

Supplementary Materials: Clinical *S. aureus* Isolates Vary in Their Virulence to Promote Adaptation to the Host

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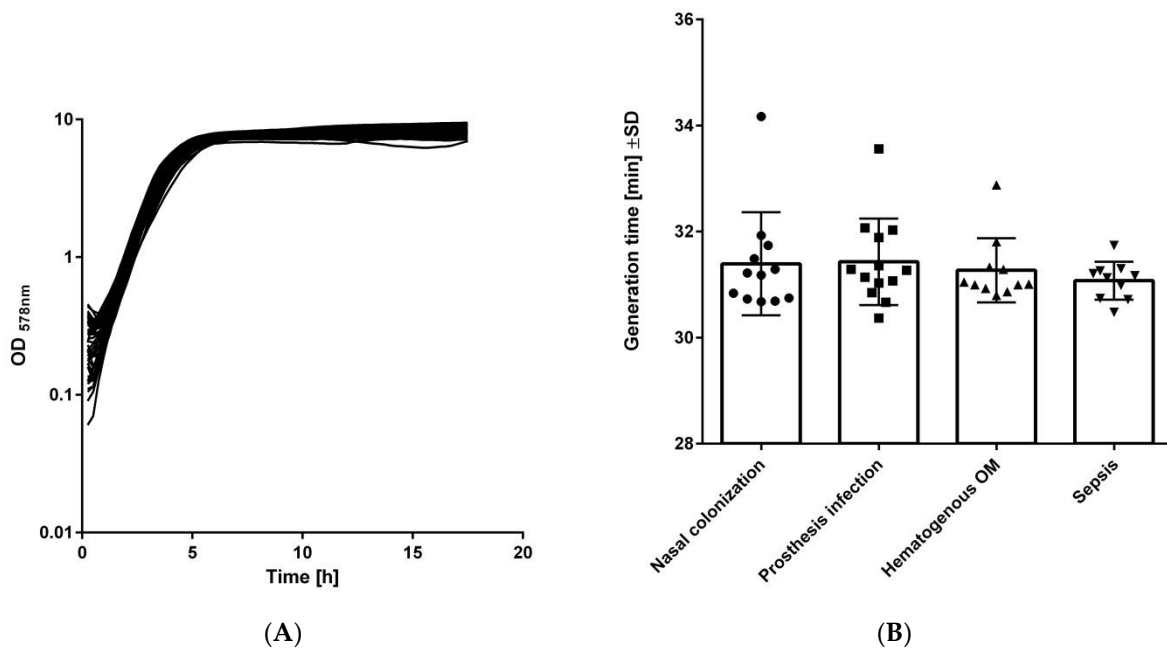


Figure S1. Growth dynamics of *S. aureus* isolates.

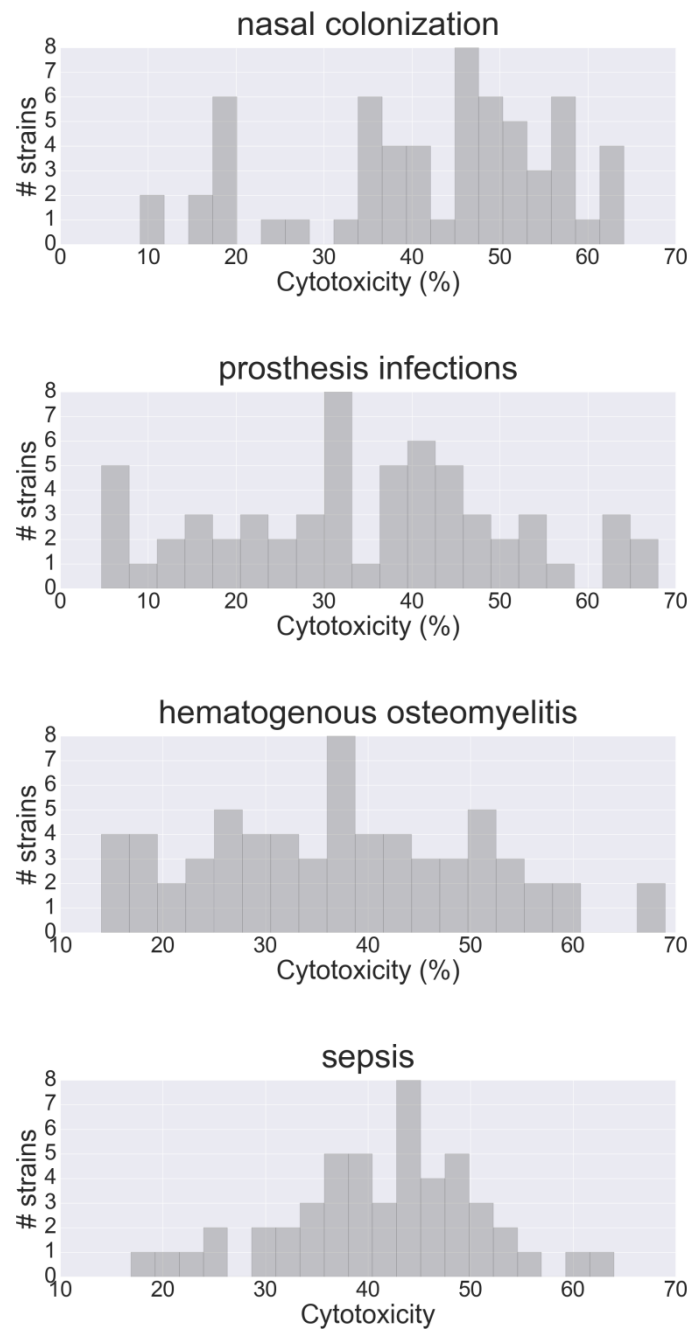


Figure S2. Cytotoxicity of *S. aureus* isolates.

Table S1. Clinical characteristics of all patients.

Characteristics	Nasal Colonization	Prosthesis Infection	Hematogenous Osteomyelitis	Sepsis
Total Number	12	12	13	10
Median Age [years]	49.08 (range 40–64)	66 (range 33–85)	75.23 (range 47–96)	62.7 (range 53–78)
Male	6 (50,0%) ^a	9 (75.0%)	8 (61.5%)	5 (50,0%)
Case Fatality	-	0	5 (38.5%)	2 (20.0%)
Underlying Condition				
Immunosuppression	-	2 (16.7%)	3 (23.1%)	3 (30.0%)
Diabetes Mellitus	-	2 (16.7%)	5 (38.5%)	7 (70.0%)
Malignant Disease	-	0	2 (15.4%)	2 (20.0%)
Indwelling Devices	-	7 (58.3%)	2 (15.4%)	1 (10.0%)
Renal Disease	-	5 (41.7%)	12 (92.3%)	7 (70.0%)
Cardiovascular Disease	-	9 (75.0%)	8 (61.5%)	9 (90.0%)
Liver Disease	-	3 (25.0%)	1 (7.7%)	4 (40.0%)
Origin of Infection/Primary Focus				
Unknown	-	0	3 (23.0%)	0
Bone	-	12 (100%)	7 (53.8%)	0
Wound/Skin	-	0	1 (7.7%)	2 (20.0%)
Intravenous Catheter	-	0	2 (15.4%)	0
Lung	-	0	0	6 (60.0%)
Gastrointestinal Tract	-	0	0	2 (20.0%)
Hospital Stays During Last 3 years (in Relation to Infection)				
None	-	3 (25%)	10 (76.9%)	9 (90.0%)
1	-	0	2 (15.4%)	0
≥ 2	-	9 (75%)	1 (7.7%)	1 (10.0%)
Median Length of Hospital Stay [days]	-	60 (range 14–1080) ^b	30 (range 9–180)	17,5 (range 2–360)

a: one sample couldn't be assigned to sex; b: no data available for one isolate from the group of prosthesis infection.

Table S2. Distribution of clonal complexes amongst staphylococcal isolates from nasal colonization, prosthesis infection, hematogenous osteomyelitis and sepsis. The number of isolates are indicated between parentheses.

Clonal Complex (CC)	Nasal Colonization	Prosthesis Infection	Hematogenous Osteomyelitis	Sepsis	<i>p</i> (Fisher exact test)
	(n = 12)	(n = 12)	(n = 13)	(n = 10)	
CC1	0	0	7.7% (1)	10% (1)	0.58
CC5	16.6% (2)	25% (3)	0	10% (1)	0.27
CC6	8.3% (1)	8.3% (1)	7.7% (1)	0	1
CC7	0	33.3% (4)	0	0	0.007
CC8	0	16.6% (2)	15.4% (2)	10% (1)	0.62
CC15	8.3% (1)	0	15.4% (2)	10% (1)	0.72
CC22	0	0	30.8% (4)	30% (3)	0.02
CC25	8.3% (1)	0	7.7% (1)	0	1
CC30	16.6% (2)	8.3% (1)	0	0	0.38
CC45	25% (3)	0	7.7% (1)	30% (3)	0.12
CC101	0	0	7.7% (1)	0	1
CC121	0	8.3% (1)	0	0	0.72
CC398	16.6% (2)	0	0	0	0.16

Table S3. Frequencies of selected genes in the defined isolate groups.

Gene	Product	Nasal colonization	Prosthesis infection	Hematogenous osteomyelitis	Sepsis	<i>P</i> (Fisher test)
Regulation						
<i>agrI</i>	Accessory gene regulator allele I	58.3% (7)	66.7% (8)	69.2% (9)	70.0% (7)	0.95
<i>agrII</i>	Accessory gene regulator allele II	25.0% (3)	25.0% (3)	15.4% (2)	20.0% (2)	0.93
<i>agrIII</i>	Accessory gene regulator allele III	16.7% (2)	8.3% (1)	7.7% (1)	10.0% (1)	0.93
<i>agrIV</i>	Accessory gene regulator allele IV	0	0	7.7% (1)	0	1
Resistance						
<i>bla</i>	β -lactamase	58.3% (7)	58.3% (7)	46.2% (6)	90.0% (9)	0.18
<i>ermA</i>	Erythromycin / clindamycin resistance gene A	0	16.7% (2)	0	20.0% (2)	0.17
<i>ermB</i>	Erythromycin / clindamycin resistance gene B	0	0	0	0	-
<i>ermC</i>	Erythromycin / clindamycin resistance gene C	0	8.3% (1)	0	0	0.72
<i>fosB</i>	Metallothiol transferase	58.3% (7)	66.7% (8)	53.8% (7)	30.0% (3)	0.42
<i>mecA</i>	Alternate penicillin binding protein 2, defining MRSA	0	16.7% (2)	7.7% (1)	10.0% (1)	0.61
<i>tetEfflux</i>	Transport-/ efflux protein	100% (12)	100% (12)	69.2% (9)	70.0% (7)	0.03
<i>tetK</i>	Tetracycline resistance gene K	0	0	7.7% (1)	10.0% (1)	0.58
<i>tetM</i>	Tetracycline resistance gene M	0	8.3% (1)	0	0	0.73
<i>vanA</i>	Vancomycin resistance gene	0	0	0	0	-
Enterotoxins						
<i>sea</i>	Staphylococcal enterotoxin A	16.7% (2)	16.7% (2)	15.4% (2)	0	0.6
<i>seb</i>	Staphylococcal enterotoxin B	8.3% (1)	0	15.4% (2)	0	0.6
<i>sec, sel</i>	Staphylococcal enterotoxin C+L	25.0% (3)	0	15.4% (2)	10.0% (1)	0.33
<i>sed, sej, ser</i>	Staphylococcal enterotoxin D+J+R	0	25.0% (3)	7.7% (1)	20.0% (2)	0.24
<i>see</i>	Staphylococcal enterotoxin E	0	0	0	0	-
<i>egc-cluster</i>	Staphylococcal enterotoxin G+I+M+N+O+U	66.7% (8)	33.3% (4)	53.8% (7)	70.0% (7)	0.29
<i>seh</i>	Staphylococcal enterotoxin H	8.3% (1)	0	7.7% (1)	10.0% (1)	0.89
<i>sek, seq</i>	Staphylococcal enterotoxin K+Q	0	8.3% (1)	7.7% (1)	0	1
<i>tst1</i>	Toxic shock syndrome toxin (TSST)-1	16.7% (2)	0	0	0	0.16
<i>entP</i>	Enterotoxin P	0 ^b	41.7% (5) ^b	0	10.0% (1)	0.003
Hemolysins						
<i>hla</i>	α -toxin	100% (12)	100% (12)	100% (13)	100% (10)	-
<i>hlb</i>	β -toxin	75.0% (9)	100% (12)	11	80.0% (8)	0.38
<i>hld</i>	δ -toxin	100% (12)	100% (12)	100% (13)	100% (10)	-
Leukocidins						
<i>lukF, hlgA</i>	γ -toxin	100% (12)	100% (12)	100% (13)	100% (10)	-
<i>lukF-PV</i>	Panton-Valentin-leukotoxin	8.3% (1)	0	0	0	0.72
<i>lukD, lukE</i>	Leukocidin D, E component	41.7% (5) ^c	91.7% (11) ^c	61.5% (8)	40.0% (4)	0.034
Exfoliative toxins						
<i>etA</i>	Exfoliative toxin A	8.3% (1)	0	0	0	0.72
<i>etB</i>	Exfoliative toxin B	0	0	0	0	-
<i>etD</i>	Exfoliative toxin D	8.3% (1)	0	7.7% (1)	0	1
Enzymes						
<i>aur</i>	Aureolysin	100% (12)	100% (12)	100% (13)	100% (10)	-
<i>chp</i>	Chemotaxis inhibitory protein (CHIP)	66.7% (8)	25.0% (3)	61.5% (8)	80.0% (8)	0.06
<i>splA, splB</i>	Serine protease A, B	41.7% (5) ^d	91.7% (11) ^d	61.5% (8)	40.0% (4) ^d	0.034
<i>splE</i>	Serine protease E	41.7% (5)	66.7% (8) ^e	46.2% (6)	10% (1) ^e	0.07
<i>sspA, sspB</i>	Glutamyl endopeptidase	100% (12)	100% (12)	100% (13)	100% (10)	-
<i>sak</i>	Staphylokinase	66.7% (8) ^f	100% (12) ^f	84.6% (11)	80.0% (8)	0.17
<i>scn</i>	Staphylococcal complement inhibitor	91.7% (11)	100% (12)	100% (13)	100% (10)	0.72

Exopolysaccharides						
<i>cap5</i>	Capsular polysaccharide 5	41.7% (5)	41.7% (5)	46.2% (6)	50.0% (5)	1
<i>cap8</i>	Capsular polysaccharide 8	58.3% (7)	58.3% (7)	53.8% (7)	50.0% (5)	1
<i>icaA, D, C</i>	Polysaccharide intracellular adhesin	100% (12)	100% (12)	100% (13)	100% (10)	-
Adhesins						
<i>bbp</i>	Bone sialoprotein-binding protein	91.7% (11)	91.7% (11)	100% (13)	100% (10)	0.71
<i>clfA, clfB</i>	Clumping factors A and B	100% (12)	100% (12)	100% (13)	100% (10)	-
<i>cna</i>	Collagen binding adhesin	58.3% (7)	25.0% (3)	61.5% (8)	70.0% (7)	0.15
<i>ebh</i>	Cell wall associated fibronectin-binding protein	100% (12)	100% (12)	69.2% (9)	70.0% (7)	0.02
<i>ebps</i>	Cell surface elastin-binding protein	100% (12)	100% (12)	100% (13)	100% (10)	-
<i>eno</i>	Enolase	100% (12)	100% (12)	100% (13)	100% (10)	-
<i>fib</i>	Fibrinogen-binding protein	100% (12)	100% (12)	100% (13)	100% (10)	-
<i>fnbA</i>	Fibronectin-binding protein A	100% (12)	100% (12)	100% (13)	100% (10)	-
<i>fnbB</i>	Fibronectin-binding protein B	83.3% (10)	83.3% (10)	69.2% (9)	100% (10)	0.32
<i>map</i>	Major histocompatibility complex class II analog protein	100% (12)	91.7% (11)	100% (13)	100% (10)	0.72
<i>sasG</i>	<i>S. aureus</i> surface protein G	33.3% (4)	58.3% (7)	69.2% (9)	70.0% (7)	0.26
<i>sdrC</i>	Serine-aspartate repeat protein C	100% (12)	100% (12)	100% (13)	100% (10)	-
<i>sdrD</i>	Serine-aspartate repeat protein D	91.7% (11)	91.7% (11)	92.3% (12)	100% (10)	1
<i>vwb</i>	Van Willebrand factor binding protein	100% (12)	100% (12)	100% (13)	100% (10)	-
Miscellaneous						
<i>edinA, edinC</i>	Epidermal cell differentiation inhibitor A+C	0	0	0	0	-
<i>edinB</i>	Epidermal cell differentiation inhibitor B	8.3% (1)	0	7.7% (1)	0	1
<i>setC*</i>	Staphylococcal exotoxin-like protein	66.7% (8) ^g	91.7% (11)	100% (13) ^g	100% (10)	0.08
<i>ssl6</i>	Staphylococcal superantigen-like protein 6	8.3% (1) ^h	58.3% (7) ^h	38.5% (5)	30.0% (3)	0.08
<i>ssl8</i>	Staphylococcal superantigen-like protein 8	41.7% (5) ⁱ	91.7% (11) ⁱ	61.5% (8)	40.0% (4) ⁱ	0.034
<i>ssl11</i>	Staphylococcal superantigen-like protein 11	75.0% (9)	100% (12) ^j	53.8% (7) ^j	40.0% (4) ^j	0.007

a: *p* values were calculated with fishers exact test; b: significant difference between nasal colonization and prosthesis infection ($p = 0.037$), and between prosthesis infection and hematogenous OM ($p = 0.015$); c: significant difference between nasal colonization and prosthesis infection ($p = 0.027$) and prosthesis infection and sepsis ($p = 0.02$); d: significant difference between nasal colonization and prosthesis infection ($p = 0.027$) and prosthesis infection and sepsis ($p = 0.02$); e: significant difference between prosthesis infection and sepsis ($p = 0.011$); f: significant difference between nasal colonization and prosthesis infection ($p = 0.047$); g: significant difference between prosthesis infection and hematogenous OM ($p = 0.039$); h: significant difference between nasal colonization and prosthesis infection ($p = 0.027$); i: significant difference between nasal colonization and prosthesis infection ($p = 0.027$) and prosthesis infection and sepsis ($p = 0.02$); j: significant difference between prosthesis infection and hematogenous OM ($p = 0.015$) and prosthesis infection and sepsis ($p = 0.03$)

Table S5. Main characteristics of selected strains for long-term cell culture and the mouse sepsis model.

	N15	Chwa42	Edbr39	N9	M1	Udmi62	D2
Group	Nasal colonization	Hematogenous osteomyelitis	Hematogenous osteomyelitis	Nasal colonization	Prosthesis infection	Sepsis	Hematogenous osteomyelitis
Hemolysis [OD 570nm]	low	low	low	high	high	high	high
Invasion [%]	high	high	high	low	low	low	low
Cytotoxicity [%]	low	low	low	high	high	high	high
cp <i>hla</i> /cp <i>gyrB</i>	low	low	low	high	high	high	high
cp <i>psma</i> /cp <i>gyrB</i>	low	low	low	high	high	high	high
cp <i>agrA</i> /cp <i>gyrB</i>	low	low	low	high	high	high	high
cp <i>rnaIII</i> /cp <i>gyrB</i>	low	low	low	high	high	high	high
Range	Hemolysis	Invasion [%]	Cytotoxicity [%]	cp <i>hla</i> /cp <i>gyrB</i>	cp <i>psma</i> /cp <i>gyrB</i>	cp <i>agrA</i> /cp <i>gyrB</i>	cp <i>rnaIII</i> /cp <i>gyrB</i>
low	0–0.13	2.4–40	7.1–40	0–7	0–250	0.01–2.3	0.1–8
high	> 0.13	> 40	> 40	> 7	> 250	> 2.3	> 8

Table S6. Primers used in this study.

Gene Name	Primer Description	Primer Sequence	Product Length
<i>agrA</i>	Forward	5'-AACTGCACATACACGCTTACA-3'	145 nt
	Reverse	5'-GGCAATGAGTCTGTGAGATTT-3'	
<i>hla</i>	Forward	5'-CAACTGATAAAAAAGTAGGCTGGAAAGTGAT-3'	201 nt
	Reverse	5'-CTGGTGAAAACCCTGAAGATAATAGAG-3'	
<i>psma</i>	Forward	5'-GCCATTACATGGAATTCGT-3'	151 nt
	Reverse	5'-CAATAGCCATCGTTTTGTCCT-3'	
<i>rnaIII</i>	Forward	5'-TTCAGTGTGTCGATAATCCA-3'	70 nt
	Reverse	5'-TGATTTCAATGGCACAAGAT-3'	
<i>gyrB</i> (*)	Forward	5'-AATTGAAGCAGGCTATGTGT-3'	138 nt
	Reverse	5'-ATAGACCATTTTGGTGTGG-3'	

* Housekeeping gene