AEM
Journals.ASM.org

Longitudinal Study of the Contamination of Air and of Soil Surfaces in the Vicinity of Pig Barns by Livestock-Associated Methicillin-Resistant *Staphylococcus aureus*

Jochen Schulz,[a] Anika Friese,[b] Sylvia Klees,[c] Bernd A. Tenhagen,[d] Alexandra Fetsch,[d] Uwe Rösler,[b] and Jörg Hartung[a]

Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour, University of Veterinary Medicine Hannover Foundation, Hannover, Germany[a]; Institute for Animal Hygiene and Environmental Health, Free University Berlin, Berlin, Germany[b]; Chemisches und Veterinäruntersuchungsamt Ostwestfalen-Lippe, Detmold, Germany[c]; and Federal Institute for Risk Assessment, Berlin, Germany[d]

During 1 year, samples were taken on 4 days, one sample in each season, from pigs, the floor, and the air inside pig barns and from the ambient air and soil at different distances outside six commercial livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA)-positive pig barns in the north and east of Germany. LA-MRSA was isolated from animals, floor, and air samples in the barn, showing a range of airborne LA-MRSA between 6 and 3,619 CFU/m^3 (median, 151 CFU/m^3). Downwind of the barns, LA-MRSA was detected in low concentrations (11 to 14 CFU/m^3) at distances of 50 and 150 m; all upwind air samples were negative. In contrast, LA-MRSA was found on soil surfaces at distances of 50, 150, and 300 m downwind from all barns, but no statistical differences could be observed between the proportions of positive soil surface samples at the three different distances. Upwind of the barns, positive soil surface samples were found only sporadically. Significantly more positive LA-MRSA samples were found in summer than in the other seasons both in air and soil samples upwind and downwind of the pig barns. *spa* typing was used to confirm the identity of LA-MRSA types found inside and outside the barns. The results show that there is regular airborne LA-MRSA transmission and deposition, which are strongly influenced by wind direction and season, of up to at least 300 m around positive pig barns. The described boot sampling method seems suitable to characterize the contamination of the vicinity of LA-MRSA-positive pig barns by the airborne route.

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) isolates harbor the staphylococcal cassette chromosome *mec* (SCC*mec*), which contains the *mecA* gene, also found in the community-acquired MRSA (CA-MRSA) and hospital-acquired MRSA (HA-MRSA) strains (8, 20), which emerged first in human medicine shortly after the introduction of methicillin in 1959 (22). Concerns are rising that LA-MRSA strains enter hospitals and the health system and cause infections in patients. Such concerns are fuelled by, e.g., studies carried out in the Netherlands and Germany showing a strong regional association between high densities of pigs and the prevalence of LA-MRSA in humans (34, 36) and the number of LA-MRSA-positive dairy farms (9, 29). Furthermore, although LA-MRSA, CA-MRSA, and HA-MRSA differ phenotypically and genotypically (5, 35) and can be distinguished by different molecular typing techniques (5), such as multilocus sequence typing (MLST) and the sequence typing of the polymorphic region X of the *S. aureus* protein A gene (*spa* typing), the *spa* types t011, t108, and t034 that are specifically associated with the LA-MRSA sequence (ST398) (6) can be isolated from animals and humans (30) and have emerged in association with animal production in many countries (14, 23, 35).

However, the spread and, particularly, the transmission pathways by air from contaminated pig buildings, as well as the deposition dynamics in the environment of LA-MRSA, are not yet well understood. It has been established that MRSA can be found in dust and in the air of pig barns and may be emitted via the exhaust air of animal houses into the environment (10, 25). Gibbs et al. (11) detected penicillin- and ampicillin-resistant *S. aureus* 150 m downwind from a swine confined-animal feeding operation. They did not, however, confirm that the isolates carried the *mecA* gene. It is still unclear if and in what concentrations MRSA is present in the air downwind from contaminated pig barns and whether culturable MRSA is deposited in the vicinity in measurable amounts. If so, it is conceivable that, e.g., other farm animals, wild animals, or people can come into direct contact with emitted LA-MRSA. This study seeks to address those issues through a longitudinal study to investigate the occurrence of MRSA in the air and on soil surfaces close to MRSA-positive pig holdings in consideration of meteorological and seasonal influences.

MATERIALS AND METHODS

Sampled pig barns. The barns sampled in this study are located on farms in the northwestern (Emsland and Cloppenburg) and eastern (Ostprignitz-Ruppin and Spree-Neiße) regions of Germany, situated in typical rural areas surrounded by arable land. Criteria for barn selection were a usual commercial stock size, a minimal distance of 1 km to the next livestock holding, forced ventilated buildings, and the willingness of farmers to participate in the study. Six barns (no. 1 to 6) (Table 1) on six different farms were selected, two barns on two breeding farms (no. 2 and 4) with 500 sows each and four pig fattening barns (no. 1, 3, 5, and 6) on farms with total stocks of pigs between 1,500 and 6,300 in different buildings. Barns 2, 4, and 6 were the only pig buildings on the farm. At farms with

Received 23 February 2012 Accepted 23 May 2012
Published ahead of print 8 June 2012
Address correspondence to Jochen Schulz, Jochen.Schulz@tiho-hannover.de.
Jochen Schulz, Anika Friese, Uwe Rösler, and Jörg Hartung contributed equally.
Supplemental material for this article may be found at http://aem.asm.org/.

doi:10.1128/AEM.00550-12
The authors have paid a fee to allow immediate free access to this article.

TABLE 1 Barn number, type of production, number of pigs per barn, and farm visits

Barn no.	Production type	No. of pigs per barn	Visit date (mo/day/yr)			
			1st	2nd	3rd	4th
1	Fattening	1,400	09/14/2009	12/14/2009	03/14/2010	06/14/2010
2	Breeding	500	11/30/2009	03/02/2010	06/07/2010	09/06/2010
3	Fattening	600	03/29/2010	06/28/2010	11/02/2010	12/01/2010
4	Breeding	500	04/26/2010	07/05/2010	09/21/2010	12/07/2010
5	Fattening	1,200	03/02/2010	07/06/2010	09/21/2010	01/24/2011
6	Fattening	1,600	03/15/2010	06/01/2010	07/09/2010	01/17/2011

more than one barn, only one barn (1, 3, and 5) and always the same barn was sampled at the visits. Each barn was sampled four times within 1 year. The fattening period in all barns was approximately 5 months. Data on farm visits, production type, and numbers of pigs in the investigated barns are given in Table 1.

Sampling in and around the pig barns. All samples from individual barns were collected on the same day. Nasal swabs from 60 randomly selected pigs (30 sows and 30 piglets at breeding farms), one pair of boot swabs, and three impinger air samples were taken inside the barns during each farm visit. Nasal swabs were taken with gloved hands by swabbing one nasal vestibule of a pig with a sterile dry swab (Sarstedt AG & Co. KG, Nümbrecht, Germany). Swabs were transferred back into their transport tubes and transported in container boxes to the laboratory under ambient temperatures. A pair of new latex gloves (Nobaglove, Wetter, Germany), a pair of plastic overboots, and a pair of boot swabs (Finnimport, Hamburg, Germany) in a stomacher closure bag (Seward Ltd., United Kingdom) was transported in a sterile sealable bag to avoid contamination of sampling material prior to use. Inside the animal house, the new gloves were used to put on the overboots before putting on the boot swabs. The center aisle of the animal house was then paced backwards and forwards. The length of the center aisles varied from barn to barn between 20 and 70 m. The boot swabs were carefully removed so as not dislodge adherent material and placed back into the stomacher bag. Outside the animal house, the stomacher bag was placed in a new sealable bag for transport to the laboratory. Boot swab handling of outside samples was performed in a similar fashion. Outside the barn, a 50-m distance was paced with one pair of boot swabs at 50, 150, and 300 m downwind and at 100 m upwind of the barn. Samples were taken approximately at a right angle to the main wind direction parallel to the pig building. We never walked with boot swabs on concrete surfaces or on field paths to avoid false-positive results from other sources, such as contaminated persons or vehicles. In some cases, no sample was taken because the sampling area was not accessible (e.g., puddles, cornfields, concrete surfaces, etc.).

The wind direction was measured 100 m upwind and monitored by a compass and an anemometer (barns 1 to 4, Oregon scientific WMR 200 anemometer from Conrad, Germany; barns 5 and 6, 3-axis ultrasonic anemometer from Gill Instruments, Hampshire, England). Distances were determined by a 50-m measuring tape (Brüder Mannesmann AG, Remscheid, Germany). Air samples were taken simultaneously inside, downwind, and upwind of the barns. One AGI 30 impinger (AGI-30; Ace Glass Inc., Vineland, NJ) was placed lengthwise in each third of the barn, and three impingers were operated outside the barns at 50 and 150 m downwind and at 100 m upwind of the barns. In all cases, the impingers were placed 1.5 m above soil and ground surfaces. A modified impingement was conducted for the detection of staphylococci. Airborne staphylococci were sampled in 30 ml phosphate-buffered saline inside the animal houses. Samplings outside were conducted in 30 ml 1:1 glycerol-phosphate-buffer solutions to extend the sampling time without seriously influencing the culturability of *Staphylococcus* spp. (24, 26). Air samples were taken only on rainless days and at temperatures above 5°C to avoid formation of ice in the sampling buffer. Temperature was measured with a HygroClip S and HygroLog-D datalogger from Rotronic (Ettlingen, Germany). Three air samples were not successful due to technical problems during the field measurements.

Bacteriological analysis of samples. Samples were stored at 4°C in the microbiological laboratory. All samples were processed within 24 h, except the boot swabs, which were stored up to 7 days before analysis. Stomacher bags containing a pair of boot swabs were filled with 225 ml of Müller-Hinton broth with 6.5% NaCl (Oxoid Ltd., Basingstoke, Hampshire, England) before they were shaken in a Stomacher 400 circulator (Seward Ltd., United Kingdom) at high speed for 120 s. The bags were subsequently incubated under aerobic conditions for 24 h at 37°C to enrich salt-tolerant staphylococci. A volume of 2.5 ml of the enrichment suspension was then added to 22.5 ml tryptone-soya-bouillon (Oxoid Ltd., Basingstoke, Hampshire, England) with 3.5 mg/liter cefoxitin and 75 mg/liter aztreonam (both from Sigma-Aldrich, Taufkirchen, Germany) to grow MRSA aerobically at 37°C for 17 h. After incubation, a loopful of broth was streaked out on CHROMagarMRSA (MAST Diagnostica GmbH, Reinfeld, Germany) and subsequently incubated at 37°C for 24 h.

Sixty nasal swabs from one herd were divided into 12 single swabs and 12 pools of four swabs each. Pooled samples from piglets and sows of the breeding farms were handled separately. Swabs were analyzed qualitatively as described by Friese et al. (10).

Impingers were shaken for 30 s at full speed with a Vortex-Genie2 (Scientific Industries Inc.), and 0.5-ml aliquots from the samples were plated on CHROMagarMRSA. The plates were incubated for 24 to 48 h at 36°C, and 10-ml aliquots of the impinger solutions were filtrated through nitrocellulose membrane filters with a pore size of 0.22 μm (Millipore). The filters were handled and incubated on CHROMagarMRSA as described by Schulz and Hartung (25). Typical MRSA colonies were counted, and the numbers of airborne CFU per cubic meter were calculated by the equation from Lin et al. (16) after suspected colonies were confirmed by biochemical and molecular biological methods as described in the following section.

Confirmation of suspected MRSA isolates and *spa* typing. Five suspected MRSA colonies from each apparently positive sample were subcultivated on sheep blood agar (CM 0331; Oxoid, Wesel, Germany) and confirmed by testing the coagulase reaction by inoculation with 0.5 ml rabbit plasma (Becton, Dickinson GmbH, Heidelberg, Germany). One randomly selected coagulase-positive isolate per sample was confirmed by using a duplex real-time PCR, which detects the *nuc* gene (specific for *S. aureus*) and the *mecA* gene (21).

To compare *spa* types found within and simultaneously outside the six investigated barns, typing was conducted with isolates from one sampling day. One MRSA isolate from each positive MRSA sample was typed using the method described by Harmsen et al. (13). Isolates from sampling days on which airborne MRSA was detected downwind from the barns were included in the analysis to investigate direct airborne transmission.

Statistical methods. The FREQ procedure of the SAS software, version 9.1.3 (SAS Institute Inc., Cary, NC), was used for statistical analyses. In the first step, statistical differences between the ratios of binominal data (positive MRSA samples, 1; negative MRSA samples, 0) at various sampling points and in different seasons were analyzed by Cochran's Q test. If statistical differences were found, the pairwise McNemar test was applied to compare the frequencies of positive and negative MRSA samples among the sampling points and between the seasons.

WIN EPISCOPE 2.0 (32) was used to calculate the MRSA intraherd prevalence in pig barns.

RESULTS

MRSA-positive and -negative findings of the study are summarized in Table 2. Inside the barns, all pooled nasal swabs and boot swab samples were MRSA positive. In detail, the number of positive pools from nasal swabs varied between 10 and 12 (out of 12) in all barns. Analyzing 12 single nasal swabs resulted in 5 to 12 positive samples (the average was 10). The minimal numbers of single swabs to detect one positive pig with a probability of >95% ranged from 1 to 5. Considering the number of animals in the investigated barns (Table 1), intraherd prevalence from 47 to

TABLE 2 MRSA detection inside and in the vicinity of six pig barns

Barn no.	MRSA detection by sample source and season[a]																																			
	Downwind from the barn																				Inside the barn												Upwind from the barn (100 m)							
	Soil												Air								Floor				Air				Pigs				Air				Soil			
	300 m				150 m				50 m				150 m				50 m																					
	Sp	S	A	W	Sp	S	A	W	Sp	S	A	W	Sp	S	A	W	Sp	S	A	W	Sp	S	A	W	Sp	S	A	W	Sp	S	A	W	Sp	S	A	W	Sp	S	A	W
1	−	+	+	+	−	+	+	−	−	−	+	+	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	0	−
2	−	+	+	−	−	+	−	−	−	+	+	+	−	−	−	−	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	−	0
3	+	+	−	+	+	+	−	+	+	+	−	+	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	−	+
4	−	−	+	−	+	+	+	+	+	+	+	−	−	−	−	−	0	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	−	−
5	0	+	0	+	+	+	+	+	0	+	+	+	−	+	+	−	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	0	−	−	−	0	+	−	+
6	+	+	+	+	+	+	+	+	0	0	+	+	−	−	−	−	+	−	−	−	+	+	+	+	+	+	−	−	+	+	+	+	−	−	0	−	0	0	0	−

[a] Findings are expressed as positive (+) or negative (−). 0, no sample was taken in this interval. Findings are given for each of the four seasons: Sp, spring; S, summer; A, autumn; and W, winter.

100% could be calculated. The detection of airborne MRSA failed in three samplings (two in winter and one in autumn) inside the barns (Table 2). Airborne MRSA was detected 15 times in three impingers, 4 times in two impingers, and 2 times in one impinger. Concentrations of positive air samples ($n = 55$) varied between 6 and 3,619 CFU/m^3. The median was 151 CFU/m^3 (lower quartile, 45 CFU/m^3; upper quartile, 821 CFU/m^3). Downwind from the barns, MRSA was detected in only five air samples at three different barns (three in summer, one in spring, and one autumn). The concentrations of MRSA in these samples were very low, ranging from only 2 CFU/m^3 at 150 m (two times) to 14 CFU/m^3 (two times) and 11 CFU/m^3 at 50 m. MRSA was not detected in air samples upwind from the animal houses.

Of the boot swab samples taken from soil surfaces downwind of the barns, 73% were positive, compared to only 33% of the upwind soil samples. Statistical differences between the ratios of positive and negative samples across various sampling points and different seasons are given in Table S1 in the supplemental material. Cochran's Q-test indicates significant differences between the sampling points of soil samples and between seasons. These differences were analyzed in detail by the pairwise McNemar test. Table 3 highlights significant differences between the number of positive upwind and downwind samples for each distance. Table 4 shows a significantly higher number of positive samples in the vicinity of the pig barns in summer.

spa typing of 41 isolates indicates that *spa* types detected within the animal houses could also be detected in air or on soil outside the barns (Table 5). Inside and outside three barns, only one *spa* type (t011) was detected. At barn 4, a second *spa* type (t034) was found only on soil surfaces downwind from the barn. At barn 5, t108 dominated, but again a second type (t1344) occurred downwind from the barn. Two isolates (t034 and t1451) were found simultaneously inside and downwind of barn 6, and a third *spa* type (t011) appeared on the soil surface 150 meters downwind of the barn. Interestingly, the same *spa* types were isolated from air samples taken simultaneously inside and downwind of three different animal houses.

DISCUSSION

Staphylococcus aureus can colonize pigs and can be emitted via ventilation systems into the ambient air of swine herd confined-animal feeding operations (12). The results of our longitudinal study demonstrate that MRSA can be isolated from ambient air and also from soil surfaces in the vicinity of pig farms. To the best of our knowledge, we show for the first time a simultaneous detection of the same *spa* types within and outside pig farm operations. All typed isolates in this study are from *spa* types associated with LA-MRSA of clonal complex CC398. Isolates of these *spa* types have been confirmed as ST398 by MLST in previous studies on pig farms (1, 6, 30). A higher proportion of LA-MRSA-positive samples within the main downwind direction compared to the upwind side of the barns indicates that the dispersion into the environment is strongly influenced by wind direction. Sporadic identification of LA-MRSA on soil surfaces at the upwind side of four pig barns is likely explained by changing wind directions. It seems rather unlikely that LA-MRSA on soil surfaces could be deposited by other animal houses, because the nearest such house is

TABLE 3 Statistical differences between the numbers of LA-MRSA-positive samplings at different sampling points (using pairwise McNemar test)

Compared samplings (n)	Results for sampling point: 1			2			P value
	Soil sample location	No. of positive samplings	% Positive	Soil sample location	No. of positive samplings	% Positive	
17	300 m downwind	11	65	100 m upwind	6	35	0.0588
18	150 m downwind	13	72	100 m upwind	5	28	0.0082
18	50 m downwind	13	72	100 m upwind	5	28	0.0082
20	50 m downwind	15	75	300 m downwind	13	65	0.3173
22	150 m downwind	16	73	300 m downwind	15	68	0.6547
16	150 m downwind	11	69	50 m downwind	11	69	1.0000

TABLE 4 Seasonal influence on LA-MRSA-positive samplings outside the pig barns (by pairwise McNemar test)

Compared samplings (*n*)	Results for season: 1			2			*P* value
	Season	No. of positive samplings	% Positive samplings	Season	No. of positive samplings	% Positive samplings	
35	Summer	19	54	Spring	9	26	0.0039
34	Autumn	12	35	Spring	9	27	0.3657
34	Winter	9	27	Spring	12	35	0.2568
37	Autumn	15	35	Summer	21	57	0.0209
39	Winter	14	36	Summer	21	54	0.0196
37	Autumn	14	38	Winter	14	38	1.0000

1,300 m away. Solid or liquid manure, which could have been a possible source of LA-MRSA on soil surfaces, was not applied around the farms during the spring, summer, and winter periods of the study. There was no manure spreading in autumn near barns 1, 3, and 6. Although we cannot fully exclude an LA-MRSA contamination by slurry around barns 2, 4, and 5 in autumn, no manure application was observed, nor were traces of manure found on soil surfaces. Furthermore, it cannot be ruled out that LA-MRSA contamination of soil surfaces around a pig barn could have been caused by human carriers or rodents (33). However, such random events would not explain the significantly higher number of positive samples on the downwind side, which changed at least two times at each farm. The higher detection rates on changing downwind sides again highlight the role of the wind as an important vector for LA-MRSA.

The number of airborne LA-MRSA organisms is relatively low compared to typical numbers of airborne mesophilic bacteria in pig barns (10, 25, 28). Although the analyses of single nose swabs indicate a relatively high intraherd prevalence (4), LA-MRSA was not detected in barn air or outside air on three occasions. Friese et al. (10) detected only a weak correlation between the number of MRSA-positive air samples and the percentage of MRSA-positive individual nasal swabs. This is probably the consequence of many factors influencing the bacterial concentration in animal house air (27). This could also explain the strong deviations from the median LA-MRSA concentration we observed in the animal house air.

LA-MRSA-positive air samples were found in only 5 of 24 measurements (21%); all of these were identified in downwind air. Air samples taken simultaneously inside and downwind from the barn showed the same *spa* types. This indicates a direct airborne transmission in these situations. The relatively low detection rates outside were most likely caused by dilution in the ambient air. Other factors that have been known to influence the tenacity of airborne bacteria (15) likely did not play a major role, because the travel times of staphylococci for a distance of up to 150 meters are too short to suffer a significant decay in viability (19, 24).

Interestingly, LA-MRSA could be detected in 49 out of 67 (73%) soil surface samples from the downwind side of the pig barns. It is likely that deposited LA-MRSA can survive for longer periods on soil surfaces. While there is no exact information about the survival time of MRSA on soil, it is known from laboratory experiments that human MRSA strains show high tenacity and can survive on hard surfaces for weeks (17). Therefore, we assume that deposited MRSA is able to accumulate on soil surfaces around the barns, provided that it is not washed away by rainfall. Other meteorological conditions (e.g., turbulences, temperature, humidity, etc.) and the number and sizes of MRSA particles can also play a role in accumulation during the samplings. A higher number of deposited MRSA can usually be expected on surfaces near the barn, because larger dust particles carry more bacteria and deposit much faster (24). This was not shown by the detection rates at different distances from the barns. However, we used a selective enrichment method to detect MRSA qualitatively in swab samples because of the higher sensitivity compared to direct isolation methods (3). Future investigations may include a quantification of LA-MRSA related to a surface area. This would give more insight in the quantitative deposition of bacteria emitted from pig barns.

Factors such as seasonal influences (e.g., temperature, UV irradiation, etc.), soil composition, water activity, and the microbial community on soil surfaces can all influence the viability of *Staphylococcus aureus* in an outdoor environment (2, 7). On the other hand, the mean dust emission rate for forced ventilated pig houses in summer was estimated to be 30% higher than in winter (31). Therefore, a higher deposition rate of LA-MRSA could be expected in the vicinity of pig barns in the summer time. That LA-MRSA was found in higher quantities during summer is more likely a result of higher ventilation rates inside the animal houses to keep favorable air and temperature conditions rather than any of the factors previously posited.

The presented investigations show a persistent LA-MRSA contamination in the vicinity of pig barns housing LA-MRSA-colonized pigs. The consequences of these contaminations are not immediately clear. The possibility of MRSA transmission to neighboring farms via the contaminated environment or to animals living in the vicinity of barns cannot be excluded. Moreover, recontamination of cleaned and disinfected animal houses may occur by reentrainment from soils or surfaces via the air (incoming air), by persons (e.g., farmers), or by animals like rodents, which come into contact with contaminated soil surfaces and subsequently enter the animal house. Therefore, there is an urgent need for further studies in order to understand the survival of LA-MRSA in the outdoor environment and the transmission pathways. This may also help to better estimate the risks involved in the environmental LA-MRSA contamination of the vicinity of positive animal houses with regard to neighboring piggeries as well as to residential dwellings. Additionally, air treatment systems should be investigated for their ability to reduce emission of MRSA from animal houses (18). Likewise, air treatment techniques may be used to purify incoming air to prevent airborne

TABLE 5 Origin of MRSA *spa* types from inside and from the vicinity of investigated barns

MRSA origin by barn no. and sampling date (mo/day/yr)	*spa* type
1 (09/14/2009)	
Nasal swab	t011
Air sample inside	t011
Boot swab inside	t011
Boot swab 50 m downwind	t011
Boot swab 150 m downwind	t011
Boot swab 300 m downwind	t011
2 (06/07/2010)	
Nasal swab	t011
Air sample inside	t011
Air sample 50 m downwind	t011
Boot swab inside	t011
Boot swab 50 m downwind	t011
Boot swab 150 m downwind	t011
Boot swab 300 m downwind	t011
Boot swab 100 m upwind	t011
3 (12/01/2010)	
Nasal swab	t011
Air sample inside	t011
Boot swab inside	t011
Boot swab 50 m downwind	t011
Boot swab 150 m downwind	t011
Boot swab 300 m downwind	t011
Boot swab 100 m upwind	t011
4 (09/21/2010)	
Nasal swab	t011
Air sample inside	t011
Boot swab inside	t011
Boot swab 50 m downwind	t034
Boot swab 150 m downwind	t034
Boot swab 300 m downwind	t011
5 (07/06/2010)	
Nasal swab	t108
Air sample inside	t108
Air sample 50 m downwind	t108
Boot swab inside	t108
Boot swab 50 m downwind	t108
Boot swab 150 m downwind	t1344
Boot swab 300 m downwind	t108
Boot swab 100 m upwind	t108
6 (03/15/2010)	
Nasal swab	t1451
Air sample inside	t034
Air sample 50 m downwind	t034
Boot swab inside	t1451
Boot swab 150 m downwind	t011
Boot swab 300 m downwind	t1451

introduction of MRSA into animal holdings in regions with high animal densities.

ACKNOWLEDGMENTS

This work was financially supported by the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) through the Federal Office for Agriculture and Food (BLE), grant numbers 2808HS029 and 08HS041.

REFERENCES

1. **Alt K, et al.** 2011. Factors associated with the occurrence of MRSA CC398 in herds of fattening pigs in Germany. BMC Vet. Res. **7**:69. doi:10.1186/1746-6148-7-69.
2. **Chapple RM, Inglis B, Stewart PR.** 1992. Lethal and mutation effects of solar and UV radiation on *Staphylococcus aureus.* Arch. Microbiol. **157**:242–248.
3. **De Boer E, et al.** 2009. Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. Int. J. Food Microbiol. **134**:52–56.
4. **De Neeling AJ, et al.** 2007. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. Vet. Microbiol. **122**:366–372.
5. **Deurenberg RH, et al.** 2007. The molecular evolution of methicillin-resistant *Staphylococcus aureus.* Clin. Microbiol. Infect. **13**:222–235.
6. **European Food Safety Authority.** 2009. Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008, part A: MRSA prevalence estimates; on request from the European Commission. EFSA J. **7**:1376. doi:10.2903/j.efsa.2009.1376.
7. **Falkinham JO, et al.** 2009. Proliferation of antibiotic-producing bacteria and concomitant antibiotic production as the basis for the antibiotic activity of Jordan's red soil. Appl. Environ. Microbiol. **75**:2735–2741.
8. **Fluit AC, Schmitz F-J.** 2003. Population structure of MRSA. *In* Fluit AC, Schmitz F-J (ed), MRSA current perspectives. Caister Academic Press, Wymondham, United Kingdom.
9. **Friedrich A, Rau J, Horlacher Spohr SM.** 2011. Prevalence of methicillin-resistant Staphylococcus aureus in milk from dairy farms in northern Württemberg. Tierärztl. Umschau **66**:195–200.
10. **Friese A, et al.** 2012. Occurrence of MRSA in air and housing environment of pig barns. Vet. Microbiol. **158**:129–135. doi:10.1016/j.vetmic.2012.01.019.
11. **Gibbs SG, et al.** 2006. Isolation of antibiotic-resistant bacteria from the air plume downwind of a swine confined or concentrated animal feeding operation. Environ. Health Perspect. **114**:1032–1037.
12. **Gibbs SG, Green CF, Tarwater PM, Scarpino PV.** 2004. Airborne antibiotic resistant and nonresistant bacteria and fungi recovered from two swine herd confined animal feeding operations. J. Occup. Environ. Hyg. **1**:699–706.
13. **Harmsen D, et al.** 2003. Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. J. Clin. Microbiol. **41**:5442–5448.
14. **Khanna T, Friendship R, Dewey C, Weese JS.** 2008. Methicillin resistant Staphylococcus aureus colonization in pigs and pig farmers. Vet. Microbiol. **128**:298–303.
15. **Lighthart B, Mohr AJ.** 1987. Estimating downwind concentrations of viable airborne microorganisms in dynamic atmospheric conditions. Appl. Environ. Microbiol. **53**:1580–1583.
16. **Lin X, et al.** 1999. Long term sampling of airborne bacteria and fungi into a non-evaporating liquid. Atmos. Environ. **33**:4291–4298.
17. **Makison C, Swan J.** 2006. The effect of humidity on the survival of MRSA on hard surfaces. Indoor Built Environ. **15**:85–91.
18. **Martens W, et al.** 2001. Reduction potential of microbial, odour and ammonia emissions from a pig facility by biofilters. Int. J. Hyg. Environ. Health **203**:335–345.
19. **Müller W, Wieser P.** 1987. Dust and microbial emissions from animal production, p 47–89. *In* Neimann-Sørensen A, Triebe DE (ed), World animal science, vol B6. Elsevier Science Publishers B.V., Amsterdam, The Netherlands.
20. **Noto MJ, Barry NK, Alastair BM, Gordon LA.** 2008. Gene acquisition at the insertion site for SSC*mec*, the genomic island conferring methicillin resistance in *Staphylococcus aureus.* J. Bacteriol. **190**:1276–1283.
21. **Pasanen T, et al.** 2010. A selective broth enrichment combined with real-time nuc-mecA-PCR in the exclusion of MRSA. APMIS **118**:74–80.
22. **Robinson DA, Enright MC.** 2004. Multilocus sequence typing and the evolution of methicillin-resistant *Staphylococcus aureus.* Clin. Microbiol. Infect. **10**:92–97.
23. **Schijffelen MJ, Boel CE, Van Strijp JA, Fluit AC.** 2010. Whole genome analysis of livestock-associated methicillin-resistant Staphylococcus au-

reus ST398 isolate from a case of human endocarditis. BMC Genomics **11**:376. doi:10.1186/1471-2164-11-376.
24. **Schulz J, Formosa L, Seedorf J, Hartung J.** 2011. Measurement of culturable airborne staphylococci downwind from a naturally ventilated broiler house. Aerobiologia **27**:311–318.
25. **Schulz J, Hartung J.** 2009. Detection of MRSA in pig house air by impingement followed by membrane filtration. Gefahrstoffe-Reinhaltung Luft **9**:348–352.
26. **Schulz J.** 2008. Estimation of airborne transmission distance for bioaerosols emitted from two types of broiler houses. Ph.D. dissertation. University of Bielefeld, Bielefeld, Germany.
27. **Seedorf J.** 2004. An emission inventory of livestock-related bioaerosols for Lower Saxony, Germany. Atmos. Environ. **38**:6565–6581.
28. **Seedorf J, et al.** 1998. Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in northern Europe. J. Agric. Eng. Res. **70:**97–109.
29. **Spohr M, et al.** 2011. Methicillin-resistant Staphylococcus aureus (MRSA) in three dairy herds in southwest Germany. Zoonoses Public Health **58**:252–261.
30. **Stegger M, et al.** 2011. Rapid PCR detection of *Staphylococcus aureus* clonal complex 398 by targeting the restriction-modification system carrying sau1-hsds1. J. Clin. Microbiol. **49**:732–734.
31. **Takai H, et al.** 1998. Concentrations and emissions of airborne dust in livestock buildings in Northern Europe. J. Agric. Eng. Res. **70**:59–77.
32. **Thrusfield M, Ortega C, DeBlas I, Noordhuizen JP, Frankena K.** 2001. WIN EPISCOPE 2.0: improved epidemiological software for veterinary medicine. Vet. Rec. **148**:567–572.
33. **Van de Giessen AW, et al.** 2009. Occurrence of methicillin-resistant *Staphylococcus aureus* in rats living on pig farm. Prev. Vet. Med. **91**:270–273.
34. **Van Loo I, et al.** 2007. Emergence of methicillin-resistant Staphylococcus aureus of animal origin in humans. Emerg. Infect. Dis. **13**:1834–1839.
35. **Wulf MWH, Voss A.** 2008. MRSA in livestock animals–an epidemic waiting to happen? Clin. Microbiol. Infect. **14**:519–521.
36. **Wulf MWH, Verduin CM, Van Nes A, Huijsdens X, Voss A.** 2012. Infection and colonization with methicillin resistant *Staphylococcus aureus* ST398 versus other MRSA in an area with a high density of pig farms. Eur. J. Clin. Microbiol. Infect. Dis. **31**:61–65.