

Supplementary Materials

Supplementary Figures and Figure Legends

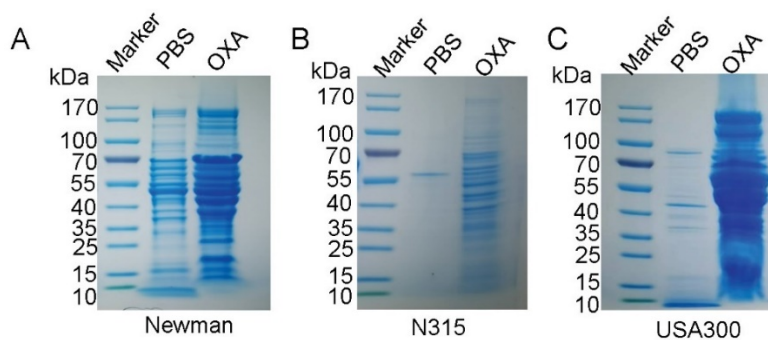


Fig. S1 SDS-PAGE analysis of MVs derived from *S. aureus* Newman (A), N315 (B), and USA300 (C) after OXA treatment. PBS served as a negative control.

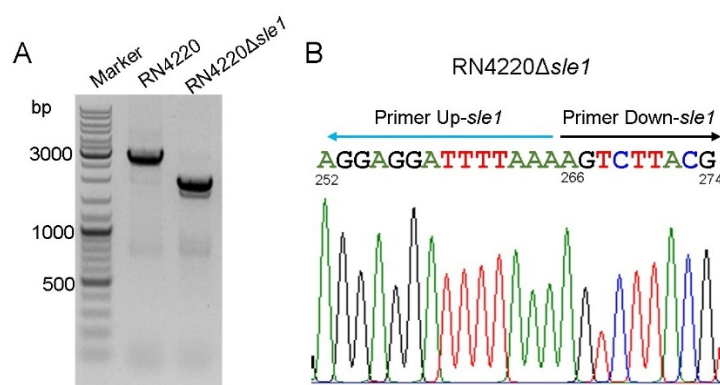


Fig. S2 Characterization of *S. aureus* RN4220 Δ *sle1*. (A) PCR identification of the RN4220 Δ *sle1* strain. The primer pair *sle1*-out-L/R used is listed in Table S4. (B) DNA sequencing showing deletion of *sle1* gene in the RN4220 Δ *sle1* strain.

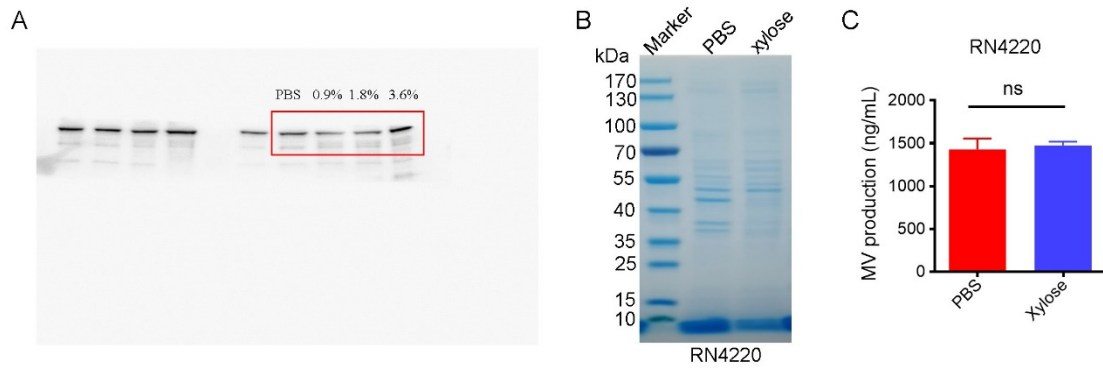


Fig. S3 The effect of xylose on *S. aureus* MV production. (A) The full-length blot for the Western blot in Fig. 3D. The red box represents the depicted part of the blot. (B) SDS-PAGE analysis of the proteins in MVs derived from *S. aureus* RN4220 with or without xylose induction. (C) BCA detection of the total xylose-induced MV proteins prepared from *S. aureus* RN4220. PBS served as a negative control. The statistical difference was calculated by Student's *t*-test, and ns represents no significance.

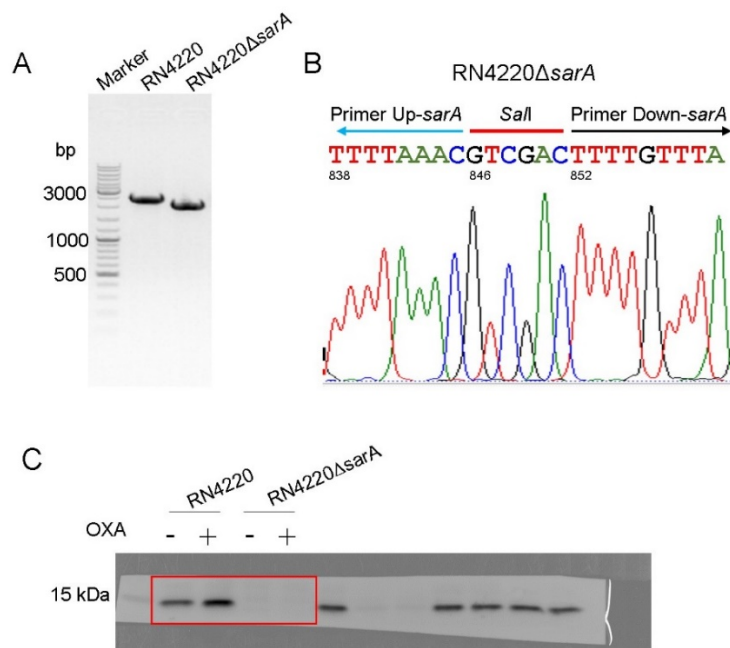


Fig. S4 Characterization of *S. aureus* RN4220 Δ *sarA*. (A) PCR identification of the RN4220 Δ *sarA* strain. The primer pair *sarA*-out-L/R used is listed in Table S4. (B) DNA sequencing showing deletion of *sle1* gene in the RN4220 Δ *sarA* strain. (C) The full-length blot for the Western blot in Fig. 4B. The red box represents the depicted part of the blot.

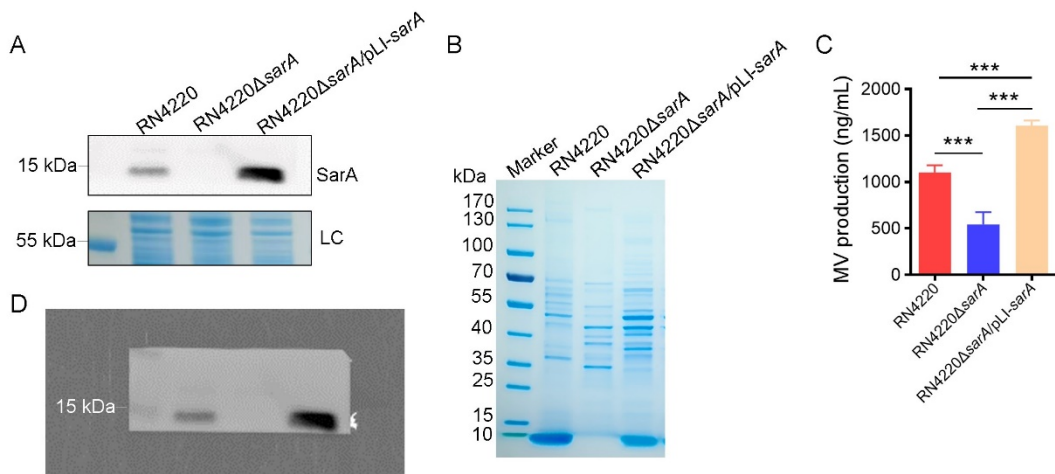


Fig. S5 The effect of SarA on *S. aureus* MV production. (A) Western blot detection of SarA expression in RN4220 and its derivatives. LC, Loading control. (B) SDS-PAGE analysis of proteins in the MVs derived from the indicated strains. (C) BCA detection of total MV proteins from *S. aureus* RN4220 and its derivatives. (D) The full-length blot for the Western blot in Fig. S5A.

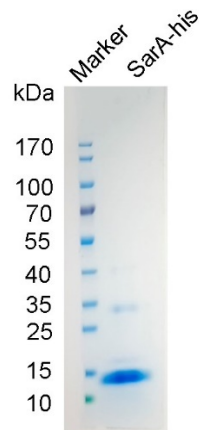


Fig. S6 SDS-PAGE analysis of the purified SarA protein.

Supplementary Tables

Table S1. The MIC_c of OXA against diverse *S. aureus* strains (mg/L)

	RN4220	RN4220Δslc1	RN4220ΔsarA	N315	USA300	Newman
MIC	0.5	0.25	0.25	>64	32	1.0
1/8 MIC	0.062 ^a	0.031 ^a	0.031 ^a	2 ^b	2 ^b	0.25 ^b

^a The MIC value of *S. aureus* strains was detected three times. As for RN4220 and its derivatives, 1/4 MIC of OXA slightly suppressed *S. aureus* growth, therefore, 1/8 MIC was used in this study.

^b The growth of high-virulent N315 was greatly inhibited by OXA at 8 mg/L^[1], therefore, 2 mg/L OXA was used to treat N315, USA300, and Newman in this study.

Table S2 · Proteins identified in MVs and MVs/OXA by proteomic analysis

Protein.ID	Protein name	Gene	AA No.	MV 1	MV2	MV3
P0C1S0	HTH-type transcriptional regulator MgrA	mgrA	147	210561.03	2771.949	129706.91
Q2F119	Probable quinol oxidase subunit 3	qoxC	201	323221.75	2771.949	2771.949
A0A0B4N9G0	Membrane protein	CV021_05915	431	79325.55	386162.4	375570.66
A0A0D1HLP4	Exported protein	A6762_13085	63	154635.17	118419.94	2771.949
A0A0E0VRM0	DUF3139 domain-containing protein	ST398NM01_2438	121	242355.55	2771.949	199138.5
A0A0H3K686	Immunoglobulin G-binding protein A	spa	508	2689895	801526.1	781901.6
A5IQ92	Large ribosomal subunit protein uL1	rplA	230	217501.34	2771.949	2771.949
A5IQ93	Large ribosomal subunit protein uL10	rplJ	166	175935.36	2771.949	770331.94
A5IQ96	DNA-directed RNA polymerase subunit beta	rpoB	1183	60536.504	2771.949	2771.949
A5IQ99	Small ribosomal subunit protein uS12	rpsL	137	169636.55	117197.2	2771.949
A5IQA0	Small ribosomal subunit protein uS7	rpsG	156	58495.805	2771.949	2771.949
A5IQA1	Elongation factor G	fusA	693	1471169	751329.9	152350.31
A5IQA2	Elongation factor Tu	tuf	394	2226721.2	487076	896022.25
A5IQX7	Phosphoglycerate kinase	pgk	396	85768.38	2771.949	2771.949
A5IRD0	Na ⁽⁺⁾ /H ⁽⁺⁾ antiporter subunit A1	mhxA1	801	2771.949	224612.2	229682.05
A5ISB7	Acyl carrier protein	acpP	77	491988.6	2771.949	181458.86
A5ISD0	Succinate--CoA ligase [ADP-forming] subunit beta	sucC	388	42696.348	2771.949	2771.949
A5ISE0	Small ribosomal subunit protein uS2	rpsB	255	95939.04	2771.949	2771.949
A5ISG9	Protein RecA	recA	347	55387.23	2771.949	2771.949
A5ISX6	Cell cycle protein GpsB	gpsB	114	1294591.2	139436.86	186101.44
A5ISY4	Asparagine--tRNA ligase	asnS	430	57160.1	2771.949	2771.949
A5ITA1	Flotillin-like protein FloA	floA	329	1864385.4	650841.44	975703.4
A0A1D5AAV8	Uncharacterized protein conserved in bacteria	NCTC5664_03517	75	387069.4	2771.949	139128.14
A5ITN4	Acetate kinase	ackA	400	49077.113	2771.949	2771.949
A5ITP0	Septation ring formation regulator EzrA	ezrA	564	149823.47	145719.81	2771.949
A5IU10	UPF0342 protein SaurJH9_1898	SaurJH9_1898	114	82869.055	79245.83	2771.949
A5IUN6	Membrane protein insertase YidC	yidC	290	889848.6	944311.94	1386828
A5IUP8	ATP synthase subunit beta	atpD	470	2257931.5	539081.75	709441.8
A5IUP9	ATP synthase gamma chain	atpG	288	253573.36	2771.949	2771.949
A5IUQ0	ATP synthase subunit alpha	atpA	502	973933.75	321758.44	253316.77
A5IUQ2	ATP synthase subunit b	atpF	173	1246272.9	422936.1	483813.7
A5IV17	Small ribosomal subunit protein uS5	rpsE	166	120557.85	2771.949	2771.949
A5IV20	Small ribosomal subunit protein uS8	rpsH	132	116917.5	2771.949	2771.949
A5IV29	Large ribosomal subunit protein uL22	rplV	117	514062.12	236150.64	209172.9
A5IV31	Large ribosomal subunit protein uL2	rplB	277	136550.88	125790.84	281521.06
A5IV35	Small ribosomal subunit protein uS10	rpsJ	102	867721.2	2771.949	2771.949
A6QDH3	PTS system glucose-specific EIICBA component	ptsG	681	184863.66	396083.38	994761.4
A6QEZZ	Undecaprenyl-diphosphatase	uppP	291	409953.12	2771.949	218151.53
A6QF98	Extracellular matrix protein-binding protein emp	ssp	340	1296887.5	317071.44	320343.22
A6QH29	Elastin-binding protein EbpS	ebpS	486	220847.95	368464.34	125722.83
Q2UX41	Elastin binding protein (Fragment)	ebpS	349	179413.72	368464.34	125722.83

Protein.ID	Protein name	Gene	AA No.	MV 1	MV2	MV3
Q2YY76	Elastin-binding protein EbpS	ebpS	483	220847.95	368464.34	125722.83
A6QI23	Foldase protein PrsA	prsA	320	13186686	5833952.5	11566480
A0A380DWX1	Foldase protein PrsA	prsA	320	13186686	5833952.5	11566480
A6QI56	Monofunctional glycosyltransferase	mgt	269	956904.9	317106.3	697656.56
A6QIQ7	Immunoglobulin-binding protein Sbi	sbi	436	377432192	72109296	216239472
A0A6B0CTK3	Immunoglobulin-binding protein Sbi (Fragment)	sbi	124	53934492	16999258	39335416
A6QK89	L-lactate dehydrogenase 2	ldh2	319	724717.8	454867.1	1182825.8
A6U1G3	UPF0154 protein SaurJH1_1431	SaurJH1_1431	80	3325858.8	2771.949	2771.949
A7WYS8	Lysine--tRNA ligase	lysS	495	58981.72	2771.949	2771.949
O08387	Protein translocase subunit SecY	secY	430	53237.48	82110.1	117238.01
O86488	Serine-aspartate repeat-containing protein D	sdrD	1315	215607.75	2771.949	126533.05
P00644	Thermonuclease	nuc	231	1585925	218662.34	733757.75
P0A029	Cell division protein FtsZ	ftsZ	390	393687.25	2771.949	320552.06
P0A036	Glyceraldehyde-3-phosphate dehydrogenase I	gapA1	336	4680806	1915411.8	1495548.1
P0A039	Glutamine synthetase	glnA	446	2411106	530508.4	900140.94
P0A071	Gamma-hemolysin component A	hlgA	309	606639	275113.56	372294.28
Q9AFA9	Leukocidin LukS component (Fragment)		286	125648.9	90321.38	2771.949
P0A0A1	Pyruvate dehydrogenase E1 component subunit beta	pdhB	325	209699.66	2771.949	2771.949
P0A0A4	Aminoacyltransferase FemA	femA	420	2771.949	128986.03	117083.61
P0A0E1	Phosphocarrier protein HPr	ptsH	88	426668.94	2771.949	162404.11
P0C1U7	N-acetylmuramoyl-L-alanine amidase sle1	sle1	334	626128.4	79615.81	117255
P10335	Lipase 2	lip2	690	10124640	1897688.9	4263664
Q2FUU5	Lipase 1	lipA	680	1349733.2	460466.12	1016998.7
P11162	PTS system lactose-specific EIICB component	lacE	570	258667.78	935314.5	1522800.8
P21224	Uncharacterized leukocidin-like protein 1	SACOL2004	338	8571573	2454146.8	2435279.5
A0A0E0VRF0	Leukocidin F subunit	ST398NM01_2060	338	7790441	560988.94	684619.3
P60089	Pyruvate dehydrogenase E1 component subunit alpha	pdhA	370	96694.99	2771.949	85239.44
P60284	DNA-directed RNA polymerase subunit beta'	rpoC	1207	88405.164	2771.949	2771.949
P63332	Putative zinc metalloprotease SAV1262	SAV1262	428	731727.5	123300.79	717165.7
P63870	Cysteine synthase	cysK	310	612309.56	83126.15	178712.42
P65424	Probable malate:quinone oxidoreductase 2	mqq2	498	236597.69	2771.949	2771.949
P65825	Staphopain A	sspP	388	146675.58	117974.62	2771.949
P66962	Transketolase	tkt	662	461657.2	2771.949	2771.949
P67370	DegV domain-containing protein SAV1425	SAV1425	279	74601.77	2771.949	2771.949
P67477	Fructose-bisphosphate aldolase	fbA	286	279504.84	116226.99	174197.84
Q2FDM1	Immunodominant staphylococcal antigen B	isaB	175	472406.62	2771.949	2771.949
Q2FDN2	Uncharacterized protein SAUSA300_2562	SAUSA300_2562	33	22148910	11430167	29937074
Q2FEC8	Uncharacterized lipoprotein SAUSA300_2315	SAUSA300_2315	209	69365.99	2771.949	2771.949
Q2FEG1	Lysostaphin resistance protein A	lyrA	419	352446.84	312388.16	304891.6
Q2FEI5	Putative formate dehydrogenase SAUSA300_2258	SAUSA300_2258	984	113120.33	2771.949	2771.949
Q2FEJ8	HTH-type transcriptional regulator SarR	sarR	115	56345.008	2771.949	2771.949
Q2FFA2	Uncharacterized leukocidin-like protein 2	SAUSA300_1975	351	10835198	4286404	8958463
Q2FFZ9	UPF0478 protein SAUSA300_1685	SAUSA300_1685	163	717478.2	2771.949	2771.949

Protein.ID	Protein name	Gene	AA No.	MV 1	MV2	MV3
Q2FG40	Pyruvate kinase	pyk	585	717632.44	194321.23	268657.25
Q2FH26	Dihydrolypoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	odhB	422	13629.768	2771.949	2771.949
Q2FH48	Phosphate-binding protein PstS	pstS	327	134352.73	2771.949	2771.949
Q2FHD8	Aerobic glycerol-3-phosphate dehydrogenase	glpD	557	1240227.4	2771.949	2771.949
Q2FHZ1	Ribonuclease J 1	rnj1	565	113176.195	2771.949	2771.949
Q2FI17	Probable quinol oxidase subunit 2	qoxA	366	12991127	1630484.2	2217011.8
Q2FI18	Probable quinol oxidase subunit 1	qoxB	662	3217389.8	931735.06	3768048
Q2FIN9	Ribosome hibernation promotion factor	hpf	190	57050.37	2771.949	2771.949
Q2FIS2	Lipoteichoic acid synthase	ltaS	646	5558898	2387369.8	3500406.2
Q2FJE0	Large ribosomal subunit protein bL25	rpLY	217	66358.305	2771.949	2771.949
Q2FK44	Formate acetyltransferase	pfIB	749	1237632.2	125425.72	168123.94
Q2FZK7	Bifunctional autolysin	atl	1256	7570411	2731115.8	3754681
Q2FZQ3	Enoyl-[acyl-carrier-protein] reductase [NADPH] FabI	fabI	256	70184.875	2771.949	2771.949
Q2FZW3	Protein DltD	dltD	391	213119.81	367541.25	2771.949
Q2G2J2	Staphylococcal secretory antigen ssaA2	ssaA2	267	7793055	1836280	4355984
Q5HFU7	Small ribosomal subunit protein bS1	rpsA	391	409899.06	75953.05	195942.69
Q5HFZ9	PTS system glucose-specific EIIA component	ctr	166	111171.86	2771.949	2771.949
Q5HHC7	NAD-specific glutamate dehydrogenase	gluD	414	24530.002	2771.949	2771.949
A0A068A2L4	Mannitol-specific phosphotransferase enzyme IIA component	fruA_2	652	2129766.8	830649.1	1231176.9
A0A6B1RN75	Mannitol-specific phosphotransferase enzyme IIA component	GAY51_04280	656	2129766.8	830649.1	1231176.9
A0A068DV34	Putative iron(III) dicitrate transporter binding lipoprotein	yfiY	330	1839987	188879.2	114425.92
A0A090LUS6	DUF1307 domain-containing protein	yehR	151	1083219.8	201212.6	690994.8
A0A0A0Q4N9	Serine protease HtrA-like	htrA1	424	795179.44	102073.78	247505.89
A0A0B4N8P6	Amino acid/peptide transporter	yjdL	501	139256.31	121982.71	2771.949
A0A0B4ND04	L-lactate permease	lctP_1	532	401276.4	590611.75	860616.8
A0A0E0VKK7	L-lactate permease	ST398NM01_0121	530	2771.949	120104.484	291243.44
A0A0D1GQ07	Exported protein	CV021_12380	168	2256514.8	67641.43	239548.9
A0A0D1H4D9	Putative transcription factor	nhaX	137	2771.949	2771.949	2771.949
A0A0D1HHU9	Alanine:cation symporter family protein	CV021_04530	486	81405.72	2771.949	2771.949
A0A0D1HK54	Multifunctional fusion protein	secD	759	1977080	1727036.5	1378956.6
A0A0D1IBJ1	Cell envelope transcriptional attenuator	lytR_1	315	53886.195	2771.949	2771.949
A0A0D1IYN8	Glucosamine-6-phosphate deaminase	CV021_04655	199	79155.59	327039.2	2771.949
A0A0D1JLA1	ABC transporter permease	yhaP	410	52085.65	2771.949	2771.949
A0A0D1JNG6	Pyruvate oxidase	G6Y24_16510	579	718897.3	2771.949	254070.58
A0A0D1K0S1	CamS family sex pheromone protein	CV021_17180	399	58112.406	97800.89	116613.03
A0A0D3Q462	Uncharacterized protein	DD547_00265	507	157650.88	77118.336	2771.949
A0A0D3Q4X2	Lipoprotein	metQ_2	280	341890.2	2771.949	206878.62
A0A0D3Q6F6	Fibrinogen-binding protein	ecb	109	226279.1	100427.38	2771.949
A0A0D3QA03	DUF805 domain-containing protein	yhal	215	119570.016	103960.836	108091.5
A0A0D3QA12	HlyD family efflux transporter periplasmic adaptor subunit	emrK	215	138983.67	365567.8	234505.1

Protein.ID	Protein name	Gene	AA No.	MV 1	MV2	MV3
A0A0D6G8E8	PTS ascorbate transporter subunit IIA	treB	475	2771.949	187188.12	2771.949
A0A0D6GCD0	Lipoprotein	DD547_00359	190	196055.89	135236.86	2771.949
A0A0D6H3T6	Molybdate ABC transporter substrate-binding protein	modA	260	373478	71124.06	407485.5
A0A0D6H9G0	Lytic regulatory protein	DD547_02182	363	622256.94	437164.16	458533.7
A0A0D6HD63	AAA family ATPase	DD547_01922	978	137902.56	82069.586	2771.949
A0A0D6HDR3	Lipoprotein	DD547_01873	208	48481.434	2771.949	2771.949
A0A0E0VLB8	NDxxF motif lipoprotein	ST398NM01_0455	208	274641.44	2771.949	2771.949
A0A0E0VLC3	YhcL	ST398NM01_0461	465	1363575.4	1108774.2	842669.44
A0A0E0VLN4	ATP-dependent zinc metalloprotease FtsH	ftsH	697	130140.97	2771.949	250539.39
A0A0E0VM22	Manganese-binding protein	ST398NM01_0710	309	4999632	741573	751071.1
A0A0E0VMK1	Putative membrane spanning protein	ST398NM01_1007	361	84856.85	122347.88	2771.949
A0A0E0VMW9	DM13 domain-containing protein	ST398NM01_0785	146	838613.7	424131.88	793357
A0A0E0VNI9	Protein translocase subunit SecA	secA	843	353670.3	2771.949	2771.949
A6QF62	Protein translocase subunit SecA 1	secA1	843	353670.3	2771.949	2771.949
A0A0E0VNI26	Lipoprotein	ST398NM01_1089	213	451576.3	115147.29	107970.18
A0A0E0VNI36	Spermidine/putrescine-binding protein	ST398NM01_1099	357	23386.076	2771.949	2771.949
A0A0E0VNI55	NupC/NupG family nucleoside CNT transporter	ST398NM01_0596	404	77816.31	2771.949	159898.36
A0A0E0VNI55	DoxX family protein	ST398NM01_0784	160	122670.92	2771.949	2771.949
A0A0E0VNIU4	IsdA	ST398NM01_1126	354	612512.9	69221.55	185644.95
A5IS16	Iron-regulated surface determinant protein A	isdA	350	404942.25	69221.55	185644.95
A0A0E0VNI1	Glycine betaine transporter	ST398NM01_1350	548	300054.97	80544.54	245288.66
A0A0E0VNI10	Phosphoesterase	ST398NM01_1910	398	64075.41	129384.766	178503.75
A0A0E0VNIH8	Lipoprotein	ST398NM01_1596	193	291222.7	2771.949	131222.69
A0A0E0VNI20	Putative membrane associated protein	ST398NM01_1975	91	169333.34	2771.949	2771.949
A0A0E0VNI70	Transaldolase	ST398NM01_1832	237	58218.098	2771.949	2771.949
A0A0E0VNI35	Ferrichrome-binding protein	ST398NM01_2335	302	7685489.5	797427	1443276.6
A0A0E0VNI77	Gluconate permease	ST398NM01_2555	452	2771.949	714048.3	742108.06
A0A0E0VNI28	Putative membrane associated protein	ST398NM01_2217	310	51532.582	2771.949	2771.949
A0A0E0VNI57	Uncharacterized protein	ST398NM01_2485	225	177512.25	104008.63	248404.4
A0A0E1VNIQ1	PTS system, N-acetylglucosamine-specific IIBC component	nagE	488	85834.52	338242.12	2771.949
A0A0E1VNIJ9	DUF4887 domain-containing protein	HMPREF0776_1781	209	15036.377	2771.949	2771.949
A0A0E1VNIJ0	Periplasmic binding protein	HMPREF0776_1746	342	438250.1	210986.55	2771.949
A0A0E1XNI91	Periplasmic binding protein	HMPREF0769_11558	342	438250.1	210986.55	2771.949
A0A0E1VNI89	Periplasmic binding protein	HMPREF0776_1614	283	323724.56	173996.45	248741.92
A0A0E1VNI55	DUF2179 domain-containing protein	HMPREF0776_1649	277	138788.88	112200.74	2771.949
A0A0E1VNI00	ABC transporter, substrate-binding protein, family 3	HMPREF0776_0469	259	4080062.2	407805.94	862674.5
A0A0E1VNI46	ABC transporter, ATP-binding protein	HMPREF0776_0467	243	81757.055	2771.949	2771.949
A0A0E1VNI84	5'-nucleotidase, lipoprotein e(P4) family	HMPREF0776_1051	296	11928486	4137550.8	5040054
P21222	30 kDa neutral phosphatase (Fragment)		35	6630391	1829448.8	2743547.2
A0A0E1VNI89	Periplasmic binding protein	HMPREF0776_0229	327	2418431.5	388316.6	571481.6
A0A0E1VNI81	RND transporter, HAE1/HME family, permease protein	HMPREF0776_0309	1055	112166.27	2771.949	125596.57
A0A0E1VNI29	ABC transporter, ATP-binding protein	HMPREF0776_0571	231	7263150.5	649189.25	4992942

Protein.ID	Protein name	Gene	AA No.	MV 1	MV2	MV3
A0A0E1VQI3	DUF4889 domain-containing protein	HMPREF0776_0437	118	233143.55	570749.4	516785.78
A0A0E1VS93	Alkaline shock protein 23	HMPREF0776_2224	124	304593.7	117651.98	159563.56
A0A0E1VT13	Lipoprotein	HMPREF0776_1830	273	886613.1	490077.75	352181.06
A0A0E1VY92	Alpha/beta hydrolase	HMPREF0776_0241	294	40437.83	2771.949	2771.949
A0A0E1X9N9	DUF5067 domain-containing protein	HMPREF0769_11505	242	415787.06	400059.94	296047.7
A0A0E1XH07	Pyruvate carboxylase	pyc	1150	188925.22	2771.949	2771.949
A0A0E8I376	Amidase domain-containing protein	EP54_05180	619	3801007.8	1510503.8	2254104.5
A0A0H2WWQ9	Penicillin-binding protein 2	pbp2	727	1944794.5	455989.12	862428.75
A0A0H2WX78	Lipoprotein, putative	SACOL2407	199	56445.535	2771.949	103077.01
A0A0H3JL84	SA0233 protein	SA0233	509	91619.3	117067.99	174380.77
A0A0U1MQG8	Putative exported protein	BN1321_270014	302	945542.44	473883.75	309639.8
A0A2C9TUB8	non-specific serine/threonine protein kinase	EP54_08500	664	724215.44	204816.05	361537.88
A0A380DVS7	SasB protein	ebh_9	440	581914.2	136579.86	2771.949
A0A7R6SNW6	Pts system, glucose-specific iiabc component	SAJPND4_02538	727	149462.45	2771.949	160081.05
A0A850FQI7	Tandem-type lipoprotein Lp110	lp110	260	171060.12	2771.949	102153.08
A0A8D9SNT7	L-lactate dehydrogenase	ldh	317	119724.125	2771.949	2771.949
A5IUP4	3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ	fabZ	146	629877.9	109503.35	95594.11
A0A068A2A0	Branched-chain amino acid transport system carrier protein	brnQ	451	91504.8	134176.2	2771.949
A0A0D1JYJ4	Membrane spanning protein	CV021_05790	152	59419.336	2771.949	2771.949
A0A0E0VKV9	Putative membrane spanning protein	ST398NM01_0230	190	248640.58	191368.22	335950.7
A0A0E0VN96	Antibacterial protein 3	ST398NM01_1170	47	738155.94	120353.05	130800.46
A0A0H3JPX7	DUF3173 domain-containing protein	SAV0393	66	2771.949	411145.9	405139.2
A0A6M1XSY5	Gfo/Iah/MocA family oxidoreductase (Fragment)	G6Y24_10920	37	1466204	2422144.5	5022991.5

Protein.ID	Protein name	Gene	Protein. Length	MV/OXA1	MV/OXA2	MV/OXA3
A5ITK3	Large ribosomal subunit protein bL20	rplT	118	917407.8	1338536.4	539593.9
A5IUQ7	Uracil phosphoribosyltransferase	upp	209	81345.45	2771.949	202180.3
A5IV27	Large ribosomal subunit protein uL16	rplP	144	168693.28	72650.234	73411.19
A6QES8	Transcriptional regulator SarA	sarA	124	1116424.9	1332845.8	1257635.9
Q2FG28	Putative universal stress protein SAUSA300_1656	SAUSA300_1656	166	87790.71	131905.06	73590.1
A0A0B4N9G0	Membrane protein	CV021_05915	431	2771.949	134195.42	2771.949
A5IQ92	Large ribosomal subunit protein uL1	rplA	230	235250.61	185141.19	283968.06
A5IQ93	Large ribosomal subunit protein uL10	rplJ	166	2771.949	195704.75	204889.52
A5IQ96	DNA-directed RNA polymerase subunit beta	rpoB	1183	2771.949	584847.9	1544407.4
A5IQA0	Small ribosomal subunit protein uS7	rpsG	156	177749.69	114139.81	68208.05
A5IQA1	Elongation factor G	fusA	693	2771.949	102991.44	299251.3
A5IQA2	Elongation factor Tu	tuf	394	285598.94	140250.08	315599.53
A5IQX2	ATP-dependent Clp protease proteolytic subunit	clpP	195	446366.16	470163.25	136447.2
A5IQX7	Phosphoglycerate kinase	pgk	396	2771.949	447226.9	213229.86
A5ISD8	ATP-dependent protease ATPase subunit HslU	hslU	467	109824.21	121840.58	2771.949
A5ISE0	Small ribosomal subunit protein uS2	rpsB	255	1472995.2	1109279.8	690524.5
A5ISG9	Protein RecA	recA	347	203805.36	73373.36	55320.258
A5ISX6	Cell cycle protein GpsB	gpsB	114	2771.949	61773.906	55991.926

Protein.ID	Protein name	Gene	Protein.	MV /OXA1	MV /OXA2	MV /OXA3
			Length			
A5ISY4	Asparagine--tRNA ligase	asnS	430	220265.17	2771.949	427283.97
A5ITA1	Flotillin-like protein FloA	floA	329	524252.28	494530.72	407197.75
A0A1D5AAV8	Uncharacterized protein conserved in bacteria	NCTC5664_03517	75	85790.586	111273.375	2771.949
A5ITN4	Acetate kinase	ackA	400	250196.73	30590.05	283423
A5IUH6	Chaperonin GroEL	groEL	538	2771.949	159821.25	82372.81
A5IUJ2	Redox-sensing transcriptional repressor Rex	rex	211	174581.11	111037.664	2771.949
A5IUP8	ATP synthase subunit beta	atpD	470	2771.949	241127.31	2771.949
A5IUP9	ATP synthase gamma chain	atpG	288	235454.72	68220.9	211303.19
A5IUQ0	ATP synthase subunit alpha	atpA	502	2771.949	106355.516	2771.949
A5IUQ8	Serine hydroxymethyltransferase	glyA	412	647881.75	629875.2	2771.949
A5IV07	Large ribosomal subunit protein bL17	rplQ	122	115708.77	71742.93	2771.949
A5IV17	Small ribosomal subunit protein uS5	rpsE	166	198939.81	238474.17	287500.12
A5IV20	Small ribosomal subunit protein uS8	rpsH	132	230077.94	125451.69	195234.9
A5IV22	Large ribosomal subunit protein uL5	rplE	179	116224.13	585508.44	332180.62
A5IV31	Large ribosomal subunit protein uL2	rplB	277	96327.945	2771.949	67104.86
A5IV35	Small ribosomal subunit protein uS10	rpsJ	102	2771.949	2771.949	93131.59
A6QEZ2	Undecaprenyl-diphosphatase	uppP	291	2771.949	2771.949	207473.08
A6QI23	Foldase protein PrsA	prsA	320	886166.4	944754.5	498347.28
A0A380DWX1	Foldase protein PrsA	prsA	320	886166.4	944754.5	498347.28
A6QI56	Monofunctional glycosyltransferase	mgt	269	210432.33	216665.42	56099.965
A6QIQ7	Immunoglobulin-binding protein Sbi	sbi	436	958975.6	4604034.5	371823.75
A6QK04	Fructose-1,6-bisphosphatase class 3	fbp	654	161746.97	2771.949	62489.523
A6QK89	L-lactate dehydrogenase 2	ldh2	319	293024.66	258402.47	201951.86
A7WYS8	Lysine--tRNA ligase	lysS	495	113479.81	123012.17	2771.949
O07325	Cell division protein FtsA	ftsA	470	2771.949	41205.773	2771.949
P00644	Thermonuclease	nuc	231	2771.949	999768.9	2771.949
P0A036	Glyceraldehyde-3-phosphate dehydrogenase 1	gapA1	336	974735.6	1762386	1671528.4
P0A039	Glutamine synthetase	glnA	446	1034330.6	809896.5	454506.72
P0A0A1	Pyruvate dehydrogenase E1 component subunit beta	pdhB	325	2771.949	30773.717	2771.949
P0A0A4	Aminoacyltransferase FemA	femA	420	2771.949	2771.949	206910.34
P0A0E1	Phosphocarrier protein HPr	ptsH	88	163507.4	2771.949	2771.949
P0C1U7	N-acetylmuramoyl-L-alanine amidase sle1	sle1	334	9771.82	12770.923	2771.949
P10335	Lipase 2	lip2	690	393674.28	2771.949	2771.949
Q2FUU5	Lipase 1	lipA	680	92722.414	27026.188	2771.949
P11162	PTS system lactose-specific EIICB component	lacE	570	111993.96	2771.949	64697.64
P60107	TelA-like protein SAV1406	SAV1406	378	101266.2	271785.72	2771.949
P60284	DNA-directed RNA polymerase subunit beta'	rpoC	1207	1497062.1	902172.1	1259174.9
P63870	Cysteine synthase	cysK	310	180190.5	167011.58	2771.949
P64227	Glutamine--fructose-6-phosphate aminotransferase [isomerizing]	glmS	601	190744.75	2771.949	76389
P65424	Probable malate:quinone oxidoreductase 2	mqq2	498	2771.949	276897.6	275899.16
P66962	Transketolase	tkt	662	102796.41	2771.949	465961.1

Protein.ID	Protein name	Gene	Protein.	MV /OXA1	MV /OXA2	MV /OXA3
			Length			
Q2FDN2	Uncharacterized protein SAUSA300_2562	SAUSA300_2562	33	2675388.5	9663968	6686242
Q2FEC8	Uncharacterized lipoprotein SAUSA300_2315	SAUSA300_2315	209	286188	422943	268643.03
Q2FEI9	Putative 2-hydroxyacid dehydrogenase SAUSA300_2254	SAUSA300_2254	317	2771.949	195941.3	249327.19
Q2FEJ8	HTH-type transcriptional regulator SarR	sarR	115	645424.75	1386130.8	995671.2
A0A2C9TQZ8	MarR family transcriptional regulator (Fragment)	EP54_15150	52	2771.949	63339.543	76536.305
Q2FG40	Pyruvate kinase	pyk	585	259234.19	183730.22	164825.73
Q2FHD8	Aerobic glycerol-3-phosphate dehydrogenase	glpD	557	890234.75	2771.949	392626.47
Q2FHZ1	Ribonuclease J I	rnjI	565	263468.5	2771.949	303030.72
Q2FI17	Probable quinol oxidase subunit 2	qoxA	366	247309.05	201739.47	218642.69
Q2FID4	Type II NADH:quinone oxidoreductase	SAUSA300_0844	402	112884.89	2771.949	53194.09
Q2FIN9	Ribosome hibernation promotion factor	hpf	190	2771.949	866404.94	585687.25
Q2FJE0	Large ribosomal subunit protein bL25	rplY	217	2771.949	2771.949	338617.62
P65176	Ribitol-5-phosphate cytidyltransferase 2	tarI2	238	119235.5	239464.64	399221.6
Q2FZQ3	Enoyl-[acyl-carrier-protein] reductase [NADPH] FabI	fabI	256	2771.949	41704.68	118818.83
Q2FZW3	Protein DltD	dltD	391	2771.949	2771.949	66369.78
Q5HFZ9	PTS system glucose-specific EIIA component	err	166	2771.949	2771.949	48888.953
Q5HH38	1,4-dihydroxy-2-naphthoyl-CoA synthase	menB	273	464883.94	423217.5	575152.2
Q5HHB4	Coenzyme A disulfide reductase	cdr	438	100670.73	2771.949	340824.78
Q5HHB9	Signal peptidase IB	spsB	191	105210.586	285326.22	270565.28
Q5HHC7	NAD-specific glutamate dehydrogenase	gluD	414	2771.949	204866.89	58135.57
A0A068A2L4	Mannitol-specific phosphotransferase enzyme IIA component	fruA_2	652	208564.8	125965.82	191208.75
A0A6B1RN75	Mannitol-specific phosphotransferase enzyme IIA component	GAY51_04280	656	208564.8	125965.82	191208.75
A0A068DV34	Putative iron(III) dicitrate transporter binding lipoprotein	yfiY	330	518814.62	346472.38	483617.5
A0A090LVU9	Signal recognition particle protein	ffh	455	303719.3	291351.16	2771.949
A0A090LZA4	3-deoxy-7-phosphoheptulonate synthase	aroX	363	92307.99	61572.87	50784.348
A0A0A0Q4N9	Serine protease HtrA-like	htrA1	424	523959.22	401179.06	458556
A0A0D11BJ1	Cell envelope transcriptional attenuator	lytR_1	315	321451.25	232881.61	536717.06
A0A0D1JNG6	Pyruvate oxidase	G6Y24_16510	579	2771.949	2771.949	96720.58
A0A0D1JPY7	Zinc metalloproteinase	CV021_08570	226	209402.67	2771.949	2771.949
A0A0D3Q462	Uncharacterized protein	DD547_00265	507	86349.734	2771.949	2771.949
A0A0D3Q4X2	Lipoprotein	metQ_2	280	241252.5	143504.95	427874.3
A0A0D3Q5Z8	Dipeptide-binding protein DppE	oppA	551	190709.72	71165.17	209561.8
A0A0D3Q8S4	Nicotinate phosphoribosyltransferase	pncB	489	88657.75	71338.63	210002.84
A0A0D6H3T6	Molybdate ABC transporter substrate-binding protein	modA	260	741773.94	915160.5	383365.75
A0A0D6HCB3	Aminotransferase class I/II-fold pyridoxal phosphate-dependent enzyme	aspC	428	97059.24	130729.336	271773.5
A0A0D6HDR3	Lipoprotein	DD547_01873	208	21898.463	2771.949	2771.949
A0A0D6HJM8	2-oxoisovalerate dehydrogenase subunit alpha	bfmBAA	330	31791.543	72003.68	2771.949
A0A0E0VLB8	NDxxF motif lipoprotein	ST398NM01_0455	208	1304282.8	1019715.75	301193.34
A0A0E0VLC3	YhcL	ST398NM01_0461	465	2771.949	2771.949	190076.48

Protein.ID	Protein name	Gene	Protein.	MV /OXA1	MV /OXA2	MV /OXA3
			Length			
A0A0E0VLN4	ATP-dependent zinc metalloprotease FtsH	ftsH	697	2771.949	53961.98	2771.949
A0A0E0VM22	Manganese-binding protein	ST398NM01_0710	309	576781.75	785012.56	680332.1
A0A0E0VMW9	DM13 domain-containing protein	ST398NM01_0785	146	2429077.5	2776897.5	3136602
A0A0E0VN19	Protein translocase subunit SecA	secA	843	211941.03	2771.949	2771.949
A6QF62	Protein translocase subunit SecA 1	secA1	843	211941.03	2771.949	2771.949
A0A0E0VN26	Lipoprotein	ST398NM01_1089	213	107091.63	462876.53	132450.61
A0A0E0VNS5	DoxX family protein	ST398NM01_0784	160	2771.949	116244.24	259800.17
A0A0E0VPM3	DUF4064 domain-containing protein	ST398NM01_1100	339	2771.949	128748.08	201159.61
A0A0E0VPM7	YajC	ST398NM01_1701	86	556981.6	606894.06	721413.4
A0A0E0VPP5	Ribosome biogenesis GTPase A	ST398NM01_1243	294	2771.949	349062.44	261803.9
A0A0E0VQH8	Lipoprotein	ST398NM01_1596	193	420976.6	834026.56	2771.949
A0A0E0VQI1	ABC transporter ATP-binding protein	ST398NM01_2052	290	189076.58	2771.949	2771.949
A0A0E0VR20	Putative membrane associated protein	ST398NM01_1975	91	2771.949	34182.3	2771.949
A0A0E0VR70	Transaldolase	ST398NM01_1832	237	2771.949	2771.949	50585.95
A0A0E0VRB3	Cell shape-determining protein MreC	ST398NM01_1712	283	2771.949	54315.87	80447.555
A0A0E0VRC5	Ferrichrome-binding protein	ST398NM01_2335	302	1047788.7	1109945	1229818.9
A0A0E0VTS7	Uncharacterized protein	ST398NM01_2485	225	308097.4	393748.97	272784.53
A0A0E1VGQ1	PTS system, N-acetylglucosamine-specific IIBC component	nagE	488	606184.4	2771.949	2771.949
A0A0E1VJF0	Periplasmic binding protein	HMPREF0776_1746	342	268452.3	184200.12	180072.72
A0A0E1X971	Periplasmic binding protein	HMPREF0769_11558	342	268452.3	184200.12	180072.72
A0A0E1VLR9	Periplasmic binding protein	HMPREF0776_1614	283	284267.4	210973.97	228153.73
A0A0E1VN00	ABC transporter, substrate-binding protein, family 3	HMPREF0776_0469	259	844037.8	1298445.5	724163.8
A0A0E1VN46	ABC transporter, ATP-binding protein	HMPREF0776_0467	243	2771.949	68001.25	72500.31
A0A0E1VNB4	5'-nucleotidase, lipoprotein e(P4) family	HMPREF0776_1051	296	2771.949	73098.19	2771.949
A0A0E1VNE9	Periplasmic binding protein	HMPREF0776_0229	327	401927	361151.56	500169.5
A0A0E1VT13	Lipoprotein	HMPREF0776_1830	273	445384.4	1020230.25	2771.949
A0A0E1VY92	Alpha/beta hydrolase	HMPREF0776_0241	294	37843.03	2771.949	232333.89
A0A0E1X9N9	DUF5067 domain-containing protein	HMPREF0769_11505	242	115986.414	30709.531	85911.44
A0A0E1XH07	Pyruvate carboxylase	pyc	1150	941845.9	398039	889696.1
A0A0H2WWQ9	Penicillin-binding protein 2	pbp2	727	414705.22	641399.9	292178.06
A0A0U1MHA7	Oligopeptide ABC transporter (ATP-binding protein)	oppF	313	561704.94	317412.75	2771.949
A0A380E2T5	Formate--tetrahydrofolate ligase	fts	581	311925.28	106445.69	332243.88
A0A6A9GV45	Alanine--tRNA ligase	alaS	876	237967.81	2771.949	303169.28
A0A850FQI7	Tandem-type lipoprotein Lp110	lp110	260	2771.949	187852.3	96470.7
Q2G026	Protein translocase subunit SecG	SAOUHSC_00801	91	224183.23	2771.949	2771.949
A0A0D6HG31	Calcium-binding protein	yokF	204	21169.787	2771.949	2771.949
A0A0D1HE14	Exported protein	A6762_09810	57	185535.33	2771.949	198583.8
A0A0H3JPX7	DUF3173 domain-containing protein	SAV0393	66	2771.949	2771.949	20613.973
A0A0E0VUE2	Choline transport protein	ST398NM01_2662	540	2771.949	34474.195	2771.949

Note: A true detection was indicated by a value greater than 2771.949 (base line), and a protein is shown with at least one detection in three biological repeats.

Table S3. Strains and plasmids used in this study.

Strains/plasmids	Description	Reference
Strains		
E. coli		
DH5 α	Laboratory strain	Tiagen
S. aureus		
Newman	A highly virulent strain	[2]
USA300	A high-virulent and multidrug-resistant strain	[3]
N315	USA100	[4]
RN4220	Restriction deficient cloning host	[4]
RN4220 Δ <i>sarA</i>	deletion of <i>sarA</i> in RN4220	This study
RN4220 Δ <i>sle1</i>	deletion of <i>sle1</i> in RN4220	This study
RN4220 Δ <i>sarA</i> /pLI- <i>sarA</i>	<i>sarA</i> overexpressed with pLI50 in RN4220 Δ <i>sarA</i>	This study
RN4220-pXR- <i>sle1</i>	<i>sle1</i> overexpressed with pXR in RN4220	This study
RN4220-pOS1- <i>sle1</i>	<i>sle1</i> reporter strain with pOS1	This study
plasmids		
pBT2	Shuttle vector, temperature sensitive	[2]
pBT2- Δ <i>sarA</i>	pBT2 recombinant plasmid for mutation of the <i>sarA</i> gene	[1]
pBT2- Δ <i>sle1</i>	pBT2 recombinant plasmid for mutation of the <i>sle1</i> gene	This study
pLI50	Shuttle overexpressed vector	[1]
pLI- <i>sarA</i>	pLI50 recombinant plasmid for overexpression of the <i>sarA</i> gene	[1]
pXR	Xylose inducible expression vector, fused with pLI50, <i>xyIR</i> and <i>xyLAB</i>	[1]
pXR- <i>sle1</i>	pXR with <i>sle1</i> fused to a 3 \times FLAG tag	This study
pOS1	Reporter vector, lacZ activity	[4]
pOS1- <i>sle1</i>	pBT2 recombinant plasmid for mutation of the <i>sle1</i> gene	This study

Table S4. Primers used in this study.

Primers	Sequence (5'-3')	Notes
<i>sarA</i> -out-L	TAGCAATGACATCCAGAAAAACT	Selecting the <i>sarA</i>
<i>sarA</i> -out-R	TAGTTTATTGAGGGAGGTGTAC	deletion
<i>sle1</i> -up-L	CACTCATCGCAGTGCAGCGGGTATAAGAAGGCACATAAAG	PCR for homologous
<i>sle1</i> -up-R	ATATTTATATACGTAAGACTTTTAAAAATCCTCCTCTTGCT	upstream of <i>sle1</i>
<i>sle1</i> -down-L	AGCAAGAGGAGGATTTTAAAAAGTCTTACGTATATAAATAT	PCR for homologous
<i>sle1</i> -down-R	CCGGGTACCGAGCTCGAATTAGCGTATATCCGTAATTTCA	downstream of <i>sle1</i>
<i>sle1</i> -in-L	AGGTGGCGGATCATACTACACAGTA	
<i>sle1</i> -in-R	TGACAATGAGTCACCTGCTGTACT	Selecting the <i>sle1</i>
<i>sle1</i> -out-L	GATGTGGAGATTAAGCACTTICCG	deletion
<i>sle1</i> -out-R	AAACTTTGACAGCAGGCGCAATC	
<i>sle1</i> -qpcr-L	GAATGCTAATAACTGGGATA	RT-qPCR
<i>sle1</i> -qpcr-R	GTTTGAGCGATAGAACCT	
<i>sarA</i> -qpcr-L	ACAGTTCTTTCATCATGCTCATTACG	RT-qPCR
<i>sarA</i> -qpcr-R	CGCTGTATTGACATACATCAGCG	
<i>atl</i> -qpcr-L	ACGCCTACACCAACACCT	RT-qPCR
<i>atl</i> -qpcr-R	TTCCACCTAAACTTGCTTCT	
<i>gyrB</i> -qpcr-L	GGTGGCGACTTTGATCTAGC	Internal reference gene,
<i>gyrB</i> -qpcr-R	TTATACAACGGTGGCTGTGC	RT-qPCR
<i>walR</i> -qpcr-L	ATGTGTACTGTGCATACGATGGTAATG	RT-qPCR
<i>walR</i> -qpcr-R	TGAATCTTTAGCAGTAAGCATTATAATTGG	
<i>graR</i> -qpcr-L	AATGTTGCTGGTATTGAAGATTTCC	RT-qPCR
<i>graR</i> -qpcr-R	AGATGATAAAAATAATATTGGTACGTTGG	
<i>sle1</i> -P-L	TAAAGACGATCCGGGATTATATTGCGTCCTACAAGC	PCR for <i>sle1</i> gene
<i>sle1</i> -P-R	CGTTGTAACGACAATTACTTTTTTTTGCACCTT	promoter region
<i>sle1</i> -EMSA-L	CATCAACCTATTATACATAATAAAATCGTATAATGATGTAGTATTCATAAATTCGGA	EMSA for <i>sle1</i> , comprising
<i>sle1</i> -EMSA-R	TCCGAATTTATGAATACTACATCATTATACGATTTTATTATGTATAATAGGTTGATG	the AT-rich fragment
<i>sle1</i> -M-L	CATCAACCTATTATACATAGGGGGGGCGTATAATGATGTAGTATTCGGGGGGCGGA	Negative control for EMSA,
<i>sle1</i> -M-R	TCCGCCCCCGAATACTACATCATTATACGCCCCCTATGTATAATAGGTTGATG	carried with the GC-rich fragment
<i>sle1</i> -flag-L	TGTAGGAGGAATATTATGCAAAAAAAGTAATTGCAGC	PCR for <i>sle1</i> gene to
<i>sle1</i> -flag-R	CATGGTCTTTGTAGTCGTGAATATATCTATAATTATTTACTTGGTAAG	recombine with pXR vector

References

1. Shang W, Rao Y, Zheng Y, Yang Y, Hu Q, Hu Z, Yuan J, Peng H, Xiong K, Tan L, Li S, Zhu J, Li M, Hu X, Mao X, Rao X. β -Lactam Antibiotics Enhance the Pathogenicity of Methicillin-Resistant *Staphylococcus aureus* via SarA-Controlled Lipoprotein-Like Cluster Expression. *mBio*. 2019 Jun 11;10(3) pii: mBio.00880-19.
2. Chen J, Lv Y, Shang W, Yang Y, Wang Y, Hu Z, Huang X, Zhang R, Yuan J, Huang J, Rao X. Loaded delta-hemolysin shapes the properties of *Staphylococcus aureus* membrane vesicles. *Front Microbiol*. 2023;14:1254367.
3. Li M, Wang Y, Liu H, Huang X, Peng H, Yang Y, Hu Z, Dou J, Xiao C, Chen J, Shang W, Rao X. *Staphylococcus Aureus* Membrane Vesicles Kill Tumor Cells Through a Caspase-1-Dependent Pyroptosis Pathway. *Int J Nanomedicine*. 2024;19:4007-4019.
4. Peng H, Hu Q, Shang W, Yuan J, Zhang X, Liu H, Zheng Y, Hu Z, Yang Y, Tan L, Li S, Hu X, Li M, Rao X. 2017. WalK(S221P), a naturally occurring mutation, confers vancomycin resistance in VISA strain XN108. *J Antimicrob Chemother* 72:1006-13.