Dynamics of methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus* carriage in pig farmers: a prospective cohort study

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Abstract

Our purpose was to determine the dynamics of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) carriage and its determinants in persons working at pig farms, in order to identify targets for interventions. This prospective cohort study surveyed 49 pig farms in the Netherlands on six sampling dates in 1 year (2010–11). Nasal and oropharyngeal swabs were collected, as well as environmental surface samples from stables and house. Of 110 pig farmers, 38% were persistent MRSA nasal carriers. The average cross-sectional MRSA prevalence was 63%. Methicillin-susceptible *S. aureus* (MSSA) nasal carriage was associated with fewer MRSA acquisitions (prevalence rate (PR) = 0.47, p 0.02). In multivariate analysis, an age of 40–49 years (PR = 2.13, p 0.01), a working week of ≥40 h (PR=1.89, p 0.01), giving birth assistance to sows (PR=2.26, p 0.03), removing manure of finisher pigs (PR=0.48, p 0.02), and wearing a facemask (PR = 0.13, p 0.02) were significantly related with persistent MRSA nasal carriage. A higher MRSA exposure in stables was associated with MRSA in pig farmers (p <0.0001). This study describes a very high prevalence of LA-MRSA carriage in pig farmers, reflecting extensive exposure during work. We identified the possible protective effects of MSSA carriage and of continuously wearing a facemask during work.

Keywords: Dynamics of carriage, epidemiology, livestock, methicillin-resistant *Staphylococcus aureus*, methicillin-susceptible *Staphylococcus aureus*, MRSA, MSSA, pigs, ST398, the Netherlands
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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-known pathogen inside and outside hospitals all around the world [1]. In the last years, the distinction between hospital-associated and community-associated MRSA has become less clear. In the Netherlands there still is a very low carriage rate of MRSA in the community (0.1%) [2]. Therefore, new strains, such as livestock-associated MRSA (LA-MRSA), are easily recognized.

The first LA-MRSA-positive pig farmer in the Netherlands was recognized in 2005 [3] and subsequent surveys showed a prevalence of LA-MRSA carriage ranging from 20–40% in people working with pigs [4–8]. Invasive infections were rarely described [9–11], and close contact with livestock was shown to be the major risk factor for LA-MRSA acquisition [6,7].

Currently there is a huge reservoir of MRSA in livestock with a relatively low impact on public health. This might change because *S. aureus* has proven to be very capable of exchanging genetic material, i.e. acquiring virulence factors [12]. To reduce the threat for public health the reservoir must be eliminated or reduced. Targets for intervention are needed, but at present unidentified. The purpose of this prospective cohort study was to determine the dynamics of LA-MRSA carriage and its determinants in persons working at pig farms.

Materials and Methods

Study design and selection of farms

This prospective cohort study surveyed persons working at 49 farrowing pig farms in the Netherlands for 1 year (2010–2011). Pig farms were randomly selected among participants from a previous study [13], which contained randomly selected farrowing pig farms from all Dutch pig farms.

Sampling occasions

During the 1-year study period, there were six sampling occasions: day 0, day 4, day 7, month 4, month 8, and month 12. Quantitative nasal and oropharyngeal swabs, extensive questionnaires, and wet wipe samples of four defined surfaces in house (backdoor handle, remote control of television, favourite chair of pig farmer, and dog neck/back) and four surfaces in the stables (farrowing and weaning stables, both sampled twice) were collected on day 0. Nasal swabs were introduced in the nostril and rotated once. Oropharyngeal swabs sampled the area of the inner cheek including the tonsils. Refrigerated swabs were transported to the laboratory, and cultured within 24 h. In addition, dry electrostatic dust collector cloths (EDCs) [14] were placed in the farrowing and weaning stables (two per stable) and on the highest cupboard in the living room of the house, and were left in place for 2 weeks before quantitative analysis.

On the remaining sampling occasions, subjects semi-quantitatively sampled their own nose and filled in a short questionnaire. Swab instructions were sent with the swabs. EDCs were placed on the same five locations in months 4, 8 and 12. An extra semi-quantitative sample of the throat was taken by the subjects themselves in month 12. Results of the individual cultures were disclosed at the end of the study.

Definitions

Persons working in pig farm stables for ≥20 h per week at the start of the study were defined as pig farmers, regardless of whether they lived on the farm premises or not. Persistent carriers were defined as persons with all nasal cultures positive for MRSA, non-carriers had no positive cultures, and intermittent carriers were the remaining persons.

Questionnaires

Extensive questionnaires were used to elucidate known and hypothetical determinants for (LA-)MRSA carriage. Data were collected on exact activities on the pig farm, contact with animals, hospital contact, personal use of antibiotics or immunosuppressive drugs, underlying disorders (e.g. eczema or other skin diseases) and presence of indwelling catheters and/or open wounds.

Laboratory analysis: cultures

Quantitative cultures were performed by diluting the elution buffer from ESwabs™ (swabs with 1 mL elution buffer; Copan, Brescia, Italy) up to 10^4 times in 0.9% NaCl, and incubating 100 μL of these dilutions on chromID *S. aureus* and chromID MRSA agar plates (BioMérieux, La Balme Les Grottes, France) overnight at 35°C. The number of CFU was counted on each agar plate, and plates with 10–100 CFU were used to calculate the original CFU number per swab. The remaining elution buffer and swabs were enriched overnight in Müller–Hinton broth with 6.5% NaCl, and subsequently cultured on *S. aureus* and MRSA selective agar plates.

Semi-quantitative cultures were performed by inoculating dry swabs (Copan) directly onto *S. aureus*, MRSA and Columbia agar plates with 5% sheep blood, and Müller–Hinton enrichment broth. Wet wipe samples (Sodibox, Nevez, France) were double-enriched in Müller–Hinton broth, followed by phenol mannitol broth with ceftizoxime (BioMérieux), which was subsequently cultured on blood and *Brilliance*™ MRSA agar plates (Oxoid, Basingstoke, UK), whether colour change occurred in the phenol mannitol broth or not.

All *S. aureus* strains were defined by green colonies in combination with a positive coagulase slide and DNase test. In case of discrepancies a tube coagulase test was performed. Methicillin susceptibility was tested for all *S. aureus* isolates, using the cefoxitin disk diffusion method according to EUCAST standards [15], followed by a duplex PCR for the *nuc* and *mec*A genes as described previously [16].

Laboratory analysis: EDC PCR

The EDC was suspended in FE-buffer (150 mM NaCl, 1 mM EDTA), mixed in a Stomacher blender (Seward Limited, London, UK), and stored at −20°C until further processing. DNA was isolated and purified with a Versant Molecular kPCR molecular system (Siemens Healthcare Diagnostics, The Hague, the Netherlands). For each sample, 5 μL was used to detect four targets with a LightCycler 480-II (Roche Diagnostics, Almere, the Netherlands): (i) *mec*A for methicillin-resis-

tance [17], (ii) C01 for livestock-association [18], (iii) *fem*A [17] and (iv) *nuc* [19] for detection of *S. aureus*.

The numbers of CFU-equivalents (eqCFU) per PCR and per EDC were calculated, using a standard control sample, which was included in each run to correct the standard curve for run-to-run variation. The concentration of *S. aureus* was the maximum of either *fem*A or *nuc*; the concentration of MRSA was the minimum of *mec*A and *S. aureus*; the concentration of LA-MRSA was the minimum of MRSA and C01.

Molecular typing

All MRSA nasal and throat isolates, as well as all wet wipe sample MRSA isolates, were genotyped. Staphylococcal protein A (*spa*) typing and multiple-locus variable number of tandem repeat analysis (MLVA) were performed as described [20,21].

Statistical analysis

Data were analysed with SAS, version 9.3 (SAS Institute Inc., Cary, NC, USA). For each person, the proportion of nasal cultures positive for MRSA, methicillin-susceptible *S. aureus* (MSSA) and *S. aureus* in general was calculated, resulting in persistent, intermittent and non-carriers. Missing samples were not taken into account in this definition, sensitivity analysis was performed to estimate the effect of the missing samples. CFU counts from nasal and oropharyngeal swabs were log-transformed and tested for association with persistent nasal MRSA carriage with a non-parametric regression model (smoothing) using PROC GAM to relax the assumption of linearity.

Numbers of LA-MRSA-positive environmental samples were counted per farm. The maximum stable-EDC LA-MRSA eqCFU count was calculated per sampling occasion per farm. The median stable-EDC LA-MRSA eqCFU count per farm was computed and compared between groups of farms with a Kruskall–Wallis test.

The effect of exclusive MSSA nasal carriage on MRSA carriage was studied in sets of two consecutive sampling occasions in persons initially without MRSA. Correction for correlation in samples from one person was performed using a compound symmetry correlation structure (repeated measurement analysis).

Univariate and multivariate calculations used PROC GENMOD, a generalized estimated equations model, with persistent nasal MRSA carriage (present or absent) in pig farmers as dependent variable. Poisson regression with log link and robust standard errors was used to calculate adjusted prevalence ratios (PR) because, as a general rule, at an outcome prevalence above 20%, odds ratios heavily overestimate the strength of an association [22]. Robust standard errors were produced with a repeated measures analysis (REPEATED statement), which we also used to adjust for potential correlation of observations (cluster effect) within farms, with a compound symmetry correlation structure. All determinants with univariate p-values of $\leq$0.20 and prevalences in pig farmers $\geq$0.10 (10%*110 = 11 pig farmers) were eligible for multivariate analysis. When Spearman's rho for two determinants was >0.70, collinearity was assumed, and the determinant with the highest PR or lowest p-value was selected for the multivariate backwards stepwise analysis.

Ethical considerations

All subjects signed an informed consent form before entering the study. The study protocol was approved by the medical ethical committee of the St Elisabeth Hospital in Tilburg, the Netherlands (protocol number 0933).

Results

MRSA carriage

This 1-year prospective cohort study included 110 pig farmers (88 men), with a median age of 45 (range 17–67 years), equally divided into farmers (n = 55) and employees (n = 55). The median number of working hours per week in the stables at the start of the study was 44 (range 20–80 h per week). Table 1 demonstrates that 38% were persistent MRSA nasal carriers. Variation in MRSA prevalence and cross-sectional prevalence per sampling occasion are shown in Fig. 1, the average MRSA prevalence was 63% (range 59–71%).

The median number of MRSA in nasal samples at the start of the study was 3.2 log-transformed CFU (log CFU, interquartile range (IQR: p25-p75) (IQR) 1.8–3.8), regarding positive samples only. For oropharyngeal samples a median of 1.0 log CFU (IQR 0.0–1.6) was found. Within this group of MRSA carriers at the start of the study, pig farmers with a higher number of CFU were significantly more often persistent nasal carriers, as shown in Fig. 2 (PR per log CFU = 1.31, 95% CI 1.12–1.54, p <0.0001 for nasal samples and PR = 1.08, 95% CI 0.91–1.28, p 0.40 for oropharyngeal samples). In addition, the presence of MRSA in oropharyngeal swabs at the start of the study (0/1) was significantly associated with persistent MRSA nasal carriage (PR = 2.89, 95% CI 1.65–5.10, p <0.0001).

MSSA carriage

When considering persons without MRSA, exclusive MSSA nasal carriage was associated with fewer MRSA acquisitions on the next sampling occasion, compared with sampling occasions without MSSA (9% vs 26%, PR = 0.47, 95% CI 0.25–0.91, p 0.02).

TABLE 1. Carriage patterns of *Staphylococcus aureus* in 110 pig farmers

	Carriage pattern[a] *n* (%)			
	Persistent	Intermittent	Non-carrier	Cross-sectional prevalence[b] % (range)
MRSA nose	42 (38)	46 (42)	22 (20)	63 (59–71)
MSSA nose	10 (9)	88 (80)	12 (11)	42 (26–78)
S. aureus nose[c]	57 (52)	48 (43)	5 (5)	79 (75–85)

[a]A persistent carrier was a person with all nasal cultures positive, non-carriers had no positive cultures, intermittent carriers were the remaining persons.
[b]On an average sampling occasion.
[c]Since MRSA and MSSA could co-exist in one sample, and *S. aureus* carriage could be a combination of MRSA and/or MSSA, the numbers do not add up. For example, a person carried MRSA on four out of six sampling occasions, and MSSA on the remaining two sampling occasions. This person was an intermittent MRSA carrier, an intermittent MSSA carrier, but a persistent *S. aureus* carrier.

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.

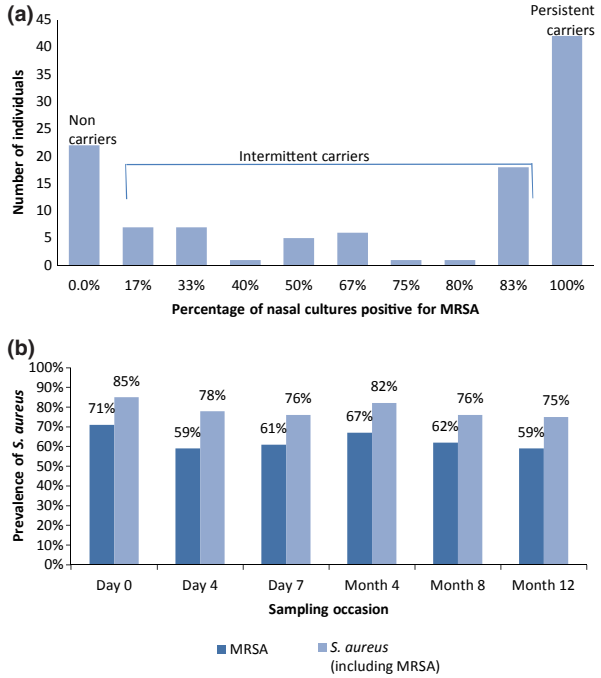

FIG. 1. Variation in methicillin-resistant *Staphylococcus aureus* (MRSA) nasal prevalence, resulting in persistent, intermittent and non-carriers (a) and mean cross-sectional nasal *S. aureus* prevalences per sampling occasion (b).

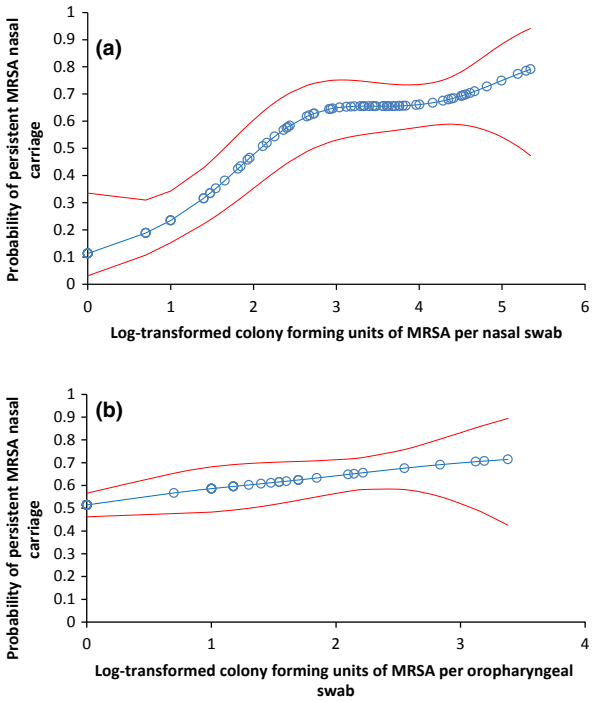

FIG. 2. Amount of methicillin-resistant *Staphylococcus aureus* (MRSA) in nasal (a) and oropharyngeal (b) swabs from start of study and probability of persistent MRSA carriage in pig farmers who were MRSA positive at the start of the study. Non-parametric regression model (smoothing) with 95% confidence bands.

Environmental samples

At least one wet wipe sample was MRSA-positive in 63% of houses and 84% of stables at the start of the study. In addition, at least one dry EDC was positive in 98% of stables, but in only 6% of houses (Table 2).

Table 2 also shows stable-EDC LA-MRSA eqCFU counts per farm during the study. Median eqCFU counts per sampling occasion did not vary significantly in time (data not shown). The median pig farm had 164 eqCFU (IQR 84–298) of LA-MRSA on its stable-EDC during the study. Farms with persistent and intermittent MRSA carriers had median stable-EDC eqCFU of 169 and 159, respectively, whereas those with non-carriers had 40 eqCFU ($p < 0.0001$).

Determinants for persistent nasal MRSA carriage

Table S1 (see Supporting information) describes determinants for persistent nasal MRSA carriage in pig farmers with a univariate $p \leq 0.20$ and a prevalence in pig farmers ≥ 0.10. No collinearity between determinants was observed. Presence of MRSA in the oropharynx at the start of the study, an age of 40–49 years, a working week of ≥40 h, giving birth assistance to sows, and moving piglets were univariately significantly associated with persistent MRSA nasal carriage. Determinants such as having a body part pierced in the last 12 months ($n = 3$), using corticosteroid medication ($n = 2$), and having

TABLE 2. Environmental samples positive for methicillin-resistant *Staphylococcus aureus* in 49 pig farms

	All farms	Farms with farmers who were[a] Persistent carriers	Intermittent carriers	Non-carriers	p value[b]
Wet wipe samples at start of study, no. positive farms/total no. farms (%)					
Stables	41/49 (84)	23/27 (85)	11/12 (92)	7/10 (70)	0.44
House	30/48 (63)	21/26 (81)	8/12 (67)	1/10 (10)	<0.0001
Dog	12/36 (33)	7/19 (37)	5/10 (50)	0/7 (0)	0.09
Dry EDCs at start of study, no. positive farms/total no. farms (%)					
Stables	48/49 (98)	27/27 (100)	11/12 (92)	10/10 (100)	0.45
House	3/48 (6)	3/26 (12)	0/12 (0)	0/10 (0)	0.41
Dry EDCs during study, median LA-MRSA eqCFU per cloth (IQR)					
Stables		169 (71–442)	159 (58–419)	40 (0–85)	<0.0001
House		0 (0–0)	0 (0–0)	0 (0–0)	0.13

[a]A persistent carrier was a person with all nasal cultures positive for MRSA, non-carriers had no positive cultures, intermittent carriers were the remaining persons. For each farm, the person carrying MRSA on most sampling occasions was selected.
[b]Differences between proportions were calculated with chi-square tests or Fisher's exact tests when 50% of the expected cell values were <5, EDC eqCFU counts were compared using Kruskall–Wallis tests.
EDC, electrostatic dust collector cloth; eqCFU, colony-forming units-equivalent; IQR, interquartile range (p25–p75); LA-MRSA, livestock-associated MRSA; MRSA, methicillin-resistant *Staphylococcus aureus*.

psoriasis (n = 3) were significantly associated with persistent MRSA carriage, but were observed sporadically and therefore not included in the multivariate analysis.

In a multivariate backwards stepwise analysis, an age of 40–49 years, a working week of ≥40 h, giving birth assistance to sows, and to a lesser extent, washing hands when leaving the stables were significantly related with persistent MRSA nasal carriage (Table 3). Lower persistent MRSA carriage rates were found in farmers who removed manure of finisher pigs, continuously wore a facemask when working in the stables, and to a lesser extent, had contact with cats.

Interactions between wearing a facemask and giving birth assistance to sows or removing manure of finisher pigs was not observed. Pig farmers continuously wearing a facemask did not work in stables with more eqCFU LA-MRSA, compared with pig farmers not always wearing a facemask (165 and 117 eqCFU, respectively, Wilcoxon Rank Sum test p 0.65).

TABLE 3. Determinants for persistent methicillin-resistant *Staphylococcus aureus* nasal carriage in pig farmers after multivariate analysis

Determinant	PR (95% CI)	P value[a]
Associated with elevated MRSA risk		
Age 17–39 years	Ref	
Age 40–49 years	2.13 (1.26–3.59)	0.01
Age 50–67 years	1.26 (0.75–2.12)	0.38
Work in stables >40 h/week	1.89 (1.19–3.01)	0.01
Give birth assistance to sows in the last 7 days	2.26 (1.10–4.67)	0.03
Wash hands when leaving stables	3.46 (0.93–12.79)	0.06
Associated with lower MRSA risk		
Remove manure of finisher pigs in the last 7 days	0.48 (0.26–0.87)	0.02
Continuously wear facemask when working in stables	0.13 (0.02–0.76)	0.02
Contact with cats in the last 12 months	0.62 (0.39–1.01)	0.05

[a]Multivariate generalized estimated equations model with persistent nasal MRSA carriage as dependent variable.
MRSA, methicillin-resistant *Staphylococcus aureus*; PR (95% CI), prevalence ratio with 95% confidence intervals; Ref, reference category.

According to Table 2, there were ten farms with farmers who did not carry MRSA during the study year, all had LA-MRSA present in the environmental samples at the start of the study. Of the 12 persons working on these farms, three (20%) wore facemasks continuously. This was more compared with persons from farms with persistent carriers (3/64 = 4%, Fisher's Exact p 0.07), but not when compared with farms with intermittent carriers (5/20 = 20%, p 1.00).

Molecular typing

In total, all 495 MRSA strains from 102 pig farmers were MLVA-typed and *spa*-typed, and are depicted in Fig. S1 (see Supporting information). All MRSA isolates from nose, oropharynx and wet wipe samples had *spa*-types and MLVA-types (MTs) corresponding to clonal complex (CC) 398, also known as the livestock-associated clade [23]. Of the MRSA isolates, 92% were MT398 (n = 248 isolates), MT572 (n = 186) and MT574 (n = 19). In addition, 94% of MRSA isolates belonged to *spa*-types t011 (n = 252), t108 (n = 187) and t034 (n = 27), where t011 generally matched with MT398 and t108 with MT572.

Of the 42 pig farmers who were persistent nasal MRSA carriers, 37 (88%) had the same MT or a single-locus variant throughout the study. Furthermore, 90% of samples from nose and oropharynx, when both positive on one sampling occasion, had the same MT or were a single-locus variant.

Discussion

MRSA carriage: persistence and prevalence

The persistent MRSA carriage rate found in pig farmers in this study (38%) was higher compared with the only other longitudinal study for MRSA in livestock farmers (18% in veal

farmers) [7]. The persistent total *S. aureus* carriage rate from pig farmers (52%) was also very high compared with the general population (20%) [1,24].

The cross-sectional *S. aureus* carriage rate in the Dutch general population is approximately 30% and a minority of the strains are MRSA [1,24]. The mean cross-sectional MRSA prevalence in pig farmers in this study was 63%, and 80% of pig farmers had at least one MRSA-positive sample during the 1-year study period. These excessive MRSA carriage rates have not been described previously. Studies on pig and veal calf farmers in Europe, USA and Canada described cross-sectional MRSA prevalences between 20 and 40% [3–8].

The high MRSA carriage rates found in pig farmers in this study can be explained by (i) a higher degree of environmental contamination in this population, or (ii) a true higher degree of colonization. Given the high number of LA-MRSA-positive stables (98% with EDCs), and the higher eqCFU counts in stables where persistent LA-MRSA carriers work, it is plausible that LA-MRSA-contaminated dust from stables is inhaled and present in the nose, but this may not necessarily represent true colonization [7,25]. Oropharyngeal carriage is significantly associated with persistent MRSA carriage [26–29]. In addition, we found high MRSA CFU counts in persistent carriers, and 88% of persistent carriers carried the same MT or single-locus variant for prolonged periods [30]. Therefore we argue that true colonization might be a plausible explanation for the majority of the persistent carriage observed.

MRSA carriage is associated with environmental contamination

It is of utmost importance to reduce MRSA exposure in pig farming, because it appears to be the most important determinant for MRSA carriage in this and other studies: working ≥40 h per week, giving birth assistance to sows, and higher eqCFU counts in stables were associated with MRSA carriage in pig farmers, confirming the existing literature [6,7,31,32].

Working with sows and piglets involves more moments with intense animal contact (birth assistance, castration, earmarking, cutting teeth and tail, etc.) than working with finisher pigs, resulting in a higher risk for LA-MRSA acquisition. The farms included in this study were all farrowing farms; the majority of farmers worked with sows and piglets, hence no comparison could be made with farmers working with finisher pigs only.

Facemasks protect for persistent MRSA carriage

Of the persons continuously wearing a facemask when working in the stables, 9% (1/11) was carrying MRSA persistently, compared with 42% (40/96) of persons not always wearing a facemask. After correction for other determinants and possible confounders, continuously wearing a facemask resulted in a relative risk reduction of 87%.

Proof for efficacy of facemasks in prevention of acquisition of *S. aureus* is lacking [33]. One small study of seven veterinarians reported no effect for facemasks on MRSA carriage [34]. However, pig farmers are generally advised to wear facemasks because of high exposure to dust pathogens [35]. This study showed a protective effect of masks, which should be confirmed in an interventional trial.

MSSA is negatively associated with MRSA carriage

Our calculations show that nasal carriage of MSSA was a significant protective factor for nasal MRSA carriage on the next sampling occasion. This effect was shown in persons who were not persistently carrying MRSA, excluding 38% of the study population. More studies have demonstrated the existence of bacterial interference, not only for *Staphylococcal* spp. among themselves, but also between genera [36,37]. A recent study in veal calf farmers found a negative association between MSSA and MRSA carriage as well [7]. A possible intervention strategy resulting from these observations is inoculation of pig farmers with MSSA species. This has been studied in the past [24,36,38], but more evidence is required. In addition, because of the 4-month time interval between the sampling occasions, other reasons for the absence of MRSA cannot be excluded (antibiotic use, nature and amount of work in the stables, status of innate immunity, etc.).

Other determinants for persistent MRSA carriage

Persons aged 40–49 years have a higher chance of being persistent MRSA nasal carriers, compared with their older and younger peers. The associations between the amount of hours worked per week in stables and MSSA carriage with age [1,24] are not strong enough to explain this finding (hours/week and age: Spearman's correlation coefficient = 0.15, p 0.14).

The borderline significant negative association of contact with cats on MRSA carriage could not be verified in literature: companion animals and their owners seem to exchange MRSA rather than protecting each other [39,40], with cats in lower amounts than with dogs [41]. The borderline risk factor of washing hands can be explained by the fact that MRSA is able to survive for days on water taps, towels and soap [42–44]. Reversed causality might have been another possibility; farmers might have washed their hands more often when working in a stable with much dust. More in-depth studies are needed for further clarification, exploring multiple hypotheses (paper versus textile towels, frequency of hand washing, location of sink), although one can wonder what the additive effect is with these huge amounts of exposure.

Study limitations

Self-sampling techniques might not have been sufficient, resulting in a possible underestimation of MRSA positivity. To validate self-sampling of both nose and oropharynx for presence of *S. aureus*, we conducted a pilot study [45]. Agreement between self-samples and investigator-samples was excellent for nasal swabs (agreement = 93%, $\kappa = 0.85$, 95% CI 0.74–0.96) and good for oropharyngeal samples (agreement = 83%, $\kappa = 0.60$, 95% CI 0.43–0.76). Moreover, the high MRSA carriage rates found in this study, compared with other studies, do not indicate an underestimation.

When a sample was MRSA-positive, the potential presence of MSSA in the same sample might have been missed, because only when two morphologically different colonies existed on the blood agar plate were both determined. The reported numbers of MRSA, isolated MSSA, or *S. aureus* carriage in general are correct, because only samples with simultaneous presence of MRSA and MSSA are affected. Therefore we believe that this effect is of negligible impact on our results.

Lastly, 32 of 660 (4.8%) nasal samples were missing, and sensitivity analysis did not reveal changes in associations.

Conclusions

This study describes very high prevalences of LA-MRSA in pig farmers, reflecting extensive exposure during work. We identified protective effects of MSSA carriage and of continuously wearing a facemask during work.

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Author Contributions

BvC, BvB, JK, HG, JW and DH designed the study. BvC collected and analysed the data, BvB, EV, MvR, MK, MB, JW, DH and JK significantly supported the data analysis. LS typed the MRSA isolates, BD analysed the EDCs. BvC wrote the manuscript, where BvB, EV, MvR, MK, HG, LS, MB, BD, JW, DH and JK supported the writing process by reviewing the manuscript.

Transparency Declaration

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Minimum spanning tree of multiple-locus variable number of tandem repeat analysis results and *spa*-types of MRSA isolates from pig farmers working in 49 pig farms.

Table S1 Determinants for persistent methicillin-resistant *Staphylococcus aureus* (MRSA) nasal carriage in pig farmers

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