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## Author Manuscript

Faculty of Biology and Medicine Publication

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Published in final edited form as:

**Title:** Antimicrobial resistance of Staphylococcus aureus strains acquired by pig farmers from pigs.

**Authors:** Oppliger A, Moreillon P, Charrière N, Giddey M, Morisset D, Sakwinska O

**Journal:** Applied and Environmental Microbiology

**Year:** 2012

**Issue:** 78

**Volume:** 22

**Pages:** 8010-8014

**DOI:** 10.1128/AEM.01902-12

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1 Antimicrobial resistance of *Staphylococcus aureus* acquired by pig farmers from  
2 pigs

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7 Running title: Resistant *S. aureus* acquired by farmers from pigs

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9 *Keywords:* CC398, MRSA, MSSA, livestock, *Staphylococcus aureus*,

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21

22 **Abstract**

23 Carriage of animal-associated MRSA CC398 is common among pig farmers. This study was  
24 conducted to investigate: 1) whether pig farmers are colonized with pig-specific *S. aureus*  
25 genotypes different than CC398, and 2) survey antimicrobial resistance of *S. aureus* isolates from  
26 pigs and pig farmers. Forty-eight *S. aureus* isolates from pig farmers and veterinarians and 130  
27 isolates from pigs collected in Western Switzerland were genotyped by spa-typing and amplified  
28 fragment length polymorphism (AFLP). Antimicrobial resistance profiles were determined for  
29 representative sample of the isolates. Obtained earlier data on healthy *S. aureus* carriers without  
30 exposure to agriculture were used for comparison. The genotype composition of *S. aureus*  
31 isolates from pig farmers and veterinarians was similar to isolates from pigs with predominant  
32 AFLP Clusters CC398, CC9 and CC49. The resistance to tetracycline and macrolides  
33 (clarithromycin) was common among the isolates from farmers and veterinarians (52% and 21%,  
34 respectively), and similar to resistance levels in isolates from pigs (39% and 23%, respectively).  
35 This was in contrast to isolates from persons without contact with agriculture, where no (0/128)  
36 isolates were resistant to tetracycline and 3% of isolates were resistant to clarithromycin. MRSA  
37 CC398 was isolated from pigs (n=11) and pig farmers (n=5). These data imply that zoonotic  
38 transmission of multidrug resistant *S. aureus* from pigs to farmers is frequent, and well-known  
39 MRSA transmission merely represents a tip of an iceberg of this phenomenon. We speculate that  
40 relatively low frequency of MRSA isolation is related to lower antimicrobial use in Switzerland  
41 compared to e.g. the Netherlands.

42 **Introduction**

43 Nasal carriage of animal associated (or livestock-associated LA) MRSA Clonal Complex 398  
44 among farmers and other persons in contact with animals has been widely reported (for recent  
45 review see (13)). First studies in the Netherlands and Denmark in the early 2000s (6, 17), were  
46 followed by reports from diverse locations, e.g. (8, 14, 20, 21, 42). The epidemiological link with  
47 nasal carriage by pigs was clear. Pigs appear to be natural host of CC398 lineage, but CC398  
48 strains can also be transmitted to humans given direct contact. CC398 had, apparently recently,  
49 acquired methicillin resistance, probably because of antimicrobial use and overuse in farming.  
50 People who have direct contact with pigs, such as pig farmers and veterinarians were reported to  
51 have very high carriage rates of MRSA, e.g. 26% in (36). Invasive infections with MRSA CC398  
52 strains have likewise been reported (5, 7, 22, 24, 35, 37, 38, 41) which is not entirely surprising  
53 given that the human nares are the reservoir of infection with *S. aureus* (39).  
54 Most studies of animal associated *S. aureus* have naturally been devoted to MRSA CC398, and  
55 its ability to colonize humans as well as pigs. There are few reports of other pig-associated  
56 MRSA genotypes which seem rare in humans: CC9, CC1 and CC49 (2, 15, 27, 34, 40). Pig-  
57 associated ST9 *S. aureus* can be transmitted to humans who were in contact with pigs (26).  
58 MSSA CC398 were reported from human carriage and infection (1, 35), but only infrequently. It  
59 is unclear whether the transmission of MSSA CC398 and other pig-specific genotypes to humans  
60 is as common as for MRSA CC398.  
61 MRSA strains isolated from pigs are often multi-resistant, mostly characteristically to  
62 tetracycline (2, 12). Interestingly, MSSA CC398 isolated from pigs also showed high level of  
63 antimicrobial resistance, predominantly to tetracycline and macrolides (34).

64 The aim of the present study was thus threefold: 1. Investigate whether pig farmers are colonized  
65 with pig-associated *S. aureus* other than MRSA CC398; 2. Examine the antimicrobial resistance  
66 of pig-associated *S. aureus*. 3. Explore links between genotype and antimicrobial resistance.

## 67 **Materials and Methods**

68 **Sample collection and processing.** The samples from pig were collected on 41 pig farms located  
69 in Western Switzerland between June 2008 and July 2009. The farms housed between 4 and 280  
70 pigs. Nasal samples from 344 pigs of different groups (suckling piglets, weanling pigs, grower-  
71 finisher pigs and sows) were collected with Amies agar transport swabs (Copan, Brescia, Italy)  
72 inserted 1 cm deep into nostrils. On average, 8.8 (range: 2-29) pigs per farm were sampled. In  
73 addition, swabs were also collected at slaughterhouse just after the slaughter from 66 pigs  
74 carcasses originating from two farms suspected positive for MRSA. Nasal samples from 67 pig  
75 farmers and 8 pig veterinarians were collected. Participants collected swab samples themselves  
76 using agar transport swabs. The ethical clearance for this study was sought and obtained from  
77 Ethical Committee of the University of Lausanne.

78 The swabs were stored at 4°C and processed within 3 weeks. Each swab was vigorously rubbed  
79 in 1 mL TE buffer and 100 µL was inoculated on SAID plate. To detect very low number of  
80 bacteria, the rest of the sample was transferred to 5 ml of Bacto m Staphylococcus Broth (Difco,  
81 BD, Allschwil, Switzerland) and incubated overnight at 37°C. The enrichment broth was then  
82 plated on SAID plate, and isolates were processed in the same manner as those from primary  
83 plates. To screen for MRSA, the enrichment broth was also plated on MRSASelect (BioRad,  
84 Reinach, Switzerland) selective chromogenic agar. Phenotypic screening for resistance to  
85 methicillin on MRSASelect plates was followed by a PCR screen for an internal fragment of  
86 *mecA* gene as described previously (31) using primers *mecA\_Sa\_fw\_865* (5'-AAA AAG CTC  
87 CAA CAT GAA GA-3') and *mecA\_Sa\_rv\_1211* (5'-GTT GAA CCTGGT GAA GTT GT-3').

88 **Genotyping.** DNA was extracted from 600 µL to 1mL overnight cultures of bacterial isolates  
89 grown in Brain Heart Infusion broth as described before (9). Amplified fragment length  
90 polymorphism (AFLP) was performed as described (31) except that only one set of selective  
91 primers was used. Electropherograms of AFLP were analyzed with GeneMapper software  
92 (Applied Biosystems, Carlsbad, CA, USA). Bayesian phylogeny was constructed with MrBayes  
93 (29). Multi-locus sequence typing (MLST) analysis was performed as previously described (11)  
94 on selected isolates. Sequence types (STs) were identified by consulting *S. aureus* MLST  
95 database (<http://www.mlst.net/>). The repeat region of *spa* gene was amplified as previously  
96 described (18, 33). The *spa*-types were assigned with the online *spa* database  
97 (<http://www.spaserver.ridom.de/>).

98 **Antimicrobial susceptibility testing.** Antibiotic susceptibility profiles were established for all  
99 isolates from farmers and veterinarians and at least one isolate of a distinct *spa*-type per farm  
100 among the animal isolates. The disk diffusion assay was used in accordance with CASFM  
101 (Antimicrobial Committee of the French Microbiology Society) guidelines (3) or, alternatively,  
102 with VITEK2 system (bioMerieux, Geneva, Switzerland). A panel of 24 compounds was used to  
103 cover antimicrobials used in both human and veterinary medicine. Antimicrobial resistance was  
104 likewise determined in a collection of *S. aureus* strains from healthy human carriers in urban  
105 environment, thus presumably without contact with pigs or other farms animals. These strains  
106 were collected in Western Switzerland in 2005-2006 (31).

## 107 **Results**

108 **Colonization with MRSA and MSSA.** 36% (123/343) of live animals carried *S. aureus* in their  
109 noses. Colonized pigs were present on 75% (31/41) of the farms. More than one strain was found  
110 in 7 animals. Among samples taken from nostrils of 66 pig carcasses, only one did not grow *S.*  
111 *aureus*. 44 out of 75 (57%) pig farmers and veterinarians were *S. aureus* carriers. MRSA was

112 found in 11 pigs from 3 farms. Five pig farmers were MRSA carriers. We did not find any signs  
113 of *S. aureus* infection, neither among animals nor people.

114 **Genotype composition.** Clustering of AFLP data and MLST genotyping of several isolates per  
115 AFLP Cluster were used to establish the identity of AFLP Clusters as in (30, 32). The genotype  
116 composition of *S. aureus* isolates from pigs and pig farmers and veterinarians was compared to  
117 those obtained earlier for healthy volunteers without contact with agriculture and from cow  
118 farmers (30, 31).

119 The genotype composition of *S. aureus* from pigs and from pig farmers and veterinarians was  
120 similar (Figure 1, Table S1, Figure S1). Pig isolates were dominated by Cluster 9, 398, and 49.  
121 More than half of the pig farmers and veterinarian's isolates belonged to these three Clusters; the  
122 rest to more typically human-associated Clusters. This was in contrast to cow farmers, who  
123 harbored *S. aureus* strains very similar to those from healthy volunteers.

124 **Antimicrobial resistance.** Farmers and veterinarians harbored resistant isolates more frequently  
125 than people without contact with pigs (Figure 2). This was particularly striking in case of  
126 tetracycline, where 50% of farmers' isolates were resistant, while people without exposure to pigs  
127 harbored none (0/123) (Yates corrected chi-square test,  $df = 1$ ;  $Chi^2 = 73.5$ ). The resistance to  
128 clindamycin, clarithromycin, oxacillin and cefoxitin was also common among farmers' isolates,  
129 around 20%, and virtually absent among non-farmers' (Yates corrected chi-square test,  $df = 1$  ;  
130  $Chi^2 = 23.6$ ,  $p < 0.001$ ;  $Chi^2 = 12.6$ ,  $p < 0.001$ ;  $Chi^2 = 5.0$ ,  $p = 0.025$  and  $Chi^2 = 5.0$ ,  $p = 0.025$   
131 respectively). Resistance to erythromycin, lincomycin, spiramycin and streptomycin was also  
132 common in farmers' and veterinarians' isolates: around 20% of isolates showed resistance to  
133 these compounds; the isolates from non-farmers were not tested. The resistance to antimicrobials  
134 was similar among isolates from pigs and pig farmers (Yates corrected chi-square test:  $p > 0.05$  for  
135 all tested antimicrobials, Figure 2, Table 1). Only resistance to penicillin was common (70%)

136 among the isolates from non-farmers and it was similar in the three groups of isolates ( $\text{Chi}^2 =$   
137 23.551,  $\text{df} = 2$ ,  $p = 0.241$ ). No resistance to vancomycin, teicoplanin, synercid, linezolid, mupirocin  
138 and rifampicin was detected. Clear link between resistance and genotype was seen. MSSA  
139 isolates from the same Cluster 398 isolated from pigs and farmers were nearly uniformly  
140 tetracycline resistant: only 1 out of 34 isolates was sensitive to tetracycline. Only three isolates of  
141 MSSA CC398 were found in non-farmers, but they were all sensitive to tetracycline (Table 1).  
142 The other genotypes were less numerous, but pig-associated Cluster 49 and 9 (from pigs and pig-  
143 farmers) showed significantly less resistance to tetracycline, clindamycin, penicillin,  
144 ciprofloxacin, erythromycin, spiramycin and clarithromycin and the same resistance to  
145 streptomycin and lincomycin compared to MSSA CC398 (from pigs and pig farmers) (Pearson  
146 chi-square,  $\text{df} = 2$  :  $\text{Chi}^2 = 66.4$ ,  $p < 0.001$ ;  $\text{Chi}^2 = 21.9$ ,  $p < 0.001$ ;  $\text{Chi}^2 = 30.2$ ,  $p < 0.001$ ;  $\text{Chi}^2 =$   
147 6.3,  $p = 0.043$ ;  $\text{Chi}^2 = 21.9$ ,  $p < 0.001$ ,  $\text{Chi}^2 = 21.9$ ,  $p < 0.001$ ;  $\text{Chi}^2 = 21.9$ ,  $p < 0.001$ ;  $\text{Chi}^2 = 5.3$ ,  
148  $p = 0.068$ ;  $\text{Chi}^2 = 4.8$ ,  $p = 0.09$  respectively). Both MSSA and MRSA CC398 strains isolated  
149 from pigs and pig farmers, displayed high level of multiresistance, typically to tetracycline,  
150 macrolides and lincosamides. 42% (55/131) and 22% (29/131) of isolates were resistant to at  
151 least two and at least three classes of antimicrobials (other than penicillin), respectively (Table 1).

## 152 **Discussion**

153 The results of our study are quite clear-cut: Pig farmers and veterinarians harbor much more  
154 resistant *S. aureus* than people without contact with pigs. Contact with pigs is a risk factor not  
155 only for MRSA carriage but also for carriage of *S. aureus* sensitive to methicillin but resistant to  
156 tetracycline and often macrolides and lincosamides. The overall prevalence of *S. aureus* carriage  
157 was also higher among farmers and veterinarians than among people without contact with pigs.  
158 The antimicrobial resistance among isolates from pigs and farmers and veterinarians was  
159 virtually the same. High antimicrobial resistance of *S. aureus* carried by farmers and veterinarians



160 was clearly linked to the fact that the genotype composition of *S. aureus* from pigs and farmers  
161 was quite similar, implying that *S. aureus* was readily transmitted from pigs to humans who  
162 remain in contact with these animals. Pigs were colonized by *S. aureus* from a few specific  
163 lineages rarely found in humans: Cluster 398, Cluster 9 and Cluster 49. All three Clusters were  
164 likewise found in farmers and veterinarians, however, Cluster 398 was both the most  
165 predominant and it displayed the highest levels of antimicrobial resistance. Strains from Clusters  
166 9 and 49, although they did get transmitted from pigs to humans, did not show considerable  
167 antimicrobial resistance. The documented route of transmission for *Staphylococcus* sp. is by  
168 direct contact. However, we suspect that inhalation of contaminated air may play a role as both  
169 MSSA and MRSA was detected in the air of animal houses (Masclaux et al, unpublished data).  
170 The real colonization rate of pigs was most likely higher than evidenced in our study. Carcasses  
171 which can be sampled without constraints yielded much higher prevalence (nearly 100%), which  
172 suggests that our sampling technique was not exhaustive enough. This, however, does not affect  
173 our conclusions about the high levels of antimicrobial resistance among the isolates which we  
174 were able to collect.

175 In our survey, MRSA prevalence in pig colonization was low compared to other European  
176 countries. This was in agreement with other studies conducted in Switzerland (19, 27, 34). We  
177 speculate that this might be due to differences in the use of antimicrobial agents in farming. The  
178 Netherlands is the biggest user of antibacterial agents in livestock in Europe with 514 tons sold in  
179 2009, while in Switzerland this was 70 tons. 188 mg of antimicrobials per kg biomass of animal  
180 meat produced is used in the Netherlands, while in Switzerland it is 86 mg (16). Although these  
181 figures concern the total volume of sales, it can be assumed that the quantity used in pigs is  
182 proportional to the total use.

183 Different patterns of antimicrobial resistance may well be due to the differences in the  
184 management of pig farming. In Switzerland the number of animals per farm is low compared to  
185 most European countries. 60% of the pig farms have less than 50 animals and pig houses are  
186 restricted to a maximum of 1000 pigs (data available on website;  
187 <http://www.agriculture.ch/fr/infos/animaux/porcs/>), while in the Netherlands, in 2010, there were  
188 700 farms with more than 2000 pigs (data available on website;  
189 <http://www.gov.mb.ca/agriculture/livestock/pork/pdf/swineseminar2011/bab25s00n.pdf>). At least  
190 in the Netherlands, the use of antimicrobial is highly associated with farm size. Farms with less  
191 than 250 sows used on average 15 daily doses, while those with more than 600 sows used 50  
192 daily doses of antimicrobials (25). Moreover, pigs grown in alternative holding systems, where  
193 the use of antimicrobials is more restrictive, appears to be less prone to colonization with MRSA  
194 (4).

195 The antimicrobial agents most used in Swiss veterinary medicine are: sulfonamides (41.7%)  
196 followed by tetracyclines (22.7 %) and penicillins (18.8%), (10). Indeed between 55% and 70 %  
197 of total quantities of veterinary antimicrobial agents sold in 8 countries (CZ, FI, DK, FR, NL,  
198 NO, SE and UK) are tetracyclines and penicillins while only 10% to 25% are sulfonamides (10).  
199 Although tetracycline and penicillin are not the most frequently sold antimicrobials in  
200 Switzerland, the resistance level against them are high. On the other hand, resistance against  
201 sulfonamides (Bactrim) which is theoretically the most used antimicrobial is absent. However, in  
202 pig farming tetracycline might be used more than sulfonamides. Unfortunately, animal-specific  
203 data are difficult to obtain, but the data from Dutch report (25), show that tetracycline  
204 administration amounted to 70% of antimicrobial use in fattening pigs and only 10 % in dairy  
205 cattle.

206 The resistance of *S. aureus* from animal sources to antimicrobials other than beta-lactams is  
207 rarely investigated. One can suspect the antimicrobial resistance among MSSA is in line with  
208 prevalence of MRSA in other European countries, and therefore probably much higher than  
209 documented here for Switzerland. Interestingly, antimicrobial resistance of bovine mastitis *S.*  
210 *aureus* isolates was low, even to penicillin (32). The reason for this is not entirely clear, but  
211 suggests that antimicrobial resistance is likely to be driven by non-therapeutic use more than  
212 directed therapeutic use. *S. aureus* in bovine mastitis is the target for eradication, while in pigs  
213 the nasal colonization is not particularly triggered with antibiotics given to pigs.

214 Widespread nasal carriage of multi-resistant *S. aureus* in pig farmers and veterinarians can be  
215 easily overlooked, especially in case of MSSA but can be of medical significance in case of  
216 hospitalization. Therefore, pig farmers and veterinary practitioners, as well as medical staff need  
217 to be aware of the possibility of MRSA and multiresistant MSSA colonization. Moreover,  
218 standard hygiene practices of pig farmers (hand washing after each contact with pig and use of  
219 piggery-specific work clothing) should be reinforced by the use of personal protective equipment  
220 such as gloves when handling animals and respirator mask (P2 type) when carrying out activities  
221 that generate lots of dust.

222 It is generally accepted that the emergence of MRSA CC398 is due to relatively recent  
223 acquisition of genomic island *SCCmec*, which confers resistance to beta-lactams, by MSSA  
224 CC398. Large variability of *SCCmec* found in MRSA CC398 (12, 23) suggests multiple  
225 acquisition events. Detailed phylogenetic comparison of CC398 strains isolated from humans and  
226 animals supported strong and diverse antimicrobial selection (28). Humans could be the original  
227 host of CC398, which acquired resistance to methicillin after the introduction to livestock from  
228 humans. Our results suggest that the acquisition of resistance to tetracycline and macrolides

229 might precede the emergence of MRSA. In this context, multiresistant MSSA heralds the arrival  
230 of multiresistant MRSA.

231 **Acknowledgments**

232 We are grateful to farmers and veterinarians for their participation. We would like to thank

233 Eulalia Semaani for her invaluable help in obtaining the samples.

234 **References**

235

Table 1. Percentage of *S. aureus* isolates resistant to antimicrobials originating from pigs, pig farmers and veterinarians, and non-farmers. ND – not done.

Genotype		N	Tetracycline	Erythromycin	Clarithromycin	Spiramycin	Lincomycin	Clindamycin	Oxacillin	Cefoxitin	Penicillin	Augmentin	Ceftriaxone	Gentamicin	Streptomycin	Kanamycin	Fusidic Acid	Bactrim	Ciprofloxacin	Levofloxacin
Pigs	All	83	<b>39</b>	<b>23</b>	<b>23</b>	<b>23</b>	<b>40</b>	<b>23</b>	<b>13</b>	<b>13</b>	<b>60</b>	<b>6</b>	<b>0</b>	<b>5</b>	<b>33</b>	<b>2</b>	<b>6</b>	<b>6</b>	<b>0</b>	<b>0</b>
	9	32	3	0	0	0	22	0	0	0	28	0	0	6	25	0	6	0	0	0
	49	11	9	0	0	0	36	0	0	0	64	0	0	0	36	0	9	0	0	0
	398MS	19	100	47	47	47	47	47	0	0	89	5	0	5	53	5	0	5	0	0
	398MR	11	100	91	91	91	91	91	100	100	100	36	0	0	27	0	0	27	0	0
	Others	10	0	0	0	0	30	0	0	0	60	0	0	10	20	10	20	10	0	0
Pigs farmers and vets	All	48	<b>52</b>	<b>27</b>	<b>21</b>	<b>17</b>	<b>23</b>	<b>23</b>	<b>10</b>	<b>10</b>	<b>73</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>25</b>	<b>2</b>	<b>4</b>	<b>4</b>	<b>8</b>	<b>8</b>
	9	6	17	0	0	0	0	0	0	0	17	0	0	0	17	0	0	0	0	0
	49	2	50	50	50	50	100	50	0	0	50	0	0	0	50	0	50	0	0	0
	398MS	15	93	33	33	33	33	33	0	0	80	0	0	0	47	0	0	0	27	4
	398MR	5	100	80	33	33	67	80	100	100	100	0	33	0	67	33	0	20	0	0
	Other	20	15	15	15	5	10	5	0	0	75	0	0	0	5	0	5	5	0	0
Non farmers	All	128	<b>0</b>	<b>ND</b>	<b>3.1</b>	<b>ND</b>	<b>ND</b>	<b>0.8</b>	<b>1.6</b>	<b>1.6</b>	<b>70</b>	<b>ND</b>	<b>0.8</b>	<b>0</b>	<b>ND</b>	<b>ND</b>	<b>0.8</b>	<b>0</b>	<b>3.1</b>	<b>3.1</b>
	398MS	3	0	ND	33	ND	ND	0	0	0	67	ND	0	0	ND	ND	0	0	0	0

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