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1        **Tandem repeat sequence analysis of staphylococcal protein A**  
2        **(*spa*) gene in methicillin-resistant *Staphylococcus pseudintermedius***

3

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12

13        **Running title:** Methicillin resistance in *Staphylococcus pseudintermedius*

14

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18 **Abstract**

19 A putative staphylococcal protein A (*spa*) gene was discovered in the genome of *S.*  
20 *pseudintermedius* and used for developing a species-specific *spa* typing protocol. Thirty-one  
21 clinical methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolates from dogs and  
22 cats in four countries were characterized by *spa* typing, pulsed-field gel electrophoresis  
23 (PFGE) and staphylococcal cassette chromosome (*SCCmec*) typing. The results indicated the  
24 occurrence of two MRSP clones that acquired distinct *SCCmec* elements in Europe (t02,  
25 PFGE type A, *SCCmec* type III,) and California (t06, PFGE type B, *SCCmec* type V).  
26 Sequence analysis of *mecA* revealed the occurrence of four alleles (*mecA1* to *mecA4*), which  
27 correlated with the geographical origin of the isolates and enabled discrimination of two  
28 distinct subtypes within European clone. The newly developed *spa* typing method appeared to  
29 be a promising tool for easy and rapid typing of MRSP, either alone or in combination with  
30 *SCCmec* and *mecA* typing for fine-structure epidemiological analysis.

31

32 **Keywords:** *S. intermedius*, antibiotic resistance, epidemiology

### 33 **Introduction**

34 *Staphylococcus intermedius* is a commensal bacterium found on the skin and mucosae of a  
35 wide range of animals, mainly dogs, cats, pigeons and horses. This species is the most  
36 common bacterial pathogen associated with pyoderma and otitis in dogs, and can cause  
37 sporadic infections in humans. Two independent studies have recently proposed to reclassify  
38 *S. intermedius* isolated from dogs, cats and humans as *S. pseudintermedius* based on DNA-  
39 DNA hybridization (Sasaki et al. 2007a) and multi-locus sequence typing (MLST) (Bannoehr  
40 et al. 2007). Accordingly, the strains described in this study are referred to as *S.*  
41 *pseudintermedius*.

42 Various studies have reported the occurrence of methicillin-resistant *S.*  
43 *pseudintermedius* (MRSP) in small animal infections in North America (Gortel et al., 1999;  
44 Jones et al., 2007; Hanselman et al., 2008) and more recently in Europe (Vengust et al., 2006;  
45 Loeffler et al., 2007; Zubeir et al., 2007; Descloux et al., 2008; Schwarz et al., 2008; van  
46 Duijkeren et al., 2008). Similarly to for methicillin-resistant *S. aureus* (MRSA), methicillin  
47 resistance in *S. pseudintermedius* is mediated by *mecA* which encodes a low-affinity penicillin  
48 binding protein (PBP2a). MRSP infections usually occur in the form of hospital outbreaks  
49 associated with epidemic clones and can be resistant to all oral antimicrobial formulations  
50 available for therapy in small animal medicine (Bengtsson et al., 2007; Loeffler et al., 2007;  
51 van Duijkeren et al., 2008).

52 There are no standard protocols for molecular typing of *S. pseudintermedius* and the  
53 recent emergence of methicillin-resistant strains has increased the need for a simple and rapid  
54 method for typing this species. This study describes a novel single-locus typing method based  
55 on tandem repeat sequence analysis of a putative staphylococcal protein A (*spa*) gene in *S.*  
56 *pseudintermedius*. The method was tested on an international collection of 31 MRSP isolates  
57 in combination with current methods for molecular typing of MRSA.

## 58 **Materials and Methods**

### 59 *Strains*

60 The 31 clinical MRSP isolates analyzed in this study originated from Germany, Sweden, UK  
61 and USA (Table 1). German isolates were isolated from skin and ear infections of dogs and  
62 cats attending a dermatology referral practice in Hamburg (Loeffler et al., 2007). Swedish  
63 isolates were mostly (n=12) isolated from post-operative infections at four different veterinary  
64 clinics (Bengtsson et al., 2007). Canine isolates from the USA were isolated from cases of  
65 community-acquired skin infections at one clinic in California. The UK strain was isolated  
66 from a canine post-operative wound infection. All isolates were coagulase-positive, mannitol-  
67 negative, haemolytic, *mecA*-positive and resistant to oxacillin (MIC  $\geq$ 254 mg/L) with the only  
68 exception of Swedish isolate S1, which was susceptible to oxacillin (MIC = 0.75 mg/L)  
69 despite the presence of *mecA*. A subset of 15 isolates was previously analyzed by MLST  
70 (Bannoehr et al. 2007).

### 71 *Sequence analysis of spa*

72 The sequence of *spa* in *S. pseudintermedius* (Accession number) was obtained from the  
73 methicillin-susceptible reference strain ED99 (Bannoehr et al. 2007). Interproscan  
74 (<http://www.ebi.ac.uk/InterProScan/>) was used for primary protein structure analysis and  
75 comparison with other conserved protein domains. BLAST sequence alignment tool  
76 (<http://www.ncbi.nlm.nih.gov/blast/>) and Align X (Vector NTI) were used to study nucleotide  
77 and amino acid sequence similarity between *S. pseudintermedius* and *S. aureus*.

### 78 *Design, performance and validation of spa typing*

79 Primers were designed for amplification and sequencing of the variable region X of *spa* in *S.*  
80 *pseudintermedius*: SIspa F (5'-AACCTGCGCCAAGTTTCGATGAAG-3') and SIspaR (5'-  
81 CGTGGTTTGCTTTAGCTTCTTGGC-3'). PCR was performed in a final volume of 50  $\mu$ L  
82 containing of 5  $\mu$ L of crude lysate, 0.5  $\mu$ M of each primer, 5  $\mu$ L of 10-fold concentrated PCR

83 Buffer without MgCl<sub>2</sub> (Applied Biosystems, California, USA), 2.5 mM MgCl<sub>2</sub>, 200 μM  
84 deoxynucleoside triphosphates, and 1.25 U of AmpliTaq DNA polymerase (Applied  
85 Biosystems, USA). Thermal cycling reactions consisted of an initial denaturation (10 min at  
86 95°C) followed by 30 cycles of denaturation (30 s at 95°C), annealing (30 s at 58°C), and  
87 extension (60 s at 72°C), with a single final extension (10 min at 72°C). PCR products were  
88 purified using the Nucleospin Extract II kit (Macherey-Nagel, Germany). DNA sequencing  
89 was performed by Macrogen Inc (Seoul, South Korea) using BigDye (Applied Biosystems) on  
90 a 3730xl DNA Analyzer (Applied Biosystems). Forward and reverse sequences were aligned  
91 using Align X (Vector NTI) and tandem repeat sequences were identified visually by  
92 matching to the repetitive unit motifs shown in Table 2. A numeric code was given to each  
93 unique repeat sequence and *spa* types were designated numerically depending on the  
94 composition and order of repeats. Eight randomly selected isolates were subcultured daily for  
95 10 days and sequencing of region X was repeated on days 0 and 10 in order to assess stability  
96 and reproducibility of *spa* typing.

### 97 **PFGE**

98 PFGE was performed using the protocol described by Murchan et al. (2003) with minor  
99 modifications. Briefly, colonies from overnight cultures were incorporated into 1.6% (w/v)  
100 agarose plugs. After 18 h lysis with lysostaphin (10 mg/L, Sigma-Aldrich, Germany) and  
101 RNase (50 mg/L, Sigma-Aldrich) at 37°C, plugs were incubated with proteinase K (20 mg/L,  
102 Sigma-Aldrich) overnight at 56°C. A 1 mm thick segment from the agarose plug was digested  
103 with *Sma*I (20 U/mL, New England Biolabs, UK) for 4 h at 25°C. PFGE was performed by  
104 clamped homogenous electric field (CHEF) electrophoresis with a CHEF-DR III System (Bio-  
105 Rad Laboratories, USA) in a 0.8% (w/v) agarose gel with a pulsing switch time of 5 to 35 s at  
106 6 V cm<sup>-1</sup> for 20 h. Gels were analyzed using Gelcompar II (Applied Maths, Belgium), and  
107 cluster analysis was performed by UPGMA based on the Dice similarity coefficient, with

108 optimization and position tolerance set at 1.5% and 2.5%, respectively. MRSP isolates were  
109 clustered using an 80% homology cut-off, above which strains were considered to be related  
110 and assigned to the same PFGE type (A, B, or C). Isolates showing minor band differences  
111 within the same PFGE type were assigned to distinct subtypes (A1-A7, B1-B3).

#### 112 *SCCmec typing and mecA sequence analysis*

113 *SCCmec* types were determined using the multiplex PCR described by Kondo et al. (2007).  
114 Only M-PCR 1 and 2 were performed in order to assign *SCCmec* types based on the type of  
115 *ccr* and *mec* elements. *mecA* sequencing was performed using primers previously described by  
116 Malik et al. (2006) and the sequences were analyzed using AlignX (Vector NTI Advance 10,  
117 Invitrogen, USA).

#### 118 *GeneBank accession numbers*

119 Novel nucleotide sequences were submitted to GeneBank and assigned the following  
120 accession numbers: EU930814 (*spa* in *S. pseudintermedius* ED99), EU929079 (*mecA1*),  
121 EU929080 (*mecA2*), EU929081 (*mecA3*) and EU929082 (*mecA4*).

## 123 **Results**

124 The *spa* gene in *S. pseudintermedius* ED99 was 1389 bp long and had 68% nucleotide and  
125 55% predicted amino acid identity to the homologous gene in the *S. aureus* reference strain  
126 8325-4 where protein A was originally described (Genbank accession code J01786). The  
127 predicted protein contained a number of functional regions and conserved domains previously  
128 described in *S. aureus*, including four immunoglobulin G (IgG)-binding domains and a  
129 polymorphic X-region (Figure 1a). Lack of IgG-binding domain region B (58 aa) partly  
130 accounted for the shorter protein in *S. pseudintermedius* ED99 (Figure 1b).

131 All MRSP isolates tested yielded *spa* PCR products (100% typeability). Sequencing of  
132 the PCR products showed the presence of variable numbers (between 6 and 10) of 30 bp



133 tandem repeats, resulting in the identification of 13 repeats (Table 2) and nine *spa* types  
134 (Tables 3) were identified, with *spa* type t01 being assigned to the methicillin-susceptible  
135 strain used for designing the primers. The same *spa* type (t02) was found in most isolates from  
136 Germany (9/12) and Sweden (8/14). Other *spa* types observed among isolates from these two  
137 countries (t03, t04, t05 and t08) were closely related to t02 and could have arisen from single  
138 genetic events, i.e. one point mutation in either the 7<sup>th</sup> codon of the last repeat (t03 and t04) or  
139 the 4<sup>th</sup> codon in the 3<sup>rd</sup> repeat (t08), or a duplication of repeat r03 (t05). All four canine  
140 isolates from the USA belonged to *spa* type t06. Singleton *spa* types were found in the  
141 Swedish isolate displaying oxacillin susceptibility (t07) and in the UK isolate (t09). The *spa*  
142 sequences remained unchanged following repeated repeated subculture over 10 days,  
143 indicating good *in vitro* stability.

144 Only twenty-one isolates were typeable by *Sma*I-PFGE (68% typeability), resulting in  
145 the detection of three PFGE types (A, B, and C) that were further subdivided into 10 subtypes  
146 (A1 to A7, B1 to B3) (Table 1, Figure 2). The PFGE results correlated with those obtained by  
147 *spa* typing. PFGE type A grouped European isolates belonging to t02-related *spa* types, while  
148 PFGE types B and C corresponded to *spa* types t06 and 07. Indistinguishable PFGE patterns  
149 were detected among isolates originating from different dogs and veterinary clinics in  
150 Germany (A4, A6 and A7), Sweden (A1), and USA (B3) (Figure 2).

151 All American isolates harbored SCC*mec* type V, whereas all European isolates  
152 contained SCC*mec* type III, except Swedish isolate 13 which displayed a combination of  
153 SCC*mec* I-II-IV cassette: type B *mec* complex (found in SCC*mec* type I and IV cassettes) and  
154 2 recombinases: *ccrA2-ccrB* (SCC*mec* II) and *ccrA4-ccrB4* (SCC*mec* IV). UK strain 3279  
155 was non-typeable and probably a novel SCC*mec* type. Four *mecA* alleles (*mecA1* to *mecA4*)  
156 showing 99% similarity to each other were detected (Table 4). The four *mecA* alleles were not  
157 associated to specific SCC*mec* elements as indicated by the finding of two distinct *mecA*

158 alleles (*mecA2* and *mec3*) within SCC*mec* type III (Table 1). The *mecA* sequences were  
159 strongly associated with the geographical origin of the isolates: *mecA1* occurred in all US  
160 isolates, *mecA2* in all Swedish isolates, *mecA3* in all Germany isolates, and *mecA4* in the UK  
161 strain. The *mecA* sequences in *S. pseudintermedius* had 99% identity to the homologous gene  
162 in MRSA Mu50 (NP 370565).

163

## 164 **Discussion**

165 A species-specific *spa* typing protocol was developed following the discovery of a  
166 staphylococcal protein A homolog in the genome of *S. pseudintermedius*. This single-locus  
167 sequence-based approach is based on the same principles used for *spa* typing of *S. aureus*  
168 (Koreen et al., 2004) and has gradually replaced PFGE for MRSA typing at national reference  
169 laboratories. The results of our study indicate that *spa* typing is a valuable tool for  
170 epidemiological surveillance of MRSP. Various DNA-based techniques have previously been  
171 used for typing *S. pseudintermedius*, including ribotyping (Hesselbarth et al., 1995), PFGE  
172 (Shimizu et al., 1996) and intergenic ribosomal DNA spacer polymorphism (Bes et al., 2002).  
173 However, such band-based methods do not facilitate communication of results between  
174 laboratories and therefore are not suitable for long-term epidemiological surveillance of  
175 MRSP at the national or international level. In addition, PFGE, which is the method most  
176 commonly used for typing of *S. pseudintermedius*, is laborious and no standardized protocol is  
177 available for this species.

178 Both PFGE and *spa* typing resulted in higher discriminatory power than MLST as they  
179 were able to discriminate between isolates belonging to the same ST (Table 1). The sequence  
180 variation in the variable region X in *S. pseudintermedius* consisted of duplications or deletions  
181 of whole repeats or point mutations within repeats. The region was shown to be stable  
182 following repeated subculture over ten days, suggesting that the genetic diversification rate is

183 relatively low but sufficient for discrimination between epidemiologically-related MRSP  
184 isolates. Typeability was higher for *spa* typing but the low typeability observed with PFGE  
185 could be attributable to the specific protocol used in this study. More importantly, *spa* typing  
186 is less time-consuming than PFGE and greatly simplifies communication between  
187 laboratories. One major advantage of *spa* typing over PFGE is the possibility to collect and  
188 harmonize data in a database freely accessible to internet users. This step will first require the  
189 development of software enabling automatic identification of repeats and assignment to *spa*  
190 types, similarly to those currently available for *S. aureus spa* typing (Ridom StaphType<sup>M</sup> and  
191 Bionumerics<sup>TM</sup>).

192         The main limitation of this study was the low number of MRSP isolates tested. The  
193 *spa* typing protocol developed in this study needs to be further validated and implemented  
194 using larger collections of MRSP isolates. Methicillin-susceptible isolates should be included  
195 to test the usefulness of the method for epidemiological studies of *S. pseudintermedius*.  
196 Preliminary data generated at this laboratory indicate that the typeability rate may be lower  
197 (58%) among methicillin-susceptible isolates (data not shown). The *spa* gene might not be  
198 present in all members of this species, or at least not detectable using the primers described in  
199 this study. Since the primers were designed based on the *spa* sequence of a single methicillin-  
200 susceptible strain, further investigation of the distribution and sequence variation of *spa* in *S.*  
201 *pseudintermedius* is needed for optimizing this typing method. Even if the low typeability rate  
202 observed in methicillin-susceptible isolates was confirmed, *spa* typing could still represent a  
203 valid tool for rapid identification of MRSP clones.

204         Broad geographic dissemination of successful MRSP genetic lineages was previously  
205 hypothesized by MLST analysis (Bannoehr et al., 2007), resolving into two predominant  
206 sequence types (ST) in Northern Europe (ST71) and in the USA (ST68). The data generated  
207 by our study provides further information on inter- and intracolonial diversity of these two

208 MRSP lineages. A single *spa* type (t06) and three PFGE subtypes (B1 to B3) were detected  
209 among the four American isolates belonging to ST68. Multiple *spa* types (t02, t03, t04, t05,  
210 and t09) and PFGE subtypes (A1 to A7) were identified within the European clone ST71. The  
211 *spa* types associated with a given PFGE type showed similar composition and organization of  
212 repeats. For example, the five *spa* types associated with PFGE type A differed primarily by  
213 single point mutations and their diversity could be explained by possible microevolution of  
214 t02, resulting in the development of the other four *spa* types. The same *spa* type (t02) was  
215 found in epidemiologically unrelated dogs attending veterinary clinics in Hamburg and in two  
216 Swedish cities located 135 km apart, indicating intra- and inter-country clonal spread of an  
217 epidemic MRSP clone. The same clone has recently been reported among clinical MRSP  
218 isolates in Switzerland (Descloux et al., 2008) and subsequently identified as *spa* type t02  
219 (Vincent Perreten, personal communication).

220 ST71 and ST68 contained distinct SCC*mec* elements, type III and type V, respectively.  
221 The same two SCC*mec* types were previously reported in MRSP occurring at a veterinary  
222 teaching clinic in Japan (Sasaki et al., 2007b). A hybrid SCC*mec* type II-III was reported by  
223 Descloux et al. (2008) among Swiss ST71 isolates but this SCC*mec* element would have been  
224 classified as type III (class A *mec* complex and *ccrAB3* recombinase) according to the criteria  
225 used in the present study. SCC*mec* type III was also described in a MRSP isolate from human  
226 bloodstream infection in Italy but no information was available on the genetic lineage of this  
227 human isolate (Campanile et al., 2007). The four observed *mecA* alleles were associated with  
228 the geographical origin of the isolates rather than with the SCC*mec* type containing the  
229 resistance gene. The fact that two distinct *mecA* alleles (*mecA2* and *mecA3*) were observed  
230 within the same clonal background and SCC*mec* type (ST71, SCC*mec* III) suggests that *mecA*  
231 polymorphism analysis could be used for high-resolution subtyping of MRSP clones.

232 This is the first study describing the presence of a staphylococcal protein A homolog  
233 in *S. pseudintermedius*. Based on the similar sequence and organization, we hypothesize that  
234 the putative *S. pseudintermedius* protein could be surface-associated with related functions,  
235 i.e. inhibition of opsonization and phagocytosis by binding to the Fc-portion of IgG. The  
236 predicted protein was shorter than in *S. aureus* reference strain 8325-4 due to lack of the IgG-  
237 binding domain B. Uhlén et al. (1984) reported that *S. aureus* can contain four to five  
238 homologous IgG-binding domains. Deletion of an IgG-binding domain seems to be common  
239 in *S. aureus* as it was observed in five out of 11 protein A sequences deposited in Genbank  
240 (data not shown).

241 In conclusion, *spa* typing showed to be a useful tool to trace dissemination of the  
242 epidemic MRSP clone spreading in Europe and more in general a promising method for easy  
243 and rapid typing of MRSP, either alone or in combination with *SCCmec* and *mecA* typing for  
244 fine-structure epidemiological analysis. Further investigation is warranted to verify the use of  
245 this method for typing methicillin-susceptible isolates and to investigate the potential role of  
246 protein A as a virulence factor in *S. pseudintermedius*.

247

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252

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329 **Figure captions**

330 **Figure 1:** Schematic representation of protein A in *S. pseudintermedius* based on nucleotide  
331 and predicted amino acid sequence analysis. **(1a)** Conserved domain structure of protein A in  
332 the methicillin-susceptible strain ED99. SSS, start signal sequence; TM, transmembrane  
333 domain; four IgG-binding domains; LysM, lysin motif; AR, anchor region. **(1b)** Predicted  
334 amino acid similarity of protein A between *S. pseudintermedius* ED99 and *S. aureus* 8325-4  
335 in the segments coding for the signal sequence (S), the immunoglobulin G-binding regions  
336 (A-E) and region X.

337

338 **Figure 2:** Dendrogram based on pulsed field gel electrophoresis analysis of methicillin-  
339 resistant *S. pseudintermedius*.

1 **Table 1:** Origins, antimicrobial resistance profiles, and genotypes of *mecA*-positive *S.*  
 2 *pseudintermedius* strains used in this study

| Strain | Country | Origin | OXA MIC (mg/L) | SCC <i>mec</i> | <i>mecA</i> | <i>spa</i> | PFGE | MLST |
|--------|---------|--------|----------------|----------------|-------------|------------|------|------|
| G1     | Germany | Canine | ≥256           | III            | 3           | t02        | A6   | 71   |
| G2     | Germany | Canine | ≥256           | III            | 3           | t03        | A7   | ND   |
| G3     | Germany | Canine | ≥256           | III            | 3           | t02        | A7   | 71   |
| G4     | Germany | Feline | ≥256           | III            | 3           | t02        | A4   | 71   |
| G5     | Germany | Canine | ≥256           | III            | 3           | t02        | A3   | ND   |
| G6     | Germany | Canine | ≥256           | III            | 3           | t02        | A6   | ND   |
| G7     | Germany | Canine | ≥256           | III            | 3           | t02        | A7   | ND   |
| G8     | Germany | Canine | ≥256           | III            | 3           | t02        | A4   | ND   |
| G9     | Germany | Canine | ≥256           | III            | 3           | t04        | A5   | ND   |
| G10    | Germany | Canine | ≥256           | III            | 3           | t02        | A4   | ND   |
| G11    | Germany | Canine | ≥256           | III            | 3           | t02        | A2   | 71   |
| G12    | Germany | Canine | ≥256           | III            | 3           | t05        | A7   | ND   |
| U1     | USA     | Canine | ≥256           | V              | 1           | t06        | B3   | 68   |
| U2     | USA     | Canine | ≥256           | V              | 1           | t06        | B3   | 68   |
| U3     | USA     | Canine | ≥256           | V              | 1           | t06        | B1   | 70   |
| U4     | USA     | Canine | ≥256           | V              | 1           | t06        | B2   | 68   |
| S1     | Sweden  | Canine | 0.75           | II+IV          | 2           | t07        | C    | 69   |
| S2     | Sweden  | Canine | ≥256           | III            | 2           | t02        | NT   | ND   |
| S3     | Sweden  | Canine | ≥256           | III            | 2           | t02        | NT   | ND   |
| S4     | Sweden  | Canine | ≥256           | III            | 2           | t02        | A1   | 71   |
| S5     | Sweden  | Canine | ≥256           | III            | 2           | t08        | A1   | 71   |
| S6     | Sweden  | Canine | ≥256           | III            | 2           | t08        | A1   | ND   |
| S7     | Sweden  | Canine | ≥256           | III            | 2           | t02        | A1   | ND   |
| S8     | Sweden  | Canine | ≥256           | III            | 2           | t02        | NT   | ND   |
| S9     | Sweden  | Canine | ≥256           | III            | 2           | t02        | NT   | ND   |
| S10    | Sweden  | Canine | ≥256           | III            | 2           | t08        | NT   | 71   |
| S11    | Sweden  | Canine | ≥256           | III            | 2           | t02        | NT   | ND   |
| S12    | Sweden  | Canine | ≥256           | III            | 2           | t02        | NT   | ND   |
| S13    | Sweden  | Canine | ≥256           | III            | 2           | t08        | NT   | 71   |
| S14    | Sweden  | Canine | ≥256           | III            | 2           | t08        | NT   | 71   |
| UK1    | UK      | Canine | ≥256           | NT             | 4           | t09        | NT   | 29   |

3 ND, not determined; NT, non-typeable;

4 CEP, cefalotin; CLI, clindamycin; ENR, enrofloxacin; ERY, erythromycin; FD, fusidic acid;

5 FOX, cefoxitin; GEN, gentamicin; MUP, mupirocin; PEN, penicillin; OXA, oxacillin; RIF,

6 rifampicin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline

7 **Table 2:** Differences in the nucleotide sequence between *mecA* alleles in *S. pseudintermedius*  
 8 and reference strain *S. aureus* Mu50

| Allele       | Location <sup>a</sup> |      |      |      |      |      |      |      |      |      |        |      |      |
|--------------|-----------------------|------|------|------|------|------|------|------|------|------|--------|------|------|
|              | Pos.                  | Pos. | Pos. | Pos. | Pos. | Pos. | Pos. | Pos. | Pos. | Pos. | Pos.   | Pos. | Pos. |
|              | 35                    | 36   | 37   | 75   | 84   | 502  | 513  | 594  | 642  | 675  | 907    | 1465 | 1830 |
| <i>mecA1</i> | A                     | G    | T    | A    | A    | G    | -    | A    | -    | A    | -      | A    | T    |
| <i>mecA2</i> | T                     | A    | G    | A    | A    | -    | T    | -    | T    | T    | AGAGTC | G    | T    |
| <i>mecA3</i> | T                     | A    | G    | A    | G    | G    | -    | A    | -    | T    | -      | G    | T    |
| <i>mecA4</i> | T                     | A    | G    | A    | A    | G    | -    | A    | -    | T    | -      | G    | A    |
| Mu50         | T                     | A    | G    | C    | A    | G    | -    | A    | -    | T    | -      | G    | T    |

9 *a.* Position relative to *mecA* in *S. aureus* Mu50 (GenBank accession no. NP 370565)

10 **Table 3:** *spa* types identified in 31 *mecA*-positive *S. pseudintermedius*

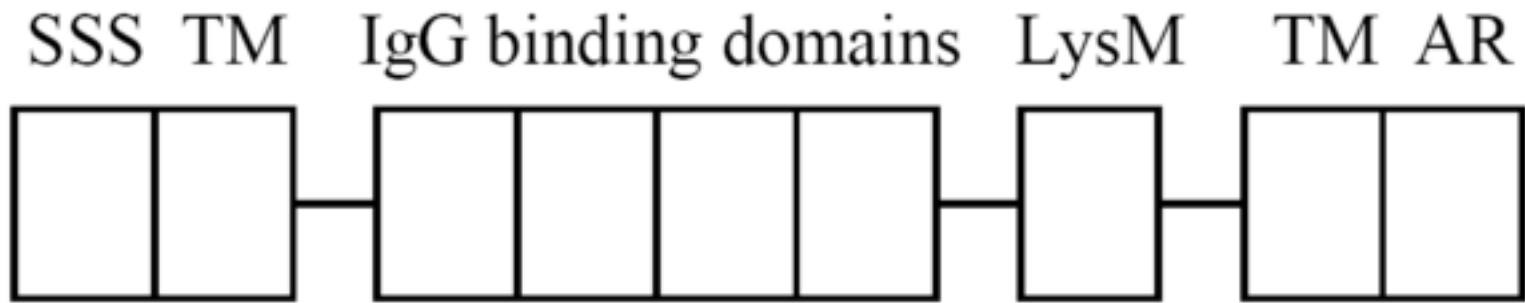
| <i>spa</i> type | Repeat sequence                     |
|-----------------|-------------------------------------|
| t01             | r01 r02 r03 r03 r03 r03 r04 r05     |
| t02             | r01 r02 r03 r03 r03 r06 r05         |
| t03             | r01 r02 r03 r03 r03 r06 r07         |
| t04             | r01 r02 r03 r03 r03 r06 r08         |
| t05             | r01 r02 r03 r03 r03 r03 r06 r05     |
| t06             | r01 r02 r03 r03 r06 r05             |
| t07             | r01 r09 r03 r10 r02 r03 r06 r05     |
| t08             | r01 r02 r11 r03 r03 r06 r05         |
| t09             | r01 r12 r02 r02 r03 r13 r03 r06 r05 |

11

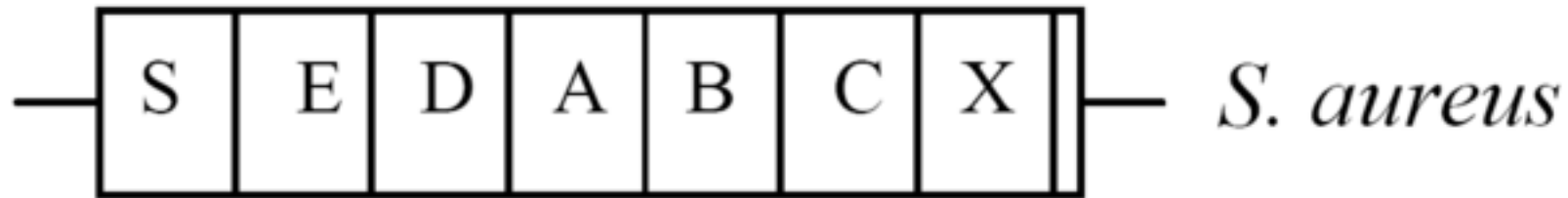
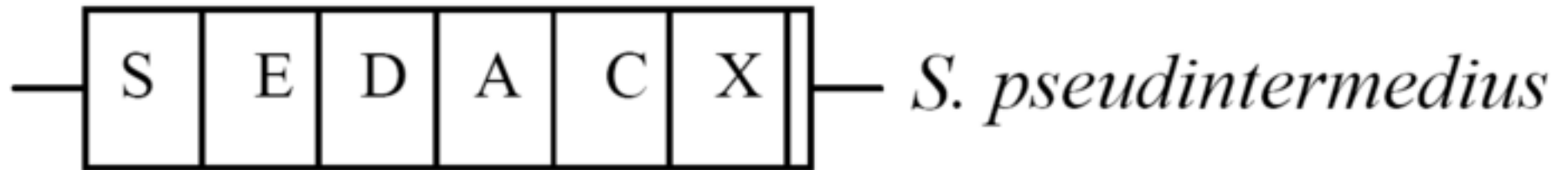
12 **Table 4:** DNA sequence of the 13 *spa* repeats identified in 31 *mecA*-positive *S.*  
 13 *pseudintermedius*. The sequences are displayed as ten coloured codons, and identical codons  
 14 in the same column are indicated with the same colour.

| <i>spa</i> repeat code | DNA sequence |     |     |     |     |     |     |     |     |     | Amino Acid sequence |
|------------------------|--------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------------------|
| r01                    | GGT          | GAA | AAC | AAA | GCT | GAA | GAC | AAA | GGC | AAC | GENKAEDKGN          |
| r02                    | AAA          | GAA | AAC | AAA | GCT | GAA | GAT | AAA | GGC | AGC | KENKAEDKGS          |
| r03                    | AAA          | GAA | GAC | AAA | GCT | GAA | GAT | AAA | GGC | AGC | KEDKAEDKGS          |
| r04                    | ATA          | GAA | GAT | AAA | GCT | AAA | GAC | AAA | GAC | AAC | IEDKAKDKDN          |
| r05                    | AAA          | GAA | GGC | AAA | GCT | GCA | GAC | AAA | GGT | ATG | KEGKAADKGM          |
| r06                    | AAA          | GAA | GAT | AAA | GCT | AAA | GAC | AAA | GAC | AAC | KEDKAKDKDN          |
| r07                    | AAA          | GAA | GGC | AAA | GCT | GCA | CAC | AAA | GGT | ATG | KEGKAAHKGM          |
| r08                    | AAA          | GAA | GGC | AAA | GCT | GCA | AAC | AAA | GGT | ATG | KEGKAANKGM          |
| r09                    | AAA          | GGC | AAC | AAA | GCT | GAA | GAT | AAA | GGC | AGC | KGNKAEDKGS          |
| r10                    | AAA          | GAA | GAC | AAA | GCT | GAA | GAT | AAA | GGC | AAC | KEDKAEDKGN          |
| r11                    | AAA          | GAA | GAC | CAA | GCT | GAA | GAT | AAA | GGC | AGC | KEDQAEDKGS          |
| r12                    | AAA          | GAA | AAC | AAA | GCT | GAA | GAC | AAA | GGC | AAC | KENKAEDKGN          |
| r13                    | AAA          | GAA | GAT | AAA | GCT | GAA | GAT | AAA | GGC | AGC | KEDKAEDKGS          |
| General Consensus      | AAA          | GAA | GAC | AAA | GCT | GAA | GAC | AAA | GGC | AXC | KEXKAEDKGX          |
| Motif 1                | AAA          | GAA | XXX | AAA | GCT | XAA | XXX | AAA | GXX | XXX |                     |
| Motif 2                | XXX          | GAA | XXX | AAA | GCT | XAA | GAC | AAA | GXX | XXX |                     |

15



a



33% 64% 58% 71% 0% 82% 41%

b

Figure 2

