

# Health and health-related quality of life in pig farmers carrying livestock-associated methicillin-resistant *Staphylococcus aureus*

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# SUMMARY

There is limited knowledge about the effect of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) carriage on health-related quality of life (QoL). With this study, we explored whether LA-MRSA causes infections or affects health-related QoL in pig farmers. This prospective cohort study surveyed persons working on 49 farrowing pig farms in The Netherlands for 1 year (2010–2011). On six sampling moments, nasal swabs, environmental samples and questionnaires on activities and infections were collected. At the end of the study year, persons were asked about their QoL using the validated SF-36 and EQ-5D questionnaires. Of 120 persons, 44 (37%) were persistent MRSA carriers. MRSA carriage was not associated with infections, use of antimicrobials, healthcare contact and health-related QoL items in univariate or multivariate analysis, most likely due to the 'healthy worker effect'. Despite high carriage rates, the impact of LA-MRSA carriage in this population of relatively healthy pig farmers on health and health-related QoL appears limited; more research is needed for confirmation.

Key words: Infectious disease epidemiology, methicillin-resistant S. aureus (MRSA), public health, zoonoses.

## **INTRODUCTION**

Staphylococcus aureus (SA) generally resides on skin and mucous membranes, like nares, pharynx, and

perineum [1, 2]. About one in three persons carry SA in their nose. Next to being a commensal bacterium, SA is also known for its pathogenic potential, ranging from harmless skin infections like impetigo and furuncles to severe infections like sepsis, osteomyelitis and pneumonia [1]. Persistent carriers are supposed to have the highest risk of infection by SA [3], whereas non-carriers appear to suffer more serious consequences when they experience a (nosocomial) SA infection [4].

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In The Netherlands, a low carriage rate of methicillinresistant *S. aureus* (MRSA) was found in the community (0.1%) [5–7]. Therefore, new MRSA clades, such as livestock-associated MRSA (LA-MRSA), were easily recognized. The prevalence of LA-MRSA carriage ranged from 20–60% in people working with pigs [8–13]. Close contact with livestock was shown to be the major risk factor for LA-MRSA acquisition [11, 13].

Like all variants of SA, LA-MRSA is able to cause a wide spectrum of infections [14–16]. Nevertheless, the proportion of LA-MRSA in serious MRSA infections appears to be low, ranging from 0.8% to 14% [17–23]. A study in livestock veterinarians found that skin infections were more prevalent in persistent SA carriers, compared to non-carriers (25% vs. 0%, relative risk 3.3, P = 0.058). Numbers were comparable for LA-MRSA and methicillin-sensitive *S. aureus* (MSSA), generally indicating a less virulent strain [24]. Several studies show that LA-MRSA has less virulence genes than other MRSA strains, but, as any other MRSA, it has the ability to exchange genetic material easily [19, 25–29].

To our knowledge, little is known about the effect on health-related quality of life (QoL) of LA-MRSA carriage. In this study, we assessed whether LA-MRSA carriage is associated with (1) a higher risk of infection or (2) reduced health-related QoL in pig farmers.

### **METHODS**

#### Study design and selection of farms

This prospective cohort study surveyed persons working on 49 farrowing pig farms in The Netherlands for a single year (2010–2011). Pig farms were randomly selected from participants in a previous study [30], which contained randomly selected farrowing pig farms from all Dutch pig farms. A detailed analysis of determinants of MRSA and MSSA carriage in the pig farmers and household members of this study has been described elsewhere [13, 31].

#### Sampling moments

Six sampling moments occurred during the 1-year study period: day 0, day 4, day 7, month 4, month 8, and month 12. On day 0, nasal and oropharyngeal swabs and extensive questionnaires with items on contact with animals, hospital contact, personal use of antimicrobials or immunosuppressive drugs, underlying disorders (e.g. eczema or other skin diseases), presence of indwelling catheters and/or open wounds were collected. Nasal swabs were introduced in the nostril and rotated once. Oropharyngeal swabs sampled the area of the inner cheek including the tonsils. House and stable environments were sampled on day 0 with wet wipe samples and dry electrostatic dust collector cloths (EDCs) [32], the latter were left in place for 2 weeks before quantitative analysis.

On the remaining sampling moments, nose selfsamples and short questionnaires on farm activities and infections were collected. Swab instructions were sent with the swabs. EDCs were placed on the same locations in months 4, 8 and 12. In month 12, an additional oropharyngeal self-sample and two healthrelated QoL questionnaires were added. Results of the individual cultures were disclosed at the end of the study.

The Short Form 36 (SF-36) [33, 34] measures eight dimensions of health: physical functioning, physical role functioning (i.e. limitations due to physical difficulties), bodily pain, social functioning, emotional role functioning, mental health, vitality, and current general health perception. Each dimension is scored from 0 (least favourable health state) to 100 (most favourable health state). An additional question on health change, compared to a year earlier, was added, which could be scored as much better (score 0), a bit better (score 25), the same (score 50), a bit worse (score 75), or much worse (score 100) [34]. Two summary scales (physical and mental) were calculated and standardized to a mean of 50 with a standard deviation of 10 in the general population for easy comparison.

The EuroQol 5D (EQ-5D) questionnaire [35] measures five domains: mobility, self-care, usual activities, pains/discomfort, and anxiety/depression. Each domain is scored as 1 (no problems), 2 (some problems), or 3 (severe problems). In addition, a visual analogue scale (EQ-VAS) on health state was added, scoring from 0 (worst imaginable health state) to 100 (best imaginable health state).

#### Definitions

Pig farmers were defined as individuals who worked in the stables for at least 20 h per week at the start of the study, regardless of whether they lived on the pig farm premises or not. Persistent MRSA carriers were defined as persons with all cultures positive for MRSA, regardless of typing results, non-carriers had no positive MRSA cultures, and intermittent carriers were the remaining persons. Nasal and oropharyngeal samples were combined, if either one of the samples was positive, that sampling moment was considered positive.

#### Laboratory analysis

The extended laboratory procedure is described elsewhere [13]. In short, cultures were performed by incubating swabs on chromID *S. aureus* and chromID MRSA agar plates (bioMérieux, France). Wet wipe samples (Sodibox, France) were double-enriched and subsequently cultured onto blood and Brilliance<sup>TM</sup> MRSA agar plates (Oxoid, UK).

All SA strains were defined by coloured colonies on selective SA agar in combination with a positive coagulase slide latex agglutination test and positive DNase test. Methicillin susceptibility was tested using the cefoxitin disk diffusion method according to EUCAST standards [36], followed by a duplex polymerase chain reaction (PCR) for the *nuc* and *mecA* genes as described previously [37].

For each EDC sample, four targets were detected with a LightCycler 480-II real-time quantitative PCR (Roche Diagnostics, The Netherlands): (i) *mecA* for methicillin resistance [38], (ii) C01 for sequence type 398 showing livestock association [39], (iii) *femA* [38] and (iv) *nuc* [40] for detection of SA [13].

#### Statistical analysis

Data were analysed with SAS v. 9.3 (SAS Institute Inc., USA). For each person, the proportion of nasal cultures positive for MRSA was calculated, resulting in persistent, intermittent and non-carriers.

Intensive pig contact was expected to be a strong confounder in the possible association between MRSA carriage and QoL [13]. Since most of the pig farmers in this study worked with sows, which implies more intensive pig contact than working with finisher pigs, we excluded persons not working with sows from the analysis. In a comparable manner, since MSSA was negatively associated with MRSA carriage in previous studies [13, 31], and we expected MSSA carriage to be related to infections and possibly QoL, we only analysed those persons who were not persistent MSSA carriers.

The effect of MRSA on acquisition of infections and use of antimicrobials was studied in sets of two consecutive sampling moments with PROC GENMOD, a generalized estimated equations model (logistic regression), with adjustment for correlations between repeated observations from the same individual and for potential confounders (age, gender, MRSA in home and stable environment, working in the stables, giving birth assistance to sows, wearing facemasks). All determinants with univariate  $\chi^2 P$  values of  $\leq 0.20$  were eligible for multivariate analysis. When Spearman's rho for two determinants was  $\ge 0.70$ , collinearity was assumed, and the determinant with the highest prevalence ratio (PR) [41] or lowest P value was selected for the multivariate analysis. Association of MRSA and healthcare contact, which was not enquired for on all sampling moments and therefore did not vary over the 1-year study period, was studied with  $\chi^2$  tests, or Fisher's exact tests when expected cell counts were <5.

Mean scores for different dimensions in SF-36 health-related QoL in persistent, intermittent and non-MRSA carriers were compared with a one-way ANOVA. Mental and physical summary scales were used as dependent variables in multivariate PROC GENMOD, this time used as a generalized linear model, with persistent MRSA carriage as a possible determinant, adjusted for correlated observations from the same farm and for expected confounders. All determinants with univariate type 3 P values of  $\leq 0.20$  were eligible for multivariate analysis. Type 3 P values were calculated using likelihood-ratio tests, and reflect the significance of the effect in the presence of interactions. Wald P values from the estimates were less powerful and did not take interaction effects into account. When Spearman's rho for two determinants was  $\geq 0.70$ , collinearity was assumed, and the determinant with the most extreme estimate or lowest P value was selected for multivariate analysis.

Problems in each domain of EQ-5D health-related QoL were compared between persistent, intermittent, and non-MRSA carriers with Fisher's exact tests. Associations between each domain and persistent MRSA carriage were tested with multivariate logistic regression, adjusted for correlated observations from the same farm and for expected confounders, with eligibility and collinearity confirmations as described above. The EQ-VAS was analysed linearly, as described above.

#### Ethical considerations

All subjects provided written informed consent before entering the study. The study protocol was approved by the medical ethical committee of St

| Characteristic                                     | Total      | Persistent MRSA | Intermittent MRSA | Non-MRSA   |
|--|------------|-----------------|-------------------|------------|
| Total number, <i>n</i> (row %)                     | 120        | 44 (37)         | 53 (44)           | 23 (19)    |
| Male gender, $n$ (row %)                           | 81         | 33 (41)         | 40 (49)           | 8 (10)     |
| Age, years, median (IQR)                           | 44 (32–49) | 46 (39–51)      | 40 (30-49)        | 39 (20-48) |
| Work in stables $\geq 20$ h/week, <i>n</i> (row %) | 88         | 40 (45)         | 40 (45)           | 8 (9)      |
| Work at farm (hours/week), median (IQR)*           | 39 (14-50) | 49 (30-60)      | 39 (18-45)        | 5 (0-35)   |
| Wear facemask, <i>n</i> (row %)                    |            |                 |                   |            |
| Always   | 12         | 1 (8)           | 7 (58)            | 4 (33)     |
| Sometimes  | 45         | 20 (44)         | 18 (40)           | 7 (16)     |
| Never/missing                                      | 63         | 23 (37)         | 28 (44)           | 12 (19)    |
| Give birth assistance to sows, $n$ (row %)         | 80         | 38 (48)         | 34 (43)           | 8 (10)     |

Table 1. General characteristics of the study population

MRSA, Methicillin-resistant Staphylococcus aureus; IQR, interquartile range (i.e. p25-p75).

Persistent, samples from all sampling moments positive; Non-carrier, all samples negative; Intermittent, the remaining persons.

Only persons working with sows, who completed the Quality-of-Life questionnaire and were not persistent methicillinsusceptible S. aureus carriers are depicted (n = 120).

\* Median amount of hours worked per week are shown for all study subjects, being both pig farmers (persons who work in the stables, n = 88) and household members (n = 32).

Elisabeth Hospital in Tilburg, The Netherlands (protocol no. 0933).

Table 2. Acquisition of infections and use ofantimicrobials associated with MRSA, in consecutivesets of sampling moments

# RESULTS

#### Study population

Of the 281 persons from 49 farms who entered the study (pig farmers, employees, household members), 198 (70%) worked with sows, mostly men of working age. Of these persons, 62 did not complete the QoL questionnaires and another 16 were persistent MSSA carriers, leaving 120 persons for analysis (Table 1).

In total, 44/120 persons (37%) were persistent MRSA carriers, 53 (44%) were intermittent MRSA carriers and 23 (19%) did not carry MRSA during the study period. Environmental sampling showed that all stables were MRSA positive at the start of the study, and 41/46 (89%) houses.

#### Infections, use of antimicrobials and healthcare contact

Of the 198 persons working with sows, 398 sets of consecutive sampling moments were available for analysis, of which 198 were initially without MSSA. Frequencies and univariate logistic regression results are shown in Table 2. In total, 23 infections were present on 198 sampling moments [12%, Wilson 95% confidence interval (CI) 8–17%, one person had both eczema and an open wound on one sampling moment]. Skin infections were found on 21/198 (11%,

|                         |                            | moment                    | sampning                    |                      |
|-------------------------|----------------------------|---------------------------|-----------------------------|----------------------|
| Current sampling moment | Total<br>( <i>N</i> = 198) | MRSA<br>( <i>n</i> = 100) | No MRSA<br>( <i>n</i> = 98) | P value <sup>a</sup> |
| Abscess                 | 0 (0)                      | 0 (0)                     | 0 (0)                       | n.a.                 |
| Bacteraemia             | 0 (0)                      | 0 (0)                     | 0 (0)                       | n.a.                 |
| Osteomyelitis           | 2 (1)                      | 1 (1)                     | 1 (1)                       | 0.58                 |
| Impetigo                | 0 (0)                      | 0 (0)                     | 0 (0)                       | n.a.                 |
| Pneumonia               | 0 (0)                      | 0 (0)                     | 0 (0)                       | n.a.                 |
| Open wounds             | 10 (5)                     | 4 (4)                     | 6 (6)                       | 0.29                 |
| Boils                   | 1 (1)                      | 0 (0)                     | 1 (1)                       | n.a.                 |
| Diabetes mellitus       | 0 (0)                      | 0 (0)                     | 0 (0)                       | n.a.                 |
| Eczema                  | 11 (6)                     | 7 (7)                     | 4 (4)                       | 0.65                 |
| Psoriasis               | 0 (0)                      | 0 (0)                     | 0 (0)                       | n.a.                 |
| Use antimicrobials      | 13 (7)                     | 7 (7)                     | 6 (6)                       | 0.25                 |

Values given are n (%).

MRSA, Methicillin-resistant *Staphylococcus aureus*; n.a., not applicable.

\* Univariate *P* values for association with logistic regression, adjusted for correlated observations from the same person.

95% CI 7–16) sampling moments, divided into 10% (10/100, 95% CI 6–17) of MRSA-positive sampling moments, and 11% (11/98, 95% CI 6–19) of MRSA-negative sampling moments in the 1-year study period. These skin infections occurred in 19 different persons (19/116 = 16%, 95% CI 11-24).

|   | MRSA carria | ge, mean score (s.d.) |           |          |
|---|-------------|-----------------------|-----------|----------|
| Dimension   | Persistent  | Intermittent          | Non-MRSA  | P value* |
| Physical functioning                                | 93 (11)     | 95 (9)                | 95 (11)   | 0.74     |
| Role functioning, physical                          | 93 (20)     | 92 (22)               | 90 (26)   | 0.87     |
| Bodily pain   | 91 (15)     | 87 (17)               | 85 (17)   | 0.42     |
| General health perception                           | 79 (14)     | 77 (16)               | 79 (15)   | 0.70     |
| Vitality  | 77 (15)     | 72 (14)               | 75 (10)   | 0.15     |
| Social functioning                                  | 92 (12)     | 91 (18)               | 91 (13)   | 0.91     |
| Role functioning, emotional                         | 97 (10)     | 94 (21)               | 93 (25)   | 0.62     |
| Mental health                                       | 84 (12)     | 85 (9)                | 81 (8)    | 0.21     |
| Physical summary scale                              | 54 (6)      | 53 (6)                | 54 (7)    | 0.84     |
| Mental summary scale                                | 55 (6)      | 55 (6)                | 54 (5)    | 0.50     |
| Health change last year                             | 47 (12)     | 51 (8)                | 50 (0)    | 0.07     |
| EQ-VAS current health status                        | 85 (11)     | 86 (9)                | 83 (9)    | 0.45     |
| Admitted to hospital last year, <i>n</i> /total (%) | 2/44 (5)    | 1/53 (2)              | 3/22 (14) | 0.13†    |
| Outpatient clinic last year, n/total (%)            | 9/44 (20)   | 8/53 (15)             | 5/22 (23) | 0.68‡    |

Table 3. SF-36 questionnaire, EQ-VAS and healthcare contact, in relation to different MRSA carriage types

SF-36, Short Form 36; EQ-VAS, EuroQol visual analogue scale; MRSA, methicillin-resistant *Staphylococcus aureus*; s.D. standard deviation.

Persistent, samples from all sampling moments positive; Non-carrier, all samples negative; Intermittent, the remaining persons.

\* One-way ANOVA. † Fisher's exact test.

 $\ddagger \chi^2$  test.

Presence of infections or use of antimicrobials was not significantly associated with MRSA in a previous sampling moment in univariate analysis. Moreover, additional multivariate analysis did not reveal associations with MRSA carriage.

Table 3 shows the absence of an association of healthcare contact and MRSA carriage groups.

## Short Form 36

Table 3 shows SF-36 data, where different MRSAcarriage types (persistent, intermittent, none) did not differ in mean scores.

When comparing persistent MRSA carriers with those who did not carry MRSA persistently, no relationship was found with physical or mental summary scales, as depicted in Table 4. Only higher age was associated with a lower physical summary scale (-1.32 points per 10 years, 95% CI -2.30 to -0.35, P = 0.03) in univariate analysis. Additional multivariate analysis did not reveal associations with persistent MRSA carriage.

## EuroQol 5D

Domain scores per MRSA carriage type are illustrated in Figure 1. Since no persons scored severe problems (score 3) on any domain, the results were dichotomized into no problems (score 1) and some problems (score 2). No differences were found between different carrier groups. The mean EQ-VAS score is shown in Table 3, where no differences were found between groups of MRSA carriers.

Univariate logistic (domains) and linear (EQ-VAS) regression is shown in Table 5. Self-care was not analysed since only one person reported problems in self-care. Persistent MRSA carriage was not associated with any EQ-5D domain or VAS score. The presence of MRSA in home samples was significantly associated with a lower score for mobility problems. Male gender and more hours working at the farm was significantly associated with a higher VAS score (4.88 points higher, 95% CI 1.69–8.08, P = 0.01; 1.01 points per 10 h, 95% CI 0.19–1.83, P = 0.03, respectively). Higher age and wearing a facemask both had a non-significant trend with problems in all domains (except for mood/anxiety), including a lower VAS score. Additional multivariate analysis also did not reveal associations.

#### DISCUSSION

#### Infections, antimicrobial use and healthcare contact

SA is a known pathogen for skin infections [1], these infections subsequently may result in prolonged

|   | Mental sun   | nmary scale                    |              | Physical sur | mmary scale                    |                   |
|---|--------------|--------------------------------|--------------|--------------|--------------------------------|-------------------|
| Determinant                             | Estimate     | 95% CI                         | Type 3 P*    | Estimate     | 95% CI                         | Type 3 <i>P</i> * |
| Persistent MRSA<br>MRSA in home samples | 0·78<br>1·27 | -1·40 to 2·97<br>-2·18 to 4·72 | 0·48<br>0·48 | 0·52<br>2·51 | -1.46 to $2.50-2.02$ to $7.03$ | 0·61<br>0·36      |

Table 4. Univariate regression on summary scales of SF-36 questionnaire

SF-36, Short Form 36; CI, confidence interval; MRSA, methicillin-resistant Staphylococcus aureus.

Persistent, samples from all sampling moments positive.

Univariate results from linear regression, adjusted for correlated observations from the same farm. Estimates (beta) with 95% CI are shown, reflecting the magnitude of change of summary scale (mental or physical) with change of the determinant value. \* Type 3 *P* values are calculated using likelihood-ratio tests.



**Fig. 1.** Persons with problems on EQ-5D domains. EQ-5D, EuroQol 5 dimensions; Persistent: samples from all sampling moments positive; Non-carrier: all samples negative; Intermittent: the remaining persons; MRSA, methicillin-resistant *Staphylococcus aureus*. Prevalence of problems per dimensions were shown, with 95% Wilson confidence interval bars. No significant differences between different groups were found with Fisher's exact tests.

carriage since the infection provides a persisting source. In the case of LA-MRSA, the repeated and extremely high exposure rates in the stables may result in prolonged carriage as well. In 11% (95% CI 7-16) of the sampling moments in this study skin infections were present (10% of MRSA-positive and 11% of MRSA-negative sampling moments). The incidence of bacterial skin infections in the general Dutch population is estimated to be 3.2% per year [42]. This is lower than the numbers reported from our study population, which consisted of livestock farmers who have an unusually high exposure to SA and high MRSA and MSSA carriage rates, compared to the general population [19]. Moreover, due to the nature of working in the stables, pig farmers are expected to have more skin abrasions than the average person.

Therefore it is likely that the study population is associated with a higher chance of skin infections [3]. Wardyn *et al.* reported an incidence rate of 6.6 skin infections per 1000 person-months (equal to  $6.6 \times 12 = 7.9\%$ per year) in a combined group of persons with livestock contact and community-based controls in the USA [18]. A study in Dutch livestock veterinarians found a prevalence of skin infections of 25% in persistent SA carriers, compared to 0% in non-carriers [24]. These numbers are more comparable to the results from our study.

Regarding serious infections, osteomyelitis was acquired on two sampling moments (2/198 = 1%, 95% CI 0·3–3·6), one with and one without previous MRSA carriage. During routine care, no cultures were performed to find the causative organism.

| 1 auto J. Univariate reg                             | Sression             | nh ac-Za 1                  | hinoma                | ane         |                               |                    |                    |                            |                   |                 |                            |                            |                                     |  |                       |
|--|----------------------|-----------------------------|-----------------------|-------------|-------------------------------|--------------------|--------------------|----------------------------|-------------------|-----------------|----------------------------|----------------------------|-------------------------------------|--|-----------------------|
|  | Mobi                 | ility                       |                       | Daily       | activities                    |                    | Mood               | l/anxiety                  |                   | Pain            |                            |                            | EQ-VAS                              |  |                       |
| Determinant  | PR                   | 95% CI                      | $\chi^2 P$            | PR          | 95% CI                        | $\chi^2 P$         | PR                 | 95% CI                     | $\chi^2 P$        | PR              | 95% CI                     | $\chi^2 P$                 | Estimate                            | 95% CI                                   | Type 3 $P^{\uparrow}$ |
| Persistent MRSA<br>MRSA in home samples              | 0·58<br>0·17         | 0.13-2.67<br>0.05-0.57      | 0.48<br><b>0-00</b>   | 2.03        | 0.75-5.49                     | 0·16<br>0.99       | 0.70               | 0.12 - 3.70<br>0.30 - 1.67 | 0.65<br>0.42      | 0-91<br>0-70    | 0.51 - 1.63<br>0.30 - 1.67 | 0·75<br>0·42               | -0.11<br>-0.80                      | -3.96-3.72<br>-6.20-4.59                 | 0-95<br>0-77          |
|  |                      |                             |                       | -<br>-<br>- |                               | È t                |                    |                            |                   |                 |                            |                            | 0000                                |  |                       |
| EQ-5D, EuroQol 5 dimer<br>Univariate results from lo | nsions;<br>ogistic r | EQ-VAS, Eu<br>egression, ad | iroVol v<br>Jjusted f | isual ar    | nalogue scale<br>dated observ | ; Ul, co<br>ations | onndenc<br>from th | te same farm               | MKSA,<br>1. The I | methic<br>30-VA | S score is and             | <i>Staphy</i><br>alvsed li | <i>tococcus au</i><br>nearly, estii | <i>eus</i> ; PK, preva<br>nates are show | lence ratio.<br>n.    |
| Persistent: samples from                             | all samı             | pling momen                 | uts posit             | ive.        |                               |                    |                    |                            |                   | ,               |                            | •                          |                                     |  |                       |
| * Self-care was not analy:                           | sed, sinc            | ce only one l               | person r              | eported     | problems in                   | self-ca            | re.                |                            |                   |                 |                            |                            |                                     |  |                       |
| $\ddagger Type 3 P$ values are cal                   | culated              | using likelih               | nood-rat              | io tests.   |                               |                    |                    |                            |                   |                 |                            |                            |                                     |  |                       |

Serious infections with LA-MRSA have been reported before in 0.8-14% of serious MRSA infections [17–23].

Development of infections, antimicrobial use and healthcare contact (admittance to hospital and visiting an outpatient clinic) were not associated with MRSA carriage in the current study in univariate and multivariate analysis. In the literature, several studies show that LA-MRSA has a low amount of virulence genes compared to other MRSA strains[19, 25–29]. The most likely explanation for the absence of an association of infections with MRSA carriage is the limited effect of SA carriage in general among healthy individuals.

As our population consisted of working persons, it is likely that the 'healthy worker effect' limits the impact of MRSA carriage seen in this study; persons who work are generally more healthy, since persons who do not work (possibly due to physical or mental problems) are excluded. In our study population and in the literature, working in the stables is strongly related to MRSA carriage [13, 31], and we did not find an association between infections and MRSA. To determine whether LA-MRSA truly has a lower pathogenicity, or that these results represent the healthy worker effect, a control group is needed with persons who work in the stables, but are MRSA negative, or who are MRSA positive, but do not work in the stables. For pig farms, the distinction between working MRSA-positive pig farmers and not-working MRSA-negative household members is so clear-cut that this does not result in a suitable control group [13, 31]. Otherwise, power limitations - despite incidences comparable to the literature - might also be possible explanations. Larger studies with longer follow-up periods might be needed to further clarify this.

#### Short Form 36

Dimension scores were not significantly different between different MRSA carriage groups, and univariate and multivariate linear regression on physical and mental summary scales did not show significant associations with persistent MRSA. Only the expected confounder of rising age was associated with a lower physical summary scale. The absence of an effect may be due to a true negligible effect on QoL of LA-MRSA, or to a low power.

#### EuroQol 5D

No participant scored severe problems on the health-related QoL domains, and no significant

associations could be found between EQ-5D domains or EQ-VAS scores and (persistent) MRSA carriage. Again, the relatively healthy working population, a true low impact of LA-MRSA on QoL or a low distinctive power might be possible explanations for these findings.

The presence of MRSA in home samples was associated with fewer mobility problems, which may best be explained by the healthy worker effect, i.e. persons with MRSA more often work in the stables, and more often contaminate their home environment than persons without MRSA. These MRSA-positive persons are in general healthier than the non-working population, and therefore have fewer mobility problems. The same explanation can be given for the association between male gender and more hours working at the farm and a higher VAS score (higher = better).

#### **Study limitations**

The most important study limitation is that differentiation between healthy worker effect and the extent of pathogenicity of LA-MRSA is not clearly possible in this study group, as mentioned above. A highly homogeneous study group and very prevalent expected confounders lead to exclusion of a large part of the study population: 83/281 (30%) persons did not work with sows and 200/398 (50%) sets of consecutive sampling moments were MSSA positive, leading to exclusion from analysis. This has likely contributed to a reduced discriminative power and difficulties in finding a correct control group. A sensitivity analysis, which placed persons with only one MRSA-positive sample in the non-carrier group, and persons with only one MRSA-negative sample in the persistent carrier group, did not result in different conclusions. Larger and more diverse study populations (e.g. different types of livestock farmers, veterinarians, people not carrying LA-MRSA or MRSA) would be preferred.

Furthermore, the culture method is limited in detecting MSSA and MRSA in one sample as different entities [13]. Therefore the MRSA samples may have had undetected MSSA. We excluded persistent MSSA carriers from our analysis, but due to this limitation the number of persistent MSSA carriers with MRSA might have been underestimated, leading to an incomplete exclusion and bias. Since MSSA can lead to infections, and the presence of MSSA is shown to be negatively associated with the presence of MRSA [13, 31], this bias is expected to result in an underestimation of the pathogenicity of MRSA.

However, since MSSA carriage numbers are comparable to the literature we do not expect this bias to be of significant relevance.

Last, the samples from this study were largely based on self-sampling techniques, which might not have been sufficient. However, a pilot study that compared self-sampling and sampling by a trained person showed excellent agreement [43].

# CONCLUSIONS

This study investigated health and health-related QoL in pig farmers carrying LA-MRSA. MRSA carriage was not associated with infections, antimicrobial use, healthcare contact, or health-related QoL problems, most probably due to the 'healthy worker effect'. Despite high carriage rates the impact of LA-MRSA carriage in this population of relatively healthy pig farmers on health and health-related QoL appears limited, more research is needed for confirmation.

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# **DECLARATION OF INTEREST**

None.

# REFERENCES

- 1. Wertheim HFL, et al. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infectious Diseases* 2005; **5**: 751–762.
- Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clinical Microbiology Reviews* 1997; 10: 505–520.
- 3. Nouwen JL, et al. Predicting the *Staphylococcus aureus* nasal carrier state: derivation and validation of a 'culture rule'. *Clinical Infectious Diseases* 2004; **39**: 806–811.

- Van Belkum A, Melles DC. Not all Staphylococcus aureus strains are equally pathogenic. Discovery medicine 2005; 5: 148–152.
- Jevons M. 'Celbenin'-resistant staphylococci. British Medical Journal 1961; 1: 124–125.
- Bode LGM, et al. Sustained low prevalence of meticillin-resistant *Staphylococcus aureus* upon admission to hospital in The Netherlands. *Journal of Hospital Infection* 2011; 79: 198–201.
- Voss A, et al. Methicillin-resistant Staphylococcus aureus in pig farming. Emerging Infectious Diseases 2005; 11: 1965–1966.
- Osadebe LU, et al. Prevalence and characteristics of Staphylococcus aureus in Connecticut swine and swine farmers. Zoonoses and Public Health 2013; 60: 234–243.
- Bisdorff B, et al. MRSA-ST398 in livestock farmers and neighbouring residents in a rural area in Germany. *Epidemiology and Infection* 2012; 140: 1800–1808.
- Van den Broek IVF, et al. Methicillin-resistant Staphylococcus aureus in people living and working in pig farms. Epidemiology and Infection 2009; 137: 700–708.
- Graveland H, et al. Persistence of livestock associated MRSA CC398 in humans is dependent on intensity of animal contact. PLoS ONE 2011; 6: e16830.
- Khanna T, et al. Methicillin resistant Staphylococcus aureus colonization in pigs and pig farmers. Veterinary Microbiology 2008; 128: 298–303.
- Van Cleef BAGL, et al. Dynamics of methicillinresistant Staphylococcus aureus and methicillinsusceptible Staphylococcus aureus carriage in pig farmers: a prospective cohort study. Clinical Microbiology and Infection. Published online 15 May 2014. doi:10.1111/1469-0691.12582.
- Ekkelenkamp MB, et al. Endocarditis due to meticillinresistant Staphylococcus aureus originating from pigs [in Dutch]. Nederlands Tijdschrift voor Geneeskunde 2006; 150: 2442–2447.
- Rasigade J-P, et al. Lethal necrotizing pneumonia caused by an ST398 Staphylococcus aureus strain. Emerging Infectious Diseases 2010; 16: 1330.
- Van der Mee-Marquet N, *et al.* Emergence of unusual bloodstream infections associated with pig-borne-like *Staphylococcus aureus* ST398 in France. *Clinical Infectious Diseases* 2011; 52: 152–153.
- Verkade EJM, Kluytmans JAJW. Livestock-associated Staphylococcus aureus CC398: animal reservoirs and human infections. Infection, Genetics and Evolution 2014; 21: 523–530.
- Wardyn SE, et al. Swine farming is a risk factor for infection with and high prevalence of carriage of multidrug-resistant *Staphylococcus aureus*. Clinical Infectious Diseases 2015; 61: 59–66.
- Van Cleef BAGL, et al. Low incidence of livestockassociated methicillin-resistant Staphylococcus aureus bacteraemia in The Netherlands in 2009. PLoS ONE 2013; 8: e73096.
- Monaco M, et al. Livestock-associated methicillinresistant *Staphylococcus aureus* responsible for human colonization and infection in an area of Italy with high density of pig farming. *BMC Infectious Diseases*. 2013; 13: 258.

- Cuny C, Köck R, Witte W. Livestock associated MRSA (LA-MRSA) and its relevance for humans in Germany. *International Journal of Medical Microbiology* 2013; 303: 331–337.
- Köck R, et al. Characteristics of hospital patients colonized with livestock-associated meticillin-resistant Staphylococcus aureus (MRSA) CC398 versus other MRSA clones. Journal of Hospital Infection 2011; 79: 292–296.
- Köck R, et al. Livestock-associated methicillin-resistant Staphylococcus aureus (MRSA) as causes of human infection and colonization in Germany. PLoS ONE 2013; 8: e55040.
- 24. Verkade EJM. Characterization of livestock-associated MRSA CC398 detection, transmission and virulence (dissertation). Amsterdam, The Netherlands. VU University Amsterdam, 2014.
- 25. Kadlec K, et al. Diversity of antimicrobial resistance pheno- and genotypes of methicillin-resistant Staphylococcus aureus ST398 from diseased swine. Journal of Antimicrobial Chemotherapy 2009; 64: 1156–1164.
- Monecke S, et al. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant Staphylococcus aureus. PloS One 2011; 6: e17936.
- Köck R, et al. Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) among pigs on German farms and import of livestockrelated MRSA into hospitals. *European Journal of Clinical Microbiology & Infectious Diseases* 2009; 28: 1375–1382.
- Zarfel G, et al. Virulence and antimicrobial resistance genes in human MRSA ST398 isolates in Austria. *Epidemiology and Infection* 2013; 141: 888–892.
- 29. Van Rijen MML, Van Keulen PH, Kluytmans JA. Increase in a Dutch hospital of methicillin-resistant *Staphylococcus aureus* related to animal farming. *Clinical Infectious Diseases* 2008; **46**: 261–263.
- Broens EM, et al. Prevalence and risk factor analysis of livestock associated MRSA-positive pig herds in The Netherlands. Preventive Veterinary Medicine 2011; 102: 41–49.
- Van Cleef BAGL, et al. Livestock-associated MRSA in household members of pig farmers: transmission and dynamics of carriage, a prospective cohort study. *PLoS ONE* 2015; 10: e0127190.
- Noss I, et al. Evaluation of a low-cost electrostatic dust fall collector for indoor air endotoxin exposure assessment. Applied and Environmental Microbiology 2008; 74: 5621–5627.
- Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). Conceptual framework and item selection. *Medical Care* 1992; 30: 473–483.
- 34. Van der Zee K, Sanderman R. Measuring general health with the RAND-36. A manual, 2nd edn [in Dutch]. Groningen. Noordelijk Centrum voor Gezondheidsvraagstukken, NCG reeks Meetinstrumenten 3. 1993.
- 35. Brooks R. EuroQol: the current state of play. *Health Policy* 1996; **37**: 53–72.
- EUCAST. EUCAST Clinical breakpoints. (www.eucast. org/clinical\_breakpoints/). Accessed 16 September 2014.

- Van Griethuysen A, et al. Rapid slide latex agglutination test for detection of methicillin resistance in *Staphylo*coccus aureus. Journal of Clinical Microbiology 1999; 37: 2789–2792.
- Francois P, et al. Rapid detection of methicillin-resistant Staphylococcus aureus directly from sterile or nonsterile clinical samples by a new molecular assay. Journal of Clinical Microbiology 2003; 41: 254–260.
- Van Meurs MLJGM, et al. Real-time PCR to distinguish livestock-associated (ST398) from non-livestockassociated (methicillin-resistant) Staphylococcus aureus. Infection 2013; 41: 339–346.
- 40. Kilic A, et al. Triplex real-time polymerase chain reaction assay for simultaneous detection of *Staphylococcus*

*aureus* and coagulase-negative staphylococci and determination of methicillin resistance directly from positive blood culture bottles. *Diagnostic Microbiology and Infectious Disease* 2010; **66**: 349–355.

- Knol MJ, *et al.* Overestimation of risk ratios by odds ratios in trials and cohort studies: alternatives to logistic regression. *Canadian Medical Association Journal* 2012; 184: 895–899.
- 42. Wielink G, et al. Dutch Society for General Practice: Bacterial skin infections [in Dutch]. Huisarts & Wetenschap 2007; 50: 426–444.
- Van Cleef BA, et al. Self-sampling is appropriate for detection of Staphylococcus aureus: a validation study. Antimicrobial Resistance and Infection Control 2012; 1: 34.