

Genotyping of 353 *Staphylococcus aureus* Bloodstream Isolates Collected between 2004 and 2009 at a Norwegian University Hospital and Potential Associations with Clinical Parameters

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We analyzed 353 *Staphylococcus aureus* bloodstream isolates from 2004 to 2009 to identify dominant genotypes, changes over time, and associations between genotype, phenotype, and clinical parameters. The isolates were genotyped with regard to *spa* type and presence of Pantone-Valentine leukocidin and toxic shock syndrome toxin 1-encoding genes. A high level of genetic diversity was detected. All but three isolates were methicillin sensitive. Interestingly, *spa* clonal complex 021 showed a weak association with higher all-cause hospital mortality.

Staphylococcus aureus is a leading cause of bacteremia often associated with metastatic infections and high levels of morbidity and mortality. In Europe, the occurrence of *S. aureus* bacteremia (SAB) caused by methicillin-sensitive *S. aureus* (MSSA) is increasing while that of SAB caused by methicillin-resistant *S. aureus* (MRSA) is decreasing (4). It is therefore important to demonstrate whether SAB due to MSSA is limited to certain bacterial clones or clusters of strains and whether they differ geographically.

The aims were to investigate (i) dominant *S. aureus* genotypes causing bacteremia, (ii) frequency shifts of genotypes over time, (iii) associations between genotypes and well-defined clinical subgroups of SAB patients, and (iv) associations between genotypes and resistance to antibiotics.

All *S. aureus* isolates ($n = 364$) collected from blood cultures in routine diagnostics from July 2004 through December 2009 at Akershus University Hospital, Norway, were obtained. The hospital is an acute somatic hospital with a catchment area of 360,000 inhabitants and more than 40,000 hospitalizations yearly in the selected period. The isolate from the first *S. aureus*-positive blood culture from each patient was included, unless consecutive blood cultures showed a different genotype; in that case (which occurred with four patients), both isolates were included, as they were regarded as unrelated infections. For one patient, only the second consecutive isolate was typeable by *spa* analysis. Five patients were excluded due to missing isolates, and six patients had untypeable *spa* genes, leaving a total of 353 isolates for further analysis.

Demographic and clinical data were extracted and reviewed retrospectively from patient charts. Hospital-acquired (HA) and community-acquired (CA) SAB were defined by a positive blood culture for *S. aureus* obtained more and less, respectively, than 48 h after hospital admission. Endocarditis was defined according to the modified Duke criteria (6). SAB associated with intravascular devices included both short- and long-term arterial and venous catheters. Deep-seated abscesses included all well-defined abscesses in musculature and inner organs, while subcutaneous abscesses were not recorded. Mortality was registered as all-cause in hospital.

spa typing was performed as described previously (1). Assignment of *spa* type and Based Upon Repeat Pattern (BURP) analysis for assignment of *spa* clonal complexes (CCs) were performed

using Ridom StaphType v.2.0.3 (<http://www.ridom.de>). The BURP parameters were set as described by Mellman and collaborators (8).

Detection of Pantone-Valentine leukocidin (PVL) genes (*lukS/F-PV*) on isolates from 2006 to 2007 and *tst* on all isolates was performed using real-time PCR (RT-PCR) and power SYBR green master mix according to the manufacturer's instructions (Applied Biosystems, Foster City, CA) (2, 3). PVL analysis of the remaining isolates was performed according to an in-house RT-PCR protocol with a minor groove binder (MGB) probe (6-carboxyfluorescein-C AGAATTTATTGGTGCCTATC), a forward primer published previously (3), and a reverse primer (TTTTGCAGCGTTTTGTT TTCG) according to the manufacturer's instructions for FAST application (Applied Biosystems).

Antibiotic susceptibility was performed by disk diffusion analysis (Bio-Rad, France) according to guidelines and breakpoints of the Norwegian Working Group of Antibiotics (<http://www.antibiotikaresistens.no>). The antibiotics tested were erythromycin, fusidic acid, gentamicin, oxacillin, penicillin, tetracycline, and trimethoprim-sulfamethoxazole. Sensitive and intermediate isolates were combined in statistical analyses.

Statistical analyses were conducted using SPSS version 15 (SPSS Inc., Chicago, IL). Fisher's exact test was performed for comparison analysis and linear regression for time trend analyses using 6 months as the time interval. A significance level of 0.05 was chosen.

The median age of patients was 70 years (range, 0 to 97 years), and 56% were men. The clinical subgroups are listed in Table 1. The isolates were assigned to 168 different *spa* types and 13 *spa* CCs (Fig. 1 and Table 1), with no significant differences between

Received 23 May 2012 Returned for modification 20 June 2012

Accepted 5 July 2012

Published ahead of print 11 July 2012

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doi:10.1128/JCM.01352-12

TABLE 1 *spa* type, *spa* clonal complexes, PVL and *tst* status of 353 *S. aureus* bloodstream isolates in relationship with patients' clinical characteristics^g

<i>spa</i> type, <i>spa</i> CC, PVL, or <i>tst</i>	No. (%) of isolates	No. (%) with						
		CA infection	HA infection	Endocarditis	Deep-seated abscess ^a	Intravascular device	Osteomyelitis/ arthritis ^a	All-cause hospital mortality ^a
<i>spa</i> type								
t084	46 (13.0%)	22 (47.8%)	24 (52.2%)	3 (6.5%)	6 (13.4%)	15 (32.6%) ^b	6 (17.6%)	14 (16.5%)
t002	18 (5.1%)	11 (61.1%)	7 (38.9%)	2 (11.1%)	0	3 (16.7%) ^b	2 (5.9%)	5 (5.9%)
t065	15 (4.2%)	11 (73.3%)	4 (26.7%)	2 (13.3%)	2 (13.3%)	0 ^b	0	3 (3.5%)
t012	14 (4.0%)	7 (50.0%)	7 (50.0%)	1 (7.1%)	0	4 (28.6%) ^b	2 (5.9%)	6 (7.1%)
Other <i>spa</i> types ^c	260 (73.7%)	141 (54.2%)	119 (45.8%)	13 (5.0%)	27 (10.4%)	62 (23.8%) ^b	24 (70.6%)	57 (67.1%)
Total	353	192 (54.4%)	161 (45.6%)	21 (5.9%)	35 (9.9%)	84 (23.8%)	34 (9.6%)	85 (24.1%)
CC								
<i>spa</i> CC084	69 (20.7%)	34 (49.3%)	35 (50.7%)	5 (7.2%)	8 (11.6%)	19 (27.5%)	8 (11.6%)	19 (27.5%) ^d
<i>spa</i> CC021	55 (16.5%)	31 (56.4%)	24 (43.6%)	2 (3.6%)	5 (9.1%)	13 (23.6%)	6 (10.9%)	20 (36.4%) ^d
<i>spa</i> CC133	32 (9.6%)	21 (65.6%)	11 (34.4%)	1 (3.1%)	4 (12.5%)	6 (18.8%)	6 (18.8%)	4 (12.5%) ^d
<i>spa</i> CC065	30 (9.0%)	19 (63.3%)	11 (36.7%)	3 (10.0%)	3 (10.0%)	3 (10.0%)	1 (3.3%)	6 (20.0%) ^d
<i>spa</i> CC002	22 (6.6%)	14 (63.6%)	8 (36.4%)	3 (13.6%)	1 (4.5%)	3 (13.6%)	2 (9.1%)	6 (27.3%) ^d
<i>spa</i> C304/024	22 (6.6%)	12 (54.2%)	10 (45.5%)	1 (4.5%)	3 (13.6%)	6 (27.3%)	1 (4.5%)	3 (13.6%) ^d
<i>spa</i> CC359	20 (6.0%)	11 (55.0%)	9 (45.0%)	2 (10.0%)	4 (20.0%)	4 (20.0%)	0	6 (30.0%) ^d
Other <i>spa</i> CCs ^e	83 (24.9%)	41 (49.4%)	42 (50.6%)	4 (4.8%)	6 (7.3%)	26 (31.3%)	9 (11.0%)	15 (18.3%) ^d
Total	333	183 (55.0%)	150 (45.0%)	21 (6.3%)	34 (10.2%)	80 (24.0%)	33 (9.9%)	79 (23.7%)
PVL	11 (3.1%) ^f	10 (90.9%) ^f	1 (9.1%) ^f	1 (9.1%)	3 (27.3%)	2 (18.2%)	1 (9.1%)	2 (18.2%)
<i>tst</i>	54 (15.3%)	28 (51.9%)	26 (48.1%)	1 (1.9%)	4 (7.4%)	16 (29.6%)	6 (11.1%)	16 (29.6%)

^a One sample was excluded due to lack of information.

^b $P = 0.027$ for *spa* type t065 versus other *spa* types: 0% versus 23.8%.

^c Consists of 164 unique *spa* types.

^d $P = 0.023$ for *spa* CC021 versus other *spa* CCs: 36.4% versus 24.9%.

^e 20 isolates (5.7%) excluded from BURP analysis, 5 CCs had no founder, 16 isolates were singletons.

^f $P = 0.014$ for PVL in CA infection versus HA infection: 90.9% versus 9.1%.

^g Only statistically significant P values are presented.

HA and CA infections. Their frequencies coincide with those in other studies from Norway on nasal *S. aureus* carriers (11–13), suggesting that there are no locally or geographically dominating *spa* types or clusters associated with invasive MSSA infections. The degree of accordance between carrier and invasive *S. aureus spa* types may be partly explained by the high frequency of endogenous infections (15).

The PVL toxin has generally been associated with localized skin and soft tissue CA infections and necrotizing pneumonia (5, 7), but its significance in SAB is less known. As only 3.1% of the isolates were positive for the PVL genes, the role of PVL as a virulence factor of invasive infections remains unproven. Accordingly, PVL showed the only significant difference between CA and HA infections, where 10 of 11 PVL-positive isolates were CA infections (Table 1).

The frequency of isolates containing the *tst* gene (15.3%) correlates well with previous findings on SAB and asymptomatic *S. aureus* carriers (9, 14).

No shifts in frequency of *spa* type, *spa* CC, PVL, *tst*, or antibiotic resistance over time during the 5.5-year study period could be established, suggesting that the common *S. aureus* population in the hospital's catchment area is relatively stable over time.

The correlations of genotypes and clinical subgroups are presented in Table 1. Of specific interest, patients with *spa* CC021 isolates had a higher all-cause hospital mortality rate than patients with other *spa* CCs (36.4% versus 21.3%, $P = 0.023$). To our

knowledge this has not been reported previously. Because other factors likely to affect mortality, such as comorbidity and disease severity, were not accounted for, it is not known if *spa* CC021 represents an independent predictor. However, the association is weak and needs to be demonstrated in future prospective studies. As the methods utilized have relatively low resolution, whole-genome analysis could provide additional insight into the possible increased virulence of *spa* CC021 strains and the population genetic structure overall. Also, virulence determinants may differ among and between genotypes, so genotyping alone may not be predictive of disease associations.

It is noteworthy that a reported association between MSSA endocarditis and multilocus sequencing type (MLST) CC30 (10) could not be confirmed in this study, as only 2 of 21 patients with endocarditis had a *spa* type corresponding to MLST CC30.

The frequencies of antibiotic resistance were low, with the exception of penicillin, to which 262 isolates (74.2%) were resistant. Only three isolates were resistant to methicillin (MRSA) (0.8%), and less than 6% of the isolates were resistant to the other antibiotics. The low MRSA frequency was to be expected due to the low prevalence of MRSA bloodstream infections reported in Norway. The overall low prevalence of antibiotic resistance suggests that in our setting SAB isolates do not represent a large reservoir of antibiotic resistance genes.

Our study confirms the large genetic diversity of MSSA bacte-

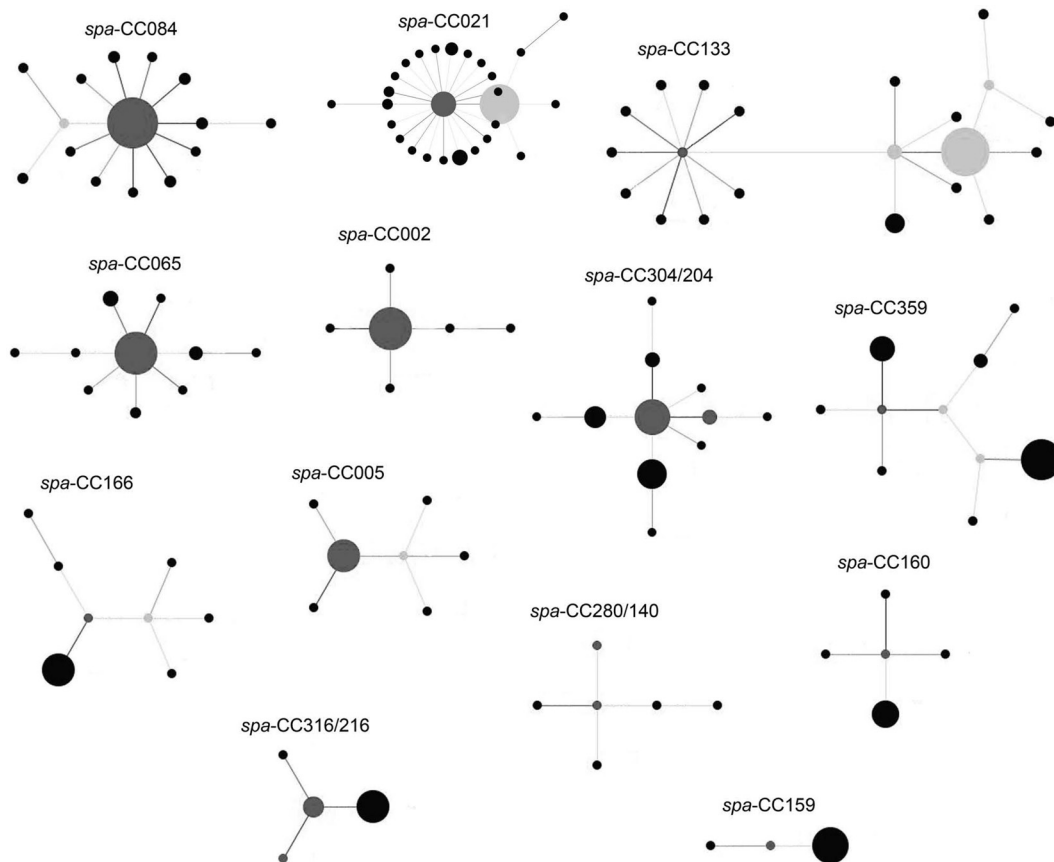


FIG 1 *spa* clonal complexes of the 353 *S. aureus* bacteremia isolates after Based Upon Repeat Pattern (BURP) analysis show genetic heterogeneity. *spa* CCs without founder, singletons, and 20 isolates not fulfilling the BURP analysis criteria (8) are not included in the figure. Clusters of linked isolates correspond to *spa* CCs. The *spa* type with the highest founder score is defined as the founder of the cluster and is marked in dark gray. Light gray indicates subfounders, which are the *spa* types with the second highest founder score. If two *spa* types have an equally high founder score, both are colored dark gray. Only the *spa* CCs are labeled. The size of the circles indicates the number of isolates that were assigned to that *spa* type. Note that the spacing between linked and unlinked *spa* types and *spa* CCs does not provide information about the genetic distance between them. The different clonal complexes consist of the following *spa* types. *spa* CC 021: t012, t017, t018, t019, t021, t037, t046, t122, t238, t318, t342, t486, t582, t584, t726, t840, t869, t1298, t1347, t1414, t2303, t3222, t3611, t4033, t6360, t6361, t6362; *spa* CC 133: t015, t050, t116, t133, t230, t295, t331, t722, t728, t908, t1078, t1081, t1510, t1996, t2519, t3537, t4604, t5025, t6259, t6368, t6414; *spa* CC 084: t084, t085, t120, t279, t328, t346, t491, t774, t853, t11034, t1116, t1492, t1877, t3603, t4714; *spa* CC 304/024: t008, t024, t190, t304, t594, t648, t701, t2842, t5160, t6269, t6363; *spa* CC 065: t004, t040, t065, t123, t130, t330, t370, t715, t880, t6413; *spa* CC 359: t044, t127, t177, t189, t224, t267, t359, t1381, t6367, t6369; *spa* CC 166: t089, t136, t153, t166, t414, t2103, t3631, t5741; *spa* CC 005: t005, t223, t309, t474, t490, t1139, t6265; *spa* CC 002: t002, t010, t045, t067, t214, t1265; *spa* CC 280/140: t140, t167, t258, t280, t1541, t3188; *spa* CC 160: t156, t160, t213, t771, t1137; *spa* CC 316/216: t172, t216, t316, t471; *spa* CC 159: t159, t171, t284.

remias and supports the perception that almost any *S. aureus* strain may cause invasive infections.

ACKNOWLEDGMENTS

We thank the Department of Microbiology and Infection Control at Akershus University Hospital, Norway, for diagnostic analyses of the *S. aureus* isolates and Anwar Kiyamow, Lydia Nyland Lineikro, and Janne Sølværnes for their technical assistance with genotyping analyses.

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