Supporting Information

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SI Text

Neutrophil Lysis Assay. Human polymorphonuclear leukocytes (or neutrophils) were isolated from heparinized venous blood of healthy individuals with a standard method (1). All work was performed in accordance with a protocol approved by the Institutional Review Board for Human Subjects, National Institute of Allergy and Infectious Diseases. Lysis of neutrophils was determined using a lactate dehydrogenase Cytotoxicity Detection Kit (Roche) according to the manufacturer's protocol and as described previously (2). Culture filtrates were diluted 1:50 for use in the assays.

PSM Measurement by RP-HPLC/Electrospray Ionization-MS. Determination of PSM production in *S. aureus* culture filtrates was performed using RP-HPLC/electrospray ionization-MS as described (3). Samples were taken from cultures inoculated from precultures and grown for 8 h (stationary growth phase).

Protease Assay. Protease activity was determined by an agar plate diffusion assay. The test agar contained 1.0% skim milk and 1.0% agar. Cultures were grown at 37 °C for 10 h, and 15 mL of culture filtrate was lyophilized. The lyophilysates were dissolved in 3 mL of 20 mmol/L Tris-HCl containing 1 mmol/L $CaCl_2$ (pH 7.8) and passed through 0.22-mm pore filters. Next, 50 μL was loaded into holes in the plates, which were incubated at 37 °C for 8 h.

Hemolysis Assay. *S. aureus* was grown to early stationary growth phase (4 h); equal amounts of cells were spotted on sheep blood agar plates and incubated at 37 °C for 24 h.

Western Blot Analysis. Culture filtrates from bacterial cultures grown to late exponential growth phase (4 h) were precipitated using 10% (vol/vol) trichloroacetic acid, separated on a 12% SDS/PAGE gel, blotted on Hybond-C-membranes (Amersham Biosciences), and incubated with α-toxin antibody (1:10,000) for 1 h. Immunoreactive protein was visualized using an ECL detection system (Amersham Biosciences).

Quantitative RT-PCR. RNAIII expression was detected by quantitative RT-PCR. For RNA isolation, overnight cultures were diluted 1:100 into 50 mL of tryptic soy broth and incubated at 37 °C with shaking at 180 rpm until grown to early stationary phase (4 h, at maximal expression of *agr*). cDNA was synthesized from total RNA using the SuperScript III first-strand synthesis system (Invitrogen) according to the manufacturer's instructions. Oligonucleotide primers and probes were designed using Primer Express (Applied Biosystems). The experiments were performed in triplicate, with *gyrB* RNA as a control. The primers and probes used were RNAIII-F 5′-TTCACTGTGTCGATAATCCA-3′, RNAIII-R 5′-GGAAGGAGTGATTTCAATGG-3′, and RNAIII-probe 5′-CAAGATATCATTTCAACAATCAGTGACTTAGT-3′ and GyrB-F 5′-CAAATGATCACAGCATTTGGTACAG-3′, GyrB-R 5′-CGGCATCAGTCATAATGACGAT-3′, and GyrB-probe 5′-AATCGGTGGCGACTTTGATCTAGCGAAAG-3′, respectively. The resulting cDNA and negative control samples were amplified with TaqMan Universal PCR Master Mix (Applied Biosystems). Reactions were performed in a MicroAmp Optical 96-well reaction plate using a 7700 Sequence Detector (Applied Biosystems). Standard curves were determined for each gene, using purified chromosomal template DNA at concentrations of 0.005–50 ng/mL. All RT-PCR experiments were performed in triplicate, with *gyrB* RNA used as a control.

Determination of Minimum Inhibitory Concentrations of Different Antimicrobial Peptides. *S. aureus* cells were grown to mid-log phase; cultures were harvested, washed twice with 10 mM sodium phosphate buffer (pH 6.5) with 100 mM NaCl, and resuspended in LB to a final concentration of 10^5/mL in each sample. The bacteria were exposed to a range of AMP concentrations and incubated at 37 °C with shaking at 180 rpm for at least 12 h, after which OD_{600} was measured. The minimum inhibitory concentration was defined as the concentration at which the OD_{600} was reduced by 50%.

Peptide Degradation Assay. *S. aureus* cells were grown to exponential growth phase (OD_{600}, 2.5–3.0); cultures were harvested, washed twice with 10 mM sodium phosphate buffer (pH 6.5) with 100 mM NaCl, and resuspended in the same buffer. The bacteria were exposed to 100 μg/mL dermcidin and 50 μg/mL melittin, respectively, and incubated at 37 °C. At different incubation time points, 1 mL of the culture was collected and supernatants were lyophilized. Degradation was analyzed on 15% Tricine SDS/PAGE gels.

Statistics and DNA Sequence Analysis. Statistical analysis was performed using GraphPad Prism version 4.0 using one-way ANOVA and Bonferroni posttests, unless noted otherwise. DNA sequences were compared using Clustal W software (www.ch.embnet.org/software/ClustalW.html).

1. Kobayashi SD, Voyich JM, Buhl CL, Stahl RM, DeLeo FR (2002) Global changes in gene expression by human polymorphonuclear leukocytes during receptor-mediated phagocytosis: Cell fate is regulated at the level of gene expression. *Proc Natl Acad Sci USA* 99:6901–6906.
2. Voyich JM, et al. (2006) Is Panton-Valentine leukocidin the major virulence determinant in community-associated methicillin-resistant *Staphylococcus aureus* disease? *J Infect Dis* 194:1761–1770.
3. Wang R, et al. (2007) Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. *Nat Med* 13:1510–1514.

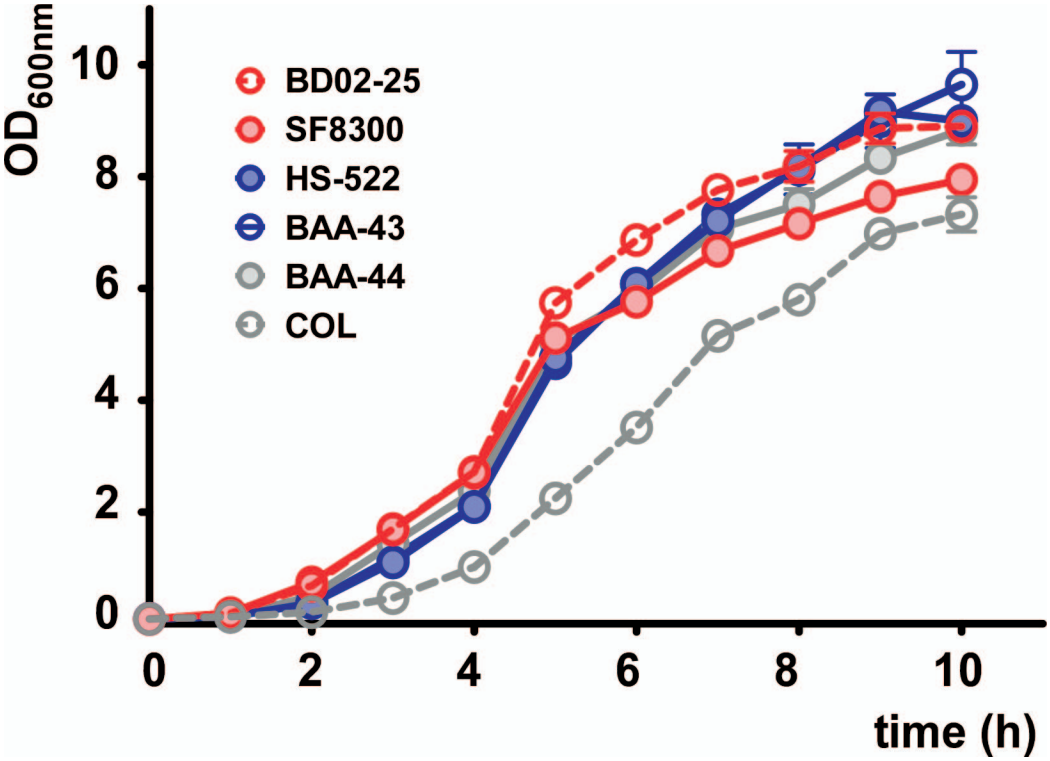

Fig. S1. In vitro growth of CC8 subclones. Strains were inoculated 1:100 from precultures grown overnight and grown with shaking in tryptic soy broth. OD_{600} was measured every hour. The analysis was performed in triplicate. Error bars depict SEM.

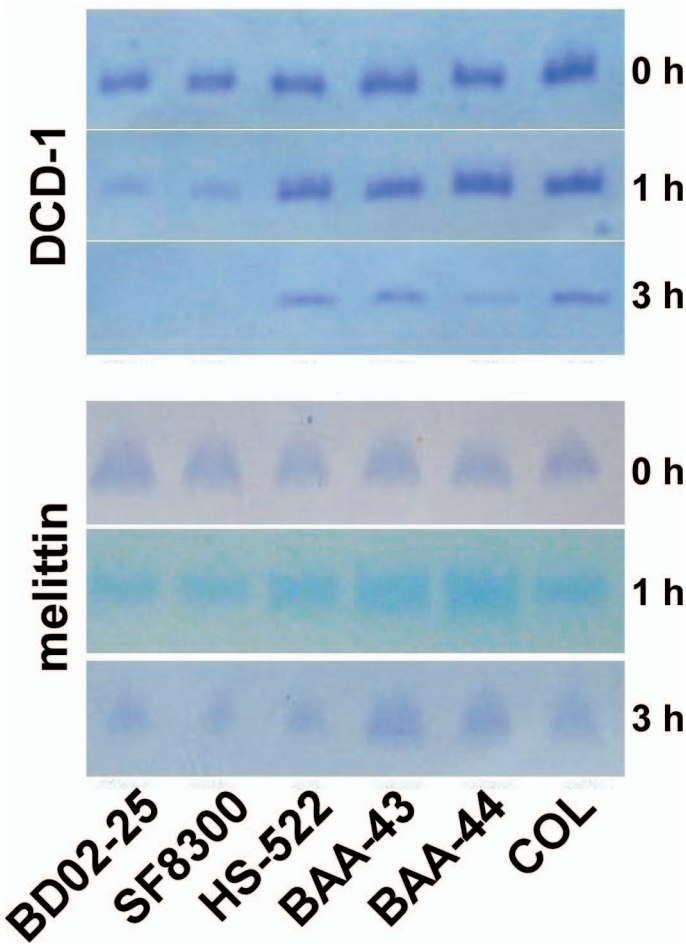

Fig. S2. AMP degradation assays. One AMP for which we found differential resistance between USA300, USA500, and the other CC8 strains [dermcidin (DCD-1)] and another for which we did not (melittin) were selected for AMP degradation assays. Equal amounts of peptides (100 μg/mL DCD-1, 50 μg/mL melittin) were incubated with culture filtrates of the strains, and aliquots were analyzed after 0, 1, and 3 h of incubation by SDS/PAGE.

Table S1. Profiles of strains used in this study

Strain ID	Clonal type	ST	Multilocus sequence typing allelic profile*	*spa* type	SCC*mec* type	*sasA*	*sasB*	*sasD*	*sasE*	*sasF*	*sasH*	*sasI*
BD02–25	USA500	8	3–3–1–1–4–4–3	YHGFMBQBLO	IV	2	2	3	1	7	8	3
BD02–26	USA500	8	3–3–1–1–4–4–3	YHGCMBQBLO	IV	2	2	3	1	7	8	3
BD02–27	USA500	8	3–3–1–1–4–4–3	YHGCMBQBLO	IV	2	2	3	1	7	8	3
FPR3757	USA300	8	3–3–1–1–4–4–3	YHGFMBQBLO	IVa	2	New	3	1	7	8	New
LAC	USA300	8	3–3–1–1–4–4–3	YHGFMBQBLO	IVa	2	New	3	1	7	8	New
SF8300†	USA300	8	3–3–1–1–4–4–3	YHGFMBQBLO	IVa	2	New	3	1	7	8	New
COL	Archaic	250	3–3–1–1–4–4–16	YHGFMBQBLO	I	2	2	3	1	7	8	3
BAA-38	Archaic	247	3–3–1–12–4–4–16	YHFGFMBQBLO	I	2	2	3	1	7	8	3
BK1953	Iberian	247	3–3–1–12–4–4–16	YHFGFMBQBLO	IA	2	2	3	1	7	8	3
BAA-44	Iberian	247	3–3–1–12–4–4–16	YHFGFMBQBLO	IA	2	2	3	1	7	8	3
BAA-40	Portuguese	239	2–3–1–1–4–4–3	WGKAOM	III var	5	2	4	1	3	6	3
BAA-43	Brazilian	239	2–3–1–1–4–4–3	WGKAOMQ	IIIA	5	2	4	1	3	6	3
HS-191	Chinese	239	2–3–1–1–4–4–3	WGKAOMQ	III.1	5	2	4	1	3	6	3
HS-340	Chinese	239	2–3–1–1–4–4–3	WGKAOMQ	III.1	5	2	4	1	3	6	3
HS-275	Chinese	239	2–3–1–1–4–4–3	WGKAOMQ	III.1	5	2	4	1	3	6	3
HS-522	Chinese	239	2–3–1–1–4–4–3	WGKAQQ	III.1	5	2	4	1	3	6	3
HS-362	Chinese	239	2–3–1–1–4–4–3	WGKMQ	III.1	5	2	4	1	3	6	3
HS-227	Chinese	239	2–3–1–1–4–4–3	WGKAOM	III.1	5	2	4	1	3	6	3
NCTC8325	lab strain	8	3–3–1–1–4–4–3	YHGGFMBQBLO	—	2	2	3	1	7	8	3
MRSA1633	USA1100	30	2–2–2–2–6–3–2	XKAKAOMQ	IV	5	5	4	25	2	6	8
MRSA1677	USA1100	30	2–2–2–2–6–3–2	XKAKAOMQ	IV	5	5	4	25	2	6	8
MRSA2865	USA1100	30	2–2–2–2–6–3–2	XKAKAOMQ	IV	5	5	4	25	2	6	8
MRSA2121	USA1100	30	2–2–2–2–6–3–2	XKAKAOMQ	IV	5	5	4	25	2	6	8
MRSA1234	USA1100	30	2–2–2–2–6–3–2	XKAKAOMQ	IV	5	5	4	25	2	6	8
MRSA1200	USA1100	30	2–2–2–2–6–3–2	XKAKAOMQ	IV	5	5	4	25	2	6	8
MRSA1162	USA1100	30	2–2–2–2–6–3–2	XKAKAOMQ	IV	5	5	4	25	2	6	8
Mu50	NY/Japanese	5	1–4–1–4–12–1–10	TJMBMDMGMK	II	2	3	2	1	6	7	5
N315	NY/Japanese	5	1–4–1–4–12–1–10	TJMBMDMGMK	II	2	3	2	1	6	7	5
MW2	USA400	1	1–1–1–1–1–1–1	UJJFKBFE	IVa	11	3	8	1	18	14	17

*Based on 7 housekeeping genes according to Enright et al. (1) (see Table S3).

†Strains used for virulence studies are underlined.

Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 38:1008–1015.

Table S2. Virulence gene presence in CC8 and control strains

	USA500	USA300	Archaic	Iberian	Portuguese-Brazilian	Chinese	ST30	NCTC 8325	Mu50	N315	MW2
lukS-PV (PVL)		+					+				+
arcA (ACME)		+									
icaA	+	+	+	+	+	+	+	+	+	+	+
psmα	+	+	+	+	+	+	+	+	+	+	+
clfB	+	+	+	+	+	+	+	+	+	+	+
clfA	+	+	+	+	+	+	+	+	+	+	+
fnbA	+	+	+	+	+	+	+	+	+	+	+
ssp	+	+	+	+	+	+	+	+	+	+	
lukE	+	+	+	+	+	+		+	+	+	+
lukM											+
hla	+	+	+	+	+	+	+	+	+	+	
hlg	+	+	+	+	+	+	+	+	+	+	+
hld	+	+	+	+	+	+	+	+			+
hlb	+	+	+	+	+	+		+			+
sbi	+	+	+	+	+	+		+	+	+	+
bsaA	+	+	+	+	+	+		+			
sdrC	+	+	+	+	+	+	+	+			+
sdrD	+	+	+	+	+*	+		+	+	+	+
sdrE	+	+	+		+	+			+	+	+
cap5	+	+	+	+				+	+	+	+
cap8					+	+	+				
can					+	+	+				+
tst									+	+	
sea			+*	+		+			+		
seb			+*								+
sec									+	+	
see											+
sed											
sej											
seg							+		+	+	
sei							+		+	+	
sem							+		+	+	
sen							+		+	+	
seo							+		+	+	
she											
sek		+	+*			+					+
seq		+	+*			+					+
sel									+	+	+
sep										+	+
splB	+	+	+	+	+	+		+	+	+	
bbp							+				+
EDIN											
ebpS							+		+	+	
efb	+	+	+	+	+	+		+	+	+	+

*Present in some analyzed strains.

Table S3. Oligonucleotides used in this study

Gene product (gene locus)	Forward primer (5′-3′)	Reverse primer (5′-3′)	PCR product length (bp)	Genome location	Reference
MLST housekeeping genes					
Carbamate kinase (*arcC*)	TTGATTCACCAGCGCGTATTGTC	AGGTATCTGCTTCAATCAGCG	570	348.2	(1)
Shikimate dehydrogenase (*aroE*)	ATCGGAAATCCTATTTCACATTC	GGTGTTGTATTAATAACGATATC	536	213.6	(1)
Glycerol kinase (*glpF*)	CTAGGAACTGCAATCTTAATCC	TGGTAAAATCGCATGTCCAATTC	576	164.5	(2)
Guanylate kinase (*gmk*)	ATCGTTTTATCGGGACCATC	TCATTAACTACAACGTAATCGTA	487	153.0	(1)
Phosphate acetyltransferase (*pta*)	GTTAAAATCGTATTACCTGAAGG	GACCCTTTTGTTGAAAAGCTTAA	575	80.7	(1)
Triosephosphate isomerase (*tpi*)	TCGTTCATTCTGAACGTCGTGAA	TTTGCACCTTCTAACAATTGTAC	475	106.0	(2)
Acetyle coenzyme A acetyltransferase (*yqi*)	CAGCATACAGGACACCTATTGGC	CGTTGAGGAATCGATACTGGAAC	598	50.9	(3)
Surface protein genes					
Staphylococcus aureus surface protein SasA (*sasA*)	TCAACATCCTCAAAGAATACTACA	ATGCGTTACTTAAGCCACCAATAC	571	353.0	(1)
Staphylococcus aureus surface protein SasB (*sasB*)	GTTGCAGCGCTTGTGACT	ATTTTTGAGATTTCTTCGTTTTTA	578	286.3	(1)
Staphylococcus aureus surface protein SasD (*sasD*)	GGCGGAGTAGTACCACAAGGAA	AATGCTAAGAATAACCCAGATACT	554	19.5	(1)
Staphylococcus aureus surface protein SasE (*sasE*)	TTACAATGCAAACAATCAAGA	GTTTAGGCGTTTCGTTATGTTTT	563	140.9	(1)
Staphylococcus aureus surface protein SasF (*sasF*)	GGATAGCAAAGACAATAAAAGTTC	TGATATGTGTAATGTTGCGTTGAG	522	350.8	(1)
Staphylococcus aureus surface protein SasH *(sasH)*	CGCACCAACTAACAAACCAACTAC	TACGCCAATAATTCCATAACGA	562	4.0	(1)
Staphylococcus aureus surface protein SasI *(sasI)*	ATACTATCACTTTTTCAGCATCAA	TCATTCGTTTTATCGTTAGTATTA	565	231.3	(4)
Virulence genes					
PVL S chain (*lukS-PV*)	ATCATTAGGTAAAATGTCTGGACATGATCCA	GCATCAACTGTATTGGATAGCAAAAGC	433	193.9	(5)
ACME (*arcA*)	GAGCCAGAAGTACGCGAG	CACGTAACTTGCTAGAACGAG	723	9.2	(1)
Polysaccharide intercellular adhesin protein A (*icaA*)	GATTATGTAATGTGCTTGGA	ACTACTGCTGCGTTAATAAT	770	354.3	(6)
PSMα (*psmα*)	ATGGGTATCATCGCTGGCATCATTAAAGTTA	TTTTGCGAAAATGTCGATAATTGCTTTGAT	406	59.7	
Fibrinogen-binding protein (*clfB*)	TGCAAGATCAAACTGTTCCT	TCGGTCTGTAAATAAAGGTA	596	347.9	(6)
Fibrinogen-binding protein (*clfA*)	GTAGGTACGTTAATCGGTT	CTCATCAGGTTGTTCAGG	1584	107.9	(6)
Fibrinogen-binding protein (*fnbA*)	CACAAACTGCACAACCAGC	GGATTTGATTCCTCAGAGGAC	1522	330.1	(6)
Serine protease (*ssp*)	GGAGGTTTTTAGATGAAAGG	CGCCATTGTCTGGATTATCAGG	988	130.3	(1)
Leukocidin E (*lukE)*	GCAACTTTGTCAGTAGGACTG	GTCTACTTCACTGACATAACTC	507	245.2	(7)
Leukocidin M (*lukM*)	TGGATGTTACCTATGCAACCTAC	GTTCGTTTCCATATAATGAATCACTAC	795	Prophage phiPV83	(8)
α -Hemolysin (*hla*)	CCAAGAATCTCTATCATATGGTC	GAAAGGTACCATTGCTGGTC	391	144.9	(8)
γ -Hemolysin (*hlg*)	ATGGATGTCACTCATGCC	GTATTTCCATTAAGTCCACCAG	642	318.9	(8)
δ -Hemolysin (*hld*)	TGTTCACTGTGTCGATAATCC	CTCTCCTYACTGTYATTATACG	342	269.0	(8)
β -Hemolysin (*hlb*)	AGTTGCAACACTTGCATTAGC	CTTCAGATTGTGTATGTGTACC	202	266.7	(8)
Immunoglobulin G-binding protein (*sbi)*	CACAGAGGAACAACGTAACC	GATTTAGCTAAGTAGCCG	957	318.4	(1)
Bacteriocin (*bsaA*)	ACAGAAGCTGTTAAAACTACCC	GATTAATATGACAATTGAAGTGGGTC	197	245.0	(7)
Ser-Asp rich fibrinogen-binding protein (*sdrC*)	ACGACTATTAAACCAAGAAC	GTACTTGAAATAAGCGGTTG	560	76.6	(1)
Ser-Asp rich fibrinogen-binding protein (*sdrD*)	GGAAATAAAGTTGAAGTTTC	ACTTTGTCATCAACTGTAAT	500	77.0	(1)
Ser-Asp rich fibrinogen-binding protein (*sdrE*)	CAGTAAATGTGTCAAAAGA	TTGACTACCAGCTATATC	767	77.6	(1)
Capsular polysaccharide type 5 (*cap5*)	ATGACGATGAGGATAGCG	CTCGGATAACACCTGTTGC	881	22.7	(9)
Capsular polysaccharide type 8 (*cap8*)	ATGACGATGAGGATAGCG	CACCTAACATAAGGCAAG	1148	19.4	(9)
Collagen-binding protein (*cna*)	AAGCATTTGCAGCACGAG	ATATGACCCATAGCCTTGTGG	740	357.1	(6)
Toxic shock syndrome toxin 1 (*tst*)	ATCGTAAGCCCTTTGTTG	GTGGATCCGTCATTCATTG	578	263.6	(8)
Staphylococcal enterotoxin A (*sea*)	CATTGCCCTAACGTTGAC	CGAAGGTTCTGTAGAAGTATGG	619	216.9	(8)
Staphylococcal enterotoxin B (*seb*)	CTAAACCAGATGAGTTGCAC	CCAAATAGTGACGAGTTAGG	489	117.5	(8)
Staphylococcal enterotoxin C (*sec*)	GTAAAGTTACAGGTGGCAAAACTTG	CATATCATACCAAAAAGTATTGCCGT	296	108.8	(8)
Staphylococcal enterotoxin E (*see*)	GCTTTAAGCAATCTTAGGC	CTATCCACAAGTTAATTGGTAC	330	Unknown	(8)
Staphylococcal enterotoxin D (*sed*)	GAATTAAGTAGTACCGCGCTAAATAATA	GCTGTATTTTTCCTCCGAGAGT	491	pIB485 plasmid	(8)
Staphylococcal enterotoxin J (*sej*)	TAACCTCAGACATATATACTTCTTTAAC	AGTATCATAAAGTTGATTGTTTTCATGC	294	pIB485 plasmid	(8)
Staphylococcal enterotoxin G (*seg*)	TCTTTATACGTCTCCACC	GTCTATTGTCGATTGTTACC	326	240.0	(4)
Staphylococcal enterotoxin I (*sei*)	CAACTCGAATTTTCAACAGGTAC	CAGGCAGTCCATCTCCTG	466	240.3	(8)
Staphylococcal enterotoxin M (*sem*)	CTATTAATCTTTGGGTTAATGGAGAAC	TTCAGTTTCGACAGTTTTGTTGTCAT	326	240.4	(8)
Staphylococcal enterotoxin N (*sen*)	ATGAGATTGTTCTACATAGCTGCAAT	AACTCTGCTCCCACTGAAC	680	240.1	(8)
Staphylococcal enterotoxin O (*seo*)	AGTTTGTGTAAGAAGTCAAGTGTAGA	ATCTTTAAATTCAGCAGATATTCCATCT	180	240.6	(8)
Staphylococcal enterotoxin H (*seh*)	CGAAAGCAGAAGATTTACACG	ACCAATCACCCTTTCCTGTG	341	7.9	(8)
Staphylococcal enterotoxin K (*sek*)	GGTGTCTCTAATAGTGCCAG	TCGTTAGTAGCTGTGACTCC	284	110.7	(7)
Staphylococcal enterotoxin Q (*seq*)	TCTAGCATATGCTGATGTAGG	CAATCTCTTGAGCAGTTACYTC	383	110.8	(7)
Staphylococcal enterotoxin L (*sel)*	ATCAATGGCAAGCATCAAACAG	TGGAAGACCGTATCCTGTG	264	108.9	(7)
Staphylococcal enterotoxin P (*sep*)	GACCTTGGTTCAAAAGACACC	TGTCTTGACTGAAGGTCTAGC	275	257.3	(7)
Serine protease-like B (*splB*)	CCATATACTGGTGTAGTTG	GGGTGTGGATAACCAATC	332	243.4	(1)
Bone sialoprotein-binding protein (*bbp*)	CAGTAAATGTGTCAAAAGA	TACACCCTGTTGAACTG	1055	76.9	(1)
Epidermal cell differentiation inhibitor (*edin*)	GAAGTATCTAATACTTCTTTAGCAGC	TCATTTGACAATTCTACACTTCCAAC	619	EDINA plasmid	
Elastin-binding protein (*ebpS*)	CAATCGATAGACACAAATTC	CAGTTACATCATCATGTTTA	526	194.2	(2)
Fibrinogen-binding protein (*efb*)	AACATTAGCGGCAATAGG	ATTCGCTCTTGTAAGACC	432	144.5	(1)
Immunoglobulin G-binding protein A (*spa*)	GACGATCCTTCGGTGAGC	CAGCAGTAGTGCCGTTTGC	342	16.1	(2)

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