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### **Paper:**

Harris, L. (2017). Characterization of nasal methicillin-resistant *Staphylococcus aureus* isolated from international human and veterinary surgeons. *Journal of Medical Microbiology*, 66(3), 360-370.

<http://dx.doi.org/10.1099/jmm.0.000415>

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1 **Characterisation of nasal methicillin-resistant *Staphylococcus aureus* isolated**  
2 **from international human and veterinary surgeons**

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14 **Running Title:** MRSA colonising Orthopaedic surgeons

15 **Keywords:** methicillin-resistant *Staphylococcus aureus*, nasal colonization, clonal lineages,  
16 orthopaedic surgeons

17 **Word count:** 4169

## 18 **Abstract**

19 Nasal colonization with methicillin-resistant *Staphylococcus aureus* is poorly described for  
20 surgeons, despite the increased exposure to nosocomial pathogens and at-risk patients. This study  
21 investigated the molecular epidemiology and antimicrobial resistance of 26 MRSA isolates  
22 cultured from the nares of an international cross-sectional study of 1,166 human and 60 veterinary  
23 surgeons. All isolates were subjected to *agr*-, *spa*- and MLST typing and the presence of 22  
24 virulence factors were screened for by PCR. Additionally, biofilm-forming ability, haemolytic  
25 activity, staphyloxanthin production and antibiotic resistance were determined. The genome of a  
26 rifampicin resistant MRSA was sequenced. Approximately half of the isolates belonged to well-  
27 described clonal lineages, ST1, ST5, ST8, ST45 and ST59, that have been previously associated  
28 with severe infections and increased patient mortality. Two of the 3 veterinarian MRSA belonged  
29 to epidemic livestock-associated MRSA clonal lineages (ST398 and ST8) previously associated  
30 with high transmission potential between animals and humans. The isolates did not display any  
31 consistent virulence gene pattern, and 35% of the isolates carried at least one of: the Panton-  
32 Valentine leukocidin *lukFS-PV*; the exfoliative toxin *eta*; or the toxic shock syndrome *tst* genes.  
33 Resistance to rifampicin was detected in one veterinarian isolate, and was found to be due to 3  
34 mutations in the *rpoB* gene. Surgeons occupy a critical position in the healthcare profession due to  
35 their close contact with patients. In this study, surgeons were found to be colonized with MRSA  
36 at low rates that are similar to the general population, and the colonising strains were often  
37 common clonal lineages.

38

## 39 **Introduction**

40 *Staphylococcus aureus* colonizes between 26-35% of healthy humans, with the moist squamous  
41 epithelium of the anterior nares considered the primary reservoir (1-4). Due to its importance as a  
42 pathogen responsible for a wide range of difficult-to-treat infections, colonization with  
43 methicillin-resistant *S. aureus* (MRSA) has been investigated extensively in patients, but to a

44 lesser extent in their primary care-givers, including health care workers (HCWs) such as  
45 orthopaedic surgeons. Studies have consistently shown that average MRSA colonization rates are  
46 between 1-3% in the general population (5, 6), with increased rates shown for certain groups such  
47 as the elderly in assisted living facilities (6) or long-term in-patients (2), and those with immunity  
48 defects such as HIV, chronic granulomatous disease (CGD), Job's syndrome, Chediak-Higashi  
49 syndrome, and Wiskott-Aldrich syndrome (7, 8).

50 Recently, the MRSA colonization rate in an international cohort of human and veterinary  
51 orthopaedic surgeons was investigated (9). From a total cohort of 1,166 human surgeons, the  
52 MRSA colonization rate was 2% (23/1,166) and was 5% (3/60) amongst the veterinary surgeons  
53 (9). What remains undocumented within this study is the molecular epidemiology of these MRSA  
54 isolates. Previous studies have identified that distinct clonal lineages belonging to different  
55 sequence types (STs) or clonal complexes (CCs) have emerged and spread across the world (10-  
56 12). For example, USA300 isolates (ST8) have been documented across the US and beyond, (11).  
57 Interestingly, livestock-associated (LA)-MRSA such as ST398 have been found on humans living  
58 and working on farms (13-15). It has also been shown that veterinary surgeons are at increased  
59 risk of carrying the same epidemic MRSA isolates as the animals (13-15). This clearly  
60 demonstrates the possibility of transmission between animal and human carrier and *vice versa*.

61 We hypothesize that the MRSA nasal isolates collected from a cross-sectional study from human  
62 and veterinary surgeons will belong to well-known MRSA lineages. Therefore the aim of this  
63 study was to phenotypically and genotypically characterise the 23 MRSA nasal isolates from  
64 human surgeons as well as 3 MRSA isolates from veterinary surgeons, and determine the clonal  
65 relationship of these nasal isolates to known globally disseminated MRSA lineages.

66

## 67 **Materials and Methods**

68 Methicillin-resistant *Staphylococcus aureus* (MRSA) collection

69 MRSA isolates were obtained from a previously described study (9). Twenty-three MRSA isolates  
70 were recovered from the nares of 1,166 human orthopaedic surgeons sampled on an anonymous  
71 basis during an educational course in Switzerland in 2013. Furthermore, 3 additional MRSA  
72 isolates were collected from 60 veterinary orthopaedic surgeons not described in the previously  
73 study (9) but were included in the present study. The study was approved by the "Ethik-  
74 Kommission der Bayerischen Landesärztekammer", Germany (Approval number 13090). Beside a  
75 nasal swab all participants gave information on basic demographic and professional questions  
76 such as location of place of work and birth (country and region). Bacterial isolation and  
77 identification was performed as described previously (9). After identification a single colony was  
78 taken and resuspended in 1 ml Tryptone Soy broth (TSB, Sigma Aldrich, Buchs, Switzerland)  
79 containing 20% vol/vol glycerol and stored at -80°C. Isolates were re-grown either on Tryptone  
80 Soy Agar (TSA, Sigma Aldrich, Buchs, Switzerland) plates or in TSB media for phenotypic  
81 characterization or for genomic DNA isolation. The 23 MRSA human orthopaedic surgeon  
82 isolates and their antimicrobial resistances were partially described by Morgenstern et al. 2016 (9).  
83 A more detailed profile of each individual isolate was analysed in this study.

84

85 Phenotypic characterisation

86 *Antibiotic susceptibility testing*

87 Antibiotic susceptibility to 28 antibiotics (amikacin, ampicillin-sulbactam, cefotaxim, ceftazidime,  
88 cefuroxime, ciprofloxacin, clindamycin, daptomycin, erythromycin, fosfomycin, fusidic acid,  
89 gentamicin, levofloxacin, linezolid, mezlocillin, moxifloxacin, netilmicin, ofloxacin, oxacillin,  
90 penicillin, piperacillin, rifampicin, tetracycline, ticarcillin/clavulanate, tigecycline, tobramycin,  
91 trimethoprim-sulfamethoxazole and vancomycin) were determined using a Vitek2 machine  
92 (bioMérieux Vitek Inc., Hazelwood, MO, USA). Multiple antibiotic resistance was defined  
93 according to the definitions of the European Committee of Antimicrobial Susceptibility Testing  
94 (EUCAST). Oxacillin resistance was considered definitive for methicillin resistant status.

95

96 *Cefoxitin disc diffusion test*

97 To confirm isolates as methicillin resistant, a cefoxitin disc diffusion test was performed at Synlab  
98 Suisse (Luzern, Switzerland) using a 30 µg disc. Zone sizes were measured and interpreted  
99 according to EUCAST interpretative criteria ([http://www.eucast.org/clinical\\_breakpoints](http://www.eucast.org/clinical_breakpoints)).

100

101 *Mupirocin disc diffusion test*

102 Resistance to mupirocin was tested on all isolates at Synlab Suisse (Luzern, Switzerland) by disc  
103 diffusion test using a 200 µg disc. Zone sizes were measured and interpreted according to  
104 EUCAST interpretative criteria ([http://www.eucast.org/clinical\\_breakpoints](http://www.eucast.org/clinical_breakpoints)).

105

106 *Staphyloxanthin production, haemolytic activity and biofilm production*

107 Staphyloxanthin production was tested as described previously (16) by two independent observers.  
108 *S. aureus* Newman and USA300 were included as positive controls for strong and *S. aureus* COL  
109 and UAMS-1 for weak staphyloxanthin production, respectively (16).

110 Haemolytic activity of each MRSA isolate was assessed as described previously (16). *S. aureus*  
111 reference isolates USA300 and UAMS-1 were used as controls for strong and absent haemolysis  
112 activity, respectively (16).

113 Biofilm formation was assayed as described previously (17, 18). Briefly, overnight cultures grown  
114 in Tryptic Soy Broth (TSB, Sigma Aldrich, Buchs, Switzerland) were suspended in fresh TSB  
115 containing 1% glucose, to approximately  $1 \times 10^6$  CFU/ml, correlating to an optical density of  
116  $0.02 \pm 0.005$  at 600nm as measured with a Multiskan Go microplate reader (Thermo Scientific,  
117 Zürich, Switzerland). A total of 200 µl of the bacterial suspension was incubated in flat-bottomed  
118 96-well tissue culture-treated polystyrene microtitre plates (Nuclon, Nunc A/S, Denmark) for 24 h

119 at 37 °C. Plates were rinsed with phosphate-buffered saline (PBS, Sigma-Aldrich, Buchs,  
120 Switzerland) and stained with 150 µl of Gram`s crystal violet solution (Sigma-Aldrich, Buchs,  
121 Switzerland). The dye bound to the attached cells was solubilized by addition of 150 µl of 95%  
122 ethanol. Optical density was measured as absorbance at 595 nm using the Multiskan Go  
123 microplate reader. All isolates were tested in triplicate in three independent experiments. Each  
124 microtitre plate also consisted of negative controls (wells without bacterial inoculation). The  
125 results were evaluated using the scale described by Stepanovic et al. (17). *S. epidermidis* reference  
126 strain RP12 (ATCC 35983) was used as a control for strong biofilm production.

127

128 Genotypic characterisation

129 *DNA extraction*

130 Whole-cell (genomic) DNA, used as template for PCR amplification, was prepared from single  
131 colonies using the Wizard® 143 Genomic DNA purification kit (Promega, Dübendorf,  
132 Switzerland) according to the manufacturer`s protocol.

133

134 *PCR amplification*

135 Specific primers, corresponding genes and PCR reaction conditions are previously described by  
136 Post et al. (Post et al., 2014) and summarized in Table 1. PCR amplification was carried out in a  
137 BioRad MyCycler Thermocycler (BioRad, Reinach Switzerland) in a total volume of 12.5 µl  
138 containing 10X Green GoTaq® 151 Reaction buffer (Promega, Dübendorf, Switzerland), 5 mM  
139 dNTP Mix (Promega, Dübendorf, Switzerland), 50 pmol of each primer, 1 unit of *Taq* DNA  
140 polymerase recombinant (Invitrogen, Zug, Switzerland) and 10-50 ng template DNA.

141

142 *Accessory gene regulator (agr) typing*

143 *agr* polymorphisms were detected by PCR as described by von Eiff et al. and Post et al. (18, 19)  
144 with primers listed in Table 1.

145

146 *Microbial surface components recognizing adhesive matrix molecules (MSCRAMM) detection*

147 Genes coding for MSCRAMMs were screened for by PCR with primers previously described (18)  
148 with primers listed in Table 1.

149

150 *Staphylococcal enterotoxins and other toxins*

151 Genes coding for staphylococcal enterotoxins (SEA-SEE), Panton-Valentine leukocidin (PVL)  
152 and toxic shock syndrome toxin (TSST) were detected by PCR using primers previously described  
153 (18) with primers listed in Table 1.

154

155 *Methicillin resistance gene*

156 The methicillin resistance gene *mecA* was detected using primers previously described (18) and  
157 listed in Table 1.

158

159 *Spa typing*

160 The polymorphic repeat region (region x) of the *S. aureus* protein A encoded by the *spa* gene was  
161 amplified as described previously (20). The *spa*-type for each isolate was determined as described  
162 by Harmsen and colleagues (21) using the online tool Ridom SpaServer  
163 (<http://spaserver.ridom.de/>) with primers listed in Table 1.

164

165 *Multi-Locus Sequence Typing (MLST)*

166 MLST was performed with published primers following the instructions on  
167 <http://saureus.mlst.net/>.

168

169 *DNA sequencing and sequence analysis*



170 PCR products were purified for sequencing using the PureLink™ Quick Gel Extraction and PCR  
171 Purification Combo Kit (Invitrogen, Zug, Switzerland) following the manufacturer`s protocol.  
172 Automated sequencing was performed at Microsynth AG, Balgach, Switzerland on an Applied  
173 Biosystems ABI3730xl Sequence Analyser 5.2 using the ABI Big Dye® 182 system V3.1.

174

#### 175 *Whole genome sequencing of MRSA-3*

176 Genomic DNA of MRSA-3 isolate was extracted using a Qiagen QiAmp DNA mini kit (Qiagen,  
177 Hilden, Germany) following the manufacturer`s protocol using 1 µg/ml lysostaphin (Sigma-  
178 Aldrich, Buchs, Switzerland and 2 µg/ml lysozyme (Sigma-Aldrich, Buchs, Switzerland) to lyse  
179 the bacteria. Sequencing was performed using an Illumina MiSeq benchtop sequencer (Illumina,  
180 San Diego, CA, USA), and the 100 bp short read paired-end data was assembled using the *de novo*  
181 assembly algorithm within *Velvet* software (version 1.2.08) (22). Resulting data was archived in  
182 the Staphylococcal Bacterial Isolate Genome Sequence database (BIGSdb) (23). The *S. aureus*  
183 MRSA252 reference genome (Genbank accession number BX571856.1) (24) was used as a basis  
184 for defining locus designations, and reference sequences. A ribosomal multilocus sequence typing  
185 (rMLST) approach was used to investigate the genetic relationship between MRSA-3 and 181  
186 published *S. aureus* isolates from the National Center for Biotechnology Information (NCBI).  
187 Orthologs for the 53 genes encoding the bacterial ribosome protein subunits (*rps* genes) were  
188 defined in all isolates by comparison to the finished genome of MRSA252. To estimate the  
189 genealogies for these alignments, ClonalFrame, a model-based approach to determining  
190 microevolution in bacteria was used (25). The consensus tree represents combined data from three  
191 independent runs with 75% consensus required for inference of relatedness. Recombination events  
192 were defined as sequences with a length of >50 bp with a probability of recombination  $\geq 75\%$  over  
193 the length, reaching 95% in at least one site.

194 The presence of genes associated with the Staphylococcal Cassette Chromosome (SCC) *mec*  
195 complex (*mecA*, *mecR*, *ccrA* and *ccrB*), the *S. aureus* chromosomal *orfX* gene located to the right

196 of the *SCCmec* integration site, ACME (arginine catabolic mobile element) locus (*speG*, *aliD* and  
197 *arcA*) and the rifampicin resistance gene (*rpoB*) were investigated for MRSA-3 by BLAST (basic  
198 local alignment search tool) comparison to reference genome MRSA252 (23, 26, 27). These genes  
199 were considered as being present when a BLAST match with a >70% nucleotide sequence identity  
200 on  $\geq 50\%$  of sequence length was recorded. Where present, the genes were mapped onto the  
201 rMLST tree to examine the significance of association between the clustering on the tree and the  
202 presence of a specific gene.

203

#### 204 Statistical analysis

205 The prevalence of MRSA and the corresponding 95% confidence interval (95% CI) were  
206 calculated using SAS software (Version 9.2; Cary, NC, USA).

207

## 208 **Results**

### 209 Phenotypic characterisation

#### 210 *Antibiotic resistance profile*

211 The 26 MRSA isolates were collected from carriers coming from different regions of Africa  
212 (2/59), Asia (14/252), Central America (1/22), Europe (6/673) and South America (3/115) (Table  
213 2). The MRSA rate was 2% (23/1,166; 95% CI 1.3;2.9) for human surgeons and 5% (3/60; 95%  
214 CI 1.0;13.9) for veterinary surgeons. All MRSA isolates had cefoxitin zone diameters <22 mm,  
215 confirming their methicillin resistance status. The *mecA* gene was also detected by PCR in all 26  
216 MRSA isolates, which is in concordance with the cefoxitin disc diffusion test and the results  
217 provided by the oxacillin test by the Vitek2 system. The antibiotic susceptibility profile of all  
218 MRSA isolates is listed in Table 2. Fifty percent (13/26 isolates) of all isolates were resistant to  
219 erythromycin and clindamycin of which 6/13 isolates showed also resistance to the 4 quinolones  
220 ciprofloxacin, ofloxacin, levofloxacin and moxifloxacin. Furthermore, 30.8% (8/26 isolates) were  
221 resistant to the 4 aminoglycosides gentamicin, tobramycin, amikacin and netilmicin. Half of the

222 aminoglycoside resistant isolates (15.4%) showed also erythromycin and clindamycin resistance.  
223 All 26 MRSA isolates were susceptible to mupirocin, the only agent approved for de-  
224 contamination of MRSA nasal carriage.

225  
226 *Haemolytic activity, staphyloxanthin production and biofilm production*

227 Forty-two percent of isolates displayed the yellow-orange or yellow colony pigmentation  
228 indicative of staphyloxanthin production (Table 3) and 69% of isolates were haemolytic (Table 3).  
229 One isolate was a strong biofilm producer, and 21 isolates did not produce a biofilm under the  
230 described experimental conditions. The remaining 4 isolates produced an intermediate biofilm.

231  
232 Genotypic characterisation

233 *Accessory gene regulator (agr) typing*

234 Fifty-four percent of isolates belonged to *agr* type I, followed by 27% *agr* type II and 12% *agr* III  
235 (Table 3). Two isolates did not belong to any of the 4 *agr* types.

236  
237 *Microbial surface components recognizing adhesive matrix molecules (MSCRAMM) detection*

238 The virulence genes *hlgC/B* (gamma-hemolysin), *icaA* (intercellular adhesion), *eno* (laminin  
239 binding protein), *clfA/clfB* (fibrinogen binding protein) and *sdrC* (beta-neurexin binding protein)  
240 were present in all isolates (Table 3). Ninety-six percent and 85% of isolates carried the *sdrE*  
241 (platelet aggregation) gene and *sdrD* (fibrinogen binding protein) gene, respectively, while 73%,  
242 62%, 39% and 39% of isolates possessed the *fib* (fibrinogen binding protein), *ebpS* (elastin  
243 binding protein), *cna* (collagen binding protein) and the *fnbB* (fibrinogen binding protein and  
244 elastin binding protein) genes, respectively (Table 3). The *bbp* (bone sialoprotein binding protein)  
245 gene was not detected in any of the MRSA isolates.

246

247 *Staphylococcal enterotoxins and other toxins*

248 Two isolates carried the Panton-Valentine leukocidin (PVL) *lukFS-PV* gene and 4 isolates  
249 possessed the *tst* (toxic shock syndrome toxin, TSST) gene; all 6 isolates originating from Asia  
250 (Table 3). Half of the isolates carried one of the 5 genes coding for staphylococcal enterotoxins  
251 (SE) A, B, C, D and E. The *eta* gene (exfoliative toxin) gene was present in 3 isolates (Table 3).

252

253 *Correlation of biofilm, antimicrobial resistance and virulence genes*

254 Only 1 of the biofilm producers was found to be resistant to most antibiotics (Table 2). All 5  
255 isolates producing a biofilm showed also haemolytic activity whereas only 1 produced  
256 staphyloxanthin (Table 3). The 2 isolates carrying the Panton-Valentine leukocidin (PVL) *lukFS-*  
257 *PV* gene produced both intermediate biofilm. The *sdrD* gene was present in all and the *cna* gene in  
258 4/5 of isolates producing a biofilm (Table 3).

259

260 *Spa-typing*

261 In total, 22 different *spa*-types and 1 novel sequence repeat (Sequence repeat: 11-19-12-21-10-34-  
262 24-24-34-22-25; Kreiswirth ID: YHGFC2BQQBLO) were identified (Table 3). Only 3 isolates  
263 belonged to the same *spa*-type t688, one each from a European, African and Asian carrier (Table  
264 3). Two isolates belonged to t437 and were collected from Asia.

265

266 *Multi-locus sequence types (MLST)*

267 MLST Sequence Types (ST) were determined for all 26 isolates. Sixteen different ST types were  
268 identified (Table 3), with the main types being ST5 (n=4), ST8 (n=3) and ST59 (n=3). All isolates  
269 within ST5 belonged to *agr* type II, whilst all ST8 and ST59 isolates were *agr* type I (Table 3).

270

271 *Whole genome sequence analysis of isolate MRSA-3*

272 MRSA-3 was isolated from a veterinary surgeon from South America and was submitted for  
273 whole genome sequencing as it had a wide range of antibiotic resistance and was the only isolate  
274 to be rifampicin resistant (Table 2). *Spa*-typing and MLST revealed that it belonged to the ST8-  
275 t064 clonal lineage. The ClonalFrame phylogenetic tree based on 53 *S. aureus* rMLST genes in  
276 Figure 1, shows the evolutionary relationship between MRSA-3 and 181 published *S. aureus*  
277 genomes. MRSA-3 was in the same cluster as other ST8 isolates. Further, the ACME locus genes  
278 *speG*, *aliD* and *arcA* and the SCCmec complex genes, *mecA*, *mecR*, *ccrA*, *ccrB* and *orfX* were all  
279 present in MRSA-3. The phylogenetic relationship of MRSA-3 to the other MRSA genomes is  
280 shown for the *mecA* gene on the neighbour joining tree (Figure 2a; MRSA-3 indicated by black  
281 arrow and dark red filled square).

282 The MRSA-3 isolate was resistant to rifampicin (MIC  $\geq 32$  mg/L). Sequence NCBI BLAST  
283 analysis against the reference sequence of rifampicin susceptible *S. aureus* strain ATCC 25923  
284 (GenBank accession number CP009361) (MIC,  $\leq 0.5$  mg/L) revealed 20 nucleotide changes in the  
285 3552 bp *rpoB* sequence. However, only 3 nucleotide changes at position 1411 (GGA  $\rightarrow$  AAC),  
286 1430 (GCT  $\rightarrow$  GAT) and 2210 (TTT  $\rightarrow$  TAT) resulted in amino acid changes 471 (D $\rightarrow$ N;  
287 Asp $\rightarrow$ Asn), 477 (A $\rightarrow$ D; Ala  $\rightarrow$  Asp) and 737 (F $\rightarrow$ Y; Phe  $\rightarrow$  Tyr), respectively (Figure 3). The  
288 changes in MRSA-3 were not observed in the other published *S. aureus* ST8 genomes (Figure 2b).

289

## 290 **Discussion**

291 In this study, 26 nasal MRSA isolates obtained from human and veterinary surgeons were  
292 phenotypically and genotypically characterised. These 26 MRSA isolates were collected from a  
293 large international cohort of 1,166 human and 60 veterinary surgeons from Africa, Asia, Central  
294 America, Europe and South America. As previously described, the MRSA colonization rate  
295 amongst the human surgeons was 2% (23/1,166; 95% CI 1.3;2.9) indicating a colonization rate  
296 equivalent to the general population and from HCWs (5, 6, 9, 28-30). The MRSA rate for the

297 veterinary surgeons was 5% (3/60; 95% CI 1.0;13.9) which lies also in the colonization rate  
298 reported for veterinary personnel (31).

299

300 The most prevalent STs in this study were ST5 and ST8. The 4 ST5 isolates were collected (one  
301 each) from a European, an African, an Asian and from a Central American surgeon. The ST5  
302 lineage is associated with the successful global HA-MRSA New York/Japan clone (ST5,  
303 USA100) and the "paediatric" clone (ST5) both belonging to one of the major MRSA clonal  
304 complexes CC5 (12, 32). However, polyclonal genesis within this lineage has been shown (12, 32)  
305 and the only consistencies within the 4 ST5 isolates in this study were that they all belonged to *agr*  
306 type II, and were negative for the genes *lukFS-PV*, *tst* and *eta*.

307 In total, 3 isolates (2 from Asian surgeons and 1 from a South American veterinary orthopaedic  
308 surgeon) belonged to the ST8 (USA300) clonal lineage. This lineage is mainly associated in the  
309 US as CA-MRSA responsible for the most frequent and severe skin and soft tissue infections in  
310 emergency departments (11, 33). The reason for this is believed to be due to higher virulence  
311 including the production of toxins (12). This is consistent with the expression of staphyloxanthin  
312 and haemolytic activity in all 3 ST8 isolates as well as the possession of the *tst* gene in the 2 Asian  
313 isolates and the *eta* gene in the South American isolate.

314 In most cases, the described ST-*spa*-type combinations identified in this study have previously  
315 been associated with increased severity and patient mortality in hospital settings. For example the  
316 ST1-t386 lineage from 1 Asian surgeon belonged to CC1 and is mainly associated with USA400  
317 ORSA IV, a CA-MRSA in the USA that caused paediatric deaths in the Midwest of the United  
318 States (11). Furthermore, the 2 ST59-t437 isolates from Asian surgeons belonged to a well  
319 described CA-MRSA clonal lineage in Asia causing skin and soft tissue infections as well as  
320 sepsis and severe pneumonia (34, 35). A recent study has also identified ST59-t437 isolates in  
321 several European countries demonstrating that *S. aureus* lineages are not restricted to particular

322 geographical regions or specific host environments (36), although in our study all ST59 isolate  
323 were associated with Asian surgeons.

324 The importance of antibiotic use in food production and the risks of emergence of multiply  
325 antibiotic resistance pathogens within the food chain has resulted in an increasing awareness of the  
326 risk of transmission of MRSA within the sector, including veterinary orthopaedic surgeons (13-  
327 15). In our study, 3 veterinary orthopaedic surgeons were culture positive for MRSA (2 from  
328 Europe, 1 from South America). Two of them were colonized with LA-MRSA clonal lineages, i.e.  
329 lineages previously cultured directly from livestock. For example, 1 veterinary orthopaedic  
330 surgeon from Europe was colonized with MRSA ST398-t011, belonging to CC398. CC398 is the  
331 most predominant LA-MRSA clonal lineage reported across the world (13-15), and has been  
332 detected in a variety of domesticated animals (13-15). CC398 has often been reported to be  
333 transmitted from animals to humans (13-15) with most human CC398 infections being superficial  
334 skin and soft tissue infections (14). The second veterinary-associated MRSA (from a South  
335 American veterinary surgeon) was also carrying a known clonal lineage, ST8-t064 (37).

336 Furthermore, this ST8-t064 (MRSA-3) isolate was multiply antibiotic resistant including  
337 resistance to rifampicin. Resistance to rifampicin is a significant challenge in the treatment of  
338 orthopaedic device related infection since rifampicin is the sole clinically available antibiotic with  
339 significant activity against staphylococcal biofilms (38, 39). Whole genome sequencing revealed 3  
340 SNPs in the *rpoB* sequence, which led to a change in the amino acid sequence at positions 471,  
341 477 and 737. The amino acid change at position 477 from Alanine to Aspartic acid has been  
342 shown to be responsible for high level resistance to rifampicin (40-42).

343 Comparison of MRSA-3 SCC*mec* complex genes, (*mecA*, *mecR*, *ccrA* and *ccrB*) and *orfX* gene to  
344 the other ST8 *S. aureus* genomes from NCBI revealed a close evolutionary relationship. In  
345 contrast, the *rpoB* neighbour joining tree showed that the relationship between MRSA-3 and the  
346 other ST8 isolates is more diverse, indicating that it has a different evolutionary history. The

347 ACME locus genes: *aliD*, *arcA* and *speG* were present in the MRSA-3 indicative for most ST8  
348 (USA300) CA-MRSA (43) while the *lukFS-PV* gene encoding PVL was absent.

349 The phenotypic evaluation of all MRSA isolates revealed that the isolates displayed a range of  
350 staphyloxanthin production, haemolytic activity and biofilm forming potential. Screening for the  
351 most prevalent MSCRAMMs demonstrated the presence of most of the tested virulence genes in  
352 the majority of the 26 MRSA isolates. For example, the surface protein clumping factor B (*clfB*)  
353 present in all 26 surgeons isolates has been shown to promote attachment of *S. aureus* to human  
354 squamous nasal epithelial cells by binding to keratin-10 (1). In total 34.6% of the nasal MRSA  
355 either carried the PVL *lukFS-PV* gene, responsible for increased severe disease and clinical  
356 symptoms, including necrotic lesions of the skin (10, 11), the *eta* gene coding for exfoliative toxin  
357 enhancing the transmission of MRSA through skin-skin contact, due to the destruction of the  
358 epidermal barrier (44) or the toxic shock syndrome *tst* gene. This highlights the highly virulent  
359 potential of these nasal colonizing MRSA isolates.

360

## 361 **Conclusion**

362 The findings of this study have shown that the MRSA isolates cultured from surgeons possessed  
363 genes for a wide range of virulence factors and toxins but also belonged to clonal lineages  
364 described for their high transmission potential and are associated with increased infection severity  
365 and mortality. This indicates not only that the surgeons are a potential risk in spreading these  
366 lineages in clinical and healthcare settings, but also that they themselves are exposed to the  
367 acquisition of such isolates.

368

369 **Acknowledgements.** The authors would like to acknowledge the contribution of Drs Ben Pascoe,  
370 Matt Hitchings and Yasmin Freidmann from Swansea University, UK for the whole genome  
371 sequencing.



372 **Potential conflicts of interest.** All authors report no conflicts of interest relevant to this article.

373 **Financial support.** This work was funded by AOTrauma as part of the Clinical Priority  
374 Programme, Bone Infection.

375

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506 **Legends**

507 **Figure 1.** Population structure of MRSA-3 (indicated by a black arrow and a dark red filled  
508 square) and 181 published *S. aureus* isolates constructed from 53 rMLST genes and implemented  
509 in ClonalFrame. Isolates labelled according to ST complex: ST1 (turquoise); ST5 (pink); ST8  
510 (red); ST22 (light blue); ST30 (blue); ST45 (green); ST398 (orange); singletons (yellow). The  
511 scale (0.1) is in coalescent units and represents the number of substitutions per site.

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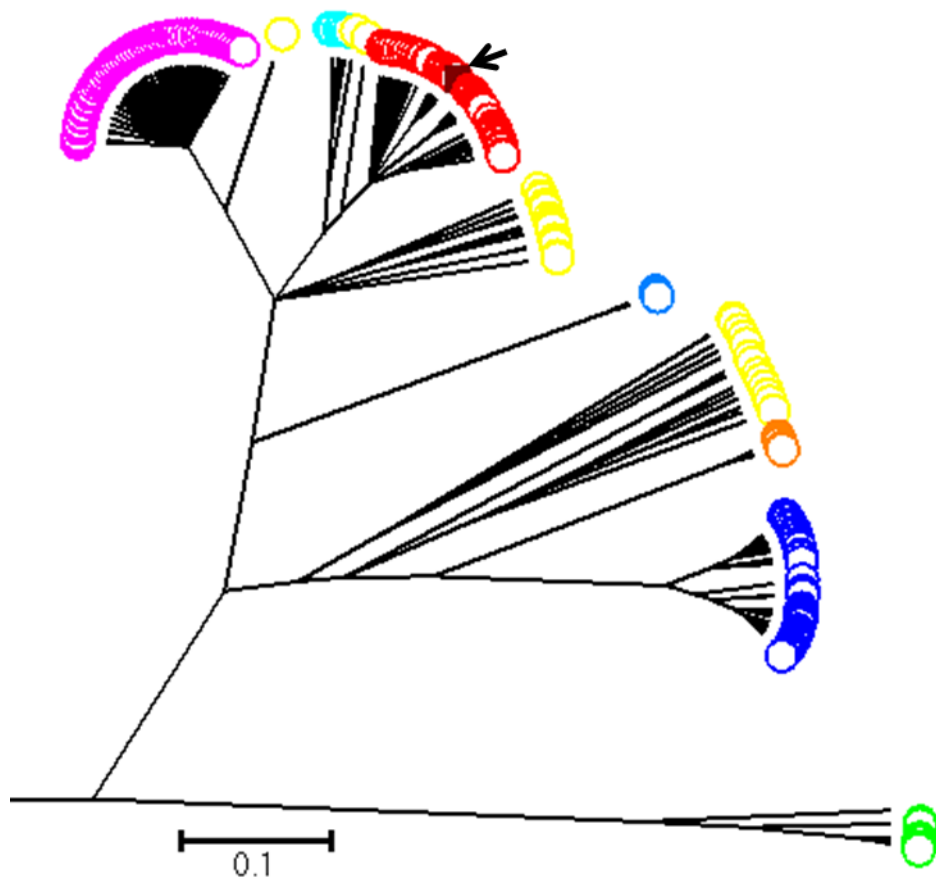
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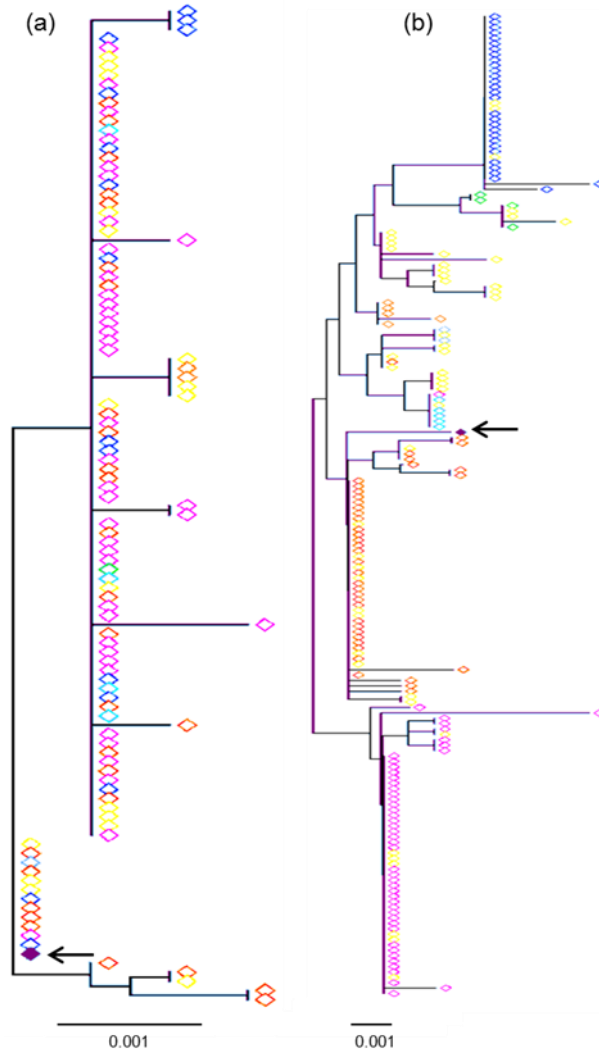
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527 **Figure 2.** Phylogenetic trees of MRSA-3 (indicated by a black arrow and a dark red filled square)  
528 and 181 published *S. aureus* genomes based on the presence of: (a) *mecA* and (b) *rpoB*. Isolates  
529 labelled according to ST complex: ST1 (turquoise); ST5 (pink); ST8 (red); ST22 (light blue);  
530 ST30 (blue); ST45 (green); ST398 (orange); singletons (yellow). The scale bar (0.001) is in  
531 coalescent units and represents the number of substitutions per site.



535 **Figure 3.** Amino acid alignment of the *rpoB* gene region (421-780) in the rifampicin resistant  
 536 MRSA-3 and the rifampicin susceptible *S. aureus* ATCC 25923 (GenBank accession number  
 537 CP009361). White letters on black indicate the 3 amino acid changes identified in MRSA-3.

538

539	MRSA-3	IGLSRMERVVRRERMSIQDTE SITPQQ LINIRPV IASIK EFFF GSSQLSQ FMDQ ANPLD ELT	480
540	ATCC 25923	IGLSRMERVVRRERMSIQDTE SITPQQ LINIRPV IASIK EFFF GSSQLSQ FMDQ ANPLA ELT	
541			
542	MRSA-3	HKRRLSALGPGGLTRERAQMEVRDVHYSHYGRMCPIETPEGPNIGLINSLSYARVNEFG	540
543	ATCC 25923	HKRRLSALGPGGLTRERAQMEVRDVHYSHYGRMCPIETPEGPNIGLINSLSYARVNEFG	
544			
545	MRSA-3	FIETPYRKVDLDTHAITDQIDYLTADEEDSYVVAQANSKLDENGRFMDDEVVCRFRGNNT	600
546	ATCC 25923	FIETPYRKVDLDTHAITDQIDYLTADEEDSYVVAQANSKLDENGRFMDDEVVCRFRGNNT	
547			
548	MRSA-3	VMAKEKMDYMDVSPKQVVSAAATACIPFLENDDSNRALMGANMQRQAVPLMNPEAPFVGTG	660
549	ATCC 25923	VMAKEKMDYMDVSPKQVVSAAATACIPFLENDDSNRALMGANMQRQAVPLMNPEAPFVGTG	
550			
551	MRSA-3	MEHVAARDSGAAITAKHRGRVEHVESNEILVRRLLVEENGVEHEGELDRYPLAKFKRSNSG	720
552	ATCC 25923	MEHVAARDSGAAITAKHRGRVEHVESNEILVRRLLVEENGVEHEGELDRYPLAKFKRSNSG	
553			
554	MRSA-3	TCYNQRPIVAVGDVVEE NEILADGPSMELGEMALGRNVVVGFM TWDG YNYEDA VIMSERL	780
555	ATCC 25923	TCYNQRPIVAVGDVVEE NEILADGPSMELGEMALGRNVVVGFM TWDG YNYEDA VIMSERL	
556			

557 Table 1. Primers used in the molecular identification of virulence factors in this study<sup>1</sup>

Gene	Primer name	Sequence (5' - 3')	Product size (bp)	Annealing temp (°C)
<i>spa</i> -typing				
<i>spa</i>	1095F	AGACGATCCTTCGGTGAGC		60
	1517R	GCTTTTGCAATGTCATTTACTG		
<i>agr</i> -typing				
<i>agr</i>	agr1-4-1	ATGCACATGGTGCWCATGC		
	agr1-2	GTCACAAGTACTATAAGCTGCGAT	439	55
	agr2-2	TATTACTAATTGAAAAGTGCCATAGC	572	55
	agr3-2	GTAATGTAATAGCTTGTATAATAATACCCAG	321	55
	agr4-2	CGATAATGCCGTAATACCCG	657	55
MSCRAMMs				
<i>cna</i>	CNA-1	GTCAAGCAGTTATTAACACCAGAC	423	55
	CNA-2	AATCAGTAATTGCACTTTGTCCACTG		
<i>eno</i>	ENO-1	ACGTGCAGCAGCTGACT	302	55
	ENO-2	CAACAGCATYCTTCAGTACCTTC		
<i>ebpS</i>	EBP-1	CATCCAGAACCAATCGAAGAC	186	55
	EBP-2	CTTAACAGTTACATCATCATGTTTATCTTTG		
<i>fnbB</i>	FNBB-1	GTAACAGCTAATGGTTCGAATTGATACT	524	55
	FNBB-2	CAAGTTCGATAGGAGTACTATGTTC		
<i>fib</i>	FIB-1	CTACAACACTACAATTGCCGTCAACAG	404	55
	FIB-2	GCTCTTGTAAGACCATTTTCTTCAC		
<i>clfA</i>	CLFA-1	ATTGGCGTGGCTTCAGTGCT	292	55
	CLFA-2	CGTTTCTTCCGTAGTTGCATTTG		
<i>clfB</i>	CLFB-1	ACATCAGTAATAGTAGGGGGAAC	205	55
	CLFB-2	TTCGCACTGTTTGTGTTTGCAC		
<i>sdrC</i>	sdrC-F	ACGACTATTAACCAAGAAC	560	45
	sdrC-R	GTAAGTAAATAAGCGGTTG		
<i>sdrD</i>	sdrD-F	GGAAATAAAGTTGAAGTTTC	500	45
	sdrD-R	ACTTTGTCATCAACTGTAAT		
<i>sdrE</i>	sdrE-F	CAGTAAATGTGTCAAAGA	767	45
	sdrE-R	TTGACTACCAGCTATATC		
<i>bbp</i>	BBP-1	AACTACATCTAGTACTCAACAACAG	575	55
	BBP-2	ATGTGCTTGAATAACACCATCATCT		
<i>icaA</i>	icaA-F	GATTATGTAATGTGCTTGGGA	770	50
	icaA-R	ACTACTGCTGCGTTAATAAT		
Toxins				
PVL	luk-PV-1	ATCATTAGGTAAAATGTCTGGACA TGATCCA	433	55
	luk-PV-2	GCATCAASTGTATTGGATAGCAAA AGC		
<i>hlg</i>	hlg-1	GCCAATCCGTTATTAGAAAATGC	937	55
	hlg-2	CCATAGACGTAGCAACGGAT		
<i>sea</i>	GSEAR-1	GGTTATCAATGTGCGGGTGG	102	57
	GSEAR-2	CGGCACTTTTTTCTCTTCGG		
<i>seb</i>	GSEBR-1	GTATGGTGGTGTAACTGAGC	164	57
	GSEBR-2	CCAAATAGTGACGAGTTAGG		
<i>sec</i>	GSECR-1	AGATGAAGTAGTTGATGTGTATGG	451	57
	GSECR-2	CACACTTTTAGAATCAACCG		
<i>sed</i>	GSEDR-1	CCAATAATAGGAGAAAATAAAAG	278	57
	GSEDR-2	ATTGGTATTTTTTTCGTTT		
<i>see</i>	GSEER-1	AGGTTTTTTCACAGGTCATCC	209	57
	GSEER-2	CTTTTTTCTTCGGTCAATC		
<i>eta</i>	GETAR-1	GCAGGTGTTGATTTAGCATT	93	57
	GETAR-2	AGATGTCCCTATTTTTGCTG		
<i>etb</i>	GETBR-1	ACAAGCAAAGAATAACAGCG	226	57
	GETBR-2	GTTTTTGGCTGCTTCTCTTG		
<i>tst</i>	GTSSSTR-1	ACCCCTGTTCCCTTATCATC	326	57
	GTSSSTR-2	TTTTTCAGTATTTGTAACGCC		

Methicillin resistance

<i>mecA</i>	MECA P4	TCCAGATTACAACCTTCACCAGG	162	53
	MECA P7	CCACTTCATATCTTGTAACG		

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558 <sup>1</sup>Table was taken and modified from Post et al. (Post et al., 2014)

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561 Table 2. Antibiotic resistance profiles of the MRSA isolates.

Region	MRSA No. <sup>1</sup>	Gentamicin	Tobramycin	Amikacin	Netilmicin	Tetracycline	Erythromycin	Clindamycin	Fusidic acid	Ciprofloxacin	Ofloxacin	Moxifloxacin	Levofloxacin	Trim/Sulf <sup>2</sup>	Rifampicin	Mupirocin
Africa	11	S	S	S	S	R	R	R	S	S	S	S	S	S	S	S
	24 <sup>4</sup>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Asia	7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	19	R	R	R	R	S	R	R	S	S	S	S	S	S	S	S
	22	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S
	8	R	R	R	R	S	S	S	S	R	ND	S	R	R	S	S
	17 <sup>4</sup>	S	S	S	S	S	R	R	S	R	R	R	R	S	S	S
	26 <sup>4</sup>	R	R	R	R	S	R	R	S	R	R	R	R	S	S	S
	4	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S
	16	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S
	1	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S
	12	S	S	S	S	R	R	R	S	R	ND	S	R	S	S	S
	6	R	R	R	R	R	R	R	S	R	R	R	R	S	S	S
	23	S	S	S	S	S	R	R	S	R	R	R	R	S	S	S
	18	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S
	25	S	S	S	S	R	R	R	S	S	S	S	S	S	S	S
Central America	5	S	S	S	S	S	R	R	S	R	R	R	R	S	S	S
Europe	2	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S
	13*	R	R	R	R	R	S	S	S	S	S	S	S	R	S	S
	15	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	9*	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S
	21	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	20 <sup>5</sup>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
South America <sup>3</sup>	3*	R	R	R	R	R	R	R	S	R	R	R	R	R	R	S
	10 <sup>4</sup>	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	14	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S
Total (n=26)		8	8	8	8	7	13	13	1	8	6	6	8	3	1	0

562 <sup>1</sup>All isolates were resistant (R) to: Penicillin, Oxacillin, Piperacillin, Mezlocillin, Ampicillin-  
563 Sulbactam, Cefotaxime, Cefuroxime, Ticarcclavulans, Cefoxitin and all isolates were susceptible  
564 (S) to: Tigecycline, Fosfomycin, Daptomycin, Vancomycin, Linezolid. ND stands for not  
565 determined.

566 <sup>2</sup>Trim/Sulf: Trimethoprim-Sulfamethoxazole.

567 <sup>3</sup>MRSA-3 isolate submitted for whole genome sequencing.

568 <sup>4</sup>Indicates intermediate biofilm production.

569 <sup>5</sup>Indicates strong biofilm production.

570 \*Indicates MRSA isolated from veterinary surgeons.

571 Table 3. Summary of the phenotypic and genotypic characterization of the MRSA isolates.

Region	Isolate No.	MLST	CC <sup>1</sup>	<i>spa</i> -type	<i>agr</i> -type <sup>2</sup>	HLG <sup>3</sup>	STX <sup>4</sup>	<i>lukFS-PV</i>	<i>tst</i>	<i>cna</i>	<i>fnbB</i>	<i>ebpS</i>	<i>fib</i>	<i>s</i>
Asia	1	ST1	1	t386	III	-	+	-	-	+	-	+	+	-
Africa	11	ST5	5	t688	II	+	-	-	-	-	-	+	+	-
Asia	12	ST5	5	t688	II	+	+	-	-	-	-	+	+	-
Central America	5	ST5	5	t895	II	-	-	-	-	-	-	+	+	-
Europe	9*	ST5	5	t1340	II	+	+	-	-	-	-	+	+	-
Asia	4	ST8	8	t1767	I	+	+	-	+	-	+	-	+	-
Asia	16	ST8	8	New <sup>5</sup>	I	+	+	-	+	-	+	-	+	-
South America	3*	ST8	8	t064	I	+	+	-	-	-	+	-	+	-
Asia	6	ST45	45	t14861	0	+	-	-	-	+	-	+	-	-
Europe	15	ST45	45	t230	I	-	-	-	-	+	-	+	-	-
Asia	19	ST59	59	t437	I	+	-	-	-	-	-	+	+	-
Asia	25	ST59	59	t437	I	+	+	-	+	-	-	-	+	-
Asia	22	ST59	59	t441	I	+	+	-	+	-	-	-	+	-
Asia	8	ST72	8	t3092	I	+	-	-	-	-	-	+	+	-
Asia	18	ST72	8	t324	I	+	-	-	-	-	-	+	+	-
Africa	24 <sup>#</sup>	ST88	88	t786	III	+	+	-	-	-	+	-	+	-
Asia	7	ST97	97	t267	I	+	+	-	-	-	+	+	+	-
South America	10 <sup>#</sup>	ST207	S	t525	III	+	-	-	-	+	+	+	+	-
Asia	17 <sup>#</sup>	ST217	S	t852	I	+	-	+	-	+	-	-	-	-
Europe	13*	ST398	398	t011	0	-	-	-	-	+	+	-	-	-
Europe	20 <sup>§</sup>	ST425	S	t6292	II	+	-	-	-	+	-	+	+	-
Europe	21	ST508	S	t1203	I	-	-	-	-	+	-	+	-	-
South America	14	ST641	S	t002	II	-	-	-	-	-	-	+	+	-
Europe	2	ST2112	S	t688	II	-	+	-	-	-	-	+	+	-
Asia	26 <sup>#</sup>	ST2124	S	t7428	I	+	-	+	-	+	-	-	-	-
Asia	23	ST2124	S	t9446	I	-	-	-	-	+	-	-	-	-

572 <sup>1</sup> CC: Clonal complex with S: S: singeltons.

573 <sup>2</sup> *agr*-type 0: no PCR product for any of the 4 *agr* types was obtained.

574 <sup>3</sup> HLG: Haemolytic activity, (+/- indicates active / not active).

575 <sup>4</sup> STX: Staphyloxanthin production (+/- indicates production / no production).

576 <sup>5</sup> Kreiswirth ID: YHGFC2BQQBLO.

577 <sup>#</sup>Indicates intermediate biofilm production.

578 <sup>§</sup>Indicates strong biofilm production.

579 NB: + / - indicates a positive / negative PCR amplification \*indicates the veterinarian nasal MRSA isolates. The *hlgC/B*, *i*  
580 present in all isolates. Ninety-six percent of isolates carried the *sdrE* gene.