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

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Abstract

Carriage of animal-associated MRSA CC398 is common among pig farmers. This study was conducted to investigate: 1) whether pig farmers are colonized with pig-specific *S. aureus* genotypes different than CC398, and 2) 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. Obtained earlier data 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 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 a tip of an iceberg of this phenomenon. We speculate that relatively low frequency of MRSA isolation is related to lower antimicrobial use in Switzerland compared to e.g. the Netherlands.

Introduction

Nasal carriage of animal associated (or livestock-associated LA) MRSA Clonal Complex 398 among farmers and other persons in contact with animals has been widely reported (for recent review see (13)). First studies in the Netherlands and Denmark in the early 2000s (6, 17), were followed by reports from diverse locations, e.g. (8, 14, 20, 21, 42). The epidemiological link with nasal carriage by pigs was clear. Pigs appear to be natural host of CC398 lineage, but CC398 strains can also be transmitted to humans given direct contact. CC398 had, apparently recently, acquired methicillin resistance, probably because of antimicrobial use and overuse in farming. People who have direct contact with pigs, such as pig farmers and veterinarians were reported to have very high carriage rates of MRSA, e.g. 26% in (36). Invasive infections with MRSA CC398 strains have likewise been reported (5, 7, 22, 24, 35, 37, 38, 41) which is not entirely surprising given that the human nares are the reservoir of infection with *S. aureus* (39).

Most studies of animal associated *S. aureu*s have naturally been devoted to MRSA CC398, and its ability to colonize humans as well as pigs. There are few reports of other pig-associated MRSA genotypes which seem rare in humans: CC9, CC1 and CC49 (2, 15, 27, 34, 40). Pig-associated ST9 *S. aureus* can be transmitted to humans who were in contact with pigs (26). MSSA CC398 were reported from human carriage and infection (1, 35), but only infrequently. It is unclear whether the transmission of MSSA CC398 and other pig-specific genotypes to humans is as common as for MRSA CC398.

MRSA strains isolated from pigs are often multi-resistant, mostly characteristically to tetracycline (2, 12). Interestingly, MSSA CC398 isolated from pigs also showed high level of antimicrobial resistance, predominantly to tetracycline and macrolides (34).

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

Materials and Methods

Sample collection and processing. The samples from pig 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 nostrils. On average, 8.8 (range: 2-29) pigs 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 collected. Participants collected swab samples themselves using agar transport swabs. The ethical clearance for this study was sought and obtained from Ethical Committee of the University of Lausanne.

The swabs were stored at 4ºC and processed within 3 weeks. Each swab was vigorously rubbed in 1 mL TE buffer and 100 µL was inoculated on SAID plate. To detect very low number of bacteria, the rest of the sample was transferred to 5 ml of Bacto m Staphylococcus Broth (Difco, BD, Allschwil, Switzerland) and incubated overnight at 37ºC. The enrichment broth was then plated on 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 (BioRad, Reinach, Switzerland) selective chromogenic agar. Phenotypic screening for resistance to methicillin on MRSASelect plates was followed by a PCR screen for an internal fragment of *mecA* gene as described previously (31) using primers mecA_Sa_fw_865 (5’-AAA AAG CTC CAA CAT GAA GA-3’) and mecA_Sa_rv_1211 (5’-GTT GAA CCTGGT GAA GTT GT-3’).

Genotyping. DNA was extracted from 600 µL to 1mL overnight cultures of bacterial isolates grown in Brain Heart Infusion broth as described before (9). Amplified fragment length polymorphism (AFLP) was performed as described (31) except that only one set of selective primers was used. Electropherograms of AFLP were analyzed with GeneMapper software (Applied Biosystems, Carlsbad, CA, USA). Bayesian phylogeny was constructed with MrBayes (29). Multi-locus sequence typing (MLST) analysis was performed as previously described (11) on selected isolates. Sequence types (STs) were identified by consulting *S. aureus* MLST database (http://www.mlst.net/). The repeat region of *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. The disk diffusion assay was used in accordance with CASFM (Antimicrobial Committee of the French Microbiology Society) guidelines (3) or, alternatively, with VITEK2 system (bioMerieux, 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 urban environment, thus presumably without contact with pigs or other farms animals. These strains were collected in Western Switzerland in 2005-2006 (31).

Results

Colonization with MRSA and MSSA. 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 7 animals. Among samples taken from nostrils of 66 pig carcasses, only one did not grow *S. aureus*. 44 out of 75 (57%) pig farmers and veterinarians were *S. aureus* carriers. MRSA was found in 11 pigs from 3 farms. Five pig farmers were MRSA carriers. We did not find any sings of *S. aureus* infection, neither among animals nor 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 in (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 (Figure 1, Table S1, Figure S1). Pig isolates were dominated by Cluster 9, 398, and 49. More than half of the pig farmers and veterinarian's isolates belonged to these three Clusters; the rest to more typically human-associated Clusters. This 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 (Figure 2). This was particularly striking in case of tetracycline, where 50% of farmers' isolates were resistant, while people without exposure to pigs harbored none (0/123) (Yates corrected chi-square test, df =1; $\text{Chi}^2 = 73.5$). The resistance to clindamycin, clarithromycin, oxacillin and cefoxitin was also common among farmers' isolates, around 20%, and virtually absent among non-farmers' (Yates corrected chi-square test, df = 1 ; $\text{Chi}^2 = 23.6$, $p < 0.001$; $\text{Chi}^2 = 12.6$, $p<0.001$; $\text{Chi}^2= 5.0$, $p= 0.025$ and $\text{Chi}^2 = 5.0$, $p= 0.025$ respectively). Resistance to erythromycin, lincomycin, spiramycin and streptomycin was also common in farmers' and veterinarians' isolates: around 20% of isolates showed resistance to these compounds; the isolates from non-farmers were not tested. The resistance to antimicrobials was similar among isolates from pigs and pig farmers (Yates corrected chi-square test: $p>0.05$ for all tested antimicrobials, Figure 2, Table 1). Only resistance to penicillin was common (70%)

among the isolates from non-farmers and it was similar in the three groups of isolates (Chi^2 = 23.551, df =2, p=0.241). No resistance to vancomycin, teicoplanin, synercid, linezolid, mupirocin and rifampicin was detected. 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 out of 34 isolates was sensitive to tetracycline. Only three isolates of MSSA CC398 were found in non-farmers, but they were all sensitive to tetracycline (Table 1). The other genotypes were less numerous, but pig-associated Cluster 49 and 9 (from pigs and pig-farmers) showed significantly less resistance to tetracycline, clindamycin, penicillin, ciprofloxacin, erythromycin, spiramycin and clarithromycin and the same resistance to streptomycin and lincomycin compared to MSSA CC398 (from pigs and pig farmers) (Pearson chi-square, df = 2 : Chi^2 = 66.4, $p<0.001$; Chi^2 = 21.9, $p< 0.001$; Chi^2 = 30.2, $p< 0.001$; Chi^2 = 6.3, p = 0.043; Chi^2 = 21.9, $p<0.001$, Chi^2 = 21.9, $p<0.001$; Chi^2 = 21.9, $p<0.001$; Chi^2 = 5.3, p = 0.068; Chi^2 = 4.8, p = 0.09 respectively). Both MSSA and MRSA CC398 strains isolated from pigs and pig farmers, displayed high level of multiresistance, typically to tetracycline, macrolides and lincosamides. 42% (55/131) and 22% (29/131) of isolates were resistant to at least wo and at least three classes of antimicrobials (other than penicillin), respectively (Table 1).

Discussion

The results of our study are quite clear-cut: Pig farmers and veterinarians harbor much more resistant *S. aureus* than people without contact with pigs. Contact with pigs is a risk factor not only for MRSA carriage but also for carriage of *S. aureus* 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 antimicrobial resistance among isolates from pigs and farmers and veterinarians was virtually the same. High antimicrobial resistance of *S. aureus* carried by farmers and veterinarians was clearly linked to the fact that the genotype composition of *S. aureus* from pigs and farmers was 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: Cluster 398, Cluster 9 and Cluster 49. All three Clusters were likewise found in farmers and veterinarians, however, Cluster 398 was both the most predominant and it displayed the highest levels of antimicrobial resistance. Strains from Clusters 9 and 49, although they did get transmitted from pigs to humans, did not show considerable antimicrobial resistance. The documented route of transmission for Staphyloccocus sp. is by direct contact. However, we suspect that inhalation of contaminated air may play a role as both MSSA and MRSA was detected in the air of animal houses (Masclaux et al, unpublished data). The real colonization rate of pigs was most likely higher than evidenced in our study. Carcasses which 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 which 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. 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. 60% of the pig farms have less than 50 animals and pig houses are restricted to a maximum of 1000 pigs (data available on website; http://www.agriculture.ch/fr/infos/animaux/porcs/), while in the Netherlands, in 2010, there were 700 farms with more than 2000 pigs (data available on website; 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 less 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, appears 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 total quantities of veterinary antimicrobial agents sold in 8 countries (CZ, FI, DK, FR, NL, NO, SE and UK) are tetracyclines and pencillins while 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 are high. On the other hand, resistance against sulfonamides (Bactrim) which is theoretically the most 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 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 the antimicrobial resistance among MSSA is in line with prevalence of MRSA in other European countries, and therefore probably much higher than documented here for Switzerland. Interestingly, antimicrobial resistance of bovine mastitis *S. aureus* isolates was low, even to penicillin (32). The reason for this is not entirely clear, but suggests that antimicrobial resistance is likely to be driven by non-therapeutic use more than directed therapeutic use. *S. aureus* in bovine mastitis is the target for eradication, while in pigs the nasal colonization is not particularly triggered with antibiotics given to pigs.

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

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

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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	Fuisidic Acid	Bactrim	Ciprofloxacin	Levofloxacin
Pigs	All	83	**39**	**23**	**23**	**23**	**40**	**23**	**13**	**13**	**60**	**6**	**0**	**5**	**33**	**2**	**6**	**6**	**0**	**0**
	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	**52**	**27**	**21**	**17**	**23**	**23**	**10**	**10**	**73**	**0**	**2**	**0**	**25**	**2**	**4**	**4**	**8**	**8**
	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	**0**	**ND**	**3.1**	**ND**	**ND**	**0.8**	**1.6**	**1.6**	**70**	**ND**	**0.8**	**0**	**ND**	**ND**	**0.8**	**0**	**3.1**	**3.1**
	398MS	3	0	ND	33	ND	ND	0	0	0	67	ND	0	0	ND	ND	0	0	0	0

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