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# Clinical and genetic risk factors for biofilm-forming *Staphylococcus aureus*

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## **Clinical and genetic risk factors for biofilm-forming *Staphylococcus aureus***

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Running Head: Genetic and Clinical Risks of MRSA Biofilms

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1 **ABSTRACT**

2 **Background.** Molecular and clinical factors associated with biofilm-forming methicillin-resistant  
3 *Staphylococcus aureus* (MRSA) are incompletely understood.

4 **Methods.** Biofilm production was quantified in 182 MRSA isolates from clinical culture sites  
5 (2004-2013). Microbiologic toxins, pigmentation, and genotypes were evaluated, and patient  
6 demographics were collected. Logistic regression was used to quantify the effect of strong biofilm  
7 production (versus weak) on clinical outcomes and independent predictors of strong biofilm.

8 **Results.** Of isolates evaluated, 25.8% (47/182) produced strong biofilm, and 40.7% (74/182)  
9 produced weak biofilm. Strong biofilm-producing isolates were more likely to be from MLST clonal  
10 complex 8 (34.0% vs. 14.9%; P=0.01), but less likely to be from MLST CC5 (48.9% vs. 73.0%,  
11 P=0.007). Predictors for strong biofilm were *spa* type t008 (aOR 4.54 95%CI 1.21-17.1), and  
12 receiving chemotherapy or immunosuppressants in the previous 90 days (aOR 33.6; 95%CI 1.68-  
13 673). Conversely, patients with high serum creatinine (aOR 0.33; 95%CI 0.15-0.72) or who  
14 previously received vancomycin (aOR 0.03; 95%CI 0.002-0.39) were less likely to harbor strong  
15 biofilm-producing MRSA. Beta-toxin producing isolates (aOR 0.31; 95%CI 0.11-0.89) and isolates  
16 with *spa* type t895 (aOR 0.02 95%CI <0.001-0.47) were less likely to produce strong biofilm.  
17 Patient outcomes also varied between the two groups. Specifically, patients with strong biofilm-  
18 forming MRSA were significantly more likely to be readmitted within 90 days (aOR 5.43; 95%CI  
19 1.69-17.4), but tended to have decreased 90-day mortality (aOR 0.36; 95%CI 0.12-1.06).

20 **Conclusions:** Patients that harbored t008 and received immunosuppressants were more likely  
21 to have a strong biofilm-producing MRSA. Clinically, patients with strong biofilm-forming MRSA  
22 were less likely to die at 90 days, but five times more likely to be re-admitted.

## 23 **BACKGROUND**

24 Biofilms are critical for the pathogenicity of most bacteria, including *Staphylococcus*. As a result,  
25 *S. aureus* infections can develop into chronic, difficult to treat infections that require long durations  
26 of antimicrobial therapy and surgical intervention. Based on previous reports and various assays  
27 used, 43 to 88% of clinical *S. aureus* isolates can form biofilms.(1-4) Biofilm in *S. aureus* has  
28 been associated with several regulatory and virulence factors such as accessory gene regulator  
29 (*agr*) downregulation and heteroresistant vancomycin intermediate susceptibility.(5) Genotypic  
30 variation among strains may also affect biofilm production, but these relationships haven't been  
31 consistently reported.(6, 7)

32  
33 Methicillin-resistant *S. aureus* (MRSA) causes significant morbidity and mortality. Risk factors for  
34 infection with MRSA are clearly defined; however, little is known about the molecular and clinical  
35 risk factors for biofilm-producing MRSA.(8-11) Defining these risk factors and understanding the  
36 clinical outcomes associated with biofilm-producing MRSA can provide critical and timely insight  
37 into the prevention and treatment of these serious infections. Further, understanding the  
38 phenotypic and genetic characteristics associated with biofilms in MRSA may enable the  
39 development of biofilm detection methods in clinical microbiology laboratories and identify  
40 therapeutic targets. Therefore, the objectives of this study were to quantify clinical outcomes  
41 among adult patients with strong biofilm-producing MRSA ( $OD \geq 2.0$ ) or weak biofilm-producing  
42 MRSA ( $OD \leq 1.0$ ) and to identify clinical and molecular independent predictors of strong biofilm-  
43 producing MRSA.

44

## 45 **MATERIALS AND METHODS**

46 **Study design, population, and bacterial isolates.** A retrospective cohort study was conducted  
47 among a sample of inpatient and outpatients with MRSA cultures from any culture site at the  
48 Providence Veterans Affairs Medical Center (PVAMC), a 119-bed federal hospital from May 2004

49 to October 2013. Nares swabs collected for infection control surveillance purposes were  
50 excluded. Duplicate isolates with the same multi-locus sequence typing (MLST) clonal complexes  
51 (CC) and collected from the same date or admission were excluded. Each isolate included was  
52 treated as an independent event, and therefore patients may have been included in the study  
53 more than once. This study was approved by the Institutional Review Board and the Research  
54 and Development Committee of the PVAMC.

55

#### 56 **Microbiological (phenotypic and genotypic) data.**

57 **Biofilm formation assay.** Biofilm formation was determined using a modified Christensen  
58 method as previously described by our group.(12-16) *Staphylococcus epidermidis* ATCC 35984  
59 and methicillin-susceptible *Staphylococcus aureus* ATCC 35556 were used as positive controls.  
60 The isogenic accumulation-negative mutant of ATCC 35984, M7, was used as a negative  
61 control.(17-19) After incubation, planktonic bacteria were removed by rinsing each well three  
62 times with sterile Millipore water. Plates were dried overnight then stained with 0.1% crystal violet  
63 for 15 minutes. Adherent stain was resolubilized with 33% glacial acetic acid for one hour before  
64 measuring optical density (OD) at 570 nm on a spectrophotometer (ELX800, Biotek, Winooski,  
65 VT). To obtain the final OD values the OD of wells containing tryptic soy broth (TSB) with 1.0%  
66 dextrose only (media control) were subtracted from wells containing isolates to remove  
67 background readings. Mean OD was calculated for each isolate, using at least four  
68 replicates.(17, 20) We used the degree of biofilm production; strong ( $OD \geq 2.0$ ), moderate ( $1.0 <$   
69  $OD < 2.0$ ) and weak ( $OD \leq 1.0$ ) as previously described.(21) For this study, we excluded  
70 moderate biofilm-producing isolates.

71

72 **Alpha- and Beta-toxin production.** Qualitative alpha-toxin production, indicated by clear zones  
73 of hemolysis, was evaluated for each strain on Mueller-Hinton agar with 5% sheep blood after

74 24h incubation at 37°C.(22) Plates were then refrigerated at 4°C for 24h to evaluate beta-toxin  
75 production, indicated by green-brown hemolysis.

76  
77 **Determination of Agr operon function.** Function of the *agr* operon was measured qualitatively  
78 by delta-toxin production.(22, 23) Delta-toxin expression was determined by streaking the MRSA  
79 test isolates adjacent to a Beta Lysin Disk (Remel, Lenexa, KS) on tryptic soy agar with 5% sheep  
80 blood and incubated at 37°C for 24 hours. The presence of synergistic hemolysis between the  
81 streak and Beta Lysin Disk indicated the production of delta-hemolysis, therefore a functional *agr*  
82 locus.(22, 23) The dysfunction of *agr* was defined as the absence of delta-hemolysis within the  
83 beta-toxin zone, as evidenced by the lack of synergistic hemolysis.(23) Reference strains  
84 RN4420 and RN6607 were used as negative and positive controls for delta-toxin, respectively.

85  
86 **Heterogeneous vancomycin intermediate Staphylococcus aureus (hVISA) presence.**  
87 Screening for hVISA was conducted using E-test glycopeptide resistance detection (GRD) strips  
88 (bioMérieux, Durham, NC).(24) Testing was conducted according to manufacturer's instructions  
89 using a standard 0.5 McFarland bacterial suspension on Mueller-Hinton agar with 5% sheep blood  
90 (BD, Sparks, MD). The results were read at 24 and 48 hours after incubation. Standard  
91 vancomycin Etests were also conducted according to manufacturer's instructions, on Mueller-  
92 Hinton agar for 24 hours. Heteroresistance was defined as a vancomycin or teicoplanin MIC of  $\geq$   
93 8  $\mu\text{g/mL}$  on the GRD Etest plus a standard vancomycin MIC  $< 4 \mu\text{g/mL}$ . Quality control of  
94 susceptibility testing was performed with reference strain ATCC700698 (Mu3, hVISA).

95  
96 **Pigmentation.** Golden pigmentation was evaluated after overnight growth on tryptic soy agar at  
97 37°C.(25, 26) Each strain was compared to a reference white strain of *S. epidermidis* ATCC35984  
98 and categorized as pigmented or non-pigmented. *S. aureus* ATCC35556 served as a pigmented

99 control. A selection of 60 strains were categorized independently by a second reviewer, with  
100 98.3% agreement.

101  
102 **Genotyping.** Staphylococcal protein A (*spa*) genotype was determined by PCR as previously  
103 described, with 1095F and 1517R primers.(27) Gene sequences were determined using Sanger  
104 sequencing with the forward primer only unless reverse primer was necessary for sequence  
105 clarification. *Spa* type was mapped to common MLST CC using the Ridom *spa* server  
106 (Spaserver.ridom.de). *Spa* types not matched to a clonal complex in the Ridom *spa* server were  
107 matched by literature search.

108  
109 **Patient data.** Data was collected through a chart review of electronic medical records and  
110 included diagnoses and procedures, clinical measurements, microbiology data, patient  
111 demographics, health-care exposure within 90 days of index culture (hospitalization >72 hours  
112 and surgical procedures), receipt of antimicrobials or medications that may influence biofilm  
113 formation in the previous 90 days (i.e. gastric acid suppressants [proton-pump inhibitors or H<sub>2</sub>-  
114 blockers], chronic corticosteroid use, non-steroidal anti-inflammatory [NSAID], HMG-CoA  
115 reductase inhibitors [statins])(28-32), presence of prosthetic/foreign devices (i.e. orthopedic,  
116 cardiovascular, urinary Foley, intravenous catheters), and infection/colonization history in the  
117 previous year.

118  
119 **Clinical outcome definitions.** Clinical outcomes of interest were all-cause mortality, admission  
120 among outpatient or re-admission among inpatients, MRSA infection and MRSA-related  
121 admission among outpatients or re-admission among inpatients. As the risk period for poor  
122 outcomes in these patients is not known, we evaluated outcomes at follow-up periods of 30 and  
123 90 days.

124



125 The index date was defined as the collection date of the MRSA isolate tested for biofilm production  
126 (index culture). MRSA infection was confirmed from microbiology data, and diagnosis of infection  
127 in the medical record. Readmission was defined as admission for any reason after the discharge  
128 date of the index culture. For index isolates collected in the outpatient setting, admission was  
129 defined as admission for any reason after the index date.

130

131 **Statistical analysis.** Between-group differences were assessed using  $\chi^2$  or Fisher exact tests  
132 for categorical variables and the T-test or Wilcoxon rank sum test for continuous variables.  
133 Logistic regression models were used to quantify the effect of strong biofilm on each clinical  
134 outcome, while controlling for confounders of the exposure-outcome relationship.(33) In  
135 multivariable modeling, a manual, non-computer-generated backward elimination approach was  
136 implemented. Logistic regression was also used to identify independent predictors associated  
137 with strong MRSA biofilm production.(33) All baseline variables were evaluated as potential  
138 confounders in the clinical outcome models, and as independent predictors of biofilms in the  
139 predictive model. Crude and adjusted odds ratios (OR) and respective 95% confidence intervals  
140 (CI) are presented. All statistical tests were conducted using SAS, version 9.2 (SAS Institute,  
141 Cary, NC), with a two-tailed  $\alpha$  value of 0.05 required for statistical significance.

142

143 **RESULTS**

144 **Isolate and clinical characteristics.** In total, 121 MRSA isolates were included for biofilm  
145 production, 38.8% (47/121) produced strong biofilms ( $OD \geq 2.0$ ) and 61.2% (74/121) produced  
146 weak biofilms ( $OD \leq 1.0$ ). Race was significantly different between the groups, with the strong  
147 biofilm group having a higher number of whites (93.6% vs. 79.7%;  $P=0.04$ ). There was no  
148 difference between the groups in age, gender or BMI. Serum creatinine and creatinine clearance  
149 was significantly different between the two groups. Median (Q1-Q3) serum creatinine was 0.9 (0.8  
150 - 1.1mg/dl) for the strong biofilm group vs 1.3 (0.9 - 2.2 mg/dL) for the weak biofilm group  
151 ( $P=0.001$ ). The median (Q1 – Q3) creatinine clearance was 92.6 (range 67.6-117.6 mL/min) in  
152 the strong biofilm group vs 58.4 (31.7 – 89.2 mL/min) for the weak biofilm group ( $P=0.001$ ).  
153 Significantly lower number of patients in the strong biofilm group had chronic renal failure (12.8%  
154 vs 31.1%;  $P=0.02$ ). There was no difference between the groups in Charlson comorbidity index  
155 or other comorbidities such as diabetes, cardiovascular disease, liver disease, malignancies and  
156 anemia. The groups did not differ in IV drug use, alcohol abuse or smoking. The presence of  
157 foreign material/device was lower in patients with a strong biofilm-producing isolate (25.5% vs  
158 50.0%;  $P=0.01$ ). Significantly lower number of patients in the strong biofilm group were  
159 hospitalized for two or more days in the previous 90 days (27.7% vs 52.7%;  $P=0.007$ ). Overall  
160 antimicrobial use in the previous 90 days from culture was not significantly different between the  
161 groups but use of vancomycin was significant with 27.0% of patients receiving vancomycin in the  
162 weak biofilm group whereas only 2.1% in the strong biofilm group ( $P=0.001$ ). There were fewer  
163 patients on hemodialysis in the strong biofilm-producing group (0% vs. 13.5%;  $P=0.006$ ). Patients  
164 in the strong biofilm group had a lower number of bacteremias (4.3% vs 17.6%;  $P=0.03$ ) and  
165 pneumonias (10.6% vs 25.7%;  $P=0.04$ ) in the year prior to the index culture. Patients in the strong  
166 biofilm group tended to present in the outpatient setting at the index culture (51.1% vs 32.4%;  
167  $P=0.04$ ). **(Table 1).**

168

169 Alpha-toxin was produced by 79.3% (n=96) of the isolates overall (74.5% strong biofilm vs  
170 82.4%weak biofilm, p=0.29). Beta-toxin production was less common, with 69.4% (n=84) of  
171 isolates (59.6% strong biofilm vs 75.7% weak biofilm, p=0.06). Presence of hVISA was rare  
172 among strong biofilm and weak biofilm-producing isolates (8.5% vs 4.4%; P=0.44). The  
173 proportion of isolates with *agr* dysfunction (61.7% vs 43.2%; P=0.05) and pigmentation (76.6%  
174 vs 54.1%; P=0.01) were significantly higher in the strong biofilm group. The distribution of  
175 vancomycin MIC was similar among both groups. MRSA isolates represented seven MLST CC,  
176 the most common were CC5 (63.6%) and CC8 (22.3%). Strong biofilm-producing isolates had  
177 significantly lower MLST CC5 (48.9% vs 73.0%; P=0.007) and significantly higher CC8 (34.0% vs  
178 14.9%; P=0.01). There were 24 different *spa* types identified among the isolates. Of those *spa*  
179 types, the most common were t002 (32.2%), t895 (15.7%), t008 (14.9%), and t1094 (5.8%).  
180 Strong biofilm-producing isolates contained significantly more *spa* type t008 (25.5% vs 8.1%;  
181 P=0.01) and less t895 (2.1% vs 24.3%; P=0.001). **(Table 2)**.

182

183 **Clinical Outcomes and Independent Predictors.** After controlling for potential confounders,  
184 patients with strong biofilm-producing MRSA were more than five times as likely to be (re)-  
185 admitted within 90 days of discharge (adjusted OR 5.43; 95% CI 1.69-17.4). The strong biofilm  
186 group was 64% less likely to die within 90 days (adjusted OR 0.36; 95% CI 0.12-1.06), but this  
187 was not statistically significant. There was no difference in 30 day mortality, 30 day (re)-admission,  
188 MRSA reinfection at 30 or 90 days, or MRSA related (re)-admission at 30 or 90 days among  
189 patients with strong or weak biofilm-producing MRSA. **(Table 3)**

190

191 Patients who were on chemotherapy and/ or used immunosuppressants within 90 days of index  
192 culture had a 33.6 times higher odds for strong biofilm-producing MRSA isolate (adjusted OR  
193 33.6; 95% CI 1.68-673). Patients harboring isolates from t008 (adjusted OR 4.54; 95% CI 1.21-  
194 17.1) also had increased risk of a strong biofilm-producing MRSA. Further, patients with isolates

195 from t895 (adjusted OR 0.02; 95% CI <0.001-0.47) or that produced beta-toxin were less likely to  
196 produce strong biofilm (adjusted OR 0.31; 95%CI 0.11-0.89). Patients who had increased serum  
197 creatinine (adjusted OR 0.33; 95% CI 0.15-0.72) or who received vancomycin in the previous 90  
198 days (adjusted OR 0.03; 95%CI 0.002-0.39) were less likely to produce strong biofilm. **(Table 4)**.  
199

200 **DISCUSSION**

201 This study demonstrated that strong biofilm formation among clinical MRSA isolates was  
202 associated with increased readmission at 90 days and a trend toward decreased 90-day mortality.  
203 Strong biofilm formation was also associated with MRSA lineage, *agr* dysfunction, pigmentation,  
204 and several patient factors including serum creatinine, race, and immunosuppressants.

205  
206 Biofilm formation has been previously associated with patient mortality. A previous study  
207 demonstrated increased mortality with biofilm-forming isolates, but the attributable mortality was  
208 low.(3) Similar to our study, included patients were primarily male, and members of military  
209 services (however they were younger than our Veterans), but whereas our study was only in  
210 MRSA isolates, this study included multiple types of bacterial cultures and found a five-fold  
211 increased association of MRSA among the biofilm-positive group (OR 5.09, 95% CI 1.12-23.1).  
212 Overall mortality with initial infection was 16% versus 5% in biofilm versus non-biofilm group  
213 ( $p=0.01$ ), with an attributable mortality of 7%.(3) Unfortunately, it is difficult to tell how many of  
214 these are due to biofilm-forming versus non-biofilm forming MRSA in the study, as opposed to  
215 other bacterial types.

216  
217 The majority of MRSA isolates in our study represented CC5, typically referred to as hospital-  
218 associated strains and CC8 historically of community origin. In the multivariate analyses, there  
219 was no association between clonal complex and biofilm formation, which has been found in other  
220 studies.(34-36) This may be due to limited number of isolates, or the clinical source of the isolates  
221 used, which may play a role in their biofilm formation. However, in univariate analyses, weak  
222 biofilm isolates had more CC5, which is traditionally hospital-associated, as well as more previous  
223 hospitalization within the previous 90 days, dialysis, bacteremia and pneumonia within the  
224 previous year, and treatment with vancomycin. Although not statistically significant, weak biofilm  
225 isolates had more antimicrobial use in the prior 90 days and more infections in the previous year

226 in all categories. This may represent a higher severity of illness, and may help to explain the  
227 increased mortality seen at 90 days. In contrast, CC8, the traditionally community-acquired clone,  
228 has been previously associated with strong biofilm production, as well as community-acquired  
229 skin infections and colonization.(34, 37) These types of infections and colonization may be  
230 associated with lower mortality, as seen in our study. The most common *spa* types were t002,  
231 t008, t895, and t1094. Though t002 and t895 are related to CC5, t002 was not related to biofilm  
232 formation. We found that isolates from *spa* type t008 were predictive for the strong biofilm  
233 phenotype, while t895 was significantly higher in weak biofilm-producing isolates. At least for this  
234 subset of isolates, *spa* type served as a better predictor of biofilm formation than MLST CC,  
235 potentially due to the greater degree of resolution in *spa* typing. This finding is consistent with  
236 previous studies evaluating genotypically different clones of MRSA in the production of biofilm.(6,  
237 7)

238  
239 Previously published data suggest that *agr* dysfunction is associated with biofilm formation in *S.*  
240 *aureus*.(5, 38-40) This is in line with our own data, which demonstrated *agr* dysfunction was  
241 present in 61.7% of strong biofilm formers versus 43.2% of weak biofilm formers. Some data  
242 demonstrate conflicting results with regard to *agr* function and biofilm, depending whether the  
243 biofilm is formed in vivo or in vitro.(41) In vitro biofilm formation may yield a different relationship  
244 with *agr* than in vivo biofilms, since there is no host response-relationship. It is suggested that  
245 the host response and *agr*-dependent virulence factors secreted in vivo regulate biofilm  
246 formation.(41) Previous studies have also suggested that *agr* dysfunction is associated with  
247 hVISA development, however because our overall numbers of hVISA were low, we could not  
248 confirm this finding.(22, 42) Beta-toxin was associated with weak biofilm formation, and was a  
249 negative predictor for strong biofilm in the logistic regression model (adjusted OR 0.31; 95%CI  
250 0.11-0.89). Although there is limited data on the connection between beta-toxin and biofilm  
251 formation, in previous studies, beta-toxin was associated with skin colonization, and colonization

252 was associated with a low biofilm phenotype, consistent with our findings.(43, 44) Alpha-toxin has  
253 also been associated with biofilm formation,(45, 46) however, we did not quantify how much  
254 alpha-toxin these isolates produce in this study, which may have correlated better to biofilm  
255 formation than a dichotomous presence or absence of alpha-toxin. Overall, these findings  
256 underscore the need for additional studies to better describe the mechanisms responsible for the  
257 presence of biofilms.

258

259 This study had several limitations. A limited sample size may have impaired the ability to find  
260 associations between biofilm production and covariates previously noted to play a role in biofilms.  
261 Of course, we cannot guarantee that in vitro biofilm formation equates to clinical biofilm formation  
262 in an infection. Due to the retrospective design of this study, not all variables or potential  
263 confounders may have been included in the analysis of clinical factors, and we are reliant on the  
264 accuracy of data as entered into the patient electronic medical record. To minimize selection bias,  
265 the investigator collecting clinical data was blinded to the biofilm status of each isolate. Biofilm  
266 formation is determined using a standard assay.(14, 15, 18, 19, 47) Additionally, we utilize a  
267 negative control isolate to ensure comparability between results. By removing the moderate  
268 biofilm category, we may have limited our power in the number of isolates, but the isolates had  
269 the most different biofilm classifications to see differences in the predictors and outcomes.

270

271 In summary, strong biofilm formation among MRSA isolates is associated with multiple features  
272 of the host and organism including phenotypic and genotypic factors, demographics, and clinical  
273 characteristics. Patients with a strong biofilm-forming MRSA isolate are 5 times more likely to be  
274 admitted or readmitted within 90 days, and tend to have decreased mortality at 90 days.

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**Table 1. Baseline Characteristics**

<b>Characteristic</b>	<b>Strong Biofilm OD ≥ 2.0 (n=47)</b>	<b>Weak Biofilm OD ≤ 1.0 (n = 74)</b>	<b>P value</b>
Age, years <sup>a</sup>	67.8 ± 13.5	68.1 ± 12.8	0.90
Male sex	44 (93.6)	72 (97.3)	0.37
Race - white	44 (93.6)	59 (79.7)	0.04
Residence – home	39 (83.0)	51 (68.9)	0.08
Weight, kg <sup>a</sup>	89.9 ± 23.0	84.4 ± 21.0	0.18
BMI <sup>a</sup>	29.4 ± 7.9	27.1 ± 6.4	0.08
SCr, (mg/dL) <sup>b</sup>	0.9 (0.8-1.1)	1.3 (0.9-2.2)	0.001
CrCl, mL/min <sup>b</sup>	92.6 (67.6-117.6)	58.4 (31.7-89.2)	0.001
Charlson Comorbidity Index <sup>b</sup>	5.0 (3- 8)	5.0 (3-8)	0.91
<b>Comorbidities</b>			
IV Drug User	2 (4.3)	2 (2.7)	0.64
Alcohol Abuse	6 (12.8)	6 (8.1)	0.53
Diabetes	17 (36.2)	35 (47.3)	0.23
Cardiovascular	36 (76.6)	59 (79.7)	0.68
Chronic respiratory disease	14 (29.8)	19 (25.7)	0.62
Liver disease	5 (10.6)	7 (9.5)	1.00
Chronic renal disease	6 (12.8)	23 (31.1)	0.02
Malignancy	14 (29.8)	21 (28.4)	0.87
Anemia	9 (19.2)	24 (32.4)	0.11
Other	13 (27.7)	11 (14.9)	0.08
Smoking Status			0.80
Non-Smoker	23 (48.9)	32 (43.2)	
Smoker	14 (29.8)	23 (31.1)	
Unknown	10 (21.3)	19 (25.7)	
Foreign material/device	12 (25.5)	37 (50.0)	0.01
Orthopedic	2 (4.3)	2 (2.7)	0.01
Other	10 (21.3)	35 (47.3)	
None	35 (74.5)	37 (50.0)	
No. Foreign material/device <sup>b</sup>	0 (0-1)	0.5 (0-1)	0.01
<b>Patient History</b>			
Hospitalization (≥ 2 days) <sup>c</sup>	13 (27.7)	39 (52.7)	0.007
Surgery <sup>c</sup>	13 (27.7)	18 (24.3)	0.68
<b>Medications <sup>c</sup></b>			
Chemotherapy/Immunosuppressants	5 (10.6)	2 (2.7)	0.11
Chronic corticosteroids <sup>d</sup>	6 (12.8)	5 (6.8)	0.33
NSAID	19 (40.4)	35 (47.3)	0.46
Gastric acid suppressor <sup>e</sup>	21 (44.7)	44 (59.5)	0.11
HMG-CoA reductase inhibitor	21 (44.7)	28 (37.8)	0.45
Antimicrobial Use <sup>c</sup>	29 (61.7)	57 (77.0)	0.07
Vancomycin	1 (2.1)	20 (27.0)	0.001

Penicillin	9 (19.2)	21 (28.4)	0.25
Cephalosporin	9 (19.2)	19 (25.7)	0.41
Beta-Lactams	14 (29.8)	30 (40.5)	0.23
Fluoroquinolone	11 (23.4)	24 (32.4)	0.29
Other	14 (29.8)	26 (35.1)	0.54
No. Antibiotic <sup>c b</sup>	1 (0-2)	1 (0-2)	0.08
Infections <sup>f</sup>			
Skin and soft tissue	5 (10.6)	9 (12.2)	0.80
Pneumonia	5 (10.6)	19 (25.7)	0.04
Urinary tract infection	14 (29.8)	24 (32.4)	0.76
Bacteremia	2 (4.3)	13 (17.6)	0.03
Other	7 (14.9)	13 (17.6)	0.70
≥1 <i>S. aureus</i> infection previous <sup>f</sup>	12 (25.5)	21 (28.4)	0.73
MRSA	11 (23.4)	18 (24.3)	0.91
Source of previous <i>S. aureus</i> infection <sup>g</sup>			
Tissue	5 (10.6)	3 (4.1)	0.26
Urine	6 (12.8)	5 (6.8)	0.33
Blood	0	5 (6.8)	0.15
Other	3 (6.4)	11 (14.9)	0.15
Index isolate same site as previous <i>S. aureus</i> infection	9 (19.2)	12 (16.2)	0.68
Previous Polymicrobial infections	13 (27.7)	23 (31.1)	0.69
MRSA nares positive <sup>f</sup>	6 (12.8)	15 (20.3)	0.29

#### Index Culture

Culture Site (%)			
Blood	9 (19.1)	23 (31.1)	0.15
Tissue	16 (34.0)	20 (27.0)	0.41
Urine	11 (23.4)	13 (17.6)	0.43
Catheter	10 (21.3)	15 (20.3)	0.89
Other	1 (2.1)	3 (4.1)	1.0
Bacteremia Source			
Foreign material	3 (6.4)	10 (13.5)	0.22
cSSTI/Osteomyelitis	0	4 (5.4)	0.16
Other	6 (12.8)	16 (21.6)	0.22
Trauma associated	5 (10.6)	9 (12.2)	0.80

#### At Index Culture

Setting			0.04
Inpatient	23 (48.9)	50 (67.6)	
Outpatient	24 (51.1)	24 (32.4)	
Inpatient admission			0.70
ICU	6 (26.1)	11 (22.0)	
Non-ICU	17 (73.9)	39 (78.0)	
Length of stay, days <sup>b</sup>	14.0 (4.0-28.0)	12.5 (7.0-20.0)	0.47

Surgery/procedure during admission	13 (27.7)	32 (43.2)	0.08
Hospital days prior to index culture <sup>b</sup>	0 (0-3)	0 (0-2)	0.85
MRSA nares positive	10 (21.3)	26 (35.1)	0.10
Urinary foley catheter	18 (38.3)	27 (36.5)	0.84
IV catheter > 48 hours	6 (12.8)	20 (27.0)	0.06
Mechanical ventilation	3 (6.4)	7 (9.5)	0.74
Dialysis	0	10 (13.5)	0.006

Data are presented as No. (%) unless otherwise specified.

Abbreviations: BMI, body mass index; SCr, Serum creatinine; CrCl, creatinine clearance (Cockcroft-gault); cSSTI, complicated skin and soft tissue infection; ICU, intensive care unit; IV, intravenous;

<sup>a</sup> Mean ± SD

<sup>b</sup> Median (Q1-Q3)

<sup>c</sup> Previous 90 days

<sup>d</sup> Prednisone 20 mg every day or equivalent for ≥ 14 days

<sup>e</sup> Proton-pump inhibitor or H<sub>2</sub>-antagonists

<sup>f</sup> Previous one year

<sup>g</sup> ≥ 1 previous infection source



**Table 2. Phenotypic and Genotypic Characteristics**

Phenotypic	Strong Biofilm	Weak Biofilm	P value
	OD ≥ 2.0 (n=47)	OD ≤ 1.0 (n=74)	
Alpha-toxin	35 (74.5)	61 (82.4)	0.29
Beta-toxin	28 (59.6)	56 (75.7)	0.06
<i>Agr</i> operon dysfunction (delta-toxin negative)	29 (61.7)	32 (43.2)	0.05
hVISA	4 (8.5)	3 (4.4)	0.44
Pigmented	36 (76.6)	40 (54.1)	0.01
Vancomycin MIC			0.37
≥ 1.5 µg/mL	28 (59.6)	50 (67.6)	
< 1.5 µg/mL	19 (40.4)	24 (32.4)	
Genotypic	Strong Biofilm	Weak Biofilm	P value
	OD ≥ 2.0 (n=47)	OD ≤ 1.0 (n=74)	
MLST CC			
CC 5	23 (48.9)	54 (73.0)	0.007
CC 8	16 (34.0)	11 (14.9)	0.01
Other <sup>a</sup>	8 (17.0)	9 (12.2)	0.45
<i>Spa</i> Type			
t002	14 (29.8)	25 (33.8)	0.65
t895	1 (2.1)	18 (24.3)	0.001
t008	12 (25.5)	6 (8.1)	0.01
t1094	4 (8.5)	3 (4.1)	0.43
Other <sup>b</sup>	16 (34.0)	22 (29.7)	0.62

Data are presented as No. (%) unless otherwise specified.

Abbreviations: *Agr*, accessory gene regulator; hVISA, heteroresistant vancomycin intermediate *S. aureus*; MLST CC, multi-locus sequence typing clonal complex

<sup>a</sup> CC1, CC4, CC20, CC30, CC45, and unable to obtain genotypic characteristics (eleven isolates)

<sup>b</sup> t004, t010, t018, t062, t064, t067, t088, t1340, t189, t1904, t2032, t242, t2666, t334, t548, t681, t693, t985, and unable to obtain genotypic characteristics (eleven isolates)

**Table 3. Clinical Outcomes**

Outcome	No. of events/No. of patients (%)			Unadjusted OR (95% CI)	Adjusted OR (95% CI)
	Strong Biofilm	Weak Biofilm	P Value		
Mortality					
30-day	3/47 (6.4)	18/74 (24.3)	0.01	0.21 (0.06-0.77)	0.32 (0.08-1.26) <sup>a</sup>
90-day	6/47 (12.8)	27/74 (36.5)	0.004	0.25 (0.10-0.68)	0.36 (0.12-1.06) <sup>a</sup>
(Re)-admission					
30-day	11/45 (24.4)	17/61 (27.9)	0.69	0.84 (0.35-2.02)	1.65 (0.58-4.65) <sup>b</sup>
90-day	20/43 (46.5)	23/57 (40.3)	0.54	1.28 (0.58-2.86)	5.43 (1.69-17.4) <sup>c</sup>
MRSA (Re)-infection					
30-day	3/47 (6.4)	8/66 (12.1)	0.36	0.49 (0.12-1.97)	0.33 (0.08-1.37) <sup>d</sup>
90-day	8/45 (17.8)	14/58 (24.1)	0.43	0.68 (0.26-1.80)	0.74 (0.25-2.18) <sup>e</sup>
MRSA related (Re)-admission					
30-day	5/44 (11.4)	8/61 (13.1)	0.79	0.85 (0.26-2.80)	1.20 (0.34-4.25) <sup>f</sup>
90-day	8/43 (18.6)	9/57 (15.8)	0.71	1.22 (0.43-3.47)	1.75 (0.56-5.45) <sup>f</sup>

Abbreviations: OR, odds ratio; CI, confidence interval; ICU, intensive care unit; BMI, body mass index

<sup>a</sup> Adjusted for hospitalized during previous 90 days for > 2days and admission type (inpatient or outpatient setting)

<sup>b</sup> Adjusted for hospitalized during previous 90 days for > 2days and infection with confirmed bacteremia at the time of index culture

<sup>c</sup> Adjusted for hospitalized during previous 90 days for > 2days, MLSTcc5, serum creatinine and infection with confirmed pneumonia at the time of index culture

<sup>d</sup> Adjusted for pigmentation

<sup>e</sup> Adjusted for MLSTcc5 and pigmentation

<sup>f</sup> Adjusted for hospitalized during previous 90 days for > 2days

**Table 4: Predictors of Strong Biofilm Producing MRSA**

<b>Variable</b>	<b>OR (95% CI)</b>
Beta-toxin	0.31 (0.11–0.89)
Chemotherapy or immunosuppressants used in previous 90 days	33.6 (1.68–673)
Serum creatinine (per unit increase)	0.33 (0.15–0.72)
<i>Spa</i> type t008	4.54 (1.21-17.1)
<i>Spa</i> type t895	0.02 (<0.001–0.47)
Vancomycin in the previous 90 days	0.03 (0.002–0.39)

Abbreviations: OR, odds ratio; CI, confidence interval