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MRSA-ST398 in livestock farmers and neighbouring residents in a rural area in Germany

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SUMMARY

Prevalence of and risk factors associated with MRSA-ST398 carriage in 1872 (response 70%) farmers and neighbouring residents in a pig- and poultry-dense area in Germany were investigated using a cross-sectional study and self-sampling nasal swabs. In the population, 1% without occupational livestock contact and 24% with occupational livestock contact tested positive for MRSA-ST398. The group without occupational livestock contact was 3·8 times [95% confidence interval (CI) 1·5–9·3] more likely to be colonized if a household member had livestock contact and 3·2 times (95% CI 1·4–7·4) more likely if they regularly made private farm visits (e.g. to buy eggs or milk). In the group with occupational livestock contact, pig contact had an odds ratio of 7·1 (95% CI 2·9–17·2) for MRSA-ST398 acquisition. This is the first study to associate private farm visits with acquisition of MRSA; more research to explore the exact transmission routes is necessary.

Key words: Factory farming dense area, livestock, MRSA-ST398, prevalence, risk factors, rural residents.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major contributor to nosocomial infections worldwide. Reports suggest that the epidemiology of MRSA is undergoing a major change following the emergence of community-acquired MRSA (CA-MRSA) [1–3]. Clinical and molecular epidemiological studies have indicated two separate evolutionary pathways for CA-MRSA and hospital-acquired MRSA (HA-MRSA) [4]. CA-MRSA can cause serious infections in otherwise healthy individuals [5]. MRSA strains belonging to various multilocus sequence types (MLST) have been associated with infection and colonization in both humans and animals, suggesting bidirectional transmission [6–8]. The increase in MRSA infections of zoonotic origin may have a significant impact on the epidemiology of CA-MRSA and on the control of MRSA, especially in countries that maintain a low prevalence by means of search-and-destroy policies [6].

In 2004, contact with livestock – especially pigs – was identified as a risk-factor for MRSA carriage in The Netherlands [9]. Since 2004, an increasing

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number of publications worldwide indicate that MRSA belongs to MLST ST398, and is also found in hospital patients with occupational livestock contact, it can also be detected in pigs as well as in their immediate environment [6–15].

Survey results of Dutch pig farmers and veterinarians revealed statistically significantly higher MRSA carriage in these groups (26% and 5%, respectively) than in the general Dutch population at the time of hospital admission (0·03%) [9–11]. Studies by Wulf *et al*. in The Netherlands [12] have shown that people in contact with pigs have a higher risk of MRSA carriage than the general population does. Isolates of closely related *spa* types corresponding to MLST ST398 were found in pig farmers, pig veterinarians and pigs themselves. The emergence of ST398, however, is not only a Dutch problem as human infections have been reported in several European countries, Canada, and Singapore [13]. A study by the German national nosocomial infections surveillance system showed that 32% of nasal swabs taken from professionals working with pigs and 13% taken from their relatives were MRSA-ST398 positive [15].

Not only can MRSA-ST398 be transmitted among family members, but also from patients to hospital staff [6, 9, 10, 13, 16]. MRSA and MRSA-ST398 can seriously affect human health and symptoms include skin infections and sepsis. Studies by Gilchrist *et al*. [17] showed that significant quantities of bacterium can be found up to 150 m away from pig farms, allowing for the conclusion that the bacteria spread through the air, e.g. dust-borne from pig and poultry farms onto neighbouring farms. If this link can be proven, all possible routes of transmission must be identified in order to prevent the spread of a new epidemic.

The aim of this study was to assess, for the first time, the prevalence of MRSA and more specifically of MRSA-ST398 in neighbouring farm residents without livestock contact in a pig- and poultry-dense area, as well as in persons with direct livestock contact; furthermore, potential risk factors associated with MRSA should be identified for both groups. Similarly to many other European countries and North America, over the past 20–30 years animal production in Lower Saxony has shifted from small family-owned farms to confined animal feeding operations (CAFOs) that house large numbers of animals [18]. The major animal production in this federal state consists of poultry (50 million animals in 2010) and swine (8 million animals in 2010) housed in about 30 000 production facilities [19].

MATERIALS AND METHODS

Study population

The chosen study population had already completed the questionnaire and had participated in the clinical part of the Lower Saxony Lung Study between 2002 and 2004 [18]. The aim of the study was to examine the potential adverse effects of environmental exposures to emissions from CAFOs on respiratory health. This study was conducted in four rural towns in Lower Saxony, northwestern Germany, with a high density of animal feeding operations. The animal production focused primarily on pigs and poultry. All adults aged 18–44 years with German citizenship, registered in the population registries of these towns, formed the target population ($n=10\,252$) of the Lower Saxony Lung Study. Before the study, the target population of each town was divided at random into two groups. All residents were sent a mailed questionnaire and additionally part of the population were randomly selected and invited to take part in the clinical examinations, of which a total of 2812 people did.

These were chosen to take part in this study and to ensure that the 2812 addresses of the participants of the Lower Saxony Lung Study were still valid the registration offices of each of the four study towns compared them with their records. Subsequently a total of six people, whose new contact details were not available, were removed from the study and an additional 50 addresses were used for the pilot study. Hence, a total of 2756 questionnaires were distributed for the main study. Participants were provided with a self-sampling kit, including an explanatory cover letter, a questionnaire, a nasal swab, instructions for taking the swab from both nostrils with a dry swab, as well as pre-paid packaging to return specimens and questionnaires. In December 2009, mailing of the self-sampling kits began and continued until April 2010. A postal reminder was sent to non-responders followed by a second postal reminder, i.e. an additional self-sampling kit and a reminder phone call to those who still had not responded. In a few cases crossovers occurred when the participant had already mailed the parcel to the Institute but it had not yet arrived when the second kit was sent to the participant. In some cases, the participant returned both kits to the Institute. These questionnaires ($n=10$) were then used to assess the repeatability of the data, by comparing the replies in the first and second questionnaires.

A possible limitation of the study might have been that sampling could have been performed incorrectly and that the lengthy transportation routes affected the quality of the nasal swabs. Therefore, unknown to laboratory personnel, on some occasions swabs already known to be MRSA positive obtained from elsewhere (tubes identical to the ones used in the study) were sent back for testing, together with some swabs from the study, to ensure that the lengthy transportation routes did not affect the quality of the nasal swabs. These samples indicated the validity of the methods.

In general samples were shipped overnight, by the study recipients from Lower Saxony to Munich (maximum distance 500 km). No temperature control was required as the study was conducted during the winter months, where the average outside temperature had no effect onto the quality of the samples.

The study was approved by the Medical Ethical Committee of the University Hospital of Munich (LMU). Participants were asked to supply written informed consent and regional health authorities were informed at the start of the study.

Questionnaire design

The majority of the 40 items of the questionnaire were taken from existing validated questionnaire instruments (a copy is available from the authors upon request).

The questionnaire was divided into the following sections:

Sociodemographic data

These included general questions on age, sex, occupation as well as whether individuals were currently participating in any form of group sport. The majority of these questions were taken from the Lower Saxony Lung Study [20].

Farm animal contact and distance to farms from home and work environment

This section included questions relating to animal contact. It identified participants and any of their family members who worked on farms, and in addition the survey assessed the distance participants lived or worked from the next farm (<500 m, $\geqslant 500$ m). This distance was chosen due to participants not being able to assess the distance to the next farm precisely, as observed in the Lower Saxony Lung Study [20]. Participants were also asked whether they kept any pets, such as cats or dogs, and also about the frequency of animal contact, if any.

Finally, participants were asked whether they had regularly (at least once a month) visited farms on a private basis, e.g. to buy eggs or milk from a farm shop. The questions were based both on the Lower Saxony Lung Study [20] and a study conducted by Anderson *et al*. [21].

Pilot study

Prior to the study, the feasibility of the questionnaire as well as the clarity of the instructions on how to take the nasal samples was assessed in 50 inhabitants of the largest study town. As a result, one question was rephrased in order to reduce confusion and to improve lucidity. Moreover, a few minor improvements were included in the questionnaire of the main study to ensure comprehension and clarity of the questions. The questionnaires and swabs of the pilot study were not included in the main analysis.

Laboratory analyses

The testing was performed in the laboratories of the Governmental Institute of Public Health of Lower Saxony. The microbiological identification of *S. aureus* was performed on a Columbia CNA (colistin-nalidixic acid) agar and an MRSA selective medium (bioMérieux, France) using direct cultures at an incubation temperature of 36 ± 1 °C for 24–48 h [14]. A coagulase test (Pastorex™ StaphPlus, Bio-Rad, France) was then conducted to verify *S. aureus* colonies. Isolated MRSA strains were sent to the University of Münster (northern Germany) for further characterization using *S. aureus* protein A gene (*spa*) typing. Cluster formation of *spa* types [*spa* clonal complexes (*spa* CC)] was performed using the based-upon-repeat-pattern (BURP) algorithm of Ridom Staph Type software (Ridom GmbH, Germany) [22].

Statistical analysis

Data were entered into an Access (Microsoft Corp., USA) database, double-checked and verified with questionnaires. Final datasets were analysed using SPSS version 17.0.2 (IBM Corporation, USA). Prevalence of MRSA was calculated first for all study participants and then stratified for those without (non-OLC) and those with (OLC) occupational

livestock contact. Bivariable and multivariate analyses were also stratified for the two groups (OLC and non-OLC). First, cross-tabulations between potential risk factors and MRSA presence were performed. For the non-OLC group the following factors were considered: working in a hospital setting (yes/no), private farm visits (yes/no), self-reported distance between home and workplace to the next farm (<500 m, $\geqslant 500$ m), household member working on a livestock farm (yes/no), and companion animal contact (yes/no).

For the OLC group, contact with each of the following animals (yes/no) was assessed: pigs, cattle, broilers, turkey, ducks and horses. The other factors, included for the non-OLC group were not considered relevant as previous studies [6, 9, 10, 15, 16, 21] had shown that direct occupational animal contact could be assumed to be more important than environmental factors.

For both groups, all factors with a $P_{\text{Fisher}} < 0{\cdot}1$ were included in the multiple logistic regression models. In addition, models were *a-priori* adjusted for age (26–44 years, 45–53 years) and sex (male/female) as potential confounders for both groups, as well as working in a hospital setting for the non-OLC group. For this group, additional sensitivity analyses were also performed to examine whether the results of the logistic regression analysis would vary if the dependent variable, presence or absence of MRSA, were divided into two separate regressions:

(*a*) for the group colonized with MRSA strains of different sequence types typically associated with hospitals;
(*b*) for those with the specific MRSA-ST398 sequence type.

For the second group, with OLC, only MRSA-ST398 was entered as for this group all strains belonged to this sequence type.

RESULTS

Response

Of the 2756 questionnaires and swabs sent out, 1966 were returned (initial response 71%). Of these, 84 study participants had moved away from the study area; their family or the new residents forwarding their mail to their new address; and were therefore excluded. Ten people had completed the questionnaire twice leaving 1872 questionnaires for the final statistical analysis, giving an overall response of 70%. Of those, 27 did not indicate their occupation and could not be included in the regression analysis due to 'occupational livestock contact' being a category of the study; a total of 1655 replies were obtained from the OLC group and a total of 190 from the non-OLC group.

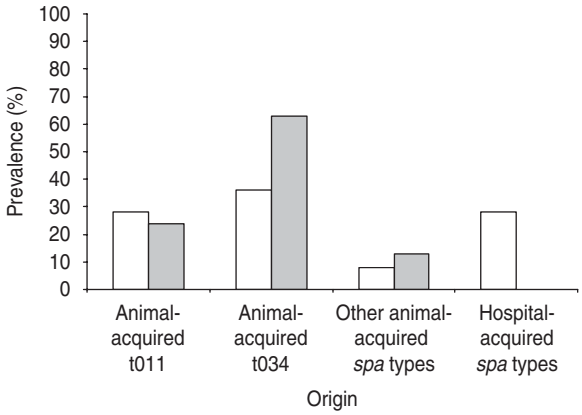

Fig. 1. Prevalence (in percent) of *spa* types found in positive MRSA samples for the group without (□) and the group with (▨) occupational livestock contact.

Average age for the two groups (OLC and non-OLC) were almost identical ($P > 0{\cdot}05$) with a mean age of 42·2 (min = 25·6, max = 52·6, S.D. = 6·9) years for the non-OLC group and 42·7 (min = 25·6, max = 52·6, S.D. = 6·8) years for OLC group. In the non-OLC group, 45% of participants were male, while in the OLC group 70% of the participants were male. Further calculations showed that the non-participants of this study did not differ in sex or age from participants of the Lower Saxony Lung Study.

Overall 3·9% of all the nasal swabs from the study participants tested positive for MRSA, with a total of 1·5% for the non-OLC group and 24% for the OLC group.

spa types

spa-typing revealed that the animal-associated t034 (ST398) was the most prevalent *spa* type colonizing both non-OLC (36%) and OLC (63%) groups (Fig. 1). The second most prevalent *spa* type was t011 (ST398), which is also animal-associated, with a prevalence of 28% for the group without and 24% for the OLC group. For the non-OLC group, the remaining 28% of *spa* types were not of animal origin but hospital-associated (R. Köck personal communication; SpaServer www.spaserver.ridom.de/)

compared to 0% in the OLC group (Fig. 1). The hospital-associated *spa* types found here were: t003 (ST225), t004 (ST45), t032 (ST22), t1107 (MLST unknown), t1344 (MLST unknown), t1708 (MLST unknown), t4395 (MLST unknown), t487 (MLST unknown). The remaining samples belonged to other animal-acquired *spa* types: t1451 (ST398), t2576 (ST398) and t899 (ST9). All of the animal-associated *spa* types for both groups belonged to the pig-associated sequence types [15].

Predictors of MRSA

Participants without occupational livestock contact

In the non-OLC group, those that tested positive for MRSA were more likely to have a household member with occupational livestock contact ($P_{\text{Fisher}} < 0{\cdot}001$) and to make private farm visits ($P_{\text{Fisher}} = 0{\cdot}001$) (Table 1).

Confirming the bivariate results, having a member of their household working on a livestock farm (OR 3·8, 95% CI 1·5–9·3), as well as making private visits to farms (OR 3·2, 95% CI 1·4–7·4) were the major predictors of MRSA positivity in the multiple logistic regression model (Table 2).

The sensitivity analyses for the sequence type MRSA-ST398 gave identical results to those of the logistic regression analysis where both groups (MRSA and MRSA-ST398) were entered.

Participants with occupational livestock contact

For the OLC group contact with pigs ($P_{\text{Fisher}} < 0{\cdot}001$) and sex ($P_{\text{Fisher}} = 0{\cdot}01$) were statistically significantly associated with MRSA positivity in the bivariate models (Table 1). Similarly, in the final multiple model contact with pigs was the major predictor for MRSA (OR 7·1, CI 2·9–17·1) (Table 2). Women with occupational livestock contact were less likely to be colonized with MRSA than men (OR 0·3, 95% CI 0·1–0·7).

DISCUSSION

The results of this study suggest private farm visits as a potential new risk factor for colonization with the bacteria. The results also imply that MRSA is being transmitted from people with livestock contact to their family members and that people working with pigs have an increased risk for MRSA-ST398 carriage.

The generated response of 71% can be considered high, especially as the participants had already taken part in the Lower Saxony Lung Study some years previously [18]. Extrapolating from the data of the Lower Saxony Lung Study, where 60% of all responders either had occupational livestock contact or grew up on a farm (note that it cannot be distinguished between these two factors), whereas in the study only 10% of all responders were currently working with livestock. It is possible that response was lower for those with occupational livestock contact, as perhaps many of them did not take part in the study because they were afraid of the consequences for their farm if they tested positive because the cover letter, included in the self-sampling kit, referred to testing for bacteria found in the farming environment. However, it is unlikely that this might have resulted in major selection bias as the study population did not know beforehand if they were colonized with MRSA or not.

For the study, nasal swabs were not only chosen because the nose is considered a prime site for MRSA colonization and studies have shown that the sensitivity of nasal swabs is around 68% [23], but also because they allowed participants to do the sampling themselves. This allowed for savings in time and money and subsequently for a larger sample size to be included. For financial reasons no MLST analysis could be performed, but the results of a study by Strommenger *et al.* [24] indicated that *spa*-typing, together with BURP clustering, was a useful tool in *S. aureus* epidemiology, especially because of its ease of use and the advantages of unambiguous sequence analysis.

In this study the overall MRSA prevalence for both groups of 4% in a rural setting, with a high density of CAFOs is almost as high as the prevalence of 5% found in a study performed in 2005 in a German university hospital [25]. However, the prevalence of MRSA in the healthcare setting is known to be much higher than in the general population. The prevalence of 24% found in the OLC group is comparable to the prevalence found in Dutch farmers (26%). In contrast, the prevalence in the neighbouring residents in our study was much higher (1·5%) than the prevalence found in Dutch patients at hospital admission (0·03%) [9–11].

Risk factors for MRSA in the latter group included regularly making private farm visits despite the fact that the individuals were merely visiting the farm store and there was rarely any farm animal contact in

Table 1. *Bivariate association between potential risk factors and MRSA positivity*

	No occupational livestock contact			With occupational livestock contact		
	MRSA– ($N=1630$)	MRSA + ($N=25$)	Fisher's exact *P* value	MRSA– ($N=144$)	MRSA + ($N=46$)	Fisher's exact *P* value
Sex: female	54·9 % ($n=895$)	72·0 % ($n=18$)	0·11	35·4 % ($n=51$)	15·2 % ($n=7$)	0·01
Age: 26–44 years	56·7 % ($n=925$)	68·0 % ($n=17$)	0·31	54·2 % ($n=78$)	52·2 % ($n=24$)	0·87
Working in a hospital setting	9·3 % ($n=153$)	12·0 % ($n=3$)	0·50	n.a.	n.a.	n.a.
Distance: *home* to next farm <500 m	57·4 % ($n=859$)	63·2 % ($n=12$)	0·65	n.a.	n.a.	n.a.
Distance: *workplace* to next farm <500 m	30·4 % ($n=449$)	33·3 % ($n=8$)	0·82	n.a.	n.a.	n.a.
Household member with occupational livestock contact	7·6 % ($n=124$)	32·0 % ($n=8$)	$<0·001$	n.a.	n.a.	n.a.
Any kind of companion animal contact	75·6 % ($n=1230$)	84·0 % ($n=21$)	0·48	93·1 % ($n=134$)	91·3 % ($n=42$)	0·747
Private visits to farms	28·8 % ($n=468$)	60·0 % ($n=15$)	0·001	n.a.	n.a.	n.a.
Occupational livestock contact with						
Pigs	n.a.	n.a.	n.a.	45·1 % ($n=65$)	84·8 % ($n=39$)	$<0·001$
Cattle	n.a.	n.a.	n.a.	28·5 % ($n=41$)	39·1 % ($n=18$)	0·201
Broiler	n.a.	n.a.	n.a.	15·3 % ($n=22$)	10·9 % ($n=5$)	0·628
Turkey	n.a.	n.a.	n.a.	20·8 % ($n=30$)	19·6 % ($n=9$)	1·000
Ducks	n.a.	n.a.	n.a.	5·6 % ($n=8$)	2·2 % ($n=1$)	0·690
Horses	n.a.	n.a.	n.a.	13·2 % ($n=19$)	6·5 % ($n=3$)	0·294

n.a., Not applicable.
Numbers might not add up due to missing data.

Table 2. *Results of the multiple logistic regression models. Risk factors associated with MRSA positivity, stratified for without and with occupational livestock contact*

Group	Factor	OR	95% CI
Without occupational livestock contact	Age (26–44 years)	0·54	0·24–1·22
	Sex (female)	2·09	0·83–5·28
	Household member with occupational livestock contact	3·81	1·55–9·33
	Private visits to farms	3·20	1·38–7·43
	Working in a hospital setting	0·89	0·25–3·17
With occupational livestock contact	Age (26–44 years)	1.36	0·65–2·84
	Sex (female)	0·29	0·12–0·75
	Occupational livestock contact with pigs	7·09	2·93–17·18

OR, Odds ratio; CI, confidence interval.

these short visits. This study is the first to recognize private farm visits as a potential risk factor. More research into establishing the exact transmission routes is required, especially because 72% of all strains in this group were in connection with the pig-associated MRSA-ST398 sequence type. A potential explanation could be that transmission from the animals to the visitors via the air occurred. Other potential sources for this MRSA acquisition could have been through the touching of contaminated surfaces within the farming environment or that the person selling the products in the shop transmitted the bacteria to the customers, as studies have shown that farmers transmitted the bacteria to their household members [7]. This risk factor also appears to be confirmed in our study, as people without occupational livestock contact were almost four times more likely to be colonized with MRSA if one of their household members did have livestock contact.

This study was not able to show any association of self-reported distance between home or workplace to the next farm and MRSA colonization, which might be due to a non-differential misclassification as distance was based on self-reports. In addition, MRSA has been shown to be spread up to 150 m from the farm [17], whereas in our study the only options given for self-reported distance to the next farm were <500 m or $\geqslant 500$ m. The reason for choosing these categories was based upon the experience that assessing self-reported distance in greater detail is even more unreliable [20]. Unfortunately, for financial reasons no objective measures of distance of the home and work environment to the next farm could be used in this study.

The analysis of this study not only suggests that pigs were the main host for ST398 but also that the presence of pigs increased the risk of MRSA-ST398 acquisition in the OLC group, confirming several previous studies from numerous European countries such as Germany, Belgium and The Netherlands [7–9, 15, 26, 27]. In these studies pigs, people working on farms as well as their household members were tested. Those studies suggested that human carriage of MRSA was associated with MRSA colonization in swine [7, 8] and showed that MRSA isolates from farmers belonged to closely related *spa* types corresponding to ST398, which are unrelated to hospital-acquired strains but identical to strains from humans in contact with pigs in other European countries [9, 26, 27]. Unfortunately for logistic reasons, farm animals could not be tested in this study.

Further, the results of this study do not suggest that companion animals play a role in the transmission process of MRSA-ST398. Whether this finding is due to a lack of power, lack of colonization of companion animals, or lack of transmission can unfortunately not be determined. Some studies have suggested that companion animals were acting as potential reservoirs or vectors for human infection of MRSA in the community [27, 28], whereas a recent review by Loeffler & Lloyd [29] concluded that available data on MRSA transmission between humans and companion animals are limited and that the public health impact on such transmission needs to be subjected to more detailed epidemiological studies.

In the OLC group males were more likely to be colonized with MRSA than females. This could be due to men performing different tasks within the farming environment than women.

This is the first study to measure MRSA prevalence within a general population without occupational

livestock contact in Germany, indicating a prevalence of 1·5%. Moreover, the study appears to confirm previously established risk factors. It also suggests that visiting farms privately is a new potential risk factor for MRSA colonization for the group without occupational contact. More research into establishing the exact transmission routes and into measures to prevent the spread of the bacterium in the farming environment is still required.

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DECLARATION OF INTEREST

None.

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