

Distribution of the Serine-Aspartate Repeat Protein-Encoding *sdr* Genes among Nasal-Carriage and Invasive *Staphylococcus aureus* Strains

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Received 28 September 2005/Returned for modification 29 November 2005/Accepted 6 January 2006

The *sdr* locus was found in all 497 investigated *Staphylococcus aureus* strains, although in 29 strains it contained only the *sdrC* gene (*sdrD* negative, *sdrE* negative). The *sdrC*-positive, *sdrD*-negative, *sdrE*-negative gene profile was exclusive to methicillin-sensitive *S. aureus* (MSSA) strains (Fisher's exact test; $P = 0.0005$) and was not found in the strains collected from bone infections ($P = 0.0019$). We also found a strong association between the presence of the *sdrD* gene and methicillin-resistant *S. aureus* strains ($P < 0.0001$). Our findings suggest that MSSA strains with the newly uncovered *sdrC*-positive, *sdrD*-negative, *sdrE*-negative gene profile have a substantially decreased potential to establish bone infection.

Staphylococcus aureus is an extremely versatile and frequent pathogen of humans both in the community and in hospitals. Infections caused by this organism involve bacterial adhesion to the host extracellular matrix. *S. aureus* adhesins are mostly cell wall-anchored proteins and are grouped into a single family named microbial surface components recognizing adhesive matrix molecules (MSCRAMM).

The Sdr proteins are members of the MSCRAMM family that are encoded by the tandemly arrayed *sdrC*, *sdrD*, and *sdrE* genes, of approximately 2.8, 3.9, and 3.5 kbp, respectively, located in the *sdr* locus (4). The Sdr proteins are members of a family of surface proteins which are characterized by the presence of an R region containing various numbers of the Ser-Asp dipeptides encoded by DNA repeats in the 3' region of the *sdr* genes. The Sdr proteins have a comparable structural organization. A signal peptide is followed by an A domain which is similar in size among the different members of the Sdr family. However, they are not closely related, with only 20 to 30% identical amino acid residues. This suggests that different Sdr proteins have different roles in *S. aureus* pathogenicity. However, a ligand was defined only for Bbp (bone sialo-binding protein), which is an allelic variant of SdrE (12). The Sdr proteins have two, three, or five additional 110- to 113-residue sequences (B motifs) that are tandemly repeated in SdrC, SdrE, and SdrD, respectively. The B repeats bind Ca²⁺ with high affinity, and their structure unfolds when calcium ions are removed. The function of the B domains remains unknown. The B motifs are followed by segments composed of the SD repeats (R region). The C-terminal end (region M) of the Sdr proteins is involved in anchoring the proteins to the bacterial cell wall.

At least two *sdr* genes are present in all tested *S. aureus* strains (4) and always include *sdrC* (8). Therefore, the lack of

sdr genes must be explained by the absence of *sdrD* or *sdrE*. Peacock and colleagues (8) demonstrated a strong correlation between *S. aureus* invasiveness and the presence of one of the allelic variants of the *sdrE* gene. Moreover, Trad et al. (10) reported a significantly higher prevalence of the *sdrD* gene in *S. aureus* strains responsible for bone infections.

S. aureus multiple-locus variable-number tandem-repeat analysis (MLVA) (7, 9) utilizes polymorphism of seven individual genes (*sspA*, *spa*, *sdrC*, *sdrD*, *sdrE*, *clfA*, and *clfB*). During MLVA characterization of *S. aureus* strains, we found several strains possessing only five bands instead of six or seven in a pattern. After analysis using simplex PCRs with primer pairs designed for the MLVA method, we determined that the subset of *S. aureus* strains had only a single gene in the *sdr* locus. Similarities in the DNA sequence flanking the SD repeats of the *sdr* genes allowed for the selection of a single pair of primers for amplification of all three individual genes in the *sdr* locus. It was not possible to determine which of the *sdr* genes is present in the *sdr* locus. Therefore, we designed a novel triplex PCR procedure to examine the distribution of the *sdr* genes among nasal-carriage and invasive *S. aureus* strains as well as methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) (Table 1).

A total of 497 strains (Table 2) were obtained during this study between 1990 and 2005, and these were selected from previously characterized collections (2, 6, 14). Only one isolate per person was tested. In addition, none of these strains were part of an outbreak, and none were determined to be from patients or personnel with obvious cross-infection. All strains in the collections were characterized by at least one genome typing method, including pulsed-field gel electrophoresis, multilocus sequence typing, and MLVA. The strains showed a large degree of diversity, and not a single clonal type was overrepresented (data not shown).

DNA was extracted with the Genomic DNA Prep Plus kit (A&A Biotechnology, Gdynia, Poland) or the MagnaPure LC

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TABLE 1. Primers used in this study^a

Gene	Primer	Nucleotide sequence	Cycling conditions ^b
<i>sdrC</i>	sdrCF	5'-AAAAGGCATGATACCAAATCGA	Den, 94°C/30 s; Ann, 53°C/30 s; Elon, 72°C/15 s
	sdrCR	5'-AATTCTCCATTCGTATGTTCTG	
<i>sdrD</i>	sdrDF	5'-AGTGGGAACAGCATCAATTTTA	
	sdrDR	5'-GTGGTAGATTGTACACTTTCTT	
<i>sdrE</i>	sdrEF	5'-AGAAAGTATACTGTAGGAACTG	
	sdrER	5'-GATGGTTTTGTAGTTACATCGT	
Azoreductase Putative glycosyltransferase	azoF glyR	5'-GTGAATAATAG(C/T)CCATATGAAC 5'-CTACACCTGTAATTTTACTTTC	Den, 94°C/30 s; Ann, 50°C/30 s; Elon, 72°C/1 min

^a The primers were designed based on *S. aureus* genomes available from the Institute for Genomic Research (<http://www.tigr.org>) (COL strain), the University of Oklahoma (<http://www.genome.ou.edu>) (strain 8325), the Sanger Institute (<http://www.sanger.ac.uk>) (strains MRSA252 and MSSA476), the National Institute of Technology and Evaluation (<http://www.bio.nite.go.jp>) (strain MW2), and Juntendo University (<http://www.juntendo.ac.jp>) (strains Mu50 and N315).

^b Den, denaturation temperature and time; Ann, annealing temperature and time; Elon, elongation temperature and time.

DNA system (DNA isolation kit III; Roche, Almere, The Netherlands) as previously described (9, 13). The triplex PCR products specific in size for *sdrC* (144 bp), for *sdrD* (272 bp), and for *sdrE* (433 bp) were analyzed by electrophoresis in 2% agarose SeaKem LE gels (FMC). The two-tailed Fisher exact test was used to analyze the distribution of the *sdr* genes among *S. aureus* strains originating from different hosts. *P* values less than 0.05 were considered a statistically significant difference.

The *sdrC* gene was present in all investigated strains ($n = 497$), which was concordant with a previous report (8). However, in 29 MSSA strains (of the total 382 MSSA strains), only the *sdrC* gene (*sdrD* negative, *sdrE* negative) was found in the *sdr* locus. The result was confirmed by PCR amplification of a sequence covering the *sdr* locus, using primer pair azoF and glyR (Table 1), targeting the sequences in flanking genes encoding azoreductase (assigned as SACOL0607 in the COL genome) and putative glycosyltransferase (SACOL0611). The amplicon sizes around 3.5 kbp confirmed the presence of only a single *sdr* gene in the *sdr* locus (data not shown). Two or three *sdr* genes were always detected in all MRSA strains ($n = 115$). A significant association between the *sdrC*-positive, *sdrD*-negative, *sdrE*-negative gene profile and MSSA strains was found (29/353 versus 0/115; Fisher's exact test; $P = 0.0005$). The strains with only the *sdrC* gene in the *sdr* locus represented different sequence types (STs) defined by multilocus sequence typing (Table 3). In the same STs we found strains with different combinations of the genes in the *sdr* locus, suggesting a high degree of variability. In contrast, the *sdrD* and *sdrE* genes were heterogeneously distributed. Among the tested strains, *sdrD* was significantly associated with MRSA strains (183/199 versus 114/1; Fisher's exact test; $P < 0.0001$), whereas the *sdrE* distribution did not differ between the MSSA and MRSA strains (344/38 versus 101/14; Fisher's exact test; $P = 0.4898$).

We checked the distribution of the *sdr* genes between the strains collected from nose swabs of asymptomatic carriers ($n = 259$) and invasive infections, osteomyelitis ($n = 89$), and blood infections ($n = 52$). The strains possessing only the *sdrC* gene in the *sdr* locus were not found in the strains collected from bone infections (23/236 versus 0/89; Fisher's exact test; $P = 0.0019$). This indicates strongly that the carriage strains with only the *sdrC* gene have abolished or substantially decreased the potential to establish bone infection. In contrast to

osteomyelitis, the *sdrC*-positive, *sdrD*-negative, *sdrE*-negative gene profile was found in the strains isolated from blood infections (23/236 versus 6/46; Fisher's exact test; $P = 0.6001$). There was no significant difference between the strains associated with nasal colonization and those associated with both types of infections ($P > 0.05$). However, the *sdrD* gene was significantly associated with osteomyelitis (112/147 versus 74/15; Fisher's exact test; $P < 0.0001$) but not with blood infections (112/147 versus 23/29; Fisher's exact test; $P = 1.0$). There was no significant correlation of *sdrE* with blood infections (231/28 versus 44/8; Fisher's exact test; $P = 0.3457$) and osteomyelitis (231/28 versus 79/10; Fisher's exact test; $P = 1.0$).

In previous investigations a contribution to the pathogenic process of the allelic variants (*sdrE/bbp*) of the *sdrE* gene was explored. Peacock and colleagues (8) have shown a possibility that one (*sdrE*) of the allelic variants of the *sdrE* gene is associated with invasive disease while another (*bbp*) is evenly distributed among isolates recovered from healthy individuals and from patients with invasive *S. aureus* disease. Tristan and colleagues (11) have investigated only one of the allelic variants of the *sdrE* gene, and they have revealed that the distribution of *bbp* is significantly associated with osteomyelitis/arthritis. There was no difference in the incidence of *bbp* when carriage-associated strains were compared to invasive isolates. During our investigations we designed the primers which had sequences corresponding to regions of both allelic variants of the *sdrE* gene. Therefore, our results strengthen the observation of Peacock et al. that the allelic variants at a given locus may have different contributions to the pathogenic process. Our data are also concordant with the studies by Trad et al. (10) in which a significantly higher prevalence of only the *sdrD* gene and not the *sdrE* gene in bone infections has been found. The sequence alignments of the *sdrC* and *sdrD* genes (sequences obtained from the *S. aureus* genomes for which web-sites are given in Table 1) show that their polymorphism level is comparable to that of the *sdrE* gene (data not shown). It is very important to address future studies to determine sequence variability of the *sdr* genes and on the basis of their polymorphism to investigate the pathogenic potential of allelic variants of the *sdrC*, *sdrD*, and *sdrE* genes.

It is not clear why *sdrC* alone seems to be limited to MSSA strains only. This might be a reflection of the fact that MRSA

TABLE 2. Distribution of *sdhD* and *sdhE* among *S. aureus* strain collections used in this study

Source of isolation	No. of strains	No. of strains carrying:						Geographic origin(s)	Reference(s)
		<i>sdhD</i>	<i>sdhE</i>	<i>sdhD</i> and <i>sdhE</i>	<i>sdhD</i> but not <i>sdhE</i>	<i>sdhE</i> but not <i>sdhD</i>	Neither <i>sdhD</i> nor <i>sdhE</i>		
Community-acquired strains from nasal swabs of asymptomatic carriers	116 MSSA 1 MRSA	53 1	99 1	51 1	2 0	48 0	15 0	United Kingdom	2
Community-acquired strains from nasal swabs of asymptomatic carriers	111 MSSA	43	104	40	3	64	4	Holland	14
Hospital-acquired strains collected from blood infections	52 MSSA	23	44	21	2	23	6	Holland	14
Hospital-acquired strains derived from a variety of noninvasive human infections	12 MSSA 85 MRSA	3 85	12 79	3 79	0 6	9 0	0 0	Bulgaria, Czech Republic, Croatia, England, Germany, Lithuania, Poland, Russia, Slovenia, Turkey	6 and this study
Community-acquired strains from nasal swabs of asymptomatic carriers	31 MSSA	15	27	15	0	12	4	Poland	This study
Community-acquired strains collected from osteomyelitis	60 MSSA 29 MRSA	46 28	58 21	44 20	2 8	14 1	0 0	Poland	This study
Summary	382 MSSA 115 MRSA	183 114	344 101	174 100	9 14	170 1	29 0		
Total	497 MSSA and MRSA	297	445	274	23	171	29		

TABLE 3. Distribution of the *sdrC*-positive, *sdrD*-negative, *sdrE*-negative gene profile among sequence types defined by multilocus sequence typing

ST (allelic profile)	No. of strains	Source of isolation
1 (1, 1, 1, 1, 1, 1, 1)	3	Nasal carriage
12 (1, 3, 1, 8, 11, 5, 11)	2	Nasal carriage (<i>n</i> = 1) Blood infection (<i>n</i> = 1)
25 (4, 1, 4, 1, 5, 5, 4)	2	Nasal carriage
30 (2, 2, 2, 2, 6, 3, 2)	6	Nasal carriage (<i>n</i> = 2) Blood infection (<i>n</i> = 4)
34 (8, 2, 2, 2, 6, 3, 2)	3	Nasal carriage
39 (2, 2, 2, 2, 2, 2, 2)	2	Nasal carriage (<i>n</i> = 1) Blood infection (<i>n</i> = 1)
45 (10, 14, 8, 6, 10, 3, 2)	2	Nasal carriage (<i>n</i> = 1) Blood infection (<i>n</i> = 1)
47 (10, 11, 8, 6, 10, 3, 2)	3	Nasal carriage
182 (18, 18, 6, 2, 13, 15, 18)	2	Nasal carriage
New-1 (3, 1, 31, 1, 29, 5, 3)	1	Nasal carriage
New-2 (3, 51, 1, 1, 4, 4, 3)	3	Nasal carriage

primarily consists of a limited number of highly successful pandemic clones. Katayama and colleagues (5) have demonstrated that genetic background profoundly influences the stability of *mecA* in *S. aureus*. We can only hypothesize now that the same genetic mechanisms could play a role in acquisition and stability of *SCCmec* and the *sdr* genes as well as other genetic elements.

Most infections caused by *S. aureus* result from the combined action of a variety of factors. However, the contribution of particular virulence factors to *S. aureus* pathogenicity in humans is poorly understood. The results obtained in experimental models suggest that *S. aureus* strains producing receptors for bone sialoprotein, collagen, and fibronectin are associated with osteomyelitis and arthritis (1, 3, 12). However, our results show that strains lacking the *sdrD* and *sdrE* genes have decreased potential to infect bones. Studies in which strains with knockouts of *sdrD* and/or *sdrE* are used in experimental models should be performed to cast light on the role of these genes in osteomyelitis. Furthermore, studies to investigate the differential distribution of genes encoding virulence factors in a larger number of *sdrD*- and/or *sdrE*-positive strains and strains lacking both *sdrD* and *sdrE* are needed. The understanding of specific pathogenetic mechanisms may have an important prophylactic and therapeutic impact. The results obtained during this study suggest that both *sdrD* and *sdrE* may play comparable and important roles in bone infections. Strains lacking the *sdrD* gene had also potential to give rise to osteomyelitis, but then they always possessed the *sdrE* gene. These findings show that most strains are capable of bone infections. For adequate and cost-effective infection prevention, it is important to distinguish nasal colonizers which may

be more aggressive from those with abolished or substantially decreased potential to establish an invasive infection.

We thank Sabina Grzeszczuk (National Institute of Public Health, Warsaw, Poland) for assistance during the course of this study.

This work was supported by grant 2 P04A 001 27 (to A.S. and W.H.) and Mikrobank (to W.H.) from the Polish Ministry of Scientific Research and Information Technology, State Committee for Scientific Research.

REFERENCES

- Elasri, M. O., J. R. Thomas, R. A. Skinner, J. S. Blevins, K. E. Beenken, C. L. Nelson, and M. S. Smelter. 2002. *Staphylococcus aureus* collagen adhesin contributes to the pathogenesis of osteomyelitis. *Bone* **30**:275–280.
- Grundmann, H., S. Hori, M. C. Enright, C. Webster, A. Tami, E. J. Feil, and T. Pitt. 2002. Determining the genetic structure of the natural population of *Staphylococcus aureus*: a comparison of multilocus sequence typing with pulsed-field gel electrophoresis, randomly amplified polymorphic DNA analysis, and phage typing. *J. Clin. Microbiol.* **40**:4544–4546.
- Johansson, A., J. I. Flock, and O. Svensson. 2001. Collagen and fibronectin binding in experimental staphylococcal osteomyelitis. *Clin. Orthop. Relat. Res.* **382**:241–246.
- Josefsson, E., K. W. McCrea, D. Ni Eidhin, D. O'Connell, J. Cox, M. Hook, and T. J. Foster. 1998. Three new members of the serine-aspartate repeat protein multigene family of *Staphylococcus aureus*. *Microbiology* **144**:3387–3395.
- Katayama, Y., D. A. Robinson, M. C. Enright, and H. F. Chambers. 2005. Genetic background affects stability of *mecA* in *Staphylococcus aureus*. *J. Clin. Microbiol.* **43**:2380–2383.
- Krzyszton-Russjan, J., J. Empel, T. Leski, M. Gniadkowski, and W. Hryniewicz. 2005. Clonal structure of the methicillin-resistant *Staphylococcus aureus* (MRSA) population in Poland: revision and update. *Microb. Drug Resist.* **11**:127–136.
- Malachowa, N., A. Sabat, M. Gniadkowski, J. Krzyszton-Russjan, J. Empel, J. Miedzobrodzki, K. Kosowska-Shick, P. C. Appelbaum, and W. Hryniewicz. 2005. Comparison of multiple-locus variable-number tandem repeat analysis with pulsed-field gel electrophoresis, *spa* typing, and multilocus sequence typing for clonal characterization of *Staphylococcus aureus* isolates. *J. Clin. Microbiol.* **43**:3095–3100.
- Peacock, S. J., C. E. Moore, A. Justice, M. Kantzanou, L. Story, K. Mackie, G. O'Neill, and N. P. Day. 2002. Virulent combinations of adhesin and toxin genes in natural populations of *Staphylococcus aureus*. *Infect. Immun.* **70**:4987–4996.
- Sabat, A., J. Krzyszton-Russjan, W. Strzalka, R. Filipek, K. Kosowska, W. Hryniewicz, J. Travis, and J. Potempa. 2003. New method for typing *Staphylococcus aureus* strains: multiple-locus variable-number tandem repeat analysis of polymorphism and genetic relationships of clinical isolates. *J. Clin. Microbiol.* **41**:1801–1804.
- Trad, S., J. Allignet, L. Frangeul, M. Davi, M. Vergassola, E. Couve, A. Morvan, A. Kechrid, C. Buchrieser, P. Glaser, and N. El Solh. 2004. DNA microarray for identification and typing of *Staphylococcus aureus* isolates. *J. Clin. Microbiol.* **42**:2054–2064.
- Tristan, A., L. Ying, M. Bes, J. Etienne, F. Vandenesch, and G. Lina. 2003. Use of multiplex PCR to identify *Staphylococcus aureus* adhesins involved in human hematogenous infections. *J. Clin. Microbiol.* **41**:4465–4467.
- Tung, H., B. Guss, U. Hellman, L. Persson, K. Rubin, and C. Ryden. 2000. A bone sialoprotein-binding protein from *Staphylococcus aureus*: a member of the staphylococcal Sdr family. *Biochem. J.* **345**:611–619.
- van Leeuwen, W. B., D. C. Melles, A. Alaidan, M. Al-Ahdal, H. A. Boelens, S. V. Snijders, H. Wertheim, E. van Duijkeren, J. K. Peeters, P. J. van der Spek, R. Gorkink, G. Simons, H. A. Verbrugh, and A. van Belkum. 2005. Host- and tissue-specific pathogenic traits of *Staphylococcus aureus*. *J. Bacteriol.* **187**:4584–4591.
- Wertheim, H. F., M. C. Vos, A. Ott, A. Voss, J. A. Kluytmans, C. M. Vandenbroucke-Grauls, M. H. Meester, P. H. van Keulen, and H. A. Verbrugh. 2004. Mupirocin prophylaxis against nosocomial *Staphylococcus aureus* infections in nonsurgical patients: a randomized study. *Ann. Intern. Med.* **140**:419–425.