ORIGINAL ARTICLE

Livestock-associated MRSA ST398 carriage in pig slaughterhouse workers related to quantitative environmental exposure

Maarten J Gilbert,[1] Marian E H Bos,[1] Birgitta Duim,[2] Bert A P Urlings,[3,4] Lourens Heres,[3] Jaap A Wagenaar,[2,5] Dick J J Heederik[1,6]

[1]Division of Environmental Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands
[2]Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
[3]VION Food Group, Eindhoven, The Netherlands
[4]Animal Sciences Group, Wageningen University, Wageningen, The Netherlands
[5]Central Veterinary Institute, Wageningen UR, Lelystad, The Netherlands
[6]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands

Correspondence to
Dr Marian E H Bos, Institute for Risk Assessment Sciences (IRAS), Utrecht University, PO BOX 80178, 3508 TD Utrecht, The Netherlands;
m.e.h.bos@uu.nl

MJG and MEHB contributed equally.

Accepted 8 March 2012
Published Online First
27 April 2012

ABSTRACT

Objectives To assess livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) carriage among workers in pig slaughterhouses and assess associated risk factors, including occupational exposure to LA-MRSA.

Methods A cross-sectional study in three Dutch pig slaughterhouses was undertaken. Nasal swabs of participants were taken. Nasal swabs and surface wipes, air and glove samples were screened for presence of methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA was quantitatively determined on gloves and in air samples by culturing and real-time PCR.

Results 11 of 341 (3.2%) participants were identified as nasal MRSA carriers. MRSA-positive workers were predominantly found at the start of the slaughter process. Major risk factors for carriage were working in the lairage and working in the scalding and dehairing area. Most nasal isolates (73%) belonged to the LA-MRSA clone ST398. MRSA ST398-positive environmental samples were found throughout the slaughter process. A clear decrease was seen along the slaughterline in the number of MRSA-positive samples and in the MRSA amount per sample.

Conclusions This study showed that working in the lairage area or scalding and dehairing area were the major risk factors for MRSA carriage in pig slaughterhouse workers, while the overall prevalence of MRSA carriage is low. Occupational exposure to MRSA decreased along the slaughterline, and the risk of carriage showed a parallel decrease.

What this paper adds

A livestock-associated strain of MRSA (ST398) emerged in the animal production chain and leads to increased human nasal carriage. This paper shows that nasal MRSA carriage risk is low in slaughterhouse workers and associated with environmental exposure on hands, in the air and on surfaces throughout the slaughter process.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) accounts for a high proportion of nosocomial and community-acquired infections in countries worldwide.[1 2] In 2005, the first livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) transmission to humans was described in a young daughter of a pig farmer,[3] which involved a previously very rare multi-locus sequence type ST398 (MLST ST398). Recent surveys among pig and veal farmers indicated MRSA ST398 carrier prevalence to be between 20% and 33%,[4–6] with a lower prevalence observed in veterinarians and people living on farms.[6–9] These surveys also showed a high prevalence of ST398 among farm animals and from this reservoir transmission to humans occurs.[6 10 11] The human-to-human transmission of MRSA ST398 is considered to be rare compared with non-livestock-associated strains.[12] Genome analysis revealed that MRSA ST398 lacks certain *S aureus* virulence factors,[13] but severe MRSA and MSSA ST398 infections have been described,[14–16] some as a result of work-related invasive accidents.[17]

Thus far, most LA-MRSA prevalence studies focused on people with frequent contact with living production animals, for example, farmers and veterinarians. The high LA-MRSA prevalence in production animals and people having contact with these animals raises the question whether LA-MRSA carriage might also occur in slaughterhouse personnel, who have contact with live animals, dead animals and derived meat products in various stages of processing. Recent studies described LA-MRSA risk factors for slaughterhouse personnel.[18 19] In both independent studies, LA-MRSA carriage in poultry and pig slaughterhouse personnel was 5.6% and contact with living animals was the major risk factor for being LA-MRSA positive.

Although it has been shown that contact with live animals is an important risk factor for LA-MRSA carriage in slaughterhouses, quantitative data on exposure of slaughterhouse workers to LA-MRSA from the animals and their derived meat products are lacking. In this paper, quantitative data on levels of LA-MRSA are presented, measured in air and glove samples collected in various pig slaughterhouse sections.

METHODS

Study design and population

Three Dutch pig slaughterhouses participated in this cross-sectional study. Between October 2009

and January 2010, sampling campaigns were conducted once in each slaughterhouse. The number of eligible workers per slaughterhouse was 150, 300 and 870. Prior to the slaughterhouse visits, total sample size was set to 375 participants. The eligible population preferably included all workers from the start of the main slaughterline (lairage area, scalding and dehairing area and evisceration area) and a sample from those cutting meat at the end of the process. Participants took two nasal swabs and filled out a short questionnaire which provided information about gender, age, current smoking, country of birth, working area, contact with livestock outside the slaughterhouse and recent antimicrobial use. Written consent was obtained from all participants. Participants worked in various sections but primarily along the main slaughterline. To minimise language-related biases and to increase response rates, questionnaires, information material and consent forms were translated into English, German, Hungarian and Polish. One of the fieldworkers was of Polish origin and assisted with recruitment.

Slaughterhouse characteristics—production process

The layout of the slaughter process is similar in all three slaughterhouses. Upon arrival, live pigs are collected in the lairage area and showered with water to calm them. The animals are stunned (electrically or by using carbon dioxide) and killed by bleeding after cutting the carotid artery. After scalding in warm water (58–60°C), carcasses are dehaired, flamed and washed with water. In the evisceration line, the carcasses are cut open, organs and fat are removed and the carcasses are cut in half over the entire length of the body. Subsequently, the heads are separated from the body and the carcasses are cooled to below 7°C. After cooling, carcasses are further divided into three parts: fore-end, middle and hind leg. These primal cuts constitute the basis for further cutting and deboning of the meat. Finally, the cut and deboned meat is processed into smaller meat cuts and packaged and shipped.

All workers wear protective equipment, including a jacket, trousers, hairnet, helmet and rubber boots and most of the workers directly involved in the slaughter process wear disposable gloves. Workers are instructed to wear a new pair of gloves after each break, for a maximum of 2 h. Upon entering and leaving work areas, workers wash and disinfect their hands. Workers wear their work clothing during the break, with exception of the jacket. Lairage workers have separate canteens. At the end of the working day, the slaughterhouse, all equipments, conveyor belts and benches are cleaned and disinfected. All workers have access to facilities to wash their hands with warm water in all slaughterhouse areas.

Environmental sampling

Personal air samples were collected using GilAir-5 air sampling pumps (Gilian) and GSP conical inhalable samplers. Cassettes containing 37 mm GFA glass fibre membrane filters (Whatman, GE Healthcare, Diegem, Belgium) were placed in the GSP samplers. Air samples were collected at a flow rate of 2 l/min for 6–10 h.

Initially, viable sampling resulted in very low colony counts. During a pilot study, different air sampling methods were compared (Andersen multistage sampler, liquid impingers and agar plate sedimentation). Both the Andersen sampler and liquid impingers had poor results compared with direct plating, probably because high air sampling volumes affected MRSA viability.[20] Best results were obtained by a GSP conical inhalable dust sampler with glass fibre or gelatine membrane filters, operated at a lower flow (2.0 instead of 3.5 l/min). Since the dissolved gelatine interfered with the real-time PCR, glass fibre filters were selected for the study. The reduced flow might have led to changes in the particle collection efficiency, but this modification had to be introduced to assure viability of MRSA from air samples. The participants who received GSP samplers were selected by the management. Samplers were attached to the lapel of their jackets. Disposable gloves worn by the workers were collected in sealed plastic bags after tasks were completed. For reasons involving standardisation, gloves worn on the left hand were collected.

Along the main slaughterline, surface samples were taken with pre-packed sterile moist wipes (Sodibox; Raisio Diagnostics, Nevez, France). The majority of the sampled surfaces had not been in direct contact with pigs and pig-derived meat products. All collected materials were transferred to the laboratory and processed the same day.

Microbiological analysis

Swabs were collected from each nostril of the participants. High-salt pre-enrichment culturing was used for LA-MRSA, with 5 ml Mueller Hinton (MH) broth with 6.5% sodium chloride (Biotrading, Mijdrecht, the Netherlands) as the pre-enrichment medium. Samples were incubated overnight at 37°C. The following day, 1 ml of culture was transferred to 9 ml phenyl mannitol broth (PHMB/ca+) (bioMérieux) with 5 μg/ml ceftizoxime and 75 μg/ml aztreonam and incubated overnight at 37°C. The other nasal swab was incubated directly in 5 ml selective culture medium (tryptic soy broth supplemented with 4% sodium chloride, 1% mannitol, ceftizoxime (5 μg/ml), aztreonam (50 μg/ml) and phenol red (16 μg/ml) (Mediaroom UMC Utrecht)) and incubated for 48 h at 37°C.[21] After incubation, 10 μl from both tryptic soy broth and PHMB cultures were plated onto Brilliance MRSA agar (Oxoid) and blood agar (Biotrading), respectively, and incubated overnight at 37°C. Colony morphology suspected *S aureus* were sub-cultured on blood agar and identified as *S aureus* with a rabbit plasma slide coagulase test and confirmed with either a ST398-specific PCR assay[22] or, for nasal isolates only, MLST and *spa*-typing for the non-ST398 strains. MRSA was confirmed with a *mecA*-specific PCR.[23 24]

Surface wipes were cultured in 200 ml MH broth (Becton Dickinson, Breda, the Netherlands) supplemented with 6.5% sodium chloride and cultured according to the pre-enrichment method as described for the nasal swabs.

Air filters and gloves were analysed with a quantitative culture method. Glass fibre filter membranes were divided into two equal parts. One half was stored at −20°C for DNA extraction and subsequent PCR analysis. After thawing, the filter material was extracted in 1.5 ml physiological saline solution with added EDTA (0.5 mM NaCl, 1 mM EDTA) by vigorous shaking for 10 min in a Stomacher, and 200 μl was used for DNA extraction as described below. The other half was extracted by vigorous vortexing in 5 ml MH broth with 6.5% sodium chloride for culturing. A 0.5 ml of this suspension was diluted 10-fold, 100-fold and 1000-fold in MH broth and further processed as described for the nasal swabs. Gloves were rinsed with 20 ml phosphate-buffered saline. Two millilitre was centrifuged for 3 min at 14 000 rpm, after which 1.8 ml supernatant was removed. The remaining pellet was stored at −20°C for DNA extraction and subsequent PCR analysis. A selection of the gloves was quantitatively cultured; 5 ml of the glove rinse was mixed 1:1 with 5 ml double-concentrated MH broth (130 g MH (Becton Dickinson) and 44 g sodium chloride per litre broth). One millilitre of this suspension was diluted 10-fold,

100-fold and 1000-fold in MH broth. Diluted suspensions were cultured according to the pre-enrichment method as described for the nasal swabs. It was established that latex gloves used did not have any growth inhibiting effects. All MRSA-positive isolates were screened for ST398, using a real-time PCR as described below.

Molecular analysis

Bacterial DNA was extracted from 200 µl rinse samples from the gloves and air filters, with a DNeasy Blood & Tissue Kit (QIAGEN Benelux, Venlo, the Netherlands) according to the manufacturer's instructions. The extract was eluted in 100 µl of elution buffer. DNA from MRSA colonies was extracted as described by Schouls *et al*.[25] In short, two colonies were incubated at 37°C in TE (10 mM Tris−HCl, 1 mM EDTA, pH 8.0), supplemented with 100 µg/ml lysostaphine (Sigma-Aldrich, Zwijndrecht, the Netherlands) followed with heating at 95°C.

Real-time PCRs were performed using oligonucleotides as described previously,[22 24 26–28] targeting *mecA* and Orf-X of *S aureus* with the Staphylococcal cassette chromosome SCC*mec* and ST398. The *mecA* gene determines methicillin resistance of MRSA, but *mecA* can also be present in coagulase-negative Staphylococcal species that can be present in the sample. The SCC*mec* real-time PCR has the advantage that it combines detection of *S aureus* with the SCC*mec* element in one PCR. The disadvantage is that SCC*mec* is highly variable and must be captured by several probes. The ST398 PCR specifically targets *S aureus* isolates belonging to ST398, including methicillin-sensitive *S aureus* ST398. For real-time detection of ST398, a Taqman probe was developed: 5′-ATTGTCAGTATGAATTGCGGT-3′. The reaction mixture was as follows: 10 µl (2×) LC480 Probe Master, 5 µl DNA and 10 pmol of each primer and 5 pmol of each probe. Thermocycling conditions of the LightCycler®480 (Roche diagnostics, Almere, the Netherlands) were as follows: 95°C for 5 min, followed by 45 cycles of 95°C for 10 s, 58°C for 20 s and 72°C for 20 s. Puc19 DNA was added to the *mecA* PCR mix for control of PCR inhibition in the sample.[29]

A standard curve of MRSA DNA consisting of five 10-fold dilutions of a stock solution of 50 000 colony-forming unit equivalents (CFUeq) followed by DNA extraction was made for each PCR. This enabled calculation of bacterial CFUeq in rinse samples from the gloves and air filter samples, adjusting for the volume used for DNA extraction (200 µl) and sample extraction volume (20 ml for gloves and 1.5 ml for air samples).

The genetic diversity of non-ST398 MRSA colonies cultured from nasal swabs was determined with MLST and *spa* typing as described previously.[25 30]

Statistical analysis

Exposure data were reported by descriptive statistics after log-transformation. Associations between MRSA carrier status and potential risk factors were explored using the GLIMMIX procedure (SAS V.9.2; SAS Institute Inc.). Slaughterhouse was included as repeated subject to account for clustering of the data on slaughterhouse level. Correlation between the results of the three quantitative PCRs (qPCRs), and between the results of the qPCRs and bacterial culturing, were analysed by means of the REG procedure (SAS V.9.2).

RESULTS

Sampling of slaughterhouse workers

In total, 341 slaughterhouse workers voluntarily participated in this study. Mean age was 42 (±10.5; range 20−65) years and was similar across all slaughterhouses. The population consisted of various nationalities; countries of birth for the participants were the Netherlands (55.8%), Poland (13.6%), Hungary (6.2%), Germany (3.2%) and other (21.2%).

Nasal swabs were considered MRSA-positive when at least one of the culturing methods (with or without pre-enrichment) yielded a MRSA-positive result. Of the 341 participants, 11 tested positive for MRSA (3.2%). Prevalence per slaughterhouse were 3/146 (2.1%), 3/135 (2.2%) and 5/60 (8.6%). Of the 11 MRSA carriers, seven were detected with the pre-enriched culturing method alone, one with the non-pre-enriched culturing method alone and three with both methods. This means that in 64% (7/11) of MRSA-positive nasal swabs tested without pre-enrichment, MRSA was not detected and resulted in false-negative results. By inoculating a standardised concentration of MRSA in the selective media, the sensitivity of these media showed to be 1.6×10^5 CFU per sample (results not shown).

All, except one, the MRSA-positive participants worked in the lairage area, scalding and dehairing area or evisceration area. One MRSA-positive participant worked for the technical service, thereby working in multiple areas. None of the participants working in the intestine cleaning area, cutting area, processing area, packing area or shipping area tested positive for MRSA. Due to cross-contamination, two MRSA-positive samples could not be assigned to a participant. Because the likelihood of a MRSA-positive nasal sample is low, it was assumed that only one of the two workers was a nasal carrier. In the statistical analysis, both results were omitted and 339 instead of 341 observations were used, including the 10 MRSA carriers. Although limited power impeded a more detailed analysis, working in the lairage area was the strongest risk factor for MRSA carriage with a statistically significant ($p<0.01$) unadjusted OR of 6.1 (95% CI 1.6 to 22.8). Working in the scalding and dehairing area resulted in a significant unadjusted OR of 4.1 ($p<0.05$, 95% CI 1.0 to 16.7). The sample was too small for a simultaneous adjustment for all potential confounders in one logistic regression model. We therefore adjusted for gender, age and smoking one by one in different models. This changed the point estimates of the ORs, but the overall pattern in the results remained the same in each of those analyses. In all cases, the risk remained highest for workers in the lairage area after adjustment for gender, age or smoking. It has previously been reported that smoking appears to have a protective effect with regard to MRSA carriage.[6] The point estimate confirmed this finding, albeit not statistically significant, with an unadjusted OR of 0.3 ($p=0.16$, 95% CI 0.07 to 1.6) for MRSA carriage in current smokers. Antimicrobial use, working for the technical service and living on a livestock farm with pigs or cattle appeared to have elevated but non-significant risks for MRSA carriage (table 1).

Exposure

In total, 150 gloves were collected and analysed by qPCR. A selection of 85 gloves was also analysed by culturing. Along the main slaughterline, from the lairage to cutting area, a decrease in MRSA was observed, both in the number of MRSA-positive gloves and in the highest MRSA-positive dilution per glove (table 2).

The relative number of MRSA-positive gloves decreased from 79% (15/19) in the lairage, scalding and dehairing areas combined to 39% (13/33) in the evisceration area and 42% (10/24) in the cutting area. Two of three gloves collected from the intestine cleaning area in one of the slaughterhouses were MRSA-positive. Higher MRSA numbers (dilution 10^{-2} to 10^{-3})

Table 1 MRSA nasal carriage prevalence and unadjusted ORs for risk factors among slaughterhouse workers

Characteristics	Total (%)	MRSA positive (%)	OR (95% CI)*	p Value
All	341/341 (100.0)	11/341 (3.2)	n.c.	n.c.
Gender (male)	304/341 (89.1)	11/304 (3.6)	n.c.	n.c.
Current smoking	146/341 (42.8)	4/146 (2.7)	0.3 (0.1 to 1.6)	0.16
Antimicrobial use	34/341 (10.0)	2/34 (5.9)	2.3 (0.5 to 11.2)	0.32
Lairage area	36/341 (10.6)	4/36 (11.1)	6.1 (1.6 to 22.8)	<0.01
Scalding and dehairing area	34/341 (10.0)	3/34 (8.8)†	4.1 (1.0 to 16.7)	0.05
Sticking	26/341 (7.6)	0/26 (0.0)	n.c.	n.c.
Combined	47/341 (13.8)	3/47 (6.4)	2.8 (0.7 to 11.3)	0.15
Evisceration area	94/341 (27.6)	4/94 (4.3)†	1.1 (0.3 to 4.4)	0.89
Intestine cleaning	22/341 (6.5)	0/22 (0.0)	n.c.	n.c.
Combined	106/341 (31.1)	4/106 (3.8)	0.9 (0.2 to 3.7)	0.92
Cutting area	109/341 (32.0)	0/109 (0.0)	n.c.	n.c.
Processing area	25/341 (7.3)	0/25 (0.0)	n.c.	n.c.
Packing area	25/341 (7.3)	0/25 (0.0)	n.c.	n.c.
Shipping area	19/341 (5.6)	0/19 (0.0)	n.c.	n.c.
Combined	146/341 (42.8)	0/146 (0.0)	n.c.	n.c.
Technical service	15/341 (4.4)	1/15 (6.7)	2.7 (0.3 to 22.9)	0.38
Other	46/341 (13.5)	0/46 (0.0)	n.c.	n.c.
Contact with livestock‡	20/341 (5.9)	2/20 (10.0)	4.3 (<0.001 to >999.9)	0.33
Pigs	10/341 (2.9)	1/10 (10.0)	3.7 (0.4 to 33.4)	0.24
Cattle	7/341 (2.1)	1/7 (14.3)	5.9 (0.6 to 55.5)	0.12

*For univariate calculation of ORs, CIs and p values, 339 observations were used, of which 10 were MRSA positive. Some participants indicated that they worked at more than one section.
†MRSA-positive participant working in both scalding and dehairing and evisceration areas (n=1).
‡Contact with livestock outside of slaughterhouse.
n.c., not computable.

were found on gloves collected in the lairage, scalding and dehairing area with the distribution shifting to medium and lower MRSA concentrations (dilution 10^0 to 10^{-2}) for gloves collected in the evisceration area and intestine cleaning area. Lower MRSA concentrations (dilution 10^0) were predominantly found in the cutting area. In samples from other areas, no MRSA could be detected.

All three qPCRs for SCC*mec*, ST398 and *mecA* showed similar trends in CFUeq per glove across the different slaughterhouse sections (table 3).

Higher CFUeq counts for each qPCR were predominantly observed in the lairage and slaughter area, decreasing to medium and lower levels in the areas further down the slaughterline. When compared with results obtained by quantitative culturing, similar trends were observed. Associations between log-transformed outcomes of the qPCR (CFUeq per qPCR sample; CFUeq/qPCR) between the qPCRs were R=0.52 for SCC*mec* and ST398, R=0.40 for SCC*mec* and *mecA* and R=0.68 for ST398 and *mecA*. Only three samples were positive for all three targets. The reasonably high correlation between the ST398 and *mecA* qPCR indicates that probably often MRSA ST398 was present in the sample. The low correlation between SCC*mec* and *mecA* indicates that *mecA* is not always associated with *S aureus* SCC*mec* elements screened for in this qPCR analysis.

Log-transformed outcomes of the qPCR (CFUeq/qPCR) were compared with the quantitative culturing method for each qPCR separately. The variable for culturing was defined as the number of MRSA-positive dilutions, from 0 for no positive dilutions to four for all dilutions positive. Associations between qPCRs and culturing were R=0.22 for SCC*mec* and culturing, R=0.70 for ST398 and culturing and R=0.57 for *mecA* and culturing. The reasonably high correlation between ST398 and culturing indicates that most of the cultured MRSA belong to ST398 and that the ST398 qPCR is a good proxy for MRSA ST398.

At each slaughterhouse, 10–15 air samples were collected, resulting in 40 samples in total. In air samples, the same pattern in MRSA levels was observed along the slaughterline. Twenty-eight per cent (11/40) air samples were MRSA-positive after culturing. For 9 of 11 MRSA-positive air samples, MRSA was only found in the undiluted sample and not in subsequent dilutions. In the two other cases, MRSA was also found in the

Table 2 MRSA distribution and concentration ranges of gloves worn by slaughterhouse workers for various slaughterhouse sections

Section	MRSA positive (%)	Highest dilution MRSA positive*			
		10^0	10^{-1}	10^{-2}	10^{-3}
Lairage/scalding and dehairing area	15/19 (79)	1	3	4	7
Evisceration area	13/33 (39)	4	4	4	1
Cutting area	10/24 (42)	8	1	0	1
Processing area	0/2 (0)	0	0	0	0
Packing area	0/2 (0)	0	0	0	0
Shipping area	0/2 (0)	0	0	0	0
Organ area	2/3 (67)	0	1	1	0

*Higher positive dilution (eg, 10^{-3}) correlates with higher concentration of MRSA in the undiluted sample.

Table 3 Total number of gloves and average CFUeq per glove for each slaughterhouse section, based on quantitative PCR results for SCC*mec*, ST398 and *mecA*

	SCC*mec*		ST398		*mecA*	
Section	**n**	**CFUeq ave**	**n**	**CFUeq ave**	**n**	**CFUeq ave**
Lairage/scalding and dehairing area	20	55 050	20	51 807	19	65 790
Evisceration area	46	225 048	46	464	46	3856
Cutting area	54	740	54	42	53	2276
Processing area	16	62	16	0	16	580
Packing area	4	0	4	0	4	254
Shipping area	4	0	4	0	4	26
Organ area	6	0	6	0	6	3832

10-fold diluted sample. Only air samples from participants working in the lairage area (7/8, 88%) and the scalding and dehairing area (4/8, 50%) were MRSA-positive, and all others were MRSA-negative.

PCR-positive samples were marginally higher above negative threshold values, indicating low CFUeq levels (0.5–5 CFUeq/qPCR). For SCC*mec*, none of the samples passed the threshold value and were all considered negative. For *mecA*, 16 samples were considered positive (9/16 in lairage/scalding and dehairing area); 44% (7/16) of the positive samples were also positive for culturing. For ST398, six samples were considered positive (5/6 in lairage/scalding and dehairing area), of which five samples were also positive for *mecA*. Of these double-positive samples, 4/5 originated in the lairage or scalding and dehairing area. Eighty-three per cent (5/6) of the ST398-positive samples was also positive for culturing. Again, the highest association was found between ST398 and culturing.

Surface wipes from the lairage area were all (9/9) MRSA-positive, three of four were positive in the scalding and dehairing area and three of 10 were positive in the evisceration area. In the cutting area, MRSA could not be detected on the sampled surfaces (0/8), indicating a clear decrease in MRSA occurrence along the main slaughterline.

Typing

As expected, the majority of the MRSA-positive isolates belonged to ST398. Ninety-five per cent (38/40) of the glove isolates, 100% (11/11) of the air isolates and 94% (15/16) of the surface samples belonged to this sequence type. For all MRSA-positive nasal isolates, *spa*-type and MLST were determined (table 4).

Seventy-three per cent (8/11) of the nasal isolates were typed as MRSA ST398. *Spa* types were t011, t064, t108 and t2330. Besides MRSA ST398, non-LA-MRSA sequence types were found: ST7 and ST8 (2/11), with associated *spa* types t091 and t064, respectively. These two isolates originated from participants working in either the lairage area, scalding and dehairing area or evisceration area and who were directly involved in the slaughter process. One isolate was identified as MRSA ST45 (*spa*-type t015), which is usually regarded as a human-related (community-acquired) MRSA sequence type. This isolate originated from a participant working for the technical service, who was not directly involved in the slaughter process. Of the three MRSA isolates other than ST398, one was detected with and without pre-enrichment (ST8) and two were detected with pre-enrichment only (ST7 and ST45). One MRSA ST398 was detected without pre-enrichment only (table 4).

DISCUSSION

Our findings confirm that direct contact with live animals largely determines LA-MRSA carriage in humans, although some MRSA carriers were found among slaughterhouse workers without contact with live animals. Carrier risk in slaughterhouse workers is considerably lower than that found in livestock farmers. MRSA is present throughout the main slaughter process, but both the proportion of MRSA-positive samples and MRSA concentration show a clear decrease along the main slaughterline. Nasal carriage is observed only in areas where MRSA concentrations on gloves and in the air are relatively high. With MRSA being found in 88% of the cultured air samples from the lairage area, it is likely that workers in this area are regularly exposed to airborne MRSA. This seems to explain higher carrier rates in workers in these areas.

Typing of the isolates showed that the majority belonged to MRSA ST398, which is the most commonly observed MRSA sequence type in pigs.[7 31 32] MRSA ST398 enters the slaughterhouse from the primary production chain and is subsequently spread throughout the whole slaughter process. Three human

Table 4 MLST and *spa* types of all nasal MRSA isolates

Nasal isolate	Pre-enrichment*	No pre-enrichment*	MLST	*spa* type
1	+	+	ST8	t064
2	–	+	ST398	t011
3	+	+	ST398	t011
4	+	–	ST398	t2330
5	+	–	ST7	t091
6	+	–	ST398	t108
7	+	–	ST398	t108
8	+	–	ST45	t015
9	+	–	ST398	t108
10	+	+	ST398	t108
11	+	–	ST398	t011

*+, positive for MRSA; –, negative for MRSA.

isolates belonged to sequence types other than MRSA ST398. Two of these, ST7 and ST8, originated from two evisceration area workers, of whom one reported to have used antimicrobials in the past 3 months. One isolate originated from a technical service employee (ST45). This was the only MRSA-positive participant who was not working on the main slaughterline. Since ST7, ST8 and ST45 are usually not regarded as pig-related strains, these most likely originate from outside the slaughterhouse.

More nasal swabs were shown to be MRSA positive with pre-enrichment indicating that this is a more sensitive MRSA detection method as observed earlier.[21] Pre-enrichment also increased the sensitivity for non-MRSA ST398 sequence types. However, salt tolerance of MRSA strains may vary.[33]

Working in the lairage area, or in the scalding and dehairing area, were the only significant risk factors for MRSA carriage in the slaughterhouse. No increased risk of MRSA carriage was found for workers in the cutting area, despite the observation that MRSA is present in low concentrations on the gloves of workers cutting the meat before packaging. We assume that MRSA concentrations found on the gloves reflect MRSA concentrations on the carcass and meat. MRSA levels found on gloves are comparable with reported presence of MRSA on meat (reported prevalence on pork meat: 1.8%–10.7%).[34 35]

LA-MRSA carriage can lead to increased LA-MRSA-related health risks. Although possible adverse health effects caused by LA-MRSA are considered minimal in healthy persons, LA-MRSA is known to cause serious infections in persons with invasive wounds and persons with an impaired immune system.[15]

A 'search and destroy' policy exists in the Netherlands and several Scandinavian countries, through which MRSA rates in hospitals are kept low by actively screening prior to admission. MRSA-positive patients are decolonised before admission or treated in isolation. In the Netherlands, persons with close work-related contact with living pigs (second highest risk category) undergo the same (isolation) procedures as proven MRSA carriers (highest risk category). In the present study, it is shown that slaughterhouse workers who come in contact with dead pigs and derived products also show an increased risk for MRSA carriage, however, the excess risk is small, and this limits efficient selection on the basis of job title.

A novel aspect of this study is that environmental exposure was measured by viable sampling and real-time PCR analysis of dust samples. Different sampling techniques have been proposed for collection and quantification of microorganisms from air,[36] each with their own advantages and disadvantages.[37] There are still several unanswered questions related to MRSA air sampling. Determining the amount of MRSA extracted from the glass fibre filters proved to be difficult, and retention of MRSA on filters after extraction cannot be ruled out. The dilution method used for quantification in air and glove samples has limited accuracy; however, it does allow a relative comparison of MRSA concentrations. The method can be optimised by diluting in triplicate,[38] but the gain in precision is limited and inefficient, given the high number of plates. In contrast to MRSA quantification by culturing, DNA fragments of non-viable microorganisms are also detected by real-time PCR. By using real-time PCR, more efficient, and for viable MRSA more stressful, air sampling methods can be used. It was decided to use both real-time PCR and culturing since carriage requires colonisation, and possible health risks caused by MRSA would require viable organisms.

Real-time PCR analysis was based on detection of the MRSA ST398 genetic targets: *S aureus* with SCC*mec* element, *mecA* and ST398. However, positive *mecA* PCR signals could not directly be assigned to MRSA ST398, since *mecA* is highly conserved and could originate from coagulase-negative *Staphylococci*. Furthermore, the SCC*mec* element is typical for MRSA but is highly heterogeneous, and therefore, variant SCC*mec* elements in LA-MRSA strains could be missed. SCC*mec* qPCR might be a poor proxy for MRSA. The ST398 qPCR is specific for *S aureus* ST398 but is not always associated with *mecA*. Nevertheless, the reasonably high correlation between ST398 and *mecA* signals, combined with the observation that the majority of MRSA isolates belonged to ST398, indicates the predominant presence of MRSA ST398. The high correlation between the ST398 PCR signal and the MRSA culturing method in both glove and air samples indicates that the ST398 signal can be considered as a good proxy for MRSA ST398.

CONCLUSIONS

The current study showed that working in the lairage area or scalding and dehairing area were significant risk factors for MRSA carriage in the slaughterhouse. MRSA is found throughout the slaughter process, but the number of MRSA colonies decreased from front to end and parallels the occupational exposure and carriage risk for MRSA for slaughterhouse workers.

Acknowledgements We would like to thank Isabella Oosting-van Schothorst, Pawel Tulinski, Laura Laarhoven, Koen Verstappen and Haitske Graveland for their contributions to this study and Virissa Lenters for her insightful comments on the manuscript.

Contributors MJG, MEHB, BAPU, LH, JAW and DJJH were involved in conception and design of the study. MJG, MEHB and LH collected the samples and data. MJG, MEHB and DJJH cleaned and statistically analysed the data. MJG, MEHB, BD, JAW and DJJH interpreted the data. MJG and MEHB performed laboratory analyses. BD and JAW developed real-time PCR targeting ST398. MJG, MEHB, BD, JAW and DJJH interpreted the results of the laboratory analysis. MJG, MEHB, JAW and DJJH drafted the paper. MJG, MEHB, BD, BAPU, LH, JAW and DJJH revised the draft paper.

Funding The BACTOPATH project was funded by NL Agency and the Dutch Product Board for Livestock and Meat (PVV).

Competing interests None declared.

Patient consent Obtained.

Ethics approval Ethics approval was provided by METC Utrecht.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. **Rybak MJ.** Resistance to antimicrobial agents: an update. *Pharmacotherapy* 2004;**24**:203S–15S.
2. **Woodford N,** Livermore DM. Infections caused by Gram-positive bacteria: a review of the global challenge. *J Infect* 2009;**59**:S4–16.
3. **Voss A,** Loeffen F, Bakker J, *et al*. Methicillin-resistant Staphylococcus aureus in pig farming. *Emerg Infect Dis* 2005;**11**:1965–6.
4. **Wulf M,** Voss A. MRSA in livestock animals-an epidemic waiting to happen? *Clin Microbiol Infect* 2008;**14**:519–21.
5. **Van Cleef BA,** Verkade EJ, Wulf MW, *et al*. Prevalence of livestock-associated MRSA in communities with high pig-densities in The Netherlands. *PLoS One* 2010;**5**: e9385.
6. **Graveland H,** Wagenaar JA, Heesterbeek H, *et al*. Methicillin resistant Staphylococcus aureus ST398 in veal calf farming: human MRSA carriage related with animal antimicrobial usage and farm hygiene. *PLoS One* 2010;**5**:e10990.
7. **Khanna T,** Friendship R, Dewey C, *et al*. Methicillin resistant Staphylococcus aureus colonization in pigs and pig farmers. *Vet Microbiol* 2008;**128**:298–303.
8. **Wulf MW,** Sorum M, van Nes A, *et al*. Prevalence of methicillin-resistant Staphylococcus aureus among veterinarians: an international study. *Clin Microbiol Infect* 2008;**14**:29–34.
9. **Wulf MW,** Tiemersma E, Kluytmans J, *et al*. MRSA carriage in healthcare personnel in contact with farm animals. *J Hosp Infect* 2008;**70**:186–90.
10. **Lewis HC,** Molbak K, Reese C, *et al*. Pigs as source of methicillin-resistant Staphylococcus aureus CC398 infections in humans, Denmark. *Emerg Infect Dis* 2008;**14**:1383–9.
11. **Huijsdens XW,** van Dijke BJ, Spalburg E, *et al*. Community-acquired MRSA and pig-farming. *Ann Clin Microbiol Antimicrob* 2006;**5**:26.
12. **Wassenberg MW,** Bootsma MC, Troelstra A, *et al*. Transmissibility of livestock-associated methicillin-resistant Staphylococcus aureus (ST398) in Dutch hospitals. *Clin Microbiol Infect* 2011;**17**:316–19.

13. **Schijffelen MJ,** Boel CH, van Strijp JA, *et al*. Whole genome analysis of a livestock-associated methicillin-resistant Staphylococcus aureus ST398 isolate from a case of human endocarditis. *BMC Genomics* 2010;**11**:376.
14. **Ekkelenkamp MB,** Sekkat M, Carpaij N, *et al*. Endocarditis due to meticillin-resistant Staphylococcus aureus originating from pigs. *Ned Tijdschr Geneeskd* 2006;**150**:2442—7.
15. **Hartmeyer GN,** Gahrn-Hansen B, Skov RL, *et al*. Pig-associated methicillin-resistant Staphylococcus aureus: family transmission and severe pneumonia in a newborn. *Scand J Infect Dis* 2010;**42**:318—20.
16. **van Belkum A,** Melles DC, Peeters JK, *et al*. Methicillin-resistant and -susceptible Staphylococcus aureus sequence type 398 in pigs and humans. *Emerg Infect Dis* 2008;**14**:479—83.
17. **Declercq P,** Petre D, Gordts B, *et al*. Complicated community-acquired soft tissue infection by MRSA from porcine origin. *Infection* 2008;**36**:590—2.
18. **Van Cleef BA,** Broens EM, Voss A, *et al*. High prevalence of nasal MRSA carriage in slaughterhouse workers in contact with live pigs in The Netherlands. *Epidemiol Infect* 2010;**138**:756—63.
19. **Mulders MN,** Haenen AP, Geenen PL, *et al*. Prevalence of livestock-associated MRSA in broiler flocks and risk factors for slaughterhouse personnel in The Netherlands. *Epidemiol Infect* 2010;**138**:743—55.
20. **Terzieva S,** Donnelly J, Ulevicius V, *et al*. Comparison of methods for detection and enumeration of airborne microorganisms collected by liquid impingement. *Appl Environ Microbiol* 1996;**62**:2264—72.
21. **Graveland H,** van Duijkeren E, van Nes A, *et al*. Evaluation of isolation procedures and chromogenic agar media for the detection of MRSA in nasal swabs from pigs and veal calves. *Vet Microbiol* 2009;**139**:121—5.
22. **van Wamel WJ,** Hansenova Manaskova S, Fluit AC, *et al*. Short term micro-evolution and PCR-detection of methicillin-resistant and -susceptible Staphylococcus aureus sequence type 398. *Eur J Clin Microbiol Infect Dis* 2010;**29**:119—22.
23. **de Neeling AJ,** van Leeuwen WJ, Schouls LM, *et al*. Resistance of staphylococci in The Netherlands: surveillance by an electronic network during 1989-1995. *J Antimicrob Chemother* 1998;**41**:93—101.
24. **Francois P,** Renzi G, Pittet D, *et al*. A novel multiplex real-time PCR assay for rapid typing of major staphylococcal cassette chromosome mec elements. *J Clin Microbiol* 2004;**42**:3309—12.
25. **Schouls LM,** Spalburg EC, van Luit M, *et al*. Multiple-locus variable number tandem repeat analysis of Staphylococcus aureus: comparison with pulsed-field gel electrophoresis and spa-typing. *PLoS One* 2009;**4**:e5082.
26. **Hagen RM,** Seegmuller I, Navai J, *et al*. Development of a real-time PCR assay for rapid identification of methicillin-resistant Staphylococcus aureus from clinical samples. *Int J Med Microbiol* 2005;**295**:77—86.
27. **Huletsky A,** Giroux R, Rossbach V, *et al*. New real-time PCR assay for rapid detection of methicillin-resistant Staphylococcus aureus directly from specimens containing a mixture of staphylococci. *J Clin Microbiol* 2004;**42**:1875—84.
28. **Melles DC,** Tenover FC, Kuehnert MJ, *et al*. Overlapping population structures of nasal isolates of Staphylococcus aureus from healthy Dutch and American individuals. *J Clin Microbiol* 2008;**46**:235—41.
29. **Abdulmawjood A,** Roth S, Bulte M. Two methods for construction of internal amplification controls for the detection of Escherichia coli O157 by polymerase chain reaction. *Mol Cell Probes* 2002;**16**:335—9.
30. **Enright MC,** Day NP, Davies CE, *et al*. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. *J Clin Microbiol* 2000;**38**:1008—15.
31. **de Neeling AJ,** van den Broek MJ, Spalburg EC, *et al*. High prevalence of methicillin resistant Staphylococcus aureus in pigs. *Vet Microbiol* 2007;**122**:366—72.
32. **Tenhagen BA,** Fetsch A, Stuhrenberg B, *et al*. Prevalence of MRSA types in slaughter pigs in different German abattoirs. *Vet Rec* 2009;**165**:589—93.
33. **Bruins MJ,** Juffer P, Wolfhagen MJ, *et al*. Salt tolerance of methicillin-resistant and methicillin-susceptible Staphylococcus aureus. *J Clin Microbiol* 2007;**45**:682—3.
34. **de Boer E,** Zwartkruis-Nahuis JT, Wit B, *et al*. Prevalence of methicillin-resistant Staphylococcus aureus in meat. *Int J Food Microbiol* 2009;**134**:52—6.
35. **Lozano C,** Lopez M, Gomez-Sanz E, *et al*. Detection of methicillin-resistant Staphylococcus aureus ST398 in food samples of animal origin in Spain. *J Antimicrob Chemother* 2009;**64**:1325—6.
36. **Lundholm IM.** Comparison of methods for quantitative determinations of airborne bacteria and evaluation of total viable counts. *Appl Environ Microbiol* 1982;**44**:179—83.
37. **Stewart SL,** Grinshpun SA, Willeke K, *et al*. Effect of impact stress on microbial recovery on an agar surface. *Appl Environ Microbiol* 1995;**61**:1232—9.
38. **de Man JC.** The probability of most probable numbers. *Appl Microbiol Biotechnol* 1975;**1**:67—78.

Livestock-associated MRSA ST398 carriage in pig slaughterhouse workers related to quantitative environmental exposure

Maarten J Gilbert, Marian E H Bos, Birgitta Duim, et al.

Occup Environ Med 2012 69: 472-478 originally published online April 27, 2012
doi: 10.1136/oemed-2011-100069