardiac valve vegetations was visually validated before being dissected from the heart. After being weight, vegetations were further mechanically homogenized in 1 mL saline. The homogenates were serially diluted and plated in triplicate on TSA plates for bacterial counting. Colonies were counted after an overnight incubation at 37 °C. Remaining vegetation homogenates were stored at -80 °C after the addition of 10% (v/v) glycerol. Phage or flucloxacillin carry over was diluted out through serial dilutions.

Phage loads in cardiac vegetations, organs, and blood. After dissection, organs were mechanically homogenized in weight-adapted volumes of saline (1 mL for cardiac vegetations, 2 mL for spleen,

liver, and kidney). Phage loads were determined using a classical DLA (see Materials and Methods). Plates were incubated at 37 °C and plaques were counted the following day.

Power calculation. We hypothesized that 100% and 30% of the placebo and phage cocktail/flucloxacillin treated rats would have infected vegetations at 24 h. These estimates, with an $\alpha = 0.05$ and a power (1-β) = 0.8 required a sample size of at least eight animals per group [55].

List of animals in groups.

Number of animals	Onset of treatment	Saline	Phage cocktail	Flucloxacillin	Phage cocktail + flucloxacillin
Considered	10	7	8	11	12
Dead after surgery	0	3	3	0	0
With not properly placed catheters	2	2	1	1	0

Determination of phage-resistance patterns of *S. aureus* clones recovered *in vivo*.

The phage-resistance patterns of the clones recovered *in vivo* from the rat cardiac vegetations were determined with diluted drop test assays. Cardiac vegetation homogenates (100 µL) were plated on TSA and incubated overnight at 37 °C. Two days later, single colonies were re-suspended in 5 mL fresh TSB and incubated overnight at 37 °C. Overnight bacterial cultures were mixed 1:100 with 15 mL of TSB soft-agar and poured into Petri dishes. The bacterial lawns were then spotted with 5 µL of serial 10-fold dilutions of each phage suspension (vB_SauH_2002, phage 66, and the phage cocktail) and incubated at 37 °C overnight. The results were scored the next day according to the observed lysis phenotypes. Absence and presence of lysis were considered definitive of a resistant phenotype (R) and a susceptible phenotype (S), respectively (Fig. S1).

Bacterial genome sequencing, assembly, and analysis.

A bacterial genomic library was prepared with an optimized protocol and standard Illumina adapter sequences, and sequencing was performed with Illumina technology, NovaSeq 6000 (read mode 2 x 150 base pairs). Both processes were performed at Eurofins Genomics Germany GmbH (Ebersberg, Germany). Reads were assembled and contigs annotated using the PATRIC pipeline

for assembly and annotation, respectively (https://www.patricbrc.org/). Comparative genomics were performed with the PATRIC variation analysis tool set to default parameters.

Table S1. *S. aureus* strains used in this study along with their EOP scores for vB_SauH_2002 and phage 66.

			EOP score			
***S. aureus* strain**	**Genbank access N°**	**ST**	**vB_SauH_2002**	**Phage 66**	**Covered by the phage cocktail**	**Reference**
Human carriage strains from healthy volunteers						
Laus102	JAETXI000000000.1	8	1	1	yes	[16]
Laus385	CP071350.1	8	1.5	1.25	yes	[16]
F60	NA	15	0.025	5.10^{-4}	yes	[56]
Human clinical strains						
VRS11b (AID1001123)*†	AHBV01000000.1	5	0.35	0.225	yes	[57]
VRS8 (71080)*†	AHBR00000000.1	5	0	0	no	[57]
VRS9 (AIS080003)*†	AHBS00000000.1	5	0	0.75	yes	[57]
VRS10 (AIS1000505)*†	AHBT00000000.1	5	0.65	0	yes	[57]
VRS11a (AIS1001095)*†	AHBU00000000.1	5	0.15	0.075	yes	[57]
VRS6 (AIS2006032)*†	AHBP00000000.1	5	0.025	0.2	yes	[57]
VRS7 (AIS2006045)*†	AHBQ00000000.1	5	0.7	0	yes	[57]
VRS4 (HIP14300)*†	AHBN00000000.1	5	0.025	0.075	yes	[57]
VRS3b (HIP13419)*†	AHBM00000000.1	5	0	0.9	yes	[57]
VRS2 (HIP11983)*†	AHBL00000000.1	5	0	0	no	[57]
VRS3a (HIP13170)*†	NBCP00000000.1	5	0	1	yes	[57]
VRS1 (HIP11714)*†	AHBK00000000.1	5	0.35	0	yes	[57]
VRS5 (HIP15178)*†	AHBO00000000.1	5	0.7	0.25	yes	[57]
ATCC 29213	LHUS00000000.2	5	0	0	no	Vicosa et al. unpublished
I37	CP071352.1	8	0.85	0.25	yes	[16]
USA300 FPR3747‡	JAFFHX000000000.1	8	0	0.2	yes	[58]
USA300 JE2‡	CP020619.1	8	0	0.8	yes	[58]
Yok80	NA	8	0.025	1	yes	This study
Yok51	NA	22	1	0.075	yes	This study
Yok49	NA	30	1	0.25	yes	This study
Yok25	NA	45	0	0	no	This study
Yok72‡	NA	105	0.7	0.0125	yes	This study
Yok53	NA	121	0.75	5.10^{-3}	yes	This study
AW10‡	NA	239	0	0.2	yes	This study
AW7‡	SRLL00000000.1	247	0.025	0.02	yes	[59]
COL‡	CP000046.1	250	0.35	0	yes	[60]
Yok45	NA	707	0	0	no	This study

Animal strains from bovine mastitis						
Jn	CP071362.1	8	0.025	0.03	yes	[61]
G04	CP071369.1	8	0.5	0.125	yes	[61]
G36	CP071366.1	8	0.8	0	yes	[61]
G57	CP071365.1	8	0.35	0	yes	[61]
O103	CP071360.1	8	0.6	0.25	yes	[61]
M160	CP071341.1	8	0.6	0	yes	[16]
M283	CP071337.1	8	0.45	0	yes	[16]
M186	CP071340.1	8	0.85	0	yes	[16]
M192	CP071339.1	8	0.025	0	yes	[16]
M385	CP071333.1	8	0.45	0	yes	[56]
M308	CP071336.1	8	0.65	0	yes	[16]
G03	CP071370.1	8	0.025	0.25	yes	[61]
Bc	CP071374.1	8	0.025	0.2	yes	[61]
O100	CP071361.1	8	0.025	0.3	yes	[61]
Je	CP071363.1	8	0.025	0.075	yes	[61]
G34	CP071367.1	8	0.025	0	yes	[61]
M222	CP071338.1	8	0.025	0	yes	[16]
M37	CP071347.1	8	0.45	0.25	yes	[16]
M5	CP071349.1	8	0.025	0	yes	[16]
M20	CP071348.1	8	0.45	1	yes	[16]
M319	CP071334.1	8	0.6	2	yes	[16]
M313	CP071335.1	8	0.6	0	yes	[16]
M124	CP071343.1	8	0.025	0	yes	[16]
M117	CP071344.1	8	0.35	0	yes	[16]
M184	NA	15	1	0	yes	[56]
M356	NA	71	0.025	0.25	yes	[16]
M159	NA	389	0.025	4.10^{-4}	yes	[16]
M323	NA	389	0.025	0	yes	[16]
M3	NA	395	0.025	0.025	yes	[16]
M75	NA	504	0.1	0.175	yes	[56]
M52	NA	504	0.025	0.9	yes	[16]
M86	CP071346.1	1650	0.6	0	yes	[16]
M126	NA	1651	0.025	3.5	yes	[16]
% coverage			**82.54**	**58.73**	**92.06**	

ST, sequence type; EOP, efficiency of plating; NA, not available, *see acknowledgements, †VRSA, ‡MRSA.

Table S2. Phage resistance patterns of clones recovered from the cardiac vegetations of rats treated with the phage cocktail/flucloxacillin combination for 24 h.

Animal N°	CFU/g vegetations	Number of clones that regrew in TSB	Phage resistance pattern (vB_SauH_2002, phage 66, phage cocktail)	
			SSS	SRS
16	5.4	21	14	6
18	3.5	15	9	7

S, susceptible; R, resistant.

Table S3. Results of the variant analysis conducted in PATRIC with default parameters between six representative SRS clones recovered from the vegetations of rats treated for 24 h with the phage cocktail/flucloxacillin combination and the Laus1002 wild-type SSS strain.

Clone 16C1

Non-synonymous mutations							
Contig	**Pos**	**Score**	**Ref_nt**	**Var_nt**	**Frameshift**	**Gene N°**	**Function**
0001	525680	5608.82	gcc	gTc		496	Transposase, IS4 family
0001	525813	75.7067	agt	Ggt		496	Transposase, IS4 family
0005	7195	525.68	ggc	gTc		1625	Transposase, IS4 family
0005	7258	844.719	gat	gGt		1625	Transposase, IS4 family
0005	7265	893.501	tgt	Ggt		1625	Transposase, IS4 family
0009	87601	3107.3	cag	Gag		2210	Transposase, IS4 family
0009	87676	8719.57	aat	Gat		2210	Transposase, IS4 family
0009	87780	13226.1	aagaaagta	AAGAAAAGta	yes	2211	Transposase, IS4 family
0009	87789	17524.8	ttggtgcgg	ttTGTGTgg		2211	Transposase, IS4 family
0009	87812	17154.9	agt	aAt		2211	Transposase, IS4 family
0009	87830	10619.7	gataattcaatttttattgatggt	AATAATTCAATTTTTATTGATGGt		2211	Transposase, IS4 family
0009	87885	8389.01	ttctat	ttCCat		2211	Transposase, IS4 family
0009	88220	2276.45	atgacccaa	TTGATCCAa		2211	Transposase, IS4 family
0009	88235	14505.1	att	aAt		2211	Transposase, IS4 family
0010	42336	648.986	aaa	Gaa		2285	Hypothetical protein, Lmo2313 homolog [phage A118]
Synonymous mutations							
0001	525712	6093.63	att	atC		496	Transposase, IS4 family
0001	525739	6269.44	aag	aaA		496	Transposase, IS4 family
0001	525793	1241.32	aag	aaA		496	Transposase, IS4 family
0001	525802	1663.27	cgt	cgA		496	Transposase, IS4 family
0005	7149	190.834	ttc	ttT		1625	Transposase, IS4 family
0009	87596	2442.06	aat	aaC		2210	Transposase, IS4 family
0009	87749	15295.7	gag	gaA		2210	Transposase, IS4 family
0009	88024	23007.5	cga	cgT		2211	Transposase, IS4 family
0009	88194	5005.21	acctctgtt	acTTCTGtt		2211	Transposase, IS4 family
0010	42286	6078.02	act	acG		2285	Hypothetical protein, Lmo2313 homolog [phage A118]

Clone 16C5

Non-synonymous mutations							
Contig	**Pos**	**Score**	**Ref_nt**	**Var_nt**	**Frameshift**	**Gene_ID**	**Function**
0001	525680	8187.98	gcc	gTc		496	Transposase, IS4 family
0005	7258	1721.74	gat	gGt		1625	Transposase, IS4 family
0005	7265	1841.36	tgt	Ggt		1625	Transposase, IS4 family
0009	87601	4777.89	cag	Gag		2210	Transposase, IS4 family
0009	87676	11201.7	aat	Gat		2210	Transposase, IS4 family
0009	87780	15992.4	aagaaagta	AAGAAAAGta	yes	2211	Transposase, IS4 family
0009	87789	20770.6	ttggtgcgg	ttTGTGTgg		2211	Transposase, IS4 family
0009	87812	21642.7	agt	aAt		2211	Transposase, IS4 family
0009	87830	11586.8	gataattcaatttttattgatggt	AATAATTCAATTTTTATTGATGGt		2211	Transposase, IS4 family
0009	87885	9779.11	ttctat	ttCCat		2211	Transposase, IS4 family
0009	88220	1090.55	atgacccaa	TTGATCCAa		2211	Transposase, IS4 family
0009	88235	17043.0	att	aAt		2211	Transposase, IS4 family
Synonymous mutations							
0001	525712	8026.6	att	atC		496	Transposase, IS4 family
0001	525739	8395.65	aag	aaA		496	Transposase, IS4 family
0001	525793	1329.23	aag	aaA		496	Transposase, IS4 family
0001	525802	1760.09	cgt	cgA		496	Transposase, IS4 family
0009	87596	3765.39	aat	aaC		2210	Transposase, IS4 family
0009	87749	17038.1	gag	gaA		2210	Transposase, IS4 family
0009	88024	21383.9	cga	cgT		2211	Transposase, IS4 family
0009	88194	4571.23	acctctgtt	acTTCTGtt		2211	Transposase, IS4 family
0010	42286	2198.73	act	acG		2285	Hypothetical protein, Lmo2313 homolog [phage A118]

Clone 16C8

Non-synonymous mutations							
Contig	**Pos**	**Score**	**Ref_nt**	**Var_nt**	**Frameshift**	**Gene_ID**	**Function**
0001	525680	5515.24	gcc	gTc		496	Transposase, IS4 family
0004	**150512**	**202.141**	**gatttttataga**	**gATTTTATAga**	**yes**	**1579**	**Glycosyl transferase family protein, putative**
0005	7258	4917.17	gat	gGt		1625	Transposase, IS4 family
0005	7265	4599.85	tgt	Ggt		1625	Transposase, IS4 family
0009	87601	6407.54	cag	Gag		2210	Transposase, IS4 family
0009	87676	10902.8	aat	Gat		2210	Transposase, IS4 family
0009	87780	16053.2	aagaaagta	AAGAAAAGta	yes	2211	Transposase, IS4 family
0009	87789	19560.9	ttggtgcgg	ttTGTGTgg		2211	Transposase, IS4 family
0009	87812	20662.2	agt	aAt		2211	Transposase, IS4 family
0009	87830	11884.0	gataattcaatttttattgatggt	AATAATTCAATTTTTATTGATGGt		2211	Transposase, IS4 family
0009	87885	9920.24	ttctat	ttCCat		2211	Transposase, IS4 family
0009	88220	2783.96	atgacccaa	TTGATCCAa		2211	Transposase, IS4 family
0009	88235	17981.6	att	aAt		2211	Transposase, IS4 family
Synonymous mutations							
0001	525712	7109.39	att	atC		496	Transposase, IS4 family
0001	525739	7076.59	aag	aaA		496	Transposase, IS4 family
0001	525793	2050.16	aag	aaA		496	Transposase, IS4 family
0001	525802	1947.28	cgt	cgA		496	Transposase, IS4 family
0009	87596	4990.44	aat	aaC		2210	Transposase, IS4 family
0009	87749	17628.2	gag	gaA		2210	Transposase, IS4 family
0009	88024	20936.6	cga	cgT		2211	Transposase, IS4 family
0009	88194	5551.04	acctctgtt	acTTCTGtt		2211	Transposase, IS4 family
0010	42286	3500.84	act	acG		2285	Hypothetical protein, Lmo2313 homolog [phage A118]

Clone 18C1

Non-synonymous mutations							
Contig	**Pos**	**Score**	**Ref_nt**	**Var_nt**	**Frameshift**	**Gene_ID**	**Function**
0001	525680	8604.29	gcc	gTc		496	Transposase, IS4 family
0005	7258	262.335	gat	gGt		1625	Transposase, IS4 family
0005	7265	822.581	tgt	Ggt		1625	Transposase, IS4 family
0009	87601	5036.85	cag	Gag		2210	Transposase, IS4 family
0009	87676	11688.4	aat	Gat		2210	Transposase, IS4 family
0009	87780	15566.7	aagaaagta	AAGAAAAGta	yes	2211	Transposase, IS4 family
0009	87789	19603.4	ttggtgcgg	ttTGTGTgg		2211	Transposase, IS4 family
0009	87812	20831.3	agt	aAt		2211	Transposase, IS4 family
0009	87830	12640.3	gataattcaatttttattgatggt	AATAATTCAATTTTTATTGATGGt		2211	Transposase, IS4 family
0009	87885	11079.3	ttctat	ttCCat		2211	Transposase, IS4 family
0009	88235	16189.7	att	aAt		2211	Transposase, IS4 family
Synonymous mutations							
0001	525712	9596.15	att	atC		496	Transposase, IS4 family
0001	525739	9276.65	aag	aaA		496	Transposase, IS4 family
0001	525793	1566.82	aag	aaA		496	Transposase, IS4 family
0001	525802	1189.01	cgt	cgA		496	Transposase, IS4 family
0009	87596	3693.58	aat	aaC		2210	Transposase, IS4 family
0009	87749	17536.0	gag	gaA		2210	Transposase, IS4 family
0009	88024	24915.3	cga	cgT		2211	Transposase, IS4 family
0009	88194	3408.42	acctctgtt	acTTCTGtt		2211	Transposase, IS4 family

Clone 18C4

Non-synonymous mutations							
Contig	**Pos**	**Score**	**Ref_nt**	**Var_nt**	**Frameshift**	**Gene_ID**	**Function**
0001	525680	10276.0	gcc	gTc		496	Transposase, IS4 family
0005	7258	1225.1	gat	gGt		1625	Transposase, IS4 family
0005	7265	1314.05	tgt	Ggt		1625	Transposase, IS4 family
0009	87601	3980.07	cag	Gag		2210	Transposase, IS4 family
0009	87676	9695.14	aat	Gat		2210	Transposase, IS4 family
0009	87780	13094.5	aagaaagta	AAGAAAAGta	yes	2211	Transposase, IS4 family
0009	87789	16499.0	ttggtgcgg	ttTGTGTgg		2211	Transposase, IS4 family
0009	87812	15960.0	agt	aAt		2211	Transposase, IS4 family
0009	87830	7869.22	gataattcaatttttattgatggt	AATAATTCAATTTTTATTGATGGt		2211	Transposase, IS4 family
0009	87885	7835.95	ttctat	ttCCat		2211	Transposase, IS4 family
0009	88220	2190.76	atgacccaa	TTGATCCAa		2211	Transposase, IS4 family
0009	88235	15953.5	att	aAt		2211	Transposase, IS4 family
Synonymous mutations							
0001	525712	10074.7	att	atC		496	Transposase, IS4 family
0001	525739	8975.51	aag	aaA		496	Transposase, IS4 family
0001	525793	1084.67	aag	aaA		496	Transposase, IS4 family
0001	525802	1072.37	cgt	cgA		496	Transposase, IS4 family
0009	87596	3262.71	aat	aaC		2210	Transposase, IS4 family
0009	87749	14795.8	gag	gaA		2210	Transposase, IS4 family
0009	88024	19495.9	cga	cgT		2211	Transposase, IS4 family
0009	88194	5599.36	acctctgtt	acTTCTGtt		2211	Transposase, IS4 family
0010	42286	570.972	act	acG		2285	Hypothetical protein, Lmo2313 homolog [phage A118]

Clone 18C10

Non-synonymous mutations							
Contig	**Pos**	**Score**	**Ref_nt**	**Var_nt**	**Frameshift**	**Gene_ID**	**Function**
0001	525680	5608.82	gcc	gTc		496	Transposase, IS4 family
0001	525813	75.7067	agt	Ggt		496	Transposase, IS4 family
0005	7195	525.68	ggc	gTc		1625	Transposase, IS4 family
0005	7258	844.719	gat	gGt		1625	Transposase, IS4 family
0005	7265	893.501	tgt	Ggt		1625	Transposase, IS4 family
0009	87601	3107.3	cag	Gag		2210	Transposase, IS4 family
0009	87676	8719.57	aat	Gat		2210	Transposase, IS4 family
0009	87780	13226.1	aagaaagta	AAGAAAAGta	yes	2211	Transposase, IS4 family
0009	87789	17524.8	ttggtgcgg	ttTGTGTgg		2211	Transposase, IS4 family
0009	87812	17154.9	agt	aAt		2211	Transposase, IS4 family
0009	87830	10619.7	gataattcaatttttattgatggt	AATAATTCAATTTTTATTGATGGt		2211	Transposase, IS4 family
0009	87885	8389.01	ttctat	ttCCat		2211	Transposase, IS4 family
0009	88220	2276.45	atgacccaa	TTGATCCAa		2211	Transposase, IS4 family
0009	88235	14505.1	att	aAt		2211	Transposase, IS4 family
0010	42336	648.986	aaa	Gaa		2285	Hypothetical protein, Lmo2313 homolog [phage A118]
Synonymous mutations							
0001	525712	6093.63	att	atC		496	Transposase, IS4 family
0001	525739	6269.44	aag	aaA		496	Transposase, IS4 family
0001	525793	1241.32	aag	aaA		496	Transposase, IS4 family
0001	525802	1663.27	cgt	cgA		496	Transposase, IS4 family
0005	7149	190.834	ttc	ttT		1625	Transposase, IS4 family
0009	87596	2442.06	aat	aaC		2210	Transposase, IS4 family
0009	87749	15295.7	gag	gaA		2210	Transposase, IS4 family
0009	88024	23007.5	cga	cgT		2211	Transposase, IS4 family
0009	88194	5005.21	acctctgtt	acTTCTGtt		2211	Transposase, IS4 family
0010	42286	6078.02	act	acG		2285	Hypothetical protein, Lmo2313 homolog [phage A118]

Figure S1. Images of representative patterns observed in diluted drop tests for *S. aureus* SSS and SRS clones isolated from the cardiac vegetations of rats treated with the phage cocktail/flucloxacillin combination for 24 h. **A.** Phage vB_SauH_2002. **B.** Phage 66. **C.** Phage cocktail. The SSS pattern observed with the wild-type (WT) strain Laus102 is indicated for comparison in the left panel. S, susceptible; R, resistant.