

# Molecular Characterization of Methicillin-Sensitive *Staphylococcus aureus* Isolates from Bacteremic Patients in a Norwegian University Hospital

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***Staphylococcus aureus* bacteremia is common in both nosocomial and community settings, and the pathogenicity of the microbe depends upon a large repertoire of virulence factors. *S. aureus* bacteremia isolates ( $n = 126$ ) were characterized using DNA microarrays. Clonal complexes 5, 8, 15, 30, and 45 accounted for 74.6% of the isolates. We identified geographical differences in dominating clones and toxin gene profiles. One isolate was methicillin resistant. Potential associations between age and genotype were detected.**

*Staphylococcus aureus* bacteremia (SAB) is often associated with increased morbidity and poor outcome (1, 2). The pathogenicity of *S. aureus* depends upon a large repertoire of virulence factors influenced by environmental conditions and host interactions (3). Associations between specific virulence factors and severe invasive infection still remain unclear. Our recent study genotyping SAB isolates (4) demonstrated a knowledge gap regarding molecular epidemiology of methicillin-sensitive *S. aureus* (MSSA) isolates despite the increasing incidence of SAB (5). Here we conducted an extensive characterization of MSSA isolates detected in blood cultures using DNA microarrays in order to determine clonal complex (CC) affiliation and carriage of clinically relevant virulence and antibiotic resistance genes.

Isolates (collected in routine diagnostics) from 126 consecutive SAB patients hospitalized in 2006–2007 at Akershus University Hospital, Lørenskog, Norway, were genotyped as described earlier (6, 7). The complete microarray hybridization data are provided in the supplemental material. Clinical parameters are listed in Table 1. The median age of patients was 65 years (range, 0 to 98), and 54.8% were men. Community-acquired (CA) and hospital-acquired (HA) SABs were defined by a positive blood culture obtained earlier or later than 48 h after hospital admission, respectively. Statistical analysis involved Fisher's exact test, with a  $P$  value of  $<0.05$  considered significant.

Twenty different CCs were detected. Five CCs (CC5, -8, -15, -30, and -45) dominated and accounted for 74.6% of the isolates (Table 1). This genetic diversity supports the perception that virtually any *S. aureus* lineage may cause invasive infection, although some clones potentially are more virulent than others (9, 10). Differences in CC distribution and frequencies were observed in comparing both various geographical origins and sampling sites. CC15 strains were more prevalent (19% versus 2.2% to 13.3%) and CC30 less prevalent (15% versus 19% to 35%) than for SAB isolates from other geographic areas (9, 11, 12) and for nasal carrier isolates and deep surgical site infections (SSI) from the same catchment area (13). The most abundant SAB lineage, CC45, was more common among nasal carriers than in invasive strains in the Netherlands (10, 14) but had a prevalence similar to those of locally found carrier and deep SSI isolates (13). A scarcity of

recombination contributes to the highly clonal structure of *S. aureus*, and the lineage preferences may be due to differences within *S. aureus* distribution geographically or in relation to host characteristics. Local frequency similarities may reflect that a substantial proportion of health care-related SABs are endogenous infections (15).

Overall, the SAB isolates showed low antibiotic resistance except that nearly 80% were penicillin resistant (*blaZ*). CC30 strains were significantly more likely to contain the *blaZ* gene (Table 2). Genes encoding resistance to chloramphenicol (*cat*), tetracyclines (*tetK* and *tetM*), fusidic acid (*far1*), macrolide-lincosamide-streptogramins (*ermA*, *ermC*, *msrA*, *mpbBM*, and *vga*), and aminoglycosides (*aacA-aphD*, *aadD*, and *aphA*) were rare, each occurring in one to four isolates. No clones harbored genes conferring resistance toward vancomycin (*vanA*, *vanB*, and *vanZ*), linezolid (*cfr*), or mupirocin (*mupR*). The same susceptibility pattern was found among other MSSA isolates originating with the same geographic area (13), demonstrating that these isolates do not represent a reservoir of various antibiotic resistance genes. Only one isolate carried *mecA*, encoding methicillin resistance (CC5-MRSA-IV), which is consistent with the low incidence of methicillin-resistant *S. aureus* (MRSA) found in blood cultures nationwide (16).

The toxin gene profiles were highly diverse among the five dominant CCs (Table 2). Panton-Valentine leukocidin (PVL)-encoding genes (*lukF-PV* and *lukS-PV*) were detected in three isolates (2.4%), belonging to CC1, CC22, and CC30. The *tst* gene, encoding toxic shock syndrome toxin 1, found in 20 (15.9%) isolates, was significantly more common in CC30 (Table 2). CC45 and CC15 strains were less likely to carry *tst* (Table 2). All main

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TABLE 1 Distribution of *S. aureus* clonal complexes in patients with bacteremia in relation to clinical parameters

<i>S. aureus</i> CC	No. (%) of patients with CC	No. of patients with condition or characteristic									
		HA infection	CA infection	Diabetes mellitus	Intravascular device <sup>b</sup>	Deep-seated abscess <sup>c</sup>	Endocarditis <sup>d</sup>	Osteomyelitis/ arthritis	All-cause hospital mortality	Under median age 65 yrs	Above median age 65 yrs
CC45	31 (24.6)	13	18	3	3	4	3	4	5	21 <sup>e</sup>	10
CC15	24 (19.0)	14	10	3	7	0	1	1	7	11	13
CC30	19 (15.1)	6	13	2	4	2	0	2	5	7	12
CC5	12 (9.5)	7	5	2	3	1	0	2	2	2	10 <sup>f</sup>
CC8	8 (6.3)	4	4	0	3	0	0	0	1	4	4
Others (15 CCs) <sup>g</sup>	32 (25.4)	14	18	5	11	4	1	3	8	17	15
Total no. (%)	126 (100.0)	58 (46.0)	68 (54.0)	15 (11.9)	31 (24.6)	11 (8.7)	5 (4.0)	12 (9.5)	28 (22.2)	62 (49.2)	64 (50.8)

<sup>a</sup> Others (*n*): CC1 (4), CC6 (1), CC7 (3), CC9 (1), CC12 (4), CC20 (3), CC22 (3), CC25 (3), CC50 (1), CC59 (2), CC97 (2), CC101 (2), CC121 (1), CC188 (1), and CC707 (1).

<sup>b</sup> Including both long-term arterial and venous catheters.

<sup>c</sup> Well-defined abscesses in musculature and inner organs.

<sup>d</sup> Defined according to the modified Duke criteria (8).

<sup>e</sup> 67.7%, versus 43.2% for all other CCs; *P* = 0.023.

<sup>f</sup> 83.3%, versus 47.4% for all other CCs; *P* = 0.030.

CCs, except CC15, carried various *S. aureus* enterotoxin (SE) and enterotoxin-like (SEL) genes. The highest frequency rates were seen for the enterotoxin gene cluster, *egc* (*seg*, *sei*, *sem*, *sen*, *seo*, and *seu*), with 57.9%, *sea*, with 27.0%, and *sec* and *sel*, with 13.5% (Table 2). The *sea* gene was significantly more prevalent in CC5 and -30 (Table 2). CC45 was more likely to contain the *sec* and *sel* genes (Table 2). The overall picture is similar with previous reports, but with some geographical differences within the lineages. CC45 isolates are found to contain both *tst* and *sea* and to lack *egc*, while CC15 can harbor *tst* and *egc* (11, 17–19). The findings support the hypothesis that the toxins are distributed mainly according to clonal lineages and that transmission is vertical. The absence of toxin genes in C15 may be attributed to the restriction modification system which prevents acquisition of mobile gene elements carrying superantigens by horizontal transfer (17, 20). Geographical toxin profile similarities may indicate that these lineages are evolutionarily old and share conserved genomic structures (21). The assessment of virulence determinants in this study are based only on gene carriage and not quantitative expression of genes or the presence of single nucleotide polymorphisms which may influence the functions of gene products.

Associations between *S. aureus* genotype and patient age have previously been described only to a small extent. Sangvik et al. found a decrease in prevalence of a specific genotype with increasing age of colonized hosts (22). Interestingly, the age preferences among CC5 and CC45 isolates found in this study (Table 1) may indicate a host and microbe matching, where factors in both parties are relevant for interactions and bacterial pathogenesis. Persistent *S. aureus* nasal carriers that tested positive for their original resident strain after inoculation with a mixture of different *S. aureus* strains demonstrated the importance of a good match between host and bacterial factors (23). No significant differences between HA and CA infections were revealed. Analysis of potential associations between bacterial characteristics and clinical manifestations was limited by a lack of comorbidity data and host factors likely to affect outcome.

In summary, our study confirms that a diversity of MSSA clones is responsible for SAB. We identified geographical frequency differences between dominating CCs and between toxin gene profiles within identical bacterial lineages. Interestingly, potential associations between age and genotype were detected. Further investigations to determine the relative role of bacterial, host,

TABLE 2 Frequencies of selected genes within the five dominant *S. aureus* clonal complexes in bacteremia patients

<i>S. aureus</i> CC	No. (%) of patients with CC	Frequency of gene(s) ( <i>n</i> )																											
		<i>agrI</i>	<i>agrII</i>	<i>agrIII</i>	<i>agrIV</i>	<i>cap5</i>	<i>cap8</i>	<i>mecA</i>	<i>blaZ</i>	<i>fosB</i>	<i>lukF-PV, lukS-PV</i>	<i>tst</i>	<i>sea</i> <sup>g</sup>	<i>seb</i>	<i>sec, sel</i>	<i>sed</i>	<i>egc</i>	<i>seh</i>	<i>sej, ser</i>	<i>sek, seq</i>	<i>eta, etb, etd</i>	<i>sak</i>	<i>chp</i>	<i>scn</i>	<i>bbp</i>	<i>cna</i>	<i>ebh</i>	<i>sdrD</i>	
CC45	31 (24.6)	30	0	0	1	0	31	0	23	0 <sup>h</sup>	0	0 <sup>d</sup>	0	1 <sup>b</sup>	15 <sup>j</sup>	0	31	0	1	0	0	31	31	31	29	31	31	31	31
CC15	24 (19.0)	0	24	0	0	0	24	0	22	24	0	0 <sup>e</sup>	0	0	0	0	0	0	0	0	0	0	0	24	24	22	0 <sup>c</sup>	24	23
CC30	19 (15.1)	0	0	19	0	0	19	0	19 <sup>a</sup>	19	1	17 <sup>f</sup>	12 <sup>h</sup>	0	0	0	18	3	0	0	0	17	15	17	18	16 <sup>c</sup>	19	19	19
CC5	12 (9.5)	0	12	0	0	12	0	1	6	12	0	0	8 <sup>i</sup>	0	0	3	12	0	2	1	0	12	8 <sup>c</sup>	12	10	0	12	11	
CC8	8 (6.3)	8	0	0	0	8	0	0	5	8	0	1	2	0	1	3	0	0	3	1	0	8	3	7 <sup>c</sup>	8	0	8	6	
Other (15 CCs)	32 (25.4)	20	5	5	2	12	19	0	23	15 <sup>c</sup>	2	2	12	4 <sup>b</sup>	1	0	12	4	0	5	4	28	13 <sup>c</sup>	30	25	16 <sup>k</sup>	27	26	
Total	126 (100)	58	41	24	3	33	93	1	98	78	3	20	34	5	17	6	73	7	6	7	4	96	94	121	112	63	121	116	

<sup>a</sup> 100%, versus 73.8% for all other CCs; *P* = 0.007.

<sup>b</sup> Three isolates with ambiguous results not included.

<sup>c</sup> One isolate with ambiguous results not included.

<sup>d</sup> 0%, versus 21.1% for all other CCs; *P* = 0.003.

<sup>e</sup> 0%, versus 19.6% for all other CCs; *P* = 0.013.

<sup>f</sup> 89.5%, versus 2.8% for all other CCs; *P* < 0.0005.

<sup>g</sup> *sea* analyzed together with *seaN315*.

<sup>h</sup> 63.2%, versus 20.6% for all other CCs; *P* < 0.0005.

<sup>i</sup> 66.7%, versus 22.8% for all other CCs; *P* = 0.003.

<sup>j</sup> 48.4%, versus 1.1% for all other CCs; *P* < 0.0005.

<sup>k</sup> Two isolates with ambiguous results not included.

and environmental factors in the pathogenesis and outcome of SAB are warranted.

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