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Occurrence of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* in Turkey and Broiler Barns and Contamination of Air and Soil Surfaces in Their Vicinity

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The emission of microorganisms, especially resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), from poultry farms is of public interest, and its occurrence and relevance are controversially discussed. So far, there are limited data on this issue. In this study, we investigated the occurrence of livestock-associated (LA)-MRSA inside and outside previously tested MRSA-positive poultry barns in Germany. In total, five turkey and two broiler fattening farms were investigated four and three times, respectively. In a longitudinal study during one fattening period, samples were collected from animals, the animals' environment inside the barn, including the air, and the barns' surroundings, such as ambient air and boot swabs of ground surfaces at different distances from the barn. Moreover, a cross-sectional study was carried out once inside the barns on five turkey and four broiler farms during the last third of the fatting period. In the cross-sectional study, LA-MRSA was detected in the air of most barns (7 of 9, 77.8%), as well as in many samples originating from animals, with detections levels of 50 to 54% in broiler and 62 to 77% in turkey farms. In the longitudinal study, LA-MRSA was found in the ambient air outside two turkey barns and on the ground surface on the downwind side of many (44.4%) turkey and broiler farms. The same *spa* types of isolates were observed inside and outside the barns. Transmission of MRSA within poultry farms, as well as emission via the airborne route, seems to be possible.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-known pathogen occurring in human and veterinary medicine. It was first described as hospital-acquired MRSA in 1961 in nosocomial infections (1). Later, the pathogen was also observed in healthy humans without hospitalization, and the term community-acquired MRSA was developed (2, 3). In 2005, MRSA was found in healthy pigs, and transmission between animals and humans was described by Voss et al. (4). Many studies on these so-called livestock-associated (LA)-MRSA isolates, especially in pigs (5–7) but also in cattle (8), calves (9), and poultry (10, 11), followed. The resistant microorganism seems to be widespread among different kinds of farm animals and is also observed in humans that have close contact with animals (12–14) or live in regions with a high pig density (15). LA-MRSA isolates usually belong to clonal complex CC398, and the majority of them to multilocus sequence type 398 (ST398) (6, 11, 13, 16). There are few data on transmission pathways of LA-MRSA in poultry farms, as well as on the emission of LA-MRSA from poultry houses. *Staphylococcus* spp. organisms were previously found in air samples from inside a naturally ventilated broiler barn and ambient air samples from upwind and downwind of this barn (17). However, the detection of MRSA was not included in the study. Schulz et al. (18) investigated six different pig barns concerning the emission of MRSA over a 1-year period and found LA-MRSA in the ambient air and regularly on soil surfaces around the barns. It was the objective of this study to estimate the prevalence of MRSA on poultry farms and to analyze the distribution of MRSA on positive farms inside and outside the barns over time.

MATERIALS AND METHODS

Sampled farms. A cross-sectional and a longitudinal study were carried out in different regions in the north, east, and southwest of Germany. Each sampled barn belonged to a different farm, and the farms were at least 2 km apart. Only poultry farms which had tested positive for MRSA previously or at the beginning of a production cycle by analysis of boot swab samples, dust samples, swabs from skin, or swabs from choana were included in the studies. In total, 85 turkey fattening farms and 40 broiler fattening farms were tested by using boot swab samples and, in most cases, dust also, as well as swabs of skin and choanae. In the following production rounds, broiler farms that tested positive were analyzed again.

Cross-sectional study. Nine of the previously screened MRSA-positive farms, five turkey and four broiler farms, were selected for a one-time sampling inside the barn in the last third of a fattening period. The turkey farm sizes varied between 10,000 and 36,000 animals (median of 23,000), and the sizes of broiler fattening farms between 35,000 and 352,000 animals (median of 250,000). The median number of animals in the turkey barns sampled was 5,250 (range of 4,500 to 6,000), and in broiler barns, the median number was 34,000 (range of 8,000 to 82,000). All farms were conventionally managed. All sampled turkey farms had open barns with passive ventilation, and all broiler farms had closed buildings with forced ventilation.

Longitudinal study. Five turkey farms, four of them also investigated in the cross-sectional study, and two broiler farms, both participating in the cross-sectional study, were analyzed four and three times, respectively, during one fattening period, with samples taken from animals and from

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TABLE 1 Sampling dates and characteristics of farms participating in the longitudinal study

Farm	Animal species	No. of animals per barn	Date (mo/day/yr) of indicated visit (age of animals in days)			
			1st	2nd	3rd	4th
1	Turkey	5,500	06/21/11 (63)	07/05/11 (77)	08/02/11 (105)	08/16/11 (119)
2	Turkey	5,250	08/30/11 (56)	09/14/11 (71)	09/27/11 (84)	10/24/11 (111)
3	Turkey	4,200	10/25/11 (63)	11/07/11 (76)	11/29/11 (98)	12/12/11 (111)
4	Turkey	4,500	08/15/11 (35)	09/20/11 (71)	10/17/11 (98)	11/07/11 (119)
5	Turkey	6,000	09/14/11 (42)	09/26/11 (54)	10/24/11 (82)	11/14/11 (103)
6	Broiler	82,000	07/12/11 (1)	07/26/11 (15)	08/09/11 (29)	
7	Broiler	50,000	10/19/11 (1)	10/31/11 (13)	11/14/11 (27)	

the barn's interior and exterior. Data on farm visits are given in Table 1. Broiler farms were investigated for the first time shortly after arrival of the hatchlings on fattening day 2, and turkey farms within the first weeks after the arrival of young birds.

Sampling in the barns. The following sampling designs were carried out inside the barns on poultry farms participating in the cross-sectional and in the longitudinal study.

(i) Air samples. Air sampling was done by impingement and filtration simultaneously, as described in detail previously (19). The samples were collected approximately 1.5 m above ground level at three different locations inside the barn, distributed as symmetrically as possible along the animal house. For impingement samples ($n = 27$ in the cross-sectional study), All-Glas-Impingers (AGI-30, Ace Glass Inc., Vineland, NJ) filled with 30 ml phosphate-buffered saline (PBS) were used. Filtration sampling was carried out ($n = 27$ in the cross-sectional study) using a personal air sampler pump (GilAir-5; Sensidyne, United States, and SKC Gulf Coast, Inc., United States) in combination with an I.O.M. dust sampler (Institute of Occupational Medicine, Edinburgh, United Kingdom, and SKC Gulf Coast Inc., United States) and a polycarbonate filter with an 8-μm pore size (Whatman, United States). The collection times were 30 min for impingement, with airflow ranging between 11.5 and 12.5 liters/min, and 150 min for the filtration technique, with airflow of 2.5 liters/min.

(ii) Environmental samples. In each barn, pooled samples of about 2.5 g of dust were collected from different locations without contact with animals by using a sterile brush. Furthermore, pooled samples of about 250 g each of feces and feed directly from the feeder, as well as a boot swab (Finnimport, Hamburg, Germany) sample of the whole length of the barn, were collected. All pooled samples of dust, feces, and feed were taken from at least five different locations inside the barn.

(iii) Animal samples. Sixty randomly selected animals were sampled by swabs of skin and choana. In broilers, samples from skin were taken under one wing, and in turkeys, samples were taken from the skin of the neck, using cotton swabs (Sarstedt AG & Co. KG, Nümbrecht, Germany) wetted with PBS. For sampling the choana, dry cotton swabs (nerbe plus GmbH, Winsen, Germany) were used.

Sampling outside the barn. Samplings outside the barn were done simultaneously with the samplings inside the animal house in farms participating in the longitudinal study. These studies were carried out in parallel with studies in and around pig barns (18).

Air samples were taken 100 m upwind and 50 and 150 m downwind from the barn at 1.5 m above the soil surface. The filtration technique was carried out as described for the interior of the barn. For the impingement sampling, the collection time was 90 min. The composition of the collection fluid was 15 ml PBS and 15 ml glycerol for the outside sampling. Furthermore, surfaces in the vicinity of the barn were sampled. Fifty meters were paced with one pair of boot swabs at 50 m, 150 m, 300 m, and if possible, 500 m downwind and at 100 m upwind from the barn. Weather conditions, including temperature, relative humidity, wind speed, and wind direction, were recorded 100 m upwind over the entire collection period and are shown in Table S1 (on farms 1, 2, 3, 6, and 7, an Oregon Scientific WMR200 weather station from Oregon scientific, Germany, was used, and on farms 4 and 5, a 3-axis ultrasonic anemometer from Gill Instruments, Hampshire, England, and Rotronic data logger Hydrolog-D HygroClipS Temperatur/RH from Rotronic GmbH, Ettlingen, Germany, were used). In rainy or windy conditions (wind speed of >5 m/s), no air sampling was performed.

Laboratory methods. All samples were transported and stored under cool conditions (4°C to 8°C) and analyzed within 24 h.

(i) Animal samples. From 60 choana and 60 skin swab samples, 12 pools built of 4 randomly chosen swabs and 12 single swabs were analyzed for each. Individually analyzed swab samples were streaked directly onto the selective agar (CHROMagar MRSA, MAST Diagnostica GmbH, Reinfeld, Germany) and incubated under aerobic conditions for 24 h at 37°C. Simultaneously, the swabs were incubated in Mueller-Hinton broth (catalog no. CM0405, Oxoid Lt., Hampshire, United Kingdom) with 6.5% NaCl (MHB+), with individual samples in 10 ml and pooled samples in 20 ml of MHB+ for 24 h at 37°C. Following incubation, 1 ml of the enrichment suspension was added to 9 ml tryptone soy broth (catalog no. CM0129, Oxoid Lt., Hampshire, United Kingdom) containing 75 mg/liter aztreonam and 3.5 mg/liter cefoxitin (TSB+) to grow MRSA aerobically at 37°C for 17 h. A loopful of TSB+ was streaked onto selective agar and then incubated at 37°C for 24 h.

(ii) Air samples. Air samples were analyzed quantitatively for the presence of MRSA, *Staphylococcus* spp., and total mesophilic bacteria as described previously (18–20). CFU counts per cubic meter of air (CFU/m^3) were determined. The detection limits for all measurements inside the animal house were 8 CFU/m^3 for impingement, via analyzing the filtrated fluid, and 89 CFU/m^3 for filtration, via plating the fluid used to wash the filter onto three agar plates simultaneously. Due to a longer collection time, the detection limit was lower (2 CFU/m^3) for impingement in samples of exhaust air.

(iii) Environmental samples. Samples of dust and boot swabs originating from inside and outside the barn were analyzed quantitatively. Therefore, 0.1 g of dust was dissolved in 10 ml PBS plus 0.01% Tween 20, and one pair of boot swabs was transferred to a sterile Stomacher bag, filled with 225 ml MHB+, and shaken at high speed for 120 s. Then, 100 μl of the MHB+ and dissolved dust and 100 μl of their first 10-fold dilution were streaked onto selective agar. In parallel, 1 ml of the dissolved dust was transferred into 9 ml MHB+. Also, 25 g of pooled feces and feed samples were inoculated into 225 ml MHB+ each and homogenized using a stomacher. MHB+ and TSB+ were handled as described above.

Confirmation of suspected MRSA isolates, *spa* typing, and CC398 identification. Up to five characteristic MRSA colonies from positive samples were transferred onto sheep blood agar (product code CM0331; Oxoid, Wesel, Germany) and analyzed by testing the coagulase reaction. One colony with a positive coagulase reaction was confirmed by duplex real-time PCR with simultaneous detection of the *nuc* gene specific for *S. aureus* and *mecA* gene specific for methicillin resistance (21). In the case of a negative PCR result, other colonies with a positive coagulase reaction were analyzed.

spa typing was performed on 80 MRSA isolates from different samples from all barns participating in the longitudinal study (22). To characterize

TABLE 2 LA-MRSA detection frequencies in samples of air, housing environment, and animals of farms participating in the cross-sectional study

Source and type of samples	No. positive/total no. (% positive)		
	All farms ($n = 9$)	Turkey farms ($n = 5$)	Broiler farms ($n = 4$)
MRSA-positive barns			
Impingement	7/9 (77.8)	4/5 (80)	3/4 (75)
Filtration	7/9 (77.8)	4/5 (80)	3/4 (75)
All air samples	7/9 (77.8)	4/5 (80)	3/4 (75)
MRSA-positive environmental samples			
Impingement	16/27 (59.3)	9/15 (60)	7/12 (58.3)
Filtration	19/27 (70.4)	9/15 (60)	9/12 (75)
Boot swabs	5/9 (55.6)	3/5 (60)	2/4 (50)
Dust	6/9 (66.7)	3/5 (60)	3/4 (75)
Feces	2/9 (22.2)	1/5 (20)	1/4 (25)
Feed	6/9 (66.7)	4/5 (80)	2/4 (50)
MRSA-positive animal samples			
Pooled choana swabs	69/108 (63.9)	43/60 (71.7)	26/48 (54.2)
Single choana swabs	61/108 (56.5)	37/60 (61.7)	24/48 (50)
Pooled skin swabs	72/108 (66.7)	48/60 (80)	24/48 (50)
Single skin swabs	70/108 (64.8)	46/60 (76.7)	24/48 (50)

a livestock association, the *spa*-typed isolates were tested concerning their association with CC398 as described previously (23).

Statistical methods. Statistical analysis was conducted using the software SPSS 16.0 (SPSS, Inc., Chicago, IL). Flocks were considered MRSA positive when at least one of the samples taken inside the barn was positive. Air samples were considered positive when at least one of the 6 samples was positive. To compare the bacterial concentrations, geometric means of positive samples were calculated. The χ^2 test was performed to calculate the differences in prevalence between samples from turkeys and broilers. In the case of low numbers of samples ($n < 10$), Fisher's exact test was applied. The pairwise McNemar test was calculated to compare the frequencies of positive samples. Differences were considered significant if the P value was <0.05. When calculating multiple pairwise comparisons, the Holm-Bonferroni correction was applied. To test the correlation between detection rates or concentrations of MRSA in different samples, Spearman's rho was calculated. To determine the association between the air load of MRSA and the numbers of positive air samples, Kendall's tau (τ) was calculated.

RESULTS

Preselection of farms. Before starting the study, poultry farms in Germany were selected based on the investigation of boot swab samples and also, in most cases, samples from dust and swabs of skin and choana. In all, 25.9% of the sampled turkey fattening farms (22/85) and 22.5% of the sampled broiler fattening farms (9/40) tested positive for MRSA by investigating once. All nine MRSA-positive broiler farms were tested again at the following production round, and MRSA was found again in only five of them. Only farms with a positive MRSA status were included in the studies.

Cross-sectional study. The results of all indoor samples of the nine farms analyzed are summarized in Table 2. LA-MRSA isolates were detected in the air of seven of the nine poultry farms, specifically, in 4 of 5 turkey flocks and 3 of 4 broiler flocks. No significant differences were found when comparing either the two collection methods or the animal species. The number of MRSA-positive animal swabs correlates positively with the number of positive air samples, including both air sampling techniques within the same barn (Spearman's rho values were 0.81 and 0.79 for pooled and single choana swabs and 0.75 and 0.77 for pooled and single skin swabs).

The differences in detection frequencies between impingement (59.3%) and filtration (70.4%) sampling were not significant (McNemar test, $P = 0.13$). The geometric mean of the MRSA count of all positive air samples was 8.8×10^2 CFU/m^3 air for impingement (2.2×10^2 CFU/m^3 in turkey barns and 5.2×10^3 CFU/m^3 in broiler barns), with ranges of 19 CFU/m^3 to 3.45×10^3 CFU/m^3 in turkey barns and 4.2×10^2 CFU/m^3 to 2.3×10^4 CFU/m^3 in broiler barns. For filtration, the geometric mean was 5.7×10^2 CFU/m^3 (3.7×10^2 CFU/m^3 in turkey barns and 8.9×10^2 CFU/m^3 in broiler farms), with ranges of 1.3×10^2 CFU/m^3 to 9.4×10^2 CFU/m^3 in turkey barns and 89 CFU/m^3 to 7.4×10^3 CFU/m^3 in broiler barns. The respective values for *Staphylococcus* spp. were 1.3×10^6 CFU/m^3 (impingement) and 7.6×10^5 CFU/m^3 (filtration). The total mesophilic bacterial counts were 2.1×10^6 CFU/m^3 using impingement and 1.2×10^6 CFU/m^3 using filtration. This results in a fraction of 0.04% LA-MRSA in the total mesophilic bacterial count using impingement compared to 0.05% using filtration. The proportions of MRSA among the total *Staphylococcus* spp. count were 0.07% for impingement and 0.08% for filtration.

Moreover, MRSA could be found in at least one of the other environmental samples, such as dust, feces, and feed, except for one broiler farm, in which only two choana swabs were positive at all (Table 2). There were no significant differences between the detection frequencies of the different environmental samples originating from turkey and broiler farms (McNemar test, $P > 0.05$). There was also no difference between turkey and broiler farms concerning the frequency of positive environmental samples (Fisher's exact test, $P > 0.05$). In five of the nine dust samples, two originating from turkey farms and three from broiler farms, MRSA could be quantified. The geometric mean of the MRSA concentration in these samples was 1.9×10^4 CFU/g (2×10^4 and 4×10^4 CFU/g in turkey barns and 1.3×10^2, 4.2×10^4, and 4.8×10^5 CFU/g in broiler barns). Whenever there were MRSA-positive dust samples, the microorganism was also found in the air. One time the dust sample was negative and the corresponding air samples were positive. In this barn, the feed sample and the majority of animal samples harbored MRSA.

MRSA was detected in animal samples on eight of the nine farms. Means are shown in Table 2. On broiler farms, the detection frequency for pooled and single choana and skin swabs varied from 0 to 100%. On one farm, MRSA was not found in any animal sample, and on another farm, MRSA was only detected in choana swabs. On the turkey farms, the prevalence of MRSA in choana swabs ranged from 25% for pooled and 8.3% for single samples to 100% when considering both kinds of swabs. For single and pooled skin swabs from turkey, the detection frequencies ranged between 33.3% and 100%. The detection frequencies did not differ significantly between skin and choana swabs when analyzing samples from turkey and broiler. However, MRSA was found less frequently in single choana swabs (56.5%) than in the corresponding pooled samples (McNemar test, $P = 0.039$). Concerning the detection level in single skin swabs (64.8%), there was no signifi-

TABLE 3 LA-MRSA detection inside and in the vicinity of barns on five turkey fattening farms and two broiler fattening farms participating in the longitudinal study

Farm	Presence of MRSA for indicated sample type, location relative to barn, and time point[a]																															
	Downwind																Inside										Upwind					
	Soil at:												Air at:																			
	500 m				300 m				150 m				50 m				150 m				50 m				Dust				Air				Choana				Air at 100 m				Soil at 100 m			
Turkey																																												
1	o	o	o	o	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	+	−	+	+	−	−	−	−	−	−	−	−
2	o	o	o	o	−	+	−	−	−	+	+	+	−	+	+	+	−	−	−	−	−	−	−	−	+	+	+	−	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	−
3	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	o	−	−	−	o	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4	o	o	o	o	+	−	−	+	+	−	−	+	o	−	+	+	o	o	−	+	o	o	+	−	+	−	−	+	−	−	+	+	+	+	+	+	o	o	−	−	+	−	−	+
5	o	o	o	o	o	−	+	+	−	−	+	+	−	+	+	+	o	−	−	+	o	−	+	+	−	−	+	+	−	+	+	+	+	−	+	+	o	−	−	−	−	−	−	+
Broiler																																												
6	o	o	o	/	−	+	−	/	−	+	−	/	−	+	−	/	o	o	o	/	−	−	−	/	−	+	+	/	−	+	+	/	−	+	+	/	−	−	−	/	−	−	−	/
7	o	+	−	/	+	+	o	/	+	+	+	/	+	+	+	/	o	−	−	/	o	−	−	/	−	+	+	/	−	−	+	/	−	−	−	/	o	−	−	/	+	+	−	/

[a] Four consecutive time points of sampling during one fattening period from the beginning to the end are represented; turkey farms were sampled four times and broiler farms three times. +, MRSA-positive sample; −, MRSA-negative sample; o, no sample was taken in this interval; /, no sampling interval on broiler farms.

cant difference compared to the detection level in pooled skin swabs (66.7%). However, the prevalence of MRSA was significantly higher in skin samples originating from turkeys than in skin samples from broilers (Fisher's exact test, $P = 0.001$ for pooled skin samples and $P = 0.004$ for single skin samples).

Longitudinal study. The results of the longitudinal study are summarized in Tables 3 and 4. On farm 3, no MRSA was detected in any sample within the barn. All other farms were MRSA positive inside the investigated barn in the course of the longitudinal study. On four turkey farms, MRSA was found at each sampling date, with a slightly increased detection frequency in animal samples over the course of time that was not significant. On the two broiler farms included (farms 6 and 7), MRSA was not detected inside the barn in any sample at the first sampling date. On farm 6, dust, air, and animal samples tested positive for MRSA at the second and third sampling dates. However, on farm 7, only environmental samples of dust (sampling dates 2 and 3) and air (sampling date 3) were positive. All animals investigated remained MRSA negative on farm 7.

Overall, airborne MRSA isolates were found inside the barn at 13 of 26 sampling dates. MRSA was not detected in air samples from the upwind side of any barn. In air samples from the downwind side, MRSA was found in five samples from two different turkey farms. The concentrations of MRSA in these samples were 33 CFU/m^3 at a 50-m distance and 11 CFU/m^3 at a 150-m distance from the barn on farm 4. On farm 5, the concentrations were 7 CFU/m^3 and 93 CFU/m^3 at 50 m and 23 CFU/m^3 at 150 m downwind.

MRSA was found in 44.4% (36/81) of all environmental boot swab samples taken from the ground surfaces on the downwind side (including the samples of all distances), compared to 26.9% (7/26) on the upwind side. Comparing the samples at the different distances from the barns to each other, no significant differences in MRSA detection frequencies between all combinations of the distances could be observed, either for the turkey farms or for the two broiler farms (McNemar test after Holm-Bonferroni-correction of α). Quantification of the bacteria after direct culturing was only possible in five out of all boot swabs taken outside the

TABLE 4 LA-MRSA detection frequencies in samples from inside the barn in the course of the longitudinal study

Sample type	% of samples MRSA positive for indicated farm(s) and time point (1–4)[a]						
	Turkey ($n = 5$ farms)				Broiler ($n = 2$ farms)		
	1	2	3	4	1	2	3
Choana swabs							
Pooled	27 (0/58.3)	17 (0/58)	50 (0/100)	58 (0/100)	0; 0	25; 0	100; 0
Single	17 (0/42)	17 (0/58)	38 (0/100)	53 (0/100)	0; 0	0; 0	100; 0
Skin swabs							
Pooled	53 (0/100)	52 (0/100)	72 (0/100)	73 (0/100)	0; 0	0; 0	100; 0
Single	53 (0/100)	54 (0/100)	60 (0/100)	70 (0/100)	0; 0	0; 0	100; 0
Boot swabs	20	0	60	40	−; −	−; −	+; −
Dust	40	40	40	40	−; −	+; +	+; +
Feces	0	0	0	60	−; −	−; −	+; −
Feed	40	20	40	80	−; −	−; −	+; −

[a] Four consecutive time points of sampling during one fattening period from the beginning to the end are shown. For turkey farms (farms 1 to 5), the detection frequencies are summarized as means, with minimum and maximum values in parentheses. For the two broiler farms investigated (farms 6 and 7), the detection frequency is shown for each. The number of individually analyzed choana or skin swab samples and pools of them, respectively, is $n = 60$ for each sampling date; for boot swabs, dust, fecal, or feed samples, $n = 1$ at each sampling date and each farm. +, tested positive for MRSA; −, tested negative for MRSA.

TABLE 5 *spa* typing of selected MRSA isolates originating from the longitudinal study

Farm	Sampling date (mo/day/yr)	*spa* type	Location(s) and sample type(s) from which isolate(s) originated[a]	
			Inside	Outside
1	06/21/2011	t011	Choana swab ($n = 2$), skin swab ($n = 2$), air sample	Boot swab 50 m and 150 m downwind
	07/05/2011	t011	Skin swab	
	08/02/2011	t011	Skin swab	
	08/16/2011	t5452	Skin swab	
2	08/30/2011	t034	Choana swab, skin swab, air sample	
		t011	Dust	
	10/24/2011	t034	Choana swab, skin swab, air sample	
	10/24/2011	t5452		Boot swab 50 m and 150 m downwind
3	11/29/2011	t1456		Boot swab 150 m downwind
	12/12/2011	t1456		Boot swab 500 m downwind
4	07/13/2011	t2577	Choana swab	
		t034	Skin swab	Boot swab 300 m downwind and boot swab 150 m downwind
		t2576	Dust	
	10/17/2011	t011	Choana swab, skin swab	
		t034		Air sample and boot swab 50 m downwind
	11/7/2011	t011	Choana swab, dust, air sample, skin swab	Boot swab 50 m downwind and boot swab 100 m upwind
		t1250		Boot swab 150 m and 300 m downwind
5	10/24/2011	t011	Choana swab, skin swab, air sample	Boot swab 50 m, 150 m, and 300 m downwind
		t002	Dust	Air sample
	11/14/2011	t011	Choana swab, skin swab	Air sample ($n = 2$), boot swab 50 m and 150 m downwind, and boot swab 100 m upwind
		t1430	Dust	
		t002	Air sample	
		t034		Boot swab 300 m downwind
6	07/26/2011	t1430	Choana swab, skin swab, dust	
		t899	Choana swab, skin swab, air sample	Boot swab 50 m, 150 m, and 300 m downwind
7	10/19/2011	t108		Boot swab 50 m, 150 m, and 300 m downwind and boot swab 100 m upwind
	10/31/2011	t108	Dust	Boot swab 50 m, 150 m, 300 m, and 500 m downwind and boot swab 100 m upwind
	11/14/2011	t108	Dust	
		t1430	Air sample	
		t034		Boot swab 50 m and 150 m downwind

[a] $n = 2$ indicates different samples.

barns. These boot swabs contained MRSA at between 2.3×10^3 and 2.7×10^5 CFU per pair.

***spa* typing and CC398 identification.** The results of *spa* typing of 80 isolates are shown in Table 5. In total, 10 different *spa* types of MRSA were found, the majority being *spa* type t011 (31/80, 38.75%). At five barns, the same *spa* types were found simultaneously inside and outside the building. Up to five different *spa* types were found in one barn. The majority of MRSA isolates from inside and outside the barns investigated (62/78) belong to clonal complex 398. Isolates which tested negative for CC398 belonged to *spa* types t011, t002, t1430, t899, and t108.

DISCUSSION

Prevalence of MRSA on poultry farms. In the present study, poultry farms were screened and only those with a positive MRSA status were included in the cross-sectional or longitudinal study. The detection frequency of 25.9% on turkey farms is in line with the results of the national zoonosis monitoring carried out in 2010, which found that 19.6% of farms were positive (24). In a regional study in the southwest of Germany, Richter et al. found that 90% (18/20) of the turkey farms were positive for MRSA (11). However, they used a different sampling protocol.

Limited data have been available so far on the prevalence of MRSA in broiler flocks. Out of 384 dust and fecal samples originating from broiler fattening farms in Germany, only 0.7% were suspected to be MRSA positive (25). We detected a substantial proportion of positive herds. Studies in other European countries also had varying results, with 35% of flocks positive at slaughter in Denmark (26) and 4 of 50 herds positive in the Netherlands (16). Another study, by Persoons et al. (27), found that 2 of 14 Belgian farms were positive for MRSA by sampling five broilers at each farm.

According to our data, the MRSA status changes from batch to batch on broiler farms. This phenomenon was also observed by Pletinckx et al. (10). They analyzed three different production cycles on three broiler farms within a time frame of 1 year. One

farm tested completely negative for MRSA, and the other two had variable MRSA status. In contrast to pig farming, on broiler farms, 1-day-old chickens which have no previous contact with older animals are brought in for new production cycles. If the hatchlings were not colonized with MRSA and the disinfection of the barn was adequate, it is most likely that the animals and the environment in the barn would remain MRSA free. In contrast, newborn piglets on breeding farms stay with the sow for at least 3 weeks. During this time, the opportunities to be colonized with MRSA via contact with the MRSA-positive sow or environment, or perhaps, via airborne contamination are frequent (28). Consequently, the piglets arrive at the weaner to grower farm or, later, at the fattening farm already colonized with MRSA.

Distribution of MRSA in poultry farms. (i) Cross-sectional study. On nine poultry farms that had previously tested positive for MRSA, the occurrence of MRSA within the barn was extensively examined once. In the majority of the barns, LA-MRSA was found in the air, as well as in the direct animal environment. To the best of our knowledge, this is the first reported detection of LA-MRSA in air samples from turkey and broiler farms worldwide. Recently, a study reported on the antibiotic resistance of 149 *S. aureus* isolates originating from feces and air of randomly chosen chicken farms in China. They found 5.4% of these to be MRSA positive but did not report the sample matrix of origin of these MRSA strains (29). In a Belgian study, air and environmental samples (floor, wall, feed, or manure) from three different broiler farms tested negative when specifically examined for the occurrence of MRSA (10). However, one of the farms in that study was completely negative for MRSA, regardless of type of sample. They collected a smaller air volume (100 liters versus >300 liters in our study) and used impaction instead of filtration and impingement for sampling. Impaction has been successfully used for MRSA detection in pig barns before (30), but its specific sensitivity for MRSA has not been compared to the sensitivities of the two sampling methods used in our study. In contrast to the results of a previous study carried out on pig farms (19), there was no significant difference between the results for the two air sampling methods in this study. The proportion of positive air samples tended to be higher on broiler farms when filtration was used. However, the number of farms studied was limited, and therefore, the difference should not be overinterpreted. The different nature of the dust on poultry farms compared to that on pig farms may influence the sensitivity of impingement and filtration sampling. So far, there are no data available on this issue.

The concentration of airborne MRSA was similar to that found in the pig barns (19). MRSA only presents a very small fraction of all *Staphylococcus* spp. and the total mesophilic bacterial count, respectively. Dust from poultry farms consists particularly of skin scale and feather particles, besides feces, as possible sources of MRSA and is probably a main factor for the occurrence of airborne MRSA since, in six of seven barns with positive air samples, the dust also tested positive for MRSA. This is in line with our results from pig barns. Likewise, the MRSA concentrations in turkey and broiler farm dust samples were similar to those in dust originating from pig barns (19).

The prevalence of MRSA in animal samples from the prescreened farms averaged between 61.7% and 80% in turkeys and 50% and 54.2% in boilers. Pletinckx et al. (10) detected between 0% and 28% MRSA-positive animals on three broiler farms. Mulders et al. (26) recorded a prevalence of only 6.9% in chicken at the abattoir and Geenen et al. (16) found 4.4% after the investigation of 250 pooled throat swabs from 50 broiler farms. Out of 200 sampled turkeys from 20 farms in southern Germany, 71.5% tested positive for MRSA using tracheal and cloacal swabs, which matches our results (11). However, in this study, we preselected MRSA-positive farms.

In our earlier study of preselected pig farms using the same sampling design (19), 79% to 88% of the animals were positive, i.e., with a similar frequency as on our turkey farms but more frequently than in the broiler flocks (19). Other studies in pigs also resulted in high within-herd prevalences (6, 31, 32). There was only one significant difference observed concerning the type of swab sample that was taken from the animals. The prevalence of MRSA was higher in skin swab samples taken from turkeys than in those taken from broilers. Swabs from turkeys were taken from the neck skin, while those from broilers were taken under the wing. Neck skin might be more exposed to environmental MRSA contamination than the skin under the wing. Moreover, differences in the dynamics of colonization in different animal species, as well as variation in the management and use of antimicrobials, could also have contributed to the difference. Furthermore, the sampled turkeys were much older than the sampled broilers and, consequently, had more time to become colonized with MRSA.

(ii) Longitudinal study. The results show that LA-MRSA can be found in the vicinity of poultry farms. These bacteria occur in ambient air and on the surfaces around the barns up to a distance of 500 m. As the same *spa* types of isolates were detected inside and outside four barns, an emission of MRSA from the barns can be assumed. However, more-discriminatory methods, such as pulsed-field gel electrophoresis, are required to confirm this finding. Up to five different *spa* types occurred in and around the barn on an individual turkey farm, and up to two in and around the barn on an individual broiler farm. *spa* types t5452, t2577, t2576, and t1250 (found only outside the barn), which have been found in samples originating from turkey farms, have not been detected in turkey or broiler samples yet. Since the farms were located in rural areas, contact of wildlife with the sampled ground surfaces is possible and could be one reason for finding these *spa* types. The other *spa* types have been found in samples of poultry or poultry meat previously (26, 27, 33). Sixty-two of 78 isolates can be assigned to the livestock-associated clonal complex 398 of MRSA. However, sequence type ST9 has also been found repeatedly in samples from broilers and broiler meat (26, 33).

MRSA was detected in boot swab samples on the downwind side, as well as on the upwind side of the farms. Probably the bacteria are carried by the wind and sediment on the ground, where they can survive for a thus-far-unknown time. Changing wind directions over time are one explanation for the MRSA detection on both sides, however, with a tendency of higher detection rates on the downwind side, as described for pig barns previously (18). Additionally, an even spread of the bacteria in every direction around a barn under windless conditions is also likely. Apart from emission from the investigated barns, commingling of microorganisms from the barns with those from surrounding animal farms is possible. Except for barn 4, there were pig farms (barns 1, 2, 3, and 5) or other poultry farms (barns 1, 2, 5, 6, and 7) at a distance of approximately 400 m to 1,000 m from the study barns. Moreover, the sampled soil surfaces often were farm land, which is generally fertilized by manure. This could be another source of resistant bacteria in the vicinity of animal farms, which

has been shown for one turkey farm previously (34). In this study, manure spreading was mostly done several weeks before the first sampling date, but for a number of farms, this could not be determined precisely, as the fields belonged to neighboring farms. However, it is certain that no fertilized farm land was sampled around farm 2 at sampling time points 1, 2, and 3 within the distances of 50 and 150 m downwind. Even then, MRSA was detected at the second and third samplings, which could not be related to a fertilization of fields as a possible source for MRSA on the ground. Other influencing parameters which were not recorded in this study, such as topographic conditions around the farms, are conceivable. An additional transportation of microorganisms via rain water could also be possible.

The very low MRSA concentrations in samples of exhaust air indicate a strong dilution of these microorganisms after leaving the barn. Factors like sedimentation of larger particles, temperature, humidity, or solar radiation will probably also have influenced the concentration of culturable MRSA in the ambient air (17).

On both broiler farms investigated, all tested chickens, as well as all other samples taken inside the barn, were negative for MRSA 1 day after arrival of the hatchlings in the barn. Over the course of time within one fattening period, the numbers of positive chickens and environmental samples increased. This may indicate that MRSA is not mainly introduced to the farms via colonized chicken hatchlings. For turkeys, this remains unknown, as the flocks were only tested within the first weeks after arrival and four of the five flocks already tested positive at the first sampling. However, in the turkey flocks also, a slight increase of MRSA detection in samples of animals was observed. This indicates a horizontal spread of MRSA within the barn during a fattening period.

In broiler barn 7, no MRSA could be detected in all animal samples, although environmental samples within and outside the barn were occasionally positive. Likewise, turkey barn 3 was completely free of MRSA, but MRSA was occasionally detected in the vicinity of the barn. In both cases, neighboring barns on the same farm tested positive for MRSA, which could be one explanation for the occurrence of MRSA in the surroundings. Moreover, both barns had tested positive for MRSA in the previous production cycle.

The relevance of the spread of MRSA from poultry houses to their environs is not clear. Neighboring residents and livestock might be exposed via the air. Transmission of MRSA via the air or contaminated surfaces is generally possible. However, if MRSA was detected in ambient air, it was at low concentrations, making direct airborne colonization of animals and people housed or living close to poultry farms unlikely. Sedimented bacteria, however, need to be studied in more detail with respect to their survival on the surfaces and potential contamination of crops used for food and feed. So far, evidence for environmental spread to neighboring residents is lacking, despite various investigations into the prevalence of MRSA in rural people without occupational exposure to livestock. Cuny et al. (35) tested pupils living in an area with a high density of pig farming in Germany and found 0.6% (3/462) to be positive for MRSA CC398. These MRSA-positive pupils were 3 of 40 pupils living on a pig farm. Another German study tested 190 residents living in a pig-and-poultry-dense area without occupational contact to livestock and found 3 (1.5%) to be MRSA positive, with one of them having MRSA CC398 (36). The potential role of emissions for the contamination of neighboring farms, as well as residents, needs to be studied more intensively.

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