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Comparison of air samples, nasal swabs, ear-skin swabs and environmental dust samples for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in pig herds

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SUMMARY

To identify a cost-effective and practical method for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in pig herds, the relative sensitivity of four sample types: nasal swabs, ear-skin (skin behind the ears) swabs, environmental dust swabs and air was compared. Moreover, dependency of sensitivity on within-herd prevalence was estimated. *spa*-typing was applied in order to study strain diversity. The sensitivity of one air sample was equal to the sensitivity of ten pools of five nasal swabs and relatively independent of within-herd prevalence [predicted to be nearly perfect (99%) for within-herd prevalence $\geqslant$25%]. The results indicate that taking swabs of skin behind the ears (ten pools of five) was even more sensitive than taking nasal swabs (ten pools of five) at the herd level and detected significantly more positive samples. *spa* types t011, t034 and t4208 were observed. In conclusion, MRSA detection by air sampling is easy to perform, reduces costs and analytical time compared to existing methods, and is recommended for initial testing of herds. Ear-skin swab sampling may be more sensitive for MRSA detection than air sampling or nasal swab sampling.

Key words: Air sampling, diagnostic, methodology, *mecA*, *spa*, swine.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) in livestock have been in focus due to zoonotic spread of livestock-associated lineages. However, the methods for sampling and detection are so far not considered optimal in terms of sensitivity and practical application. MRSA was isolated in the Danish pig production for the first time in 2006, and MRSA clonal complex (CC) 398 was retrospectively found in a Danish patient in 2003 [1, 2]. The prevalence of MRSA in Danish pig herds in 2008 was considered low compared to some European countries [breeding herds 0% (n=95) and production herds 3·5% (n=198)] [3]. In recent years 16% (n=79) of pig herds were found positive based on one pool of nasal swabs and 44% (n=777) of pigs were found positive at slaughter [4–6].

Although CC398 is the most common CC in pigs in Europe [3] other CCs and *spa* types have been observed [4, 7–9]. In Danish pigs, MRSA CC398 and CC30 (*spa* type t1333) have been identified, both CC types are common in methicillin-susceptible

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Table 1. *Description of herds included in the study*

Animal age groups in the herd	No. of herds	Age group sampled	% MRSA positive herds by			
			Nasal swab pools	Ear-skin swab pools	Dust swabs	Air sampling (direct growth)
Slaughter pigs only (*n*)	**16**	Slaughter pigs	44% (7/16)	71% (5/7)	31% (5/16)	47% (7/15)
<1000	7					
1000 to <2500	8					
⩾2500	1					
Slaughter and weaning pigs (*n*)	**6**	Weaning pigs	69% (22/32)	72% (13/18)	34% (11/32)	71% (22/31)
<1000	1					
1000 to <2500	2					
⩾2500	3					
Sows, weaning and slaughter pigs (*n*)	**26**	Weaning pigs				
<250	11					
250 to <500	5					
⩾500	10					

S. aureus (MSSA) from pigs in Denmark. MSSA can potentially act as a reservoir for novel MRSA types [4, 10].

Buying pigs from MRSA-positive farms is a risk factor for introduction and further spread of MRSA to other pigs in a herd [11–13]. In 2011 Broens *et al.* [14] showed that a herd receiving pigs from a MRSA-positive herd had 11 time higher odds of becoming contaminated/colonized than herds receiving pigs from a negative herd. Mathematical models based on Danish data on movement of pigs and a survey made in 2008 also showed movement of animals to be an important risk factor [12].

The sampling methods currently available are labour intensive and expensive as many samples are needed in order to increase the sensitivity of MRSA detection in herds. Broens *et al.* [15] found testing of pools of nasal swabs in combination with single dust swabs gave the highest sensitivity, resulting in 72·8% ($n=147$) positive herds. Recently, Friese *et al.* [16] tested air sampling and found a sensitivity of 85·2% ($n=27$) of herds tested positive by use of nasal or skin swabs (taken behind one ear) (12 pools of four swabs). Furthermore, they found that skin swabs detected MRSA at the same sensitivity as nasal swabs. Additionally, Pletinckx *et al.* [17] found that skin (behind the ears) swab sampling increased sensitivity compared to nasal swabs in Belgian pig herds.

This study aimed to develop a sensitive and cost-effective sampling method able to detect MRSA contaminated/colonized herds with a high probability.

METHODS

Selection of herds

Forty-eight conventional herds were included in the study, 46 herds were found positive in the EU baseline survey in 2008 [3] in a sampling of nasal swabs of individual pigs collected at slaughter [4], or by pools of nasal swabs of pigs in herds in 2010 and 2011 [5, 6]. The herds are described in Table 1.

Sampling

Samples were collected from September to November 2011 by personnel from the regional microbiology laboratory under the Danish Food and Veterinary Administration. To avoid contamination carry-over between herds, only one herd was sampled per day. The barn sections were selected so that they represented pig age groups as close to slaughter or transmission to another herd as possible, with preference for weaning pigs. In herds containing slaughter pigs, sampling was performed on the oldest pigs closest to time of slaughter (Supplementary Table S1).

Animal sampling

Nasal swabs

In the 48 pig herds, nasal swabs were taken from animals in 10 individual pens as follows: Five pigs per pen were swabbed with a dry cotton swab in both nostrils. Only one swab was used per animal. The swabs from animals in the same pen were placed in

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a transport tube [with 10 ml Mueller–Hinton (MH) broth + 6·5% NaCl] and this pooled sample represented a pen. The samples were analysed in the regional microbiology laboratory on the day of sampling.

Ear-skin swabs

From 25 of the herds, ear-skin (skin behind the ears) swabs were taken from the same pigs as the nasal swabs were collected from. Each pig was swabbed with a dry cotton swab over a width of ~2 cm behind both ears, from the edge of the ear from one side to the other, in the area where the ear attaches to the head. The ear-skin swabs were pooled in the same manner as the nasal swabs. The pooled samples were sent by ordinary mail to the National Food Institute for analysis.

Sampling in the environment

Collection of dust swabs

From each herd, dust swabs were collected from the same sections in the barns, where the nasal swabs were collected by use of sterile Sodibox cloths in Ringer solution (Food Diagnostics, Denmark). The cloths were transferred individually to sterile stomacher bags. The samples were taken from the horizontal surfaces of the pen separators, i.e. areas in contact with pigs. Pen separators from five different pens were sampled by swabbing 1–2 m on each separator. The samples were taken as being as representative of the barn section as possible, and were not necessarily from the pen the nasal swabs were taken. Dust swabs were delivered for analysis to the regional microbiology laboratory on the day of sampling.

Air samples

From each of the 48 pig herds, three air samples were taken by use of an air sampler AirPort MD8 (Sartorius Stedim Biotech, Denmark) as follows: a sterile filter cartridge was placed in the air sampler, and it was hung in an open area in the barn where the nasal swabs were taken. A total air volume of 750 l (air flow of 50 l/min) was sampled, corresponding to a 15-min sampling period. The filter was covered with a protective lid and transferred to a sterile plastic bag. The sample was packed in an envelope and sent by ordinary mail to the National Food Institute.

Sample treatment

Isolation and identification of MRSA from nasal and ear-skin swabs

Upon arrival at the laboratory, the tubes with swabs were incubated at 37 ± 1 °C for 18–24 h. After incubation 1 ml MH broth was transferred into 9 ml tryptone soya broth (TSB) containing 4 mg/l cefoxitin and 75 mg/l aztreonam and incubated at 37 ± 1 °C for 18–24 h. From the TSB broth 10 μl was streaked onto Brilliance MRSA 2 agar (Oxoid, Denmark) and incubated at 37 ± 1 °C for 24 h. Blue or bluish colonies were subcultured on Brilliance MRSA 2 agar (up to two colonies). Up to two blue colonies were subcultured on blood agar plates and incubated at 37 ± 1 °C for 24 h. Up to two suspect *S. aureus* were analysed by multiplex PCR for 16S, *mecA* and *nuc* genes [18]. A sample was determined MRSA positive if one of these isolates was positive by PCR for both *nuc* (*S. aureus* identification) and *mecA* (methicillin resistance) [4].

Dust swabs

Upon arrival at the laboratory the dust swabs were transferred to 100 ml MH broth + 6·5% NaCl and incubated at 37 ± 1 °C for 18–24 h. After incubation the same procedure as for nasal and ear-skin swabs was followed [4].

Filters from air sampling

The three filters per herd were analysed for the presence of MRSA according to three different protocols: (1) direct real-time PCR, (2) direct growth on selective agar, and (3) pre- and selective enrichment followed by growth on selective agar.

Direct real-time PCR

The filter was transferred to a sterile Petri dish and, according to existing in-house protocol, using a sterile scalpel ¼ of the filter was excised and transferred to an Eppendorf tube. Next, 875 μl of 0·9% saline and 25 μl Protex 6L (Genencor International BV, The Netherlands) was added to the tube to dissolve the filter. The filter sample was heat-treated at 30 °C for 2–4 min until the filter was completely dissolved, mixed thoroughly, and centrifuged at 14 000 ***g*** for 5 min. The supernatant was decanted and the pellet washed twice with 1 ml of 0·9% saline (14 000 ***g*** for 5 min). The washed pellet was re-suspended in 100 μl of 1 × TE buffer and heat-treated at 95 °C for 8 min

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to lyse cells and make DNA available for PCR. For MRSA real-time PCR 5 μl of this lysate was used as template.

Direct growth on selective agar

The filter was transferred directly onto Brilliance MRSA 2 agar and subsequently incubated at 37 ± 1 °C for 18–24 h. If possible, up to five presumptive MRSA were subcultured on sheep blood agar (Oxoid) and incubated at 37 ± 1 °C for 18–24 h. One colony, and following a MRSA-negative real-time PCR response the other four, were analysed by MRSA real-time PCR as follows. A small amount of colony material was transferred to 100 μl of 1 × TE buffer, and heat-treated at 95 ° C for 8 min. For MRSA real-time PCR 5 μl of this lysate was used as template.

Pre- and selective enrichment followed by growth on selective agar

The filter was transferred to a 50-ml centrifuge tube containing 10 ml MH broth + 6·5% NaCl, thoroughly mixed, and incubated at 37 ± 1 °C for 18–24 h. From this pre-enrichment 1 ml was transferred to 9 ml TSB with 3·5 mg/l cefoxitin and 75 mg/l aztreonam and the procedure used for the nasal and ear-skin swabs was followed. If possible, up to five presumptive MRSA colonies were subcultured on sheep blood agar and incubated at 37 ± 1 °C for 18–24 h. The colonies were analysed by MRSA real-time PCR as described in the 'Direct growth on selective agar' subsection.

MRSA real-time PCR

Real-time PCR analysis was performed on a Mx3005P (Stratagene, USA) with a GeneSig kit for the quantification of MRSA and associated precision MasterMix 2 × (PrimerDesign, UK). The kit is designed to detect *mecA* (penicillin-binding protein 2A), and the chromosomal gene *femB* of *S. aureus* in two separate reactions.

The samples were analysed in a total volume of 20 μl consisting of 10 μl master mix, 1 μl primer/probe mix, 4 μl water and 5 μl of sample with the following thermal profile: 10 min of primary denaturation followed by 40 cycles of 10 s denaturation at 95 °C and 60 s annealing and extension at 60 °C. Each run included one negative (pure water) and one positive (*mecA*/*femB*) control.

All samples that gave a positive result in both *mecA* and *femB* reaction were considered MRSA positive. A prevalence based only on this method can overestimate the positivity rate of samples, and also misclassify 'true negative' herds.

spa-typing

spa-typing was performed for all MRSA-positive herds, with two exceptions. From each nasal, ear-skin and dust swab sample up to two isolates were *spa*-typed, while only one isolate from each air sample was typed. Additionally, all MRSA isolates from three selected herds with 16–25 positive samples were *spa*-typed in order to investigate the within-herd diversity on a larger number of isolates. *spa*-typing was performed by PCR amplification and sequencing using the primers 1794 (5′-AGACGATCCWTCAGTGAGC-3′) and 1827 (5′-TAATCCACCAAATACAGTTGTACC-3′) according to the SeqNet protocols (www.SeqNet.org) [19]. A *spa* type was assigned based on the sequencing results by use of the *spa* plugin included in BioNumerics v. 4.6 software (Applied Maths, Belgium) connected to the Ridom *spa* server (http://spaserver.ridom.de/).

Analysis of data

Initially, the relative sensitivity of each of the four sampling methods to correctly classify herds as contaminated/colonized was estimated as the proportion of contaminated/colonized herds testing positive by the specific sampling method out of herds testing positive by either air samples (cultured by direct plating or direct real-time PCR), dust or nasal swabs. The precision of the estimated proportion was estimated by exact binomial 95% confidence intervals (CIs).

Whether the herds were contaminated/colonized at the sampling time was not known for all herds with certainty. Estimation of sensitivity was initially conducted using data from herds with at least one positive sample (air, dust, nasal swab). In supplementary analyses, the sensitivity was also estimated assuming all herds included initially in the study were contaminated with MRSA.

Second, we assessed the dependency of sensitivity of environmental sampling methods on the proportion of MRSA-positive animals in the stable. This was done by analysing the association between animal prevalence and the result obtained by the environmental sampling methods.

The prevalence of contaminated/colonized pigs in each herd was estimated based on the results

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(number of positive samples) of the ten pooled nasal swabs with five animals per pool, and the assumption of the use of a laboratory test with perfect analytical sensitivity and specificity. The formula used to estimate the prevalence of contaminated/colonized pigs was: pig prevalence = 1 – (1 – no. positive pools/no. pools)$^{(1/\text{no. animals per pool})}$ and estimated using maximum likelihood [20]. The algorithm used decreases when either all pools are negative or positive. In the case with all pools negative the prevalence was assumed to be very low and the estimated pig prevalence was set to 0%. In the case with all pools positive the prevalence was assumed to be higher than in the case of nine positive pools and it may actually be 100%. Logistic regression was used to estimate the effect of prevalence of contaminated/colonized pigs in a herd on the probability of detecting MRSA in air filters and dust samples, respectively. The data from herds with all pools positive was not included in the analysis, because the value that must be allocated to the pig prevalence in these herds (0·38–1) will have large influence on the estimated effect of prevalence on the sensitivity. Beyond this, the uncertainty in the estimated pig prevalence was not integrated into the logistic regression. The fit of the model to the observed data was assessed using the Hosmer–Lemeshow goodness-of-fit test.

The dependency of the diagnostic sensitivity of environmental sampling methods on the occurrence of infected pigs in the herd was also estimated in regression analyses using the proportion of positive pools as an explanatory variable instead of the predicted pig prevalence. The results from these supplementary analyses were compared with the results of the regression analysis based on pig prevalence.

Furthermore, to assess the performance of detecting infected pigs with ear-skin swabs compared to nasal swabs, the distribution of herds across estimated within-herd prevalence obtained by ear-skin swab pools was compared to the distribution of herds across estimated within-herd prevalence based on nasal swab pools using Fisher's exact test.

The agreement in the test results between the different methods was assessed by estimating the kappa value. Interpretation of the kappa value is arbitrary. We used the following scale: <0, no agreement; 0–0·4, slight agreement, 0·4–0·6, moderate agreement; 0·6–0·8, good agreement; 0·8–1, almost perfect agreement.

RESULTS

Sample types

Air, dust and nasal swab were collected from the 48 herds. From 25 of these herds ear-skin swabs were also collected. The results obtained are presented in Supplementary Table S1 (available online). As shown, 29 herds were found positive for MRSA with nasal swab sampling, 18/25 herds with ear-skin swab sampling and 16 herds with dust sampling. For air sampling, 29/46 herds were found MRSA positive when the air filter was cultured directly on selective agar. With pre- and selective enrichment of the air filters followed by culture on selective agar 25 herds were found positive, while direct real-time PCR of air filters resulted in only 13 MRSA-positive herds. Thirty-seven herds had at least one positive sample (air, dust, nasal swab), and were classified as MRSA positive. In the herds where ear-skin swabs were sampled, 20 had at least one positive sample (air, dust, nasal swab).

At the individual herd level, the number of positive nasal and ear-skin-swab pool samples ranged from 1 to 10/10. For most MRSA-positive herds, half or more of the nasal and ear-skin swab pools were positive. For the dust swab samples, the number of positive samples ranged from 1 to 5/5 taken in each herd. In several herds only one or two of the dust swab samples were found positive.

The calculated values for sensitivity of the different methods are given in Table 2. Under the assumption that only herds with at least one positive sample of nasal, air or dust were contaminated/colonized with MRSA in the regression models, we found a statistically significant increase in the probability of isolating MRSA in environmental samples (air filters and dust samples) with increasing prevalence of contaminated/colonized pigs in the herd. How the predicted values of the sensitivity of dust and air sampling, respectively, are dependent on within-herd animal prevalence in the estimated model is illustrated in Figure 1. Figure 1 also includes the observed proportion of herds with positive air and dust samples, respectively, for each specific pig prevalence. Visually the model was fairly good in predicting the observed data at all levels of pig prevalence between 0% and 37%, which also was confirmed in the goodness-of-fit test. According to the fitted models, air sampling was more sensitive to detect contaminated/colonized herds irrespective of within-herd prevalence compared to dust swab samples, and performed almost perfectly

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Table 2. *Sensitivity of different sampling methods under the assumption that positive herds have at least one MRSA-positive sample of nasal swab, dust or air samples, whereas herds with no positive samples are assumed MRSA negative*

Method	Positive herds/ tested herds	Sensitivity, % (95% CI)
Air filters (direct selective plating)	29/37	78 (62–90)
Air filters (pre- and selective enrichment)	25/37	68 (50–82)
Air filters (direct PCR detection)	13/37	35 (20–53)
Dust samples (pre- and selective enrichment)	16/37	43 (27–61)
Nasal swabs (pre- and selective enrichment)	29/37	78 (62–90)
Ear-skin swabs (pre-and selective enrichment)	18/20	90 (68–99)

CI, Confidence interval.

at a prevalence of 25% contaminated/colonized pigs (sensitivity 99%). This relationship was strongest for dust where the sensitivity was almost linearly related to the prevalence of contaminated/colonized pigs, increasing from 25% at 1% contaminated/colonized pigs, to 60% at 25% contaminated/colonized pigs. When assuming all 48 herds were truly positive, the results were similar. The results of the regression analyses of the association between proportion of positive pools and the probability of detecting MRSA in the environment showed the same dependency between the occurrence of infected animals (measured as proportion of positive pools) and the sensitivity of the air filters and dust samples as the results based on pig prevalence (results not shown).

In MRSA-positive herds, based on nasal and ear-skin swab pools, the contaminated/colonized apparent within-herd prevalence varied between zero (all ten pools were negative) and 100% (all ten pools were positive) (Table 3). Based on the estimated within-herd prevalence using the nasal swab pools, the herds were distributed relatively evenly between 0% and 100%. Whereas within-herd prevalence was estimated using the ear-skin swabs, the herds had typically relatively higher prevalence (more positive pools) of contaminated/colonized animals (Table 3, Supplementary Table S1) compared to nasal swab pools, and this difference was statistical significant. Moreover, significantly more samples tested positive by use of ear-skin swab pools (151/250, 60%, 95% CI 54–67) than by use of nasal swab pools (98/250, 39% 95% CI 33–46). By comparing the results from the air filters that were grown directly on selective agar with the nasal swab pools (Table 4), we found a kappa value of 0·7, indicating a good agreement between the methods. For comparison of results of ear-skin swab pools and nasal swab pools (Table 5) a good agreement between methods was also found (kappa value of 0·7). In order to evaluate the necessity of verifying MRSA by use of PCR the percentage of verified MRSA out of the presumptive MRSA detected by Brilliance MRSA 2 agar was calculated for dust samples (49/50, 98%, 95% CI 89–100), nasal swabs (184/194, 95%, CI 91–98) and ear-skin swabs (151/160, 94%, 95% CI 90–97), respectively.

spa-typing

MRSA isolates from 33/35 positive herds were *spa*-typed ($n = 192$). From three herds all the collected MRSA isolates were *spa*-typed (16, 23 and 25 isolates, respectively). In two of the herds all isolates were *spa* type t034 and in one herd all isolates were *spa* type t011. The remaining 29 herds had between two and seven isolates *spa*-typed and one herd had only one isolate *spa*-typed. In 73% (22/30) of the herds t034 was found, in 13% (4/30) t011 was found, in 7% (2/30) both t011 and t034 were found and in two unrelated herds (7%) a *spa* type t4208 not previously detected in Denmark, but detected in Germany and the Netherlands was found [Ridom *spa* server (http://spaserver.ridom.de/)]. t4208 has previously been reported from pork in The Netherlands [21]. Both t034, t011 and t4208 are *spa* types related to CC398.

DISCUSSION

MRSA could be detected by all the applied sampling methods, but the sensitivity varied. One air sample analysed by direct plating of the filter had the same sensitivity as ten pools of five nasal swabs. Ten pools of five ear-skin swab samples were at least as sensitive as ten pools of five nasal swab, and sampling of five dust swabs was the least sensitive method. Air sampling analysed by direct plating of the filter reduced the time of analysis by 24 h compared to traditional culture. The cefoxitin concentration of 4 mg/l used for pools of nasal and ear-skin swabs differed from the concentration used for selective enrichment

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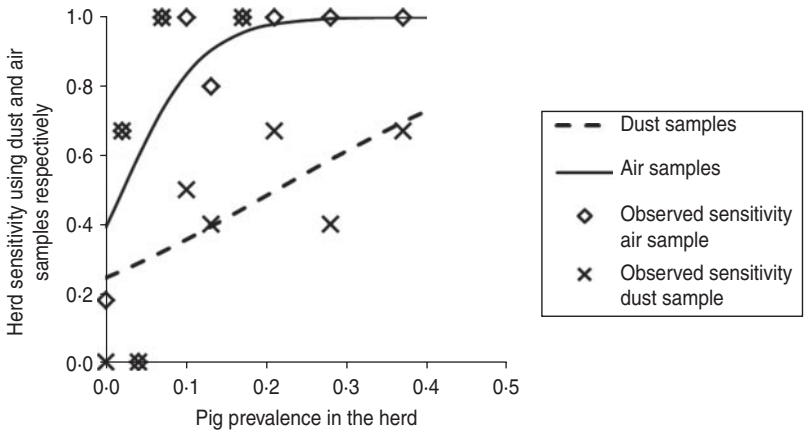

Fig. 1. Predicted values for the sensitivity on dust and air sampling at various within-herd prevalences. The prediction is based on logistic regression models using data from herds with at least one MRSA-positive sample of nasal swabs, air or dust samples.

Table 3. *Estimated within-herd prevalence in positive herds based on pools of ear-skin swabs and nasal swabs, respectively, in the 20 herds where both sampling methods were used and, based on pools of nasal swabs in all herds*

No. of positive pools/no. of pools	Estimated individual animal prevalence	% herds based on ear-skin swab pools (no. of herds)	% herds based on nasal swab pools (no. of herds)	% herds based on nasal swab pools (no. of herds)
0/10	0	10 (2)	25 (5)	22 (8)
1/10	0·02	5 (1)	10 (2)	8 (3)
2/10	0·04	0 (0)	0 (0)	3 (1)
3/10	0·07	0 (0)	0 (0)	3 (1)
4/10	0·1	5 (1)	0 (0)	5 (2)
5/10	0·13	5 (1)	20 (4)	13 (5)
6/10	0·17	5 (1)	5 (1)	3 (1)
7/10	0·21	5 (1)	10 (2)	8 (3)
8/10	0·28	5 (1)	5 (1)	14 (5)
9/10	0·37	0 (0)	10 (2)	8 (3)
10/10	1	60 (12)	15 (3)	13 (5)

of air filters (3·5 mg/l). In previous studies we detected MRSA by use of both concentrations and do not suspect a concentration of 3·5 mg/l to be less sensitive than 4 mg/l. Moreover, culturing of air samples by direct plating was found to be more sensitive than both direct PCR on the air samples and culturing based on selective enrichment, therefore the results based on direct plating were used for comparison of air sampling with the other methods (nasal, ear-skin and environmental swabs) (all done with cefoxitin concentration 4 mg/l). Therefore, we conclude that the difference in the cefoxitin concentration should not affect comparison of the sampling methods. The isolation of MRSA was done by different laboratories; however, all PCR verification was done in the same laboratory. Therefore, we conclude that this should have little effect on the results. The samples were taken from weaning pigs or slaughter pigs (Table 1, Supplementary Table S1) and from dust swabs and air in the same barn section as the animal samples were collected. Therefore, we conclude that the sample types are comparable independently of the age group sampled.

The sensitivity of the various methods was calculated based on the herds where at least one sample of air, dust swab or nasal swab was found positive. Thirteen of the herds tested negative. Of these, two previously tested negative and the remaining 11 tested positive only in a study testing pigs sampled at slaughter in 2009. Hence, these MRSA-positive pigs could have been contaminated/colonized during transport or at the slaughterhouse. The transmission of

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Table 4. *Agreement of herd classification between results obtained with air filter sampling with direct cultivation and pools based on nasal swabs*

	Nasal swabs	
Air sampling	Positive	Negative
Positive	26	3
Negative	3	14

Table 5. *Agreement of herd classification between results from pooled swab samples: nasal and ear-skin swab methods*

	Nasal swabs	
Ear-skin swabs	Positive	Negative
Positive	15	3
Negative	0	7

MRSA between pigs during transportation has been shown as a route of contamination/colonization [9], meaning that herds tested at slaughter can only be considered possible positive herds and may explain why the herds were found negative in this study. The remaining 37 herds included all the herds that tested positive at the farm level at least once in the period 2008–2011, indicating that once a herd has become contaminated/colonized it is likely to remain so if no control or eradication strategy is implemented as the herds operate in a continuous production system.

It was thus assumed that the negative herds were 'true' negative herds. It appears, however, that one of these 14 herds that tested negative by nasal swabs, air or dust samples (Supplementary Table S1) was positive by of ear-skin sampling suggesting that the 'true' number of positive herds could be higher. Therefore, the values of the sensitivity of different methods should be interpreted relatively (the relative sensitivity) and not as precise estimates of the sensitivity.

Moreover, the interpretation of the dependency between sensitivity and prevalence of MRSA in the herd should be interpreted relatively. We found that the pattern of dependence of the sensitivity of environment-based detection methods on the number of MRSA-infected pigs in the herd was the same whether comparing the association with the proportion of positive pooled samples or the within-herd prevalence predicted from the proportion of positive pooled samples.

Detection methods used for air samples

A good agreement between nasal swabs and air sampling was found, and the air sampling method can probably be further optimized by changing the sampling time, placing the air sampler at various locations in the barn during sampling and increasing the sensitivity of the analysis of air filters in the laboratory. According to the model, the air sampling performed almost perfectly at individual prevalences >25%. The air sampling method also had the advantage of being very simple to perform, and the direct plating of the filter onto a selective agar plate makes it possible to reduce time of analysis by 24 h to a total assay time of 2 days. The direct PCR detection method was significantly less sensitive than the culture-based methods, and had the further disadvantage of finding only presumptive MRSA as the method measures the presence of *S. aureus* and *mecA* independently of each other. Therefore, applying this particular real-time PCR method it is recommended to use subcultured isolates only, and not crude samples.

Comparison of sensitivity of the different methods

The number of test-positive herds using direct plating of air filters and the method with pools of nasal swabs was equal (equal sensitivity), even though it was not the same herds that tested positive. Additionally, no significant difference between the direct method and the method based on pre- and selective enrichment was observed. Direct plating of nasal pools was not tested in the present study, but other studies have shown this method to be less efficient than methods based on pre-enrichment and selective enrichment [22, 23]. Based on these results, the probability of classifying a truly contaminated/colonized herd correctly is very similar when using ten nasal swab pools compared to using one air filter. Applying air sampling holds several advantages compared to using ten pools of nasal swabs by being more cost-effective, more rapid and less labour intensive. The use of air sampling for MRSA has also been evaluated in another study [16]. In this study air sampling in 27 herds, previously found MRSA positive by use of dust sampling, resulted in a sensitivity of 85% by use of impingement into phosphate buffered saline (air volume 345 l) and a sensitivity of 56% by use of filtration (air volume 375 l). Although different equipment

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and a smaller air volume was used, and the study was performed in herds with a suspected high prevalence, the air sampling method was found to be equivalent to sampling of 12 pools of nasal swabs; this supports our findings [16]. In our study the use of environmental dust sampling was found to be less sensitive compared to other methods tested. This finding was in agreement with a study by Broens *et al.* [15].

In herds where both nasal and ear-skin swabs were sampled, more herds tested positive by use of ear-skin swab pools than by use of nasal swabbing. However, no significant difference in the sensitivity of the two sampling methods was observed at the herd level (Tables 2 and 3). However, significantly more pools of samples tested positive by use of ear-skin swabs compared to nasal swabs and the estimated herd prevalence was significantly higher when using ear-skin swabs compared to nasal swabs. This indicates that sampling of ear-skin swabs may potentially have a higher sensitivity than nasal swabs at the herd level. These results are supported by a study performed in Belgium [17]. However, more data are needed to confirm this and to optimize the number of ear-skin swabs taken in each herd. Friese *et al.* [16] found pools of ear-skin swabs to result in the same sensitivity as pools of nasal swabs, but they investigated herds with suspected high within-herd prevalence as the included herds were previously found positive by environmental dust swabbing.

Specificity of the testing method and diversity of isolates

The specificity of the various isolation techniques is believed to be close to 100%, as presumptive MRSA were isolated on MRSA-specific media, checked for *S. aureus* morphological characteristics on sheep blood agar and verified by PCR. This is, in principle, using both phenotypic and genotypic verification. For dust samples, nasal swabs and ear-skin swabs the specificity of Brilliance MRSA 2 agar was high, i.e. 94–98%, but not totally specific. Therefore, PCR-based verification of presumptive MRSA is still necessary in case of doubt, or very low within-herd prevalence. The multiplex PCR used did not include *mecC*, but since none of the tested presumptive MRSA were negative for *mecA* and positive for *femB*/*nuc* no isolates were suspected for *mecC*.

In the 32 herds where two or more isolates were *spa*-typed (including those where all MRSA isolates were *spa*-typed) only one *spa* type [mostly t011 or t034 belonging to CC398 (94%)] was found. This is in accordance with previous studies [3, 4, 24] and indicates that the spread of MRSA is mostly clonal, both in and between herds, but this needs to be confirmed by use of other typing techniques. The finding of *spa* type t4208 in two herds indicates that MRSA CC398 can develop or spread as seen recently when t1333 belonging to CC30 was found in Danish pigs at slaughter [4].

In conclusion, air sampling is easy to perform, less expensive than testing pools of nasal swabs, rapid and sensitive, and can be used initially to screen for MRSA-positive herds. To further increase the diagnostic sensitivity, it is recommended that herds testing MRSA negative by air sampling are supplemented by ear-skin swab sampling. Concurrently, the sensitivity of air sampling and ear-skin sampling should be further improved.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S095026881300280X.

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DECLARATION OF INTEREST

None.

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