Carriage of animal-associated methicillin-resistant *Staphylococcus aureus* (MRSA) clonal complex 398 (CC398) is common among pig farmers. This study was conducted (i) to investigate whether pig farmers are colonized with pig-specific *S. aureus* genotypes other than CC398 and (ii) to survey antimicrobial resistance of *S. aureus* isolates from pigs and pig farmers. Forty-eight *S. aureus* isolates from pig farmers and veterinarians and 130 isolates from pigs collected in Western Switzerland were genotyped by spa typing and amplified fragment length polymorphism (AFLP). Antimicrobial resistance profiles were determined for representative sample of the isolates. The data obtained earlier on healthy *S. aureus* carriers without exposure to agriculture were used for comparison. The genotype composition of *S. aureus* isolates from pig farmers and veterinarians was similar to isolates from pigs with predominant AFLP clusters CC398, CC9, and CC49. The resistance to tetracycline and macrolides (clarithromycin) was common among the isolates from farmers and veterinarians (52 and 21%, respectively) and similar to resistance levels in isolates from pigs (39 and 23%, respectively). This was in contrast to isolates from persons without contact with agriculture, where no (0/128) isolates were resistant to tetracycline and 3% of the isolates were resistant to clarithromycin. MRSA CC398 was isolated from pigs (n = 11) and pig farmers (n = 5). These data imply that zoonotic transmission of multidrug-resistant *S. aureus* from pigs to farmers is frequent, and well-known MRSA transmission merely represents the tip of the iceberg for this phenomenon. We speculate that the relatively low frequency of MRSA isolation is related to lower antimicrobial use in Switzerland compared to, for example, the Netherlands.

The aim of the present study was thus 3-fold: (i) to investigate whether pig farmers are colonized with pig-associated *S. aureus* strains other than MRSA CC398, (ii) to examine the antimicrobial resistance of pig-associated *S. aureus*, and (iii) to explore links between genotype and antimicrobial resistance.

### MATERIALS AND METHODS

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

The swabs were stored at 4°C and processed within 3 weeks. Each swab...
was vigorously rubbed in 1 ml of Tris-EDTA buffer, and 100 µl was inoculated on an *S. aureus* identification agar plate (SAID plates; bioMérieux, Geneva, Switzerland). To detect very low number of bacteria, the rest of the sample was transferred to 5 ml of Bacto *Staphylococcus* broth (Difco/BD, Allschwil, Switzerland), followed by incubation overnight at 37°C. The enrichment broth was then plated on a SAID plate, and isolates were processed in the same manner as those from primary plates. To screen for MRSA, the enrichment broth was also plated on MRSASelect (Bio-Rad, Reinchach, Switzerland) selective chromogenic agar. Phenotypic screening for resistance to meticillin on MRSASelect plates was followed by a PCR screen for an internal fragment of the *mecA* gene as previously described (31) using the primers mecA_Sa_fw_865 (5'-AAA ACG TCA GAA CAT GAA GA-3') and mecA_Sa_rev_1211 (5'-GTG GAA CCTTGTT GAA GTT GT-3').

Genotyping. DNA was extracted from 600-µl to 1-ml overnight cultures of bacterial isolates grown in brain heart infusion broth as described before (9). Amplified fragment length polymorphism (AFLP) was performed as described previously (31), except that only one set of selective primers was used. Electropherograms of AFLP were analyzed using GeneMapper software (Applied Biosystems, Carlsbad, CA). Bayesian phylogeny was constructed with MrBayes (29). Multilocus sequence typing (MLST) analysis was performed as previously described (11) on selected isolates. Sequence types (STs) were identified by consulting the *S. aureus* MLST database (http://www.mlst.net/). The repeat region of the *spa* gene was amplified as previously described (18, 33). The *spa* types were assigned with the online *spa* database (http://www.spaserver.ridom.de/).

Antimicrobial susceptibility testing. Antibiotic susceptibility profiles were established for all isolates from farmers and veterinarians and at least one isolate of a distinct *spa* type per farm among the animal isolates. A disk diffusion assay was used in accordance with CASFM (Antimicrobial Committee of the French Microbiology Society) guidelines (3) or, alternatively, with the Vitek2 system (bioMérieux, Geneva, Switzerland). A panel of 24 compounds was used to cover antimicrobials used in both human and veterinary medicine. Antimicrobial resistance was likewise determined in a collection of *S. aureus* strains from healthy human carriers in an urban environment, thus presumably without contact with pigs or other farm animals. These strains were collected in Western Switzerland from 2005 to 2006 (31).

RESULTS

Colonization with MRSA and MSSA. A total of 36% (123/343) of live animals carried *S. aureus* in their noses. Colonized pigs were present on 75% (31/41) of the farms. More than one strain was found in seven animals. Among samples taken from the nostrils of 66 pig carcasses, only one did not grow *S. aureus*. Of 75 pig farmers and veterinarians tested, 44 (57%) were *S. aureus* carriers. MRSA was found in eleven pigs from three farms. Five pig farmers were MRSA carriers. We did not find any signs of *S. aureus* infection among animals or people.

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

The genotype composition of *S. aureus* from pigs and from pig farmers and veterinarians was similar (Fig. 1; see also Table S1 and Fig. S1 in the supplemental material). Pig isolates were dominated by clusters 9, 398, and 49. More than half of the pig farmer and veterinarian isolates belonged to these three clusters; the remainder belonged to more typically human-associated clusters. This finding was in contrast to cow farmers, who harbored *S. aureus* strains very similar to those from healthy volunteers.

Antimicrobial resistance. Farmers and veterinarians harbored resistant isolates more frequently than people without contact with pigs (Fig. 2). This was particularly striking in case of tetracycline, where 50% of farmers' isolates were resistant, whereas people without exposure to pigs harbored none (0/123) (Yates corrected chi-square test, df = 1; \( \chi^2 = 73.5 \)). Resistance to clindamycin, clarithromycin, oxacillin, and cefoxitin was also common among isolates from pig farmers (ca. 20%) and virtually absent among nonfarmers, and it was similar in the three groups of isolates (\( \chi^2 = 23.6, P < 0.001; \chi^2 = 12.6, P < 0.001; \chi^2 = 5.0, P = 0.025; \) and \( \chi^2 = 5.0, P = 0.025 \), respectively [Yates corrected chi-square test]). Resistance to erythromycin, lincomycin, spironamycin, and streptomycin was also common in farmer and veterinarian isolates (ca. 20% of the isolates showed resistance to these compounds); the isolates from nonfarmers were not tested. Resistance to antimicrobials was similar among isolates from pigs and pig farmers (\( P > 0.05 \) for all tested antimicrobials [Yates corrected chi-square test], Fig. 2 and Table 1). Only resistance to penicillin was common (70%) among the isolates from nonfarmers, and it was similar in the three groups of isolates (\( \chi^2 = 23.551, df = 2, P = 0.241 \)). No resistance to vancomycin, teicoplanin, synergicin, linezolid, mupirocin, and rifampin was detected. A clear link between resistance and genotype was seen. MSSA isolates from the same cluster 398 isolated from pigs and farmers were nearly uniformly tetracycline resistant: only 1 of 34 isolates was sensitive to tetracycline. Only three isolates of MSSA CC398 were found in nonfarmers, but they were all sensitive to tetracycline (Table 1). The other genotypes were less numerous, but pig-associated clusters 49 and 9 (from pigs and pig farmers) showed significantly less resistance to tetracycline, erythromycin, clarithromycin, spironamycin, clindamycin, penicillin, and ciprofloxacin and the same resistance to streptomycin and lincomycin compared to MSSA CC398 (from pigs and pig farmers) (\( df = 2; \chi^2 = 66.4, P < 0.001; \chi^2 = 21.9, P < 0.001; \chi^2 = 21.9, P < 0.001; \chi^2 = 21.9, P < 0.001; \chi^2 = 30.2, P < 0.001; \chi^2 = 6.3, P = 0.043; \chi^2 = 5.3, P = 0.068; \) and \( \chi^2 = 4.8, P = 0.09 \), respectively [Pearson chi-square]). Both MSSA and MRSA CC398 strains isolated from pigs and pig farmers displayed high level of multiresistance, typically to tetracycline, mac-

![FIG 1 Genotype compositions of *S. aureus* isolates from nasal carriage. The colors represent clusters defined by AFLP analysis.](http://aem.asm.org/.../aem.asm.org)
DISCUSSION

The results of our study are quite clear-cut: pig farmers and veterinarians harbor much more resistant strains of *S. aureus* than people who do not have contact with pigs. Contact with pigs is a risk factor not only for MRSA carriage but also for carriage of *S. aureus* strains sensitive to methicillin but resistant to tetracycline and often macrolides and lincosamides. The overall prevalence of *S. aureus* carriage was also higher among farmers and veterinarians than among people without contact with pigs. The levels of antimicrobial resistance among isolates from pigs, farmers, and veterinarians were virtually the same. The high antimicrobial resistance of the *S. aureus* strains carried by farmers and veterinarians was clearly linked to the fact that the genotype compositions of the *S. aureus* from pigs and farmers were quite similar, implying that *S. aureus* was readily transmitted from pigs to humans who remain in contact with these animals. Pigs were colonized by *S. aureus* from a few specific lineages rarely found in humans: clusters 398, 9, and 49. All three clusters were likewise found in farmers and veterinarians; however, cluster 398 was not only the most predominant cluster but also displayed the highest levels of antimicrobial resistance. Strains from clusters 9 and 49, although they were transmitted from pigs to humans, did not show considerable antimicrobial resistance. The documented route of transmission for *Staphylococcus* spp. is by direct contact. However, we suspect that inhalation of contaminated air may play a role since both MSSA and MRSA were detected in the air of animal houses (F. Masclaux et al., unpublished data). The real colonization rate of pigs was most likely higher than evidenced in our study. Carcasses that can be sampled without constraints yielded much higher prevalence (nearly 100%), which suggests that our sampling technique was not exhaustive enough. This, however, does not affect our conclusions about the high levels of antimicrobial resistance among the isolates that we were able to collect.

In our survey, MRSA prevalence in pig colonization was low compared to other European countries. This was in agreement with other studies conducted in Switzerland (19, 27, 34). We speculate that this might be due to differences in the use of antimicrobial agents in farming. The Netherlands is the biggest user of antibacterial agents in livestock in Europe, with 514 tons sold in 2009, while in Switzerland this was 70 tons. A total of 188 mg of antimicrobials per kg (biomass) of animal meat produced is used in the Netherlands, while in Switzerland it is 86 mg (16). Although these figures concern the total volume of sales, it can be assumed that the quantity used in pigs is proportional to the total use. Different patterns of antimicrobial resistance may well be due to the differences in the management of pig farming. In Switzerland, the number of animals per farm is low compared to most European countries. A total of 60% of the pig farms have fewer than 50 animals, and pig houses are restricted to a maximum of 1,000 pigs (http://www.agriculture.ch/fr/infos/animal/porc/), whereas in the Nether-
lands, in 2010, there were 700 farms with more than 2,000 pigs (http://www.gov.mb.ca/agriculture/livestock/pork/pdf/swineseminar2011/bab25s00n.pdf). At least in the Netherlands, the use of antimicrobial is highly associated with farm size. Farms with fewer than 250 sows used on average 15 daily doses, while those with more than 600 sows used 50 daily doses of antimicrobials (25). Moreover, pigs grown in alternative holding systems, where the use of antimicrobials is more restrictive, appear to be less prone to colonization with MRSA (4).

The antimicrobial agents most used in Swiss veterinary medicine are sulfonamides (41.7%), followed by tetracyclines (22.7%) and penicillins (18.8%) (10). Indeed, between 55 and 70% of the total quantities of veterinary antimicrobial agents sold in eight countries (the Czech Republic, Finland, Denmark, France, the Netherlands, Norway, Sweden, and the United Kingdom) are tetracyclines and penicillins, whereas only 10 to 25% are sulfonamides (10). Although tetracycline and penicillin are not the most frequently sold antimicrobials in Switzerland, the resistance level against them is high. On the other hand, resistance to sulfonamides (bactrim), which is theoretically the most commonly used antimicrobial, is absent. However, in pig farming, tetracycline might be used more than sulfonamides. Unfortunately, animal-specific data are difficult to obtain, but the data from a Dutch report (25), show that tetracycline administration amounted to 70% of antimicrobial use in fattening pigs and only 10% in dairy cattle.

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

The widespread nasal carriage of multiresistant *S. aureus* in pig farmers and veterinarians can be easily overlooked, especially in the case of MSSA, but can be of medical significance in case of MRSA. In this context, multiresistant MSSA heralds the arrival of multiresistant MRSA.

**ACKNOWLEDGMENTS**

We are grateful to the farmers and veterinarians involved in this study for their participation. We thank Eulalia Semanii for her invaluable help in obtaining the samples.

**REFERENCES**


