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MRSA in persons not living or working on a farm in a livestock-dense area: prevalence and risk factors

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Objectives: MRSA emerged in livestock and persons in contact with livestock is referred to as livestock-associated MRSA (LA-MRSA). We assessed the prevalence and risk factors for MRSA carriage in persons not living or working on a farm.

Methods: A cross-sectional study was performed among 2492 adults living in close proximity of livestock farms. Persons working and/or living on farms were excluded. Nasal swabs were cultured using selective media. Participants completed questionnaires and the distance from the residential address to the nearest farm was calculated. The Mann–Whitney *U*-test was used to compare median distances. Risk factors were explored with logistic regression.

Results: Fourteen persons carried MRSA (0.56%; 95% CI 0.32%–0.92%), 10 of which carried LA-MRSA of multiple-locus variable-number tandem repeat analysis complex (MC) 398 (0.40%; 95% CI 0.20%–0.71%). MRSA MC 398 carriers lived significantly closer to the nearest farm than non-carriers (median: 184 versus 402 m; $P < 0.01$). In bivariate analyses correcting for contact with livestock, this difference remained significant.

Conclusions: Although the prevalence was low, living near farms increased the risk of MRSA MC 398 carriage for persons not living or working on a farm. Further research is necessary to identify the transmission routes.

Introduction

In the past decade, a specific clone of MRSA, MLST clonal complex (CC) 398, which corresponds to multiple-locus variable-number tandem repeat analysis (MLVA) complex (MC) 398, has emerged in livestock and persons in contact with livestock in the Netherlands and many countries inside and outside Europe. MRSA belonging to this CC are referred to as livestock-associated MRSA (LA-MRSA).[1] In the Netherlands, LA-MRSA belong predominantly to MLST CC 398, but MRSA of other CCs (i.e. CC 5, CC 9, CC 30 and CC 97) have also been found in livestock in the Netherlands and other countries.[2] LA-MRSA has been found in various animal species, such as swine, veal calves, horses and poultry.[3–7] Prevalence of LA-MRSA, especially MRSA CC 398, in persons in contact with livestock is high in the Netherlands. In pig farmers, an average cross-sectional prevalence of 63% has been reported.[8] The prevalence of LA-MRSA was 33% in veal farmers and 8% in their family members[5] and 8.5% and 3.4% in poultry farmers and their family members, respectively.[4] LA-MRSA has also been found in veterinarians and slaughterhouse personnel.[9,10] MRSA-positive workers were predominantly found at the start of the slaughter process and working in the lairage or the scalding and dehairing area.[10] The risk of MRSA carriage decreased along the slaughterline.[10] Prevalence of MRSA carriage in the Dutch general population is low (i.e. ∼0.1%) due to a search-and-destroy policy together with restrictive use of antibiotics.[11] The search-and-destroy policy implies that at hospital admission patients with known risk factors for MRSA carriage (i.e. contact with living pigs, veal calves or broilers) are screened and isolated.[11]

The Netherlands is one of the most densely populated countries of the world: on a surface of 41 000 km^2 live 17 million people together with 107 million chickens, 12 million pigs and 4 million cows.[12] We hypothesized that persons living in close proximity of livestock farms, even if they do not have professional contact with livestock, may be at increased risk of MRSA carriage. Carriage of LA-MRSA has mainly been studied among farmers and other persons in direct contact with livestock. The objective of the present study was to assess the prevalence and risk factors for MRSA MC 398 carriage in persons not working or living on a farm in areas with high livestock densities.

Methods

A cross-sectional study on MRSA carriage was performed among adults living in the vicinity of livestock farms in the Netherlands. This study was part of a larger population-based study on the health of residents living in a highly populated rural area with a high density of livestock farms in the south of the Netherlands: the Livestock Farming and Neighbouring Residents' Health study (Dutch acronym: VGO). The methodology of the VGO study is described in detail by Borlée et al.[13] Briefly, in 2012, participants were recruited via general practitioners (GPs). Included were persons: aged 18–70 years; living in the province of Noord-Brabant or Limburg; and living in municipalities with < 30 000 inhabitants. Only one eligible participant per household was (randomly) invited to participate. Exclusion criteria were living or working on an animal farm.

Our study was conducted from March 2014 to February 2015. Participants were invited for a medical examination at 1 of 12 research centres in the study area. The medical examination included taking a rayon nasal swab (Medical Wire & Equipment, No. MW102) from both nostrils to determine MRSA carriage. Participants were asked to complete a questionnaire on demographic characteristics, current and past profession(s), hospitalization, travel history, contact with patients, children or animals during work and/or study, recent and past exposure to livestock farms and contact with pets and/or farm animals at home or during farm visits. In addition to the questionnaire, data from the provincial database of mandatory environmental licences for keeping livestock in 2012 were used. From this database, information was collected on the precise coordinates of all livestock farms in the study area, including the animal species and number of animals. Using a geographic information system (ArcGis 10.1, Esri, Redlands, CA, USA), the distances between participants' residential addresses and livestock farms were calculated.[14] The distance to the nearest livestock farm and the number of livestock farms and animals (overall and specifically for pigs, poultry, cattle, goats and sheep) within 500 and 1000 m from the participant's residential address were studied as livestock farm exposure variables. For the distance variable, the minimum number of animals per farm type was as follows: 25 pigs, 250 chickens, 5 cows, 5 horses, 50 goats and 50 sheep. The NIVEL Primary Care Database[15] was used to collect data on prescriptions of antibiotics according to the Anatomical Therapeutic Chemical classification in the 3, 6 and 12 months preceding the medical examination. In addition, this database was used to collect data on comorbidity in the 12 months preceding the medical examination. Data from the NIVEL Primary Care Database were included when the GP registered prescriptions and morbidity (International Classification of Primary Care codes) for ≥46 weeks during the calendar year and if the patient was registered at the particular GP for at least three-quarters of the year. Data regarding use of proton pump inhibitors (PPIs) were collected during the medical examination.

Nasal swabs were cultured for MRSA using pre-enrichment and selective enrichment. Briefly, the swab was transferred into 10 mL of high-salt enrichment broth [Mueller–Hinton (BD, France) with 6.5% sodium chloride] and after 18 h of incubation at 37 °C, 1 mL was transferred into 9 mL of phenol red mannitol broth with 5 mg/L ceftizoxime and 75 mg/L aztreonam (bioMérieux, France). After 18 h of incubation at 37 °C, 10 μL of this enrichment was plated on Brilliance MRSA 2 Agar (Oxoid, Germany) and incubated for 18 h at 37 °C.[6] Suspected (blue) colonies were subcultured on Columbia agar with 5% sheep blood (Oxoid, Germany) and confirmed as MRSA by quantitative PCR (qPCR) (for detection of the *mecA* gene and specific *Staphylococcus aureus* DNA fragments: *nuc* and *tuf*). All MRSA isolates were typed by *spa* typing and MLVA typing as described previously.[16,17]

Data were analysed using SPSS version 22.0 (SPSS, Chicago, IL, USA). Prevalence of MRSA carriage was calculated with 95% CI. The Mann–Whitney *U*-test was used to compare the median distance to livestock farms, median number of livestock farms within 500 and 1000 m and median number of animals within 1000 m from the participant's address. Using univariate logistic regression analyses, ORs with 95% CIs were obtained for possible risk factors for carriage of MRSA MC 398, including exposure to livestock farms. Bivariate logistic regression analysis was performed to correct for possible confounders of the association between MRSA MC 398 and distance to the nearest livestock farm. Variables with a univariate $P < 0.2$ were included in the bivariate analysis. In this analysis, the variable distance to the nearest farm was log transformed. Multivariate logistic regression analysis including more than two independent variables was not possible due to the small number of MRSA carriers.

Ethics

The study was approved by the medical ethics committee of the University Medical Centre Utrecht (number 13/533). Participants signed an informed consent.

Results

The VGO medical examination was conducted among 2494 participants. The response rate was 34% (2494/7251). The median age of participants was 58 years (range 20–72 years) and 45.3% were male. A total of 2492 nasal swabs were analysed. Fourteen isolates were positive for *mecA* and the *nuc* and *tuf* genes and therefore were confirmed as being MRSA. The prevalence of MRSA carriage was 0.56% (95% CI 0.32%–0.92%). The prevalence varied between the 12 research centres (range 0.00%–1.07%). MLVA of the MRSA isolates showed that 10 isolates belonged to the livestock-associated MC 398, which corresponds to MLST CC 398. The prevalence of MRSA MC 398 carriage was 0.40% (95% CI 0.20%–0.71%). The predominant *spa* type of isolates belonging to MC 398 was t011 ($n = 6$); other *spa* types within MC 398 were t034 ($n = 1$), t108 ($n = 1$), t1451 ($n = 1$) and t4208 ($n = 1$). One isolate belonged to MC 22 (congruent to MLST CC 22) and *spa* type t022, two isolates to MC 30 (congruent to MLST CC 30) and *spa* type t007 and one isolate to MC 282 (MLST type unknown) and *spa* type t692.

Descriptive analyses of the questionnaire data demonstrated no difference in reported skin problems in the month preceding the medical examination between MRSA carriers and non-carriers (14% versus 21%, $P = 0.747$). The median distance to the nearest farm computed including all 14 MRSA carriers was 201 versus 402 m for non-carriers ($P < 0.01$). The 10 MRSA MC 398 carriers also lived significantly closer to the nearest livestock farm compared with non-carriers (median: 184 versus 402 m; $P < 0.01$) (Table 1). Considering the different types of farms, MRSA MC 398 carriers lived significantly closer to pig, poultry and horse farms compared with non-carriers. The difference in median distance to farms ranged from 62 m for sheep farms to 447 m for poultry farms. Also, the number of farms within 500 and 1000 m was significantly higher for MRSA MC 398 carriers versus non-carriers. When comparing the number of farm animals within 1000 m, there were significantly more poultry (median: 45 672 versus 1052; $P < 0.05$), cattle (1117 versus 566; $P < 0.05$) and horses (median: 62 versus 29; $P < 0.05$) near the homes of carriers compared with non-carriers (Table 1).

Further risk factor analyses based on questionnaire data and GP registered data on antibiotic exposure and comorbidities did not

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Table 1. Distance from residential address to the nearest farm and number of farms, and number of farm animals among carriers of MRSA MC 398 living near livestock farms in the Netherlands

	MRSA MC 398 carriers, median (range min–max); *N*=10	MRSA MC 398 non-carriers, median (range min–max); *N*=2484	*P*[a]
Distance (m) from patient to nearest			
farm	184 (38–605)	402 (3–1709)	0.002
pig farm	393 (38–1022)	689 (11–2500)	0.014
poultry farm	487 (185–1256)	934 (39–4145)	0.003
cattle farm	396 (80–675)	483 (3–1709)	0.272
horse farm	479 (272–930)	761 (11–3432)	0.010
goat farm	2209 (406–5351)	2490 (99–11 477)	0.416
sheep farm	1234 (522–1944)	1296 (13–5047)	0.662
Number of farms			
within 500 m	4 (0–7)	1 (0–12)	0.010
within 1000 m	14 (8–22)	9 (0–32)	0.003
Number of animals within 1000 m			
pigs	4696 (13–11688)	2701 (0–79 057)	0.460
poultry	45 672 (0–444010)	1052 (0–9 11 052)	0.021
cattle	1117 (147–3274)	566 (0–6804)	0.028
horses	62 (18–666)	29 (0–708)	0.021
goats	0 (0–2480)	0 (0–5015)	0.768
sheep	10 (0–160)	10 (0–1410)	0.933

[a]Mann–Whitney *U*-test.

show any additional risk factors for MRSA MC 398 (Table 2). Bivariate logistic regression analyses were performed to correct for possible confounders of the association between MRSA MC 398 and distance to the nearest livestock farm. The univariate log-transformed OR for distance to the nearest farm was 0.13 (95% CI 0.04–0.43). The following variables with a univariate $P < 0.2$ were included in bivariate analyses: comorbidity, use of PPIs, travel and contact with farm animals. In all bivariate models, the association between MRSA MC 398 and distance remained statistically significant, while the other variables were not significantly associated with MRSA MC 398. Table 3 provides an overview of the risk factors for each of the 14 MRSA carriers.

Discussion

Generally, the prevalence of MRSA among persons without professional contact with livestock who live near livestock farms was relatively low (0.56%). This is in line with the low MRSA prevalence in the Netherlands. Ten out of 14 of the MRSA isolates belonged to the livestock-associated MC 398, resulting in a prevalence for this type of MRSA of 0.40%. It should be noted, however, that the most important risk group for LA-MRSA carriage, namely persons living and/or working on livestock farms, were excluded from the present study and including these would probably have resulted in a higher prevalence.

Comparison with MRSA prevalences determined in previous studies is difficult due to different study populations and/or laboratory methods. In 1999–2000, the prevalence of MRSA carriage among patients without risk factors at the time of admission to Dutch hospitals was 0.03%.[18] In 2005–07, this prevalence increased to 0.11%, although this was not significantly different from the prevalence in 1999–2000.[11] Another study performed in 2007 in a Dutch hospital reported an MRSA prevalence of 0.5% among inpatients.[19] In long-term care facilities, a prevalence of 0.3% has been reported.[20] Among patients visiting a GP for a non-infectious disease in the Netherlands, an MRSA prevalence of 0.2% was found.[21] However, in this study, no selective pre-enrichment/culture for MRSA was done and therefore this prevalence might be an underestimation. A study performed in the Netherlands in a similar area, with a high density of pig farms, reported a prevalence of LA-MRSA of 0.2% (1/534) among persons not in direct contact with livestock.[22] In the present study, the MRSA MC 398 prevalence (0.40%) was higher, although comparison of the CIs shows no significant difference. Another study performed in an area with a high density of livestock farms reported an MRSA prevalence of 0.8% among GP patients who had used antibiotics in the previous 3 months.[23] The higher prevalence in the latter study can be explained by the fact that farmers and persons in direct contact with livestock were not excluded and three of the four MRSA carriers were farmers.

In our study, 10 out of 14 (71%) MRSA isolates belonged to the livestock-associated MC 398. In a study by den Heijer *et al.*[21] among persons in all parts of the Netherlands, 56% of MRSA isolates belonged to MRSA CC 398. The high percentage of MRSA MC 398 in the present study suggests livestock as a possible reservoir for these human cases.

An important finding of the present study was that living in the vicinity of livestock farms was a risk factor for carriage of LA-MRSA,

Table 2. Univariate logistic regression analyses of risk factors for MRSA MC 398 among people living near livestock farms in the Netherlands

Risk factor	MRSA MC 398 positive, % (*n*); *N*=10	MRSA MC 398 negative, % (*n*); *N*=2482	OR (95% CI)
Male	70 (7)	45.2 (1124)	2.82 (0.73–10.95)
Age (years)			
20–29	0	2.2 (54)	NA
30–39	10 (1)	7.3 (181)	2.09 (0.22–20.20)
40–49	30 (3)	17.7 (439)	2.59 (0.52–12.86)
50–59	30 (3)	27.1 (673)	1.69 (0.34–8.38)
≥60	30 (3)	45.7 (1135)	ref.
Level of accomplished education			
low	20 (2)	25.5 (633)	0.78 (0.13–4.69)
medium	50 (5)	44.6 (1107)	1.12 (0.27–4.69)
high	30 (3)	29.9 (742)	ref.
Specific diet (without meat or fish or animal products)	10 (1)	5.6 (137)	1.89 (0.24–15.03)
Hospitalized in last 12 months[a]	0	0.3 (7)	NA
Antibiotics in last 6 months[b]	14.3 (1)	10.2 (211)	1.47 (0.18–12.26)
Antibiotics in last 3 months[b]	0	4.7 (98)	NA
Comorbidity[c]	42.9 (3)	20.2 (418)	2.97 (0.66–13.30)
PPIs	30.0 (3)	11.8 (291)	3.20 (0.82–2.44)
During work/study contact with			
patients	0	12.1 (284)	NA
residents of nursing homes	0	15.7 (369)	NA
children	0	12.7 (299)	NA
animals	0	6.4 (150)	NA
Travelled in last 12 months to			
Southern/Eastern Europe	30 (3)	29.5 (724)	1.43 (0.32–6.41)
Africa, Asia, Latin America	30 (3)	14.2 (349)	2.97 (0.66–13.31)
no travel or travel to Western/Northern Europe, Australia, New Zealand, North America	40 (4)	56.3 (1380)	ref.
Kept pets now or in last 5 years[d]	50 (5)	52.3 (1288)	0.91 (0.26–3.16)
Visited a farm in last 12 months	80.0 (8)	62.4 (1540)	2.41 (0.51–11.38)
Contact at home or during farm visit with farm animals[e]	66.7 (6)	41.5 (1008)	2.82 (0.70–11.31)

NA, not applicable.
[a]Hospitalized in the Netherlands and/or abroad.
[b]For 415 persons, data on antibiotics and comorbidity are missing.
[c]Includes cerebrovascular disease, chronic cardiovascular disease, liver disease, chronic lung disease, chronic renal disease, autoimmune disease, neurological comorbidity, diabetes and malignancy.
[d]Dog, cat, bird, rabbit, guinea pig, hamster, mouse, rat, fish and turtle.
[e]Horses, pigs, poultry, cows, goats and sheep. Contact was defined as touching the animal or droppings of the animal.

although farmers and persons living or working on a farm were excluded. We did not find direct animal contact to be a risk factor for MRSA MC 398, but this might be due to the small number of cases. Additional analyses showed that when including all 14 MRSA carriers, contact with livestock was borderline significant (results not shown). Four MRSA MC 398 carriers did not report any contact with farm animals, one reported only contact with horses and two reported contact with pigs during farm visits. One only had contact with poultry. A previous study reported that 15%–20% of persons in the Netherlands carrying or infected with LA-MRSA did not have direct contact with pigs or veal calves.[24,25] Moreover, 38% of patients with LA-MRSA CC 398 in four German hospitals did not report direct contact with livestock.[26] The transmission route of these cases remains unknown. Possible routes are: direct animal-to-human transmission from animal species not included as risk factors in the Dutch MRSA guideline (e.g. turkeys, horses or companion animals); indirect animal-to-human transmission through the environment, e.g. by dust or air; transmission through animal products such as meat; or human-to-human transmission. In a previous study, persons without livestock contact were more likely to test positive for LA-MRSA if a household member had livestock contact or if they visited farms privately.[27] In our study, visiting farms was not associated with MRSA MC 398 carriage and only one household member was included; therefore, we could not assess human-to-human transmission within the household. Person-to-person transmission was thought to be uncommon for MRSA MC 398, but has recently been described among livestock veterinarians and their household members.[28] A genetic analysis also suggested person-to-person transmission as a possible explanation for persons without contact with livestock

Table 3. Distribution of risk factors and MC among 14 MRSA cases living near livestock farms in the Netherlands

MRSA-positive subjects	MC	Job history relevant for MRSA	Travel history in last 12 months	Contact pets	Contact farm animals at home	Contact animals during farm visit	Distance from residence to the nearest farm (m)	Type of nearest farm
1	398	healthcare worker	Northern Africa, Western Europe	cat, rabbit/guinea pig/hamster	horse, poultry	horse, poultry, rabbit/guinea pig/hamster	150	pig
2	398	not relevant	Southern Europe	dog	no contact	no contact	76	pig
3	398	not relevant	Western Asia	no contact	no contact	cow, pig	112	pig
4	398	not relevant	Southern Europe, Western Europe	no contact	no contact	horse, pig	324	cattle
5	398	not relevant	Western Asia	no contact	no contact	no contact	80	cattle
6	398	not relevant	Southern Europe, Western Europe	dog	poultry	no contact	218	pig
7	398	not relevant	no	cat, dog	horse	no contact	38	pig
8	398	not relevant	Western Europe	no contact	no contact	no contact	266	cattle
9	398	unknown	Western Europe	dog	no contact	horse, poultry	249	poultry
10	398	unknown	no	no contact	no contact	no contact	605	cattle
11	282	Elderly care worker	no	no contact	no contact	horse, poultry, rabbit/guinea pig/hamster, sheep, goat	78	poultry
12	22	technician in animal food industry	Eastern Europe, Western Europe	bird, dog, mouse/rat, rabbit/guinea pig/hamster	horse, poultry	horse, pig, poultry, rabbit/guinea pig/hamster	184	horse and pig[a]
13	30	healthcare worker, GP assistant	Eastern Asia, Western Europe	dog, rabbit/guinea pig/hamster	no contact	no contact	332	cattle
14	30	business development in poultry industry	Latin America, Western Europe	no contact	no contact	horse, cow, sheep	409	cattle

[a]Two separate farms exactly the same distance from the participant's residential address.

who carried LA-MRSA.[24] The fact that MRSA MC 398 carriers lived significantly closer to livestock farms than non-carriers, however, suggests transmission through environmental exposure. More research is necessary to assess risk factors for MRSA MC 398, including person-to-person transmission and environmental exposures possibly by contaminated dust in the air or on surfaces surrounding farms.

Most previous studies have used community-level livestock density as a marker of livestock exposure.[22,27,29] An advantage of the present study is that exposure to livestock and farms was estimated at the individual level and farmers and their family members were excluded. A study in Pennsylvania (USA) also calculated individual's exposure to livestock and found that proximity to swine manure application to crop fields and livestock farms was associated with MRSA and skin and soft-tissue infection.[30] In that study, farmers and their family members were not excluded. Contact with livestock has previously been identified as an important risk factor for LA-MRSA.[31] Farmers often live in livestock-dense areas and therefore the increased risk found in these areas might partly be attributed to the high percentage of persons with professional contact with livestock living in these areas.

Due to the low prevalence, our study lacked power to assess risk factors using multivariate logistic regression to control for possible confounders. However, additional bivariate analyses were performed to evaluate the strength of associations between distance to the nearest farm and MRSA MC 398 carriage. In all of the bivariate models that corrected for comorbidity, PPIs, travel and contact with farm animals, the distance to the nearest farm remained significantly associated with MRSA MC 398 carriage. However, it might be that other factors that were not assessed are also associated with MRSA, such as visiting horse riding schools, application of manure to local crop fields[30] or having a household member with occupational contact with livestock.[27] More research is necessary to assess these possible risk factors.

Strengths of the present study are the large sample size, the sensitive laboratory method used and the large amount of data collected by combining laboratory data with questionnaire data and data from the NIVEL Primary Care Database. Moreover, data on exposure to farms were available at the individual level in contrast to ecological studies, which only take into account density of farms in a specific area.

In conclusion, our study shows that the overall prevalence of MRSA was low. However, there seems to be a slight increase in the prevalence of MRSA among persons living in the vicinity of livestock farms. In addition, MRSA MC 398 carriers lived significantly closer to the nearest livestock farm than non-carriers, suggesting that living in the vicinity of livestock farms is a risk factor for MRSA MC 398 carriage, probably through environmental exposure. Despite the large amount of data collected, we could not identify how transmission of MRSA MC 398 from livestock farms into the general population takes place. More research on the contribution of several transmission routes is necessary in order to decide on public health measures to be taken.

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Transparency declarations

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