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MRSA

IN PIG FARMS

human epidemiology



Brigitte van Cleef

MRSA

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human epidemiology

Brigitte van Cleef
2016

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VRIJE UNIVERSITEIT

MRSA in pig farms: human epidemiology

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De Boelelaan 1105

door

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geboren te Oss

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Chapter 1

Introduction



Chapter 1.1

General introduction



Background

Staphylococcus aureus (*S. aureus*) is a commensal bacterium for humans, and generally resides on skin and mucous membranes, like nares, pharynx, and perineum (**Figure 1.1.1**) (1,2). In cross-sectional studies, about one in three persons carries *S. aureus* in the nose; the nose is thought to be the most prevalent carriage site. When tested in longitudinal studies, about 20% of individuals carry *S. aureus* persistently in their nose (1,2). The prevalence of non-carriers varies between 20% (2) and 50% (1). The remaining 30% to 60% are intermittent carriers.

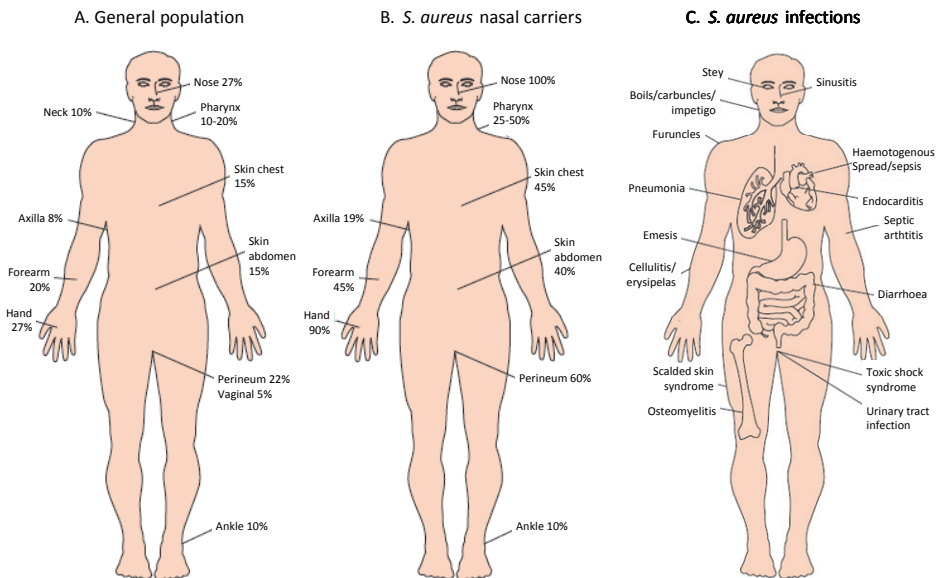


Figure 1.1.1 *S. aureus* carriage rates and infections per body site. Reprinted with permission from Wertheim *et al.* 2005 (1).

The most important transmission route from one person to another is by direct hand contact, where nose-picking probably plays a role (3), or via airborne transmission and contaminated surfaces, since *S. aureus* is known to survive very well in dry environments (**Figure 1.1.2**) (4–6). Next to being a commensal bacterium, *S. aureus* is also known for its pathogenic potential, with a range of harmless skin infections like impetigo and furuncles to severe infections like sepsis, osteomyelitis and pneumonia (**Figure 1.1.1C**) (1). Persistent carriers are thought to have the highest risk of infection by *S. aureus* (7), whereas non-carriers appear to suffer more serious consequences when they experience a (nosocomial) *S. aureus* infection (8).

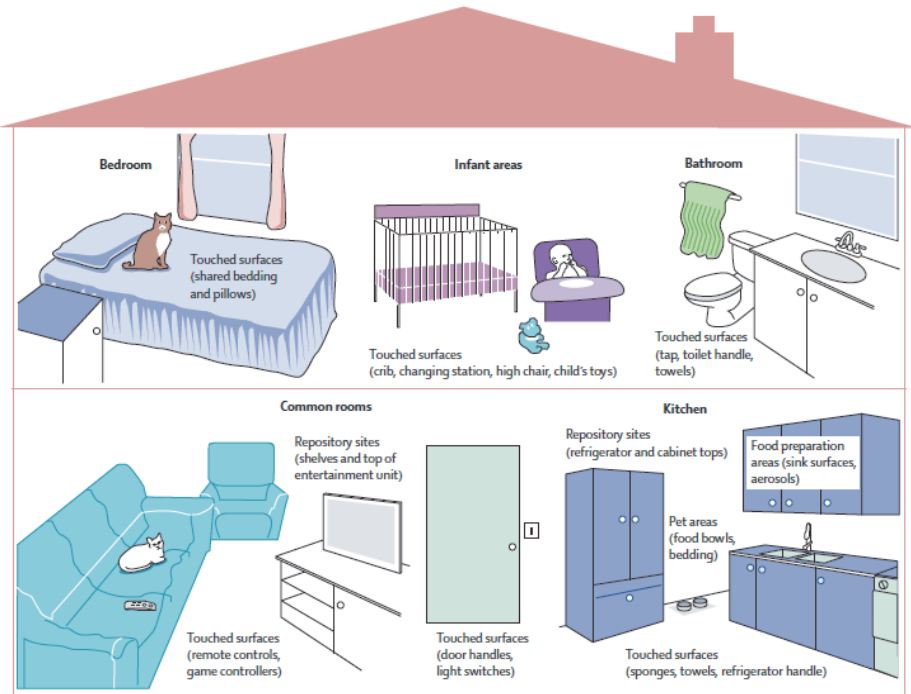


Figure 1.1.2 Potential points of transmission of staphylococci within households. Reprinted with permission from Davis *et al.* 2012 (5).

Shortly after the introduction of methicillin for the treatment of penicillin-resistant *S. aureus* infections in 1959, the first methicillin-resistant *S. aureus* (MRSA) was reported (1961) (9). At first the problems concentrated in hospitals (hospital-associated MRSA: HA-MRSA), however, in the last decades MRSA caused infections in the community in relatively young and previously healthy persons as well (community-associated MRSA: CA-MRSA).

Due to a strict 'search and destroy' (S&D) strategy and restrictive antibiotic use in the Netherlands (**Figure 1.1.3A**), the prevalence of MRSA in the general population has remained below 0.2% (10), which is low compared to other countries (**Figure 1.1.3B**). This S&D strategy comprises an active screening regimen of selected risk groups when entering hospitals, for example persons recently admitted to foreign hospitals or persons previously found MRSA positive (search), and decolonization where possible with antibiotics and disinfectants (destroy). For complete Dutch guidelines, see www.wip.nl.

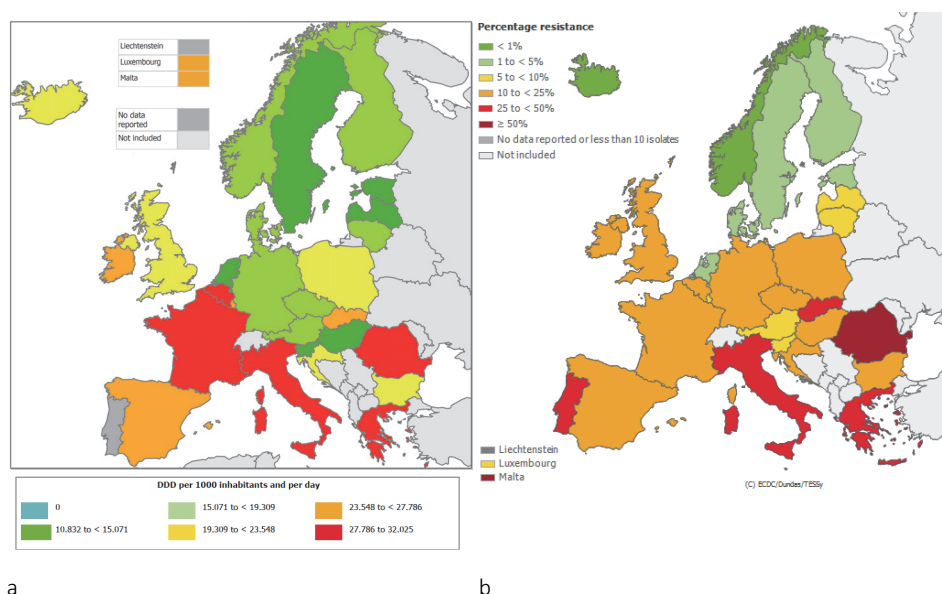


Figure 1.1.3 A. Consumption of antibiotics for systemic use in the community in Europe in 2013, expressed in DDD per 1000 inhabitants per day. Source: ESAC-Net interactive database, <http://ecdc.europa.eu>, accessed April 2015.

B. Proportion of MRSA in *S. aureus* blood cultures in Europe in 2013. Source: EARS-Net interactive database, <http://ecdc.europa.eu>, accessed April 2015.

In 2003 the association of MRSA with livestock was first reported in the Netherlands in a standard pre-operative screening of a 6-month old girl without known risk factors for MRSA (11). Both of her parents, who were pig farmers, were MRSA positive, as were some of their pigs. Subsequent investigations revealed a huge reservoir of MRSA in pigs and veal calves (12,13), which consisted of a highly clonal population of MRSA (14,15), which was later described as livestock-associated MRSA (LA-MRSA). Pig farmers and pig veterinarians were frequently carrying these strains, with an initially reported prevalence of approximately 25% (11). This is considerably higher than the estimated general MRSA prevalence of 0.1% in the Netherlands (10).

In the Netherlands, the S&D strategy was modified during 2006; persons in contact with live pigs and veal calves were added as a risk group and actively screened upon admission to the hospital. This resulted in a large rise in the number of MRSA isolates found each year, signifying the magnitude of this phenomenon (Figure 1.1.4). After the initial findings in the Netherlands, other countries reported LA-MRSA in pigs and persons in contact with pigs as well, indicating an international problem (16–19).

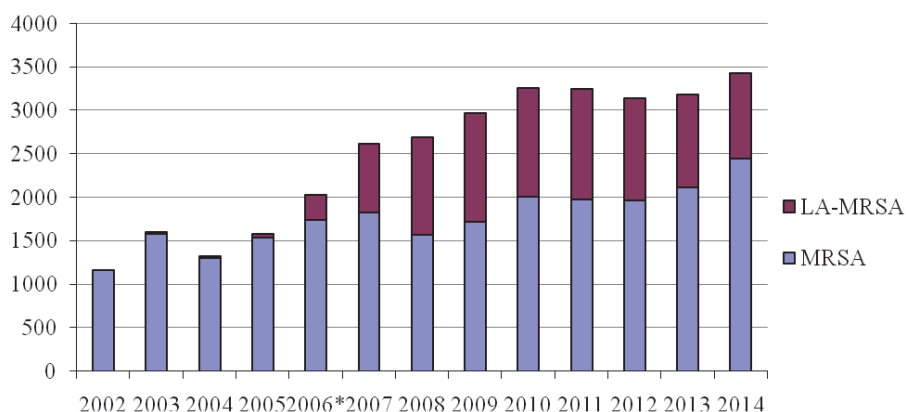


Figure 1.1.4 Number of isolates per year in the Netherlands, and the proportion of LA-MRSA isolates. * In 2006 the search and destroy strategy was modified, including pig and veal farmers as a risk group. Reprinted with permission from Haenen *et al.* 2012, 2014, 2015 (20–22).

Aims of this thesis

The research described in this thesis aimed to gain more insight into the prevalence, determinants, spread, dynamics and public health threat of LA-MRSA carriage in persons in contact with pigs.

Outline of this thesis

Part 1 is introductory, containing this general introduction to LA-MRSA, and a more thorough description in **Chapter 1.2**, reviewing history, evolution and prevalence of MRSA in general and in food animal production, indicating a potential threat to public health from LA-MRSA. This chapter indicates knowledge gaps, where further studies are needed, and stresses the importance of collaboration of human and veterinary scientists.

Part 2 describes the prevalence of LA-MRSA in different groups of people, and is divided into five chapters. **Chapter 2.1** studies the prevalence of and determinants for MRSA carriage in 50 pig farmers and their household members in the Netherlands. **Chapter 2.2** elaborates on 249 employees of three large Dutch pig slaughterhouses. **Chapter 2.3** describes the spread of LA-MRSA to 534 persons without pig contact, using a random sample of inhabitants of three pig-dense municipalities in the Netherlands. If LA-MRSA had spread to persons without pig contact,

we expected to find it first in these districts. In **Chapter 2.4**, 40 field workers are studied, who had intense pig or veal calf contact for a short duration, providing an excellent opportunity to study the risk of LA-MRSA acquisition after short-during animal contact. Lastly, **Chapter 2.5** describes the prevalence of LA-MRSA in different European countries.

Part 3 illustrates the clinical impact of MRSA and LA-MRSA in the Netherlands, based on blood cultures. In **Chapter 3.1**, the occurrence of MRSA in blood cultures from surveillance systems was compared between the Netherlands and North Rhine-Westphalia in Germany. **Chapter 3.2** investigated the public health problem of MRSA in general and LA-MRSA in specific, using the extrapolated incidence of *S. aureus* bacteraemia episodes in the Netherlands. **Chapter 3.3** studied the consequences of LA-MRSA carriage on health and health-related quality of life in pig farmers.

Part 4 consists of four chapters, describing dynamics of LA-MRSA carriage in pig farmers and their household members. **Chapter 4.1** is an international pilot study, where persons from 12 pig farms in Belgium, Denmark and the Netherlands (four farms per country) were studied and compared, and possible determinants and sampling methods were explored. Another preparation for larger studies is described in **Chapter 4.2**, where self-sampling of nose and throat for the presence of *S. aureus* was validated in 105 nursing and technical hospital personnel. **Chapters 4.3** and **4.4** describe a large longitudinal study, where persons working at or living in 49 pig farms were prospectively followed for one year. Dynamics and determinants of LA-MRSA carriage were studied using various sampling techniques, typing methods and statistical analyses.

The last part of this thesis, the *General Discussion*, discusses the most important results of the previous parts, focusing on dynamics of LA-MRSA carriage. The epidemiologic studies presented in this thesis give handles for interventions and recommendations for the future, aimed at controlling the public health threat of LA-MRSA.

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Chapter 1.2

Transmission of methicillin resistant *Staphylococcus aureus* from food production animals to humans: a review

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CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 2008 3, No. 095



Abstract

International surveillance of antimicrobial use in food animal production shows that methicillin-resistant *Staphylococcus aureus* (MRSA), traditionally a human pathogen associated with hospitals, has emerged in the community and animals. Since 1961, MRSA has been causing human infections in hospitals worldwide and a vast majority of them were caused by five major epidemic clones. After 1990, other clones have emerged in the community, leading to infections in relatively young and healthy individuals. The origin of these clones is largely unknown, and extensive diversity among isolates exists. Companion animals have been indicated as a reservoir. However, most studies suggest that they are initially infected by humans and subsequently animals re-colonize humans. More recently, a new zoonotic reservoir in food production animals was found. This involves a specific clone, MRSA ST398, which spreads extensively in animals and is also found in retail meat. It poses a potential threat to public health, as people in contact with food production animals are at much higher risk of colonization. The most probable transmission route seems to be by (in)direct contact, as dust in stables was found positive for MRSA ST398. The role of MRSA ST398 as a food pathogen needs more research. To prevent colonization in humans, it is important to investigate transmission routes and transmission dynamics between animals, between animals and humans and between humans. Collaboration of human and veterinary epidemiologists and microbiologists is needed to identify the implications of this strain for public health and to develop cost-effective control strategies.

Introduction

Antimicrobial resistance is a public health issue of growing concern. The use of antimicrobials can lead to development of antimicrobial resistance in bacterial species [1,2]. Antimicrobial use in food animal production may become a public health issue when resistant organisms or their resistance genes spread from animals to humans by (in)direct contact or through the food chain [3,4]. Surveillance of antimicrobial resistance in human and veterinary pathogenic and indicator bacteria intends to reveal trends in the evolution of resistant organisms [5]. An important, traditionally human pathogen, methicillin-resistant *Staphylococcus aureus* (MRSA) is currently causing a pandemic in hospitals around the world and is also emerging in the community [6]. For example, in the USA, MRSA was responsible for an estimated 94 000 life-threatening infections and 18 650 deaths in 2005, which is more than the 16 268 deaths caused by HIV/AIDS in that same period [7,8]. Recently, MRSA has been identified in food production animals and people in contact with these animals [9]. This involves a specific clone, multilocus sequence type 398, which seems to spread extensively among animals [10-13]. The finding of this new zoonotic reservoir of MRSA has led to several research initiatives to investigate its implications. This paper intends (1) to describe the evolution of MRSA in general and specifically of the ST398 strain and (2) to review the transmission between animals and humans in order to assess its impact on veterinary and public health.

Antimicrobial resistance and food animal production

Resistance of bacteria to a particular antimicrobial agent can be mediated by a pre-existing phenotype in natural bacterial populations or by acquired resistance. Two genetic mechanisms are involved in acquiring and disseminating resistance: (1) *de novo* mutations or (2) horizontal transmission of resistance genes between individual bacteria or between bacterial species. Resistance acquired through either mechanism is subsequently transmitted, and the frequency of resistance in populations may increase as a result of selective advantage under the pressure of antimicrobial use [1,14].

Antimicrobial agents are widely used in humans, animal husbandry and other agricultural activities. Since any use of antimicrobial agents can result in the selection for resistance, antimicrobial usage in animals has contributed to the development of resistance in bacterial species [4,15,16]. Transmission of resistant bacteria from farm animals to humans can occur not only by (in)direct contact, but also through food products of animal origin [17-19]. If these are zoonotic pathogens, this can lead to human disease with potential treatment failure. Additionally, resistant bacteria can

transfer their resistance genes to other bacteria belonging to human commensal flora [1,20].

Modern food animal production has high production levels, high stock densities and small profit margins. An environment has been created where infectious disease can have disastrous consequences. Rapid dissemination of pathogens is facilitated by the high contact rate between animals and by animal transport. To prevent outbreaks of infectious diseases, hygiene measures are being improved, management is optimized and vaccines are applied. Despite all these interventions, the use of antimicrobials in food production animals is often inevitable. Antimicrobials are not only used to treat diseases, but antimicrobials are also applied strategically to prevent infections and, in several parts of the world, as growth promoters [3,4,21].

Although the evidence for resistant bacterial infections in humans as a consequence of antibiotic use in food-producing animals is sparse, there is a general belief that prudent use of antimicrobials in food animal production should be given high priority. In 1969, the Swann Committee had already made this recommendation to the British government [22]. Only after increasing reports of resistant zoonotic human infections, antimicrobial growth promoters (AGPs) were phased out in Sweden, Norway and Denmark in 1986, 1995 and 1998-99, respectively, followed by a total ban from January 2006 onwards in all countries of the European Union [23]. Experiences in the Scandinavian countries are promising; despite an initial increase in antimicrobial use for therapeutic reasons, the total use of antimicrobial agents declined substantially after the ban on AGPs [24-26].

The increased attention being given to antimicrobial use in food animal production has led to the creation of several national and international surveillance systems [27-32]. In general, these surveys collect and present yearly data on usage of antimicrobial agents and the occurrence of resistance in bacteria from animals, food and humans. Most surveys discriminate between three groups of bacteria: (1) indicator bacteria, e.g. enterococci and *Escherichia coli*, (2) zoonotic bacteria, e.g. *Salmonella* spp. and *Campylobacter* spp., and (3) human pathogenic bacteria, e.g. staphylococci and streptococci. A finding in these reports is that MRSA, traditionally a human pathogen associated with hospitals, has emerged more and more into the community and also into animals in the last decades, which implicates a new zoonotic reservoir of MRSA [5,30,32,33].

Colonization and infection with *S. aureus*

S. aureus is a Gram-positive, coagulase-positive coccus in the family *Staphylococcaceae*. Staphylococcal species occur worldwide as commensal colonizers of the skin of animals and humans. They are additionally found on mucous membranes of the upper respiratory tract and lower urogenital tract and transiently in the digestive tract. Staphylococci are resistant to dehydration and are stable for months in the environment [34].

It is important to note that a distinction must be drawn between colonization and infection by *S. aureus* and its methicillin-resistant variant. Colonization with *S. aureus* may occur on mucous membranes of the respiratory and/ or intestinal tract, or on other body surfaces, without causing disease or harming their hosts [17,35]. Some individuals are colonized transiently and some persistently [36]. Colonization with *S. aureus* usually precedes infection, and is mostly caused by the same subtype [37,38]. The prevalence of nasal colonization with *S. aureus* among the human population is relatively high (>24%), while the prevalence of nasal colonization with MRSA among the same group is low (<1.5%) [39,40]. When the opportunity arises, *S. aureus* can contaminate wounds, bloodstream or other tissues, causing serious and even life-threatening infections [34, 41-44]. A US study on *S. aureus* infections in patients reported an increase in prevalence from 0.74% in 1998 to 1.0% in 2003 [44].

Infected and colonized human individuals constitute a major reservoir of *S. aureus*, and the primary route of *S. aureus* transmission seems to be direct contact with infected or colonized individuals [17,45,46]. However, environmental spread may be a substantially underestimated route for *S. aureus* transmission in hospitals [47,48].

From *S. aureus* to MRSA

Soon after the introduction of methicillin in 1961, the first MRSA was described [49]. This resistance is present when *S. aureus* has acquired the *mecA* gene, which codes for a variant of the penicillin-binding protein (PBP), PBP2a. PBP, normally present at the cell membrane of *S. aureus*, is bound by penicillin, and consequently cell membrane synthesis is discontinued, resulting in bacterial death. However, PBP2a has a reduced affinity for betalactam antibiotics, leaving the cell membrane intact and the organism alive [50].

The *mecA* gene resides on a mobile genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*) [51]. SCC*mec* contains a *mec* complex, which includes the

mecA gene and one or two regulatory genes, and a cassette chromosome recombinase (*ccr*) gene complex, which regulates the insertion and excision of the cassette into the bacterial chromosome. So far, five different *mec* complexes and three different *ccr* genes have been described, combining into five different SCC*mec* types [51-53].

The origin of the SCC*mec* element is unknown. SCC*mec* elements have been described in methicillin-resistant coagulase-negative staphylococci, although not in other genera. A homologue of the *mecA* gene is found in the species *Staphylococcus sciuri*, which occurs in animals. Most likely the *mecA* gene evolved out of a recombination of a normal PBP gene and an inducible β -lactamase gene [54,55]. The most acceptable theory is that the *mec* and *ccr* genes were combined in coagulase-negative staphylococci (most likely *Staphylococcus epidermidis*). Subsequently, a deletion in the *mec*-regulatory genes took place and the SCC*mec* complex was acquired by a methicillin-susceptible *S. aureus* (MSSA), creating the first MRSA [56-58].

The evolution of MRSA

The investigation of the evolution of MRSA relies on typing methods as tools for the characterization of and the distinction between different isolates. The historically applied phenotypic methods have their limitations and are now out of favour because of newly developed genotypic methods, which usually provide better discriminatory power [59,60].

Molecular typing methods

S. aureus isolates, including MRSA, can be typed using several molecular methods. The most important typing methods include pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), amplified fragment length polymorphism (AFLP), SCC*mec* typing and staphylococcal protein A typing (*spa* typing). The most appropriate method to use depends on the intended purpose; a combination of different techniques is often used [60,61].

For PFGE, genomic bacterial DNA is digested using *Sma*I (a restriction enzyme). Different strains are typed by comparing the presence and length of digestion products using gel electrophoresis. PFGE is a highly discriminatory method, and is very useful in outbreak situations, but reproducibility and inter-laboratory agreement are insufficient to be useful for long-term epidemiological surveillance [61-63].

With MLST, the allelic profile of seven housekeeping genes can be summarized in a sequence type (ST), a group of strains with seven identical loci. These housekeeping genes are highly conserved, generating a method very useful in the study of clonal evolution [64-66]. With 'based upon related sequence types' (BURST) analysis, different

STs can be grouped; all the strains in one clonal complex (CC) share at least five out of seven housekeeping genes. The CC is numbered after the ST that gave rise to it, the clonal ancestor. Single locus variants (SLVs) are strains with only one locus different from its ancestor [67] (details available from <http://www.mlst.net>). MLST provides a combination of discriminatory power and clonal stability, which makes its results unambiguous and easily exchangeable between laboratories [66,68].

The AFLP technique is based on the amplification of subsets of genomic restriction fragments using polymerase chain reaction (PCR) and documents nucleotide sequence variation, insertions and deletions across the entire genome. Results of AFLP are easy to obtain and highly reproducible, and the obtained typing patterns seem to cluster according to the *SCCmec* types of the strains [63,69,70].

Typing of the *SCCmec* element is done in various ways. The most appropriate method has not yet been determined [60,71-73].

In *spa* typing, one single locus (staphylococcal protein A) is sequenced, making interpretation of the results very simple and exchangeable, also internationally. The discriminatory power of *spa* typing is in between PFGE and MLST. Therefore, it is suitable for both outbreak situations and evolutionary studies [74-76].

Molecular evolution of MRSA

Two theories exist on the molecular evolution of MRSA. The *single clone theory* states that all MRSAs have one common ancestor; the *SCCmec* element was introduced into SA only once [77]. The *multiclonal theory* states that *SCCmec* was introduced multiple times in different SA lineages, after which horizontal spread and recombination were important mechanisms of resistance transmission [78,79]. Using the different techniques stated above, the theories have been tested and the following can be concluded: (1) prevalent MSSA strains that were successful in causing disease on a global scale have evolved into MRSA on multiple, but independent occasions, (2) horizontal transfer of the *SCCmec* has occurred a limited number of times compared with other bacteria and (3) clonal spread after acquiring the *SCCmec* appears to be the most important mechanism of dissemination of resistance [57,64,80,81].

Since the first human MRSA was isolated in 1961, at least five major clonal types (CC8, CC5, CC45, CC22 and CC30) of MRSA have been identified using PFGE [82-84]. The five types predominantly harbour *SCCmec* type I, II or III and are often multidrug-resistant [85]. These clones are responsible for the vast majority of MRSA infections in hospitals all over the world. MLST revealed two distinct ancestors for these five, so-called, epidemic clones [58].

Besides the epidemic clones, there are also clones that occur only in single hospitals or even only in single patients (sporadic isolates) and isolates that cause infections in the

community (community-acquired (CA) isolates) [82,86-88]. The characterization of these clones revealed extensive diversity among isolates. Several studies observed strong similarities between sporadic isolates and CA-MRSA, which implies that MRSA strains described as CA may actually originate from hospitals [82,85,87]. CA-MRSA isolates frequently carry *SCCmec* IV or V, are susceptible to a limited number of antimicrobials and may contain additional virulence factors [88-90]. *SCCmec* IV and V are much smaller than *SCCmec* I, II and III, which may lead to a more efficient transfer of the element between bacteria and less fitness cost in everyday metabolism [51,52,55,84]. With this greater ability for transmission and virulence, MRSA clones in the community might be an even larger threat to patients and healthcare workers than hospital-acquired clones. Until now, resistance to more than just betalactam antibiotics is relatively infrequent in these community clones, but future mutations or gene transfer may change this [47-49].

Just a few studies have been done on molecular characterization of (MR)SA in animals, which hampers the investigation of their relatedness and origin. The fact that *S. aureus* is found in different animal species makes it even more difficult, since information on (MR)SA in one species might not be comparable with information on (MR)SA in another species. Several human MLST types have been found in companion animals, suggesting interspecies transmission [91-94].

Recently, an MRSA strain was detected that appeared not typable by PFGE, owing to resistance to *Sma*I digestion [95]. These isolates predominantly originate from livestock and are found in humans as well. This strain belongs to MLST type 398 and harbours *SCCmec* IV or V, is mostly associated with *spa* types t034, t011, t108, t567, t571 and t899 and does not contain toxin genes [9,10,96-98]. Different antibiotic resistance patterns have been found, which seem to be correlated with the antibiotics used in animal practice, predominantly tetracyclins [89,96]. MRSA ST398 might originally have been a highly prevalent strain of MSSA among livestock that acquired *mecA* from other staphylococci that colonize pigs [20,91]. The fact that several different *SCCmec* elements have been found in MRSA ST398 suggests that this event must have occurred on several occasions.

MRSA in humans

Infections in humans with so-called hospital-acquired (HA)MRSA were confined to patients with established risk factors, including recent hospitalization, admission to an Intensive Care Unit, surgery, exposure to individuals who are colonized or infected with MRSA and prolonged antibacterial therapy [99-101]. However, a modelling study

showed that in low-prevalence countries, outbreaks of HA-MRSA might also be initiated by strains that circulate (without clinical signs) among the general population [102].

Worldwide, clinical cases of MRSA are increasing over the years. In the USA, MRSA prevalence among SA isolates in hospitals has increased from 2.4% in 1975 to 29% in 1991 [103]. Between 1992 and 2003, the percentage of SA isolates from patients in US Intensive Care Units that were methicillin-resistant rose from 35.9% to 64.4% [104]. In England and Wales, the percentage of SA bacteraemia caused by MRSA increased from 1–2% in 1990-1992 to approximately 40% in 2000 [105]. The European Antimicrobial Resistance Surveillance System (EARSS) monitors antimicrobial resistance continuously in most European countries. The proportion of methicillin resistance in invasive SA isolates varies largely across Europe, with the highest proportions (>40%) in Southern Europe and parts of Western Europe and the lowest proportions (<1%) in Northern Europe. MRSA in low-prevalence countries (<3%) was most frequently related to patients with a recent history of being admitted in a foreign hospital. The prevalence of MRSA in clinical isolates in these low-prevalence countries remained relatively stable over time. However, since 1999 small increases were found in the Netherlands (0.34-0.93%), Denmark (0.28-1.70%) and Finland (0.95-2.91%) (data available from <http://www.rivm.nl/EARSS>).

In the last two decades, several reports appeared about MRSA infections in healthy individuals in the community without healthcare-associated risk factors [6,89,106]. Such infections are referred to as CA and are distinct from HA-MRSA infections in terms of genetic background, epidemiology, clinical spectrum and antibacterial resistance [89,107]. Several definitions have been proposed, but no standard definition has been created for CA-MRSA yet, which makes the overall prevalence of CA-MRSA hard to ascertain [108]. However, according to several studies, MRSA infections in healthy people without the established risk factors seem to be increasing in frequency [109-111]. Children, elderly people and people in groups with close physical contact, e.g. US football teams and military recruits, seem to be at risk [41,112-116]. Food-borne infections occur as well, where the source might be the food animal itself or the food-processing person or equipment [18,117-119].

Current control strategies in countries where the spread of MRSA is still under control, such as the Netherlands and Scandinavia, have focused on the healthcare sector and are based on active surveillance for detection of MRSA carriers among patients ('search'), and isolation of colonized and infected patients and decolonization therapy ('destroy') [120,121]. Decolonization consists of topical and systemic administration of antimicrobials, and although protocols have been developed that can eradicate MRSA, re-colonization can occur [122,123]. As MRSA is able to survive for weeks or months in the environment, decontamination is a very important component of the control strategy [45,48,123]. In the low-prevalence countries, this so-called search-and-destroy

strategy prevents MRSA from becoming endemic [40]. However, the same strategies have failed in the past in countries where MRSA is endemic at a high level [124]. Recent findings suggest that effective control should be possible by stringent implementation of this search-and-destroy strategy, even in countries where MRSA already is an important nosocomial pathogen [102].

The increasing frequency of MRSA infections in the community implies an adjustment of the guidelines for the 'search' part of existing control strategies as the 'at risk' groups are expanding.

MRSA in animals

The isolation of MRSA from animals was first reported in 1972 following its detection in milk from mastitic cows [125]. Since then, MRSA has been isolated from many different animal species, including dogs [126], cats [43], horses [127], sheep [128], pigs [9], dairy cows [129], veal calves [12,130] and poultry [118,131,132]. The increasing number of publications on MRSA in, mainly, companion animals and horses was reviewed by Leonard and Markey [133] and they suggest that MRSA may be an emerging pathogen in these species. However, data on MRSA in food production animals were not reviewed. A distinction should be made between food production animals, which are housed in an industrialized way at high stocking densities, and animals that are, predominantly, kept for companionship and leisure purposes.

In food production animals, a new strain of MRSA (ST398) turned up recently [97,130,131], whereas companion animals most often are colonized or infected with classical human strains of MRSA [134-136].

In pigs, MRSA ST398 seems to spread extensively. Dutch studies report prevalences of positive farms varying from 23% to 81%, whereas the prevalence in individual pigs varies from 11% to 39% [10,98,137]. After the initial findings in The Netherlands [9,10,97], also Belgium [138], Denmark [139], Germany [140], France [91], the USA [141] and Canada [11] reported the occurrence of MRSA in pigs.

In veal production, high prevalences of MRSA ST398 were found as well: 88% of the farms and 28% of the calves tested positive [12]. Furthermore, a human case report on MRSA ST398 linked to poultry and the finding of MRSA ST398 in SA isolates from poultry has been published [131,132]. This, however, has not yet been confirmed in larger surveys.

Studies on risk factors for MRSA infections in animals have been performed for small animals and horses admitted to veterinary hospitals. Similar to the factors in human cases, prolonged hospitalization, surgery, contact with MRSA colonized individuals and antimicrobial use were found to be significant factors [142-144]. Preliminary results of

studies on pig farms reveal that farms where finishing pigs are present were more often MRSA-positive than farms without finishing pigs and that MRSA seems to spread downwards through the pig production chain by the purchase of positive pigs [98,137,145]. Few studies have investigated dust inside pig stables. In these studies a strong association between results from animal samples and environmental samples was found, which might indicate the possibility of spread of MRSA ST398 between animals within a farm by air [137,138]. However, so far no controlled studies on population dynamics and risk factors for MRSA in farm animals have been published. Just a few studies on eradication and control of MRSA in pets and horses have been conducted [143,146]. The British Small Animal Veterinary Association has drawn up guidelines on how to manage MRSA (available from [http:// www.bsava.com](http://www.bsava.com)). These are all control measures and guidelines for individual veterinary hospitals and practices. No studies, however, have been conducted to test these strategies on livestock farms so far.

Transmission between humans and animals

The role of animal populations in the transmission of pathogens to humans is obligatorily dependent not only on the possibility of transmission from animals to humans, but also on the possibility of transmission between animals.

Dogs and cats were initially indicated as a reservoir for MRSA years ago [147,148]. The strains involved in canine and feline cases are usually similar to those infecting humans, and the most obvious explanation for this veterinary problem is that the pets acquired the resistant bacteria from humans and that these strains can also be passed back from animals to humans [134,136,144]. This does not necessarily imply that companion animals are a reservoir.

In several studies, MRSA was isolated from horses and horse personnel. All the isolates appeared to be identical or closely related and differed from common human isolates [127,149]. Therefore, MRSA in horses has zoonotic potential. However, quantitative risk assessment of this reservoir has not been done.

Several studies on pig farmers showed a significant higher risk for *S. aureus* carriage in this group. Nasal *S. aureus* colonization was found significantly more often in French pig farmers (45%) than in non-farming controls (24%), suggesting transfer of these bacteria from farm animals to farmers [20,91]. Dutch pig farmers and veterinarians were screened at different occasions and showed prevalences of MRSA carriage of >20% and 4.6%, respectively, whereas the prevalence in the general population upon hospital admission in The Netherlands is 0.03% [9,40,150,151]. Moreover, it was found that the density of MRSA ST398 in a particular area corresponds to the density of pig farming,

whereas the density of other MRSA corresponds to the density of the human population [96]. An international study among pig veterinarians revealed an overall prevalence of 12.5%; MRSA carriers originated from nine different countries [152].

Hospital surveys that investigated the association of non-typable MRSA in human patients with a reservoir in animals identified cattle as another source for human carriage, next to pigs. Carriers of MRSA ST398 were more often people in contact with pigs or cattle than carriers of other strains of MRSA [96,153]. Surveys on Dutch pig farms supported the finding that contact of humans with the animals is an important risk factor for human MRSA colonization. The intensity of contact was strongly associated with increased prevalence. People with intensive contact with pigs were more often MRSA carriers (29%) than people who lived on these farms and had no contact with the animals (2%) [151]. The association between intensity of human-animal contact and MRSA prevalence of humans was confirmed on veal farms, although no exact figures have been published yet [12].

So far, just a few studies reported clinical infections in humans caused by MRSA ST398 [154–156]. Nevertheless, the first hospital outbreak caused by MRSA ST398 has already been described [157]. To investigate the differences between typable and non-typable MRSA, a limited hospital survey was performed. The frequency of an infection caused by MRSA was higher in patients who carried a typable strain than in patients carrying a non-typable strain: 42% and 13%, respectively. For typable MRSA, the number of secondary cases was 22 out of 2139 contact persons (1%), whereas, for non-typable MRSA, no secondary cases were found in the 408 contact persons [153]. These findings might indicate that transmission between humans is less likely and that this clone is less adaptive for humans than it is for animals.

The presence of MRSA ST398 in environmental swabs indicates that direct contact with animals is not necessarily needed for transmission to humans [137,138,151]. Based on the above-stated findings in pig and veal farming, people who are professionally in contact with live pigs and veal calves are now included in the high-risk group for the Dutch MRSA ‘search-and-destroy’ policy in hospitals (available from <http://www.wip.nl>). Other food production animals are reported to be zoonotic sources for MRSA as well. A recent Dutch case report suggests that poultry may be a source of human MRSA infection. People living on a poultry farm and chicken droppings from the same farm were positive for an identical MRSA strain [131].

In the past, SA strains isolated from mastitic cows and from human infections were generally found to be different strains [158]. However, a recent Hungarian study has found clear evidence that certain MRSA strains can be passed between cattle and humans [129].

Studies on MRSA in meat products demonstrate that MRSA originating from animal sources has entered the food chain [118,158–160]. MRSA was found in meat products

originating from animals. A recent survey of the Dutch Food and Safety Authority in almost 1300 meat products from retail showed a prevalence of 11%. The highest prevalences were found in products of turkey (31%), chicken (27%) and veal (17%), whereas 10% of pig products were positive [160]. So far, the risk for public health of eating MRSA-positive meat is assumed to be of minor importance [30,159,160].

Summary of conclusions and recommendations

Over the past decades the epidemiology of MRSA has changed significantly. MRSA, traditionally a primarily nosocomial pathogen, has entered the community, causing serious infections. Additionally, MRSA infection and colonization has been documented in several animal species. Although several reports have presented information suggesting that animals may act as a source for zoonotic staphylococcal infections in humans, no transmission studies have been done for MRSA yet.

Recently, MRSA ST398, a novel clone linked to food production animals, has emerged in humans. Molecular typing methods support the relationship between this particular strain in food production animals and humans who have been in contact with these animals. From the animal reservoir, MRSA can be introduced into hospitals and serious infections and outbreaks may occur. Since farm animals are usually housed together in groups, frequent contact between group members is likely. As (in)direct contact is a major route for MRSA transmission between animals, the prevention and control of MRSA in food production animals should focus on the control of the spread of MRSA between animals within a farm and between farms rather than only controlling the organism in individual hosts. In order to design an effective intervention programme for decreasing the risk for public health, experimental and longitudinal research is needed to gain insights into the transmission dynamics of MRSA between animals within a farm and between farms.

Considering the huge spread of MRSA ST398 among food production animals, it is unlikely that this will be eradicated easily. To prevent the occurrence of disease in humans, it is important to investigate the transmission routes from animals to humans and from humans to humans as well. The most probable transmission route seems to be by (in)direct contact, but the role of MRSA as a food pathogen needs more research. Human microbiologists should investigate the pathogenicity and the capacity for transmission between humans of this particular novel strain to assess the potential threat for public health. At the same time, cooperation between epidemiologists and microbiologists in the human and veterinary field will be required to create a complete overview of all aspects of this problem and to develop cost-effective prevention strategies in both the human and animal populations.

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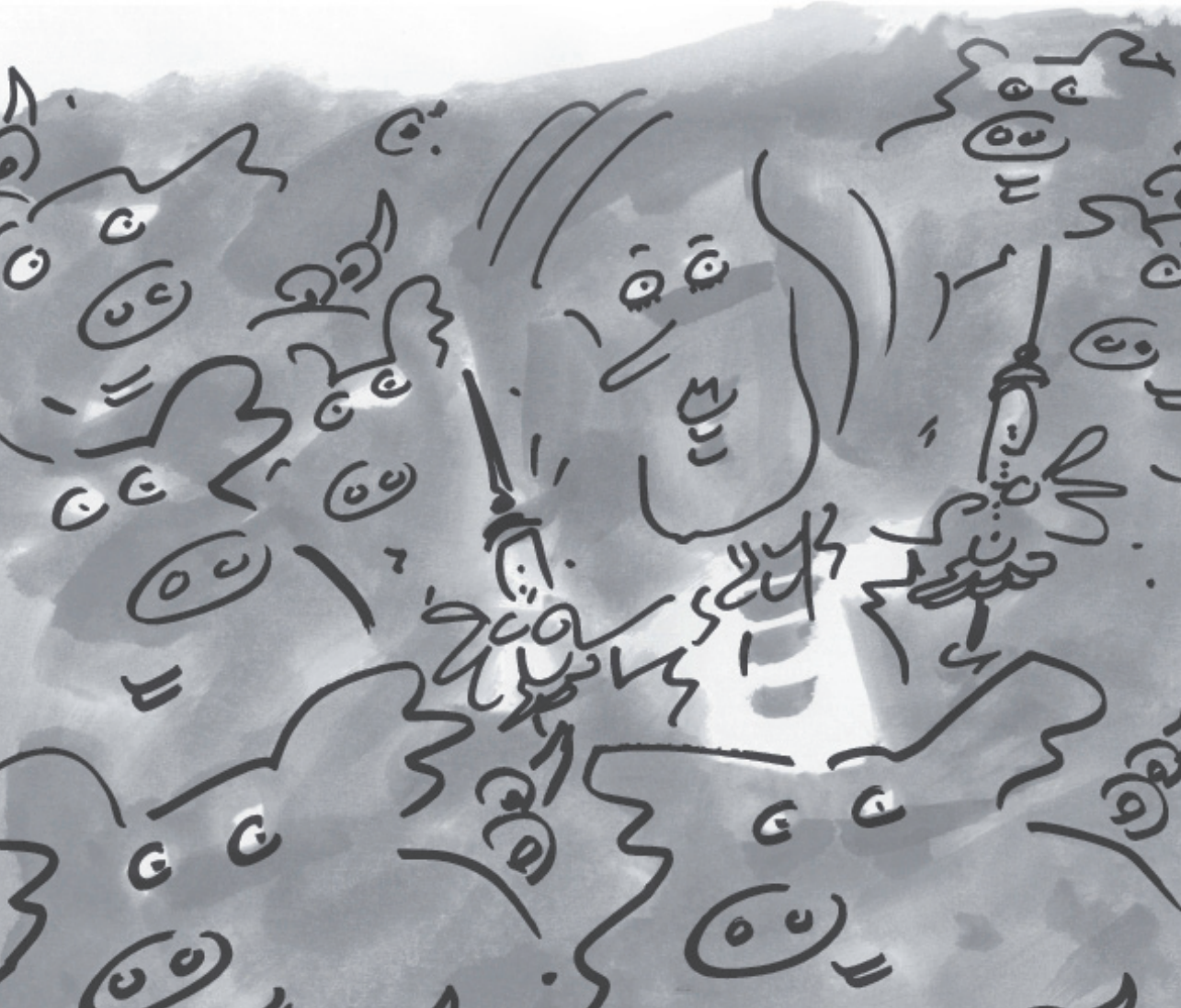
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Chapter 2

Prevalence of livestock-associated MRSA



Chapter 2.1

Methicillin-resistant *Staphylococcus aureus* in people living and working in pig farms

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Abstract

We compared the prevalence of human and animal methicillin-resistant *Staphylococcus aureus* (MRSA) at pig farms in the Netherlands, and related this to individual and farm-level characteristics. More than half of the farms investigated (28/50) had MRSA in pigs or stable dust and about one third (15/50) of person(s) were identified as MRSA carriers. Human carriage was found only on farms with MRSA-positive pigs or dust. MRSA strains in human samples were the same *spa*-type as found in pigs and all were not typable by pulsed-field gel electrophoresis (NT-MRSA). Multivariate analyses showed that risk factors for human MRSA carriage were: working in pig stables (OR 40, 95% CI 8-209) and the presence of sows and finishing pigs (OR 9, 95% CI 3-30). Veterinary sample collectors sampling the pigs showed transient MRSA carriage only during the day of the farm visit. Working in pig stables with MRSA-positive pigs poses a high risk for acquiring MRSA, increasingly so when contact with live pigs is more intensive or long lasting.

Introduction

The antimicrobial agent methicillin was introduced into clinical practice in 1960 and methicillin-resistant *Staphylococcus aureus* (MRSA) was described 1 year later [1]. Worldwide, MRSA is now responsible for considerable mortality, morbidity and healthcare expenditure both in hospital and community settings [2,3]. In the Netherlands MRSA has been controlled effectively in hospitals by a search and destroy strategy [4]. In 2006, 2% of the *S. aureus* invasive isolates in the Netherlands were resistant to methicillin, compared to 20-40% in surrounding countries [5].

The Netherlands was the first country to report patients with a specific MRSA variant associated with pigs [6]. This strain was not typable by pulsed-field gel electrophoresis (PFGE) and was therefore referred to as NT-MRSA [7]. Since then an increasing number of Dutch studies have reported NT-MRSA in people in contact with pigs, i.e. pig farmers and family, co-workers and veterinarians [8-11]. In a pilot survey, Voss et al. [6] found a 23% prevalence of NT-MRSA in 26 pig farmers. A study of pigs in slaughterhouses revealed that more than one third of them carried NT-MRSA [12]. Cattle (veal) farming was also identified as a risk factor for NT-MRSA carriage in humans [13,14].

Since mid-2006, pig farmers and their family members are screened for MRSA at ambulatory care or before hospitalization. As a result thereof, the proportion of NT-MRSA in the national surveillance increased by 20% in the second half of 2006 and by 30% in 2007 [13] (unpublished data, RIVM) and even a threefold increase was reported from a hospital in the south of the country [15]. Up to now, NT-MRSA has been found in France, Germany, Austria, Denmark, Belgium and Canada [16-21].

Despite published reports, no representative estimate of the prevalence of NT-MRSA on Dutch farms is available. Our objective was to obtain further insight into the prevalence and determinants of human carriage of NT-MRSA in relation to the presence of NT-MRSA in pigs on the farm.

Materials and methods

Selection of farms

A cross-sectional prevalence survey was conducted during the period January–October 2007. Farms were randomly selected from a complete list of pig farms in the Netherlands. On 50 farms we expected to include 140 persons, i.e. 50 farmers and their family (average Dutch family size 2.8), sufficient to estimate the MRSA point-prevalence, assuming 23% positivity [6], accepting a 5% risk of type I error with a precision of 10% and a design effect of 2 [anticipating clustering of cases ; calculation with Epi-Info 6.04 (CDC, Atlanta, GA, USA)]. The study protocol was approved by the medical ethical

committee of the Therapeutic Drug Evaluation Foundation (STEG, Almere, The Netherlands).

Sampling and questionnaires at pig farms

Procedures for human subjects

Written informed consent to participate in the research was obtained; for children aged <18 years parental consent was requested. A short questionnaire and nasal swabs (from both anterior nares) were taken by the research assistant visiting the farm. The questionnaire addressed individual factors such as age, sex, intensity of contact with pigs or other animals, potential other risk factors for MRSA carriage and self-reported medical history related to MRSA infection or (skin) problems.

Research assistants took their own nasal swabs just before they visited the farm, immediately after and the morning following the visit.

Procedures for animal data

Data on farm-related factors included size and type of farm, age groups of pigs present (sows, suckling piglets, weaned piglets, gilts and finishing pigs), measures of hygiene implemented, feeding method and housing characteristics. Pigs were sampled by nasal swabs and 60 samples were collected per farm, representative for the age group(s) of pigs present. These swabs were pooled into 10 pools of six swabs. Additionally, at each farm five environmental samples of dust were collected by wiping the top of the pen separations in different compartments of the pig-houses. Visits were separated by at least 1 day and limited to two farms per week.

Laboratory analysis

Samples were cultured at the hospital laboratory in Breda (human samples) and the RIVM laboratory (pig and dust samples). Samples were first enriched in Mueller–Hinton broth with 6.5% NaCl, followed by selective enrichment in Phenol Red mannitol broth with 75 mg/l aztreonam and 4 mg/l oxacillin or ceftizoxime. After culturing on sheep blood agar and MRSA screen agar (Oxoid, Basingstoke, Hants, UK), suspected colonies were confirmed by polymerase chain reaction (PCR) for the *S. aureus*-specific DNA fragment [22], the *mecA* gene [23] and the Panton Valentine leukocidin toxin genes [24].

For the human isolates, methicillin resistance was screened by a disk diffusion test using a cefoxitin disk and confirmed by the presence of the *mecA* gene by PCR. All MRSA strains were typed by *spa* typing [25] and strains in human samples not yet identified as NT-MRSA were checked for typability by PFGE [26]. Furthermore, in human samples,

susceptibility was determined for 21 antimicrobial agents with the VITEK system (bioMérieux SA, Craponne, France) according to the manufacturer's instructions.

Data analysis

Data were entered into Access, double-checked and verified with questionnaires and analysed with SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) and SAS version 9.1 (SAS Institute, Cary, NC, USA). A dataset was created with individual human results and aggregated animal and farm data. Summary variables were created, such as a score for 'personal hygiene in the stable' based on the following hygiene measures: separate entrance and exit, changing room, showers, water, soap, boots, overalls and disinfection footbath.

The relationship between MRSA presence in human and animal and/or dust samples was investigated by chi-square test. Risk factors for MRSA carriage were first identified by univariate logistic regression analysis. For further analyses we considered exposure to MRSA-positive pigs or dust a prerequisite or 'necessary cause' for MRSA positivity in humans and therefore further analyses were limited to data from farms where pigs or dust tested positive. Multivariate regression analysis by stepwise, forward entry included factors influencing MRSA positivity in humans ($P < 0.2$ in univariate analysis) and logical interaction factors thereof. A random cluster effect was included in the model to adjust for the fact that observations of humans on the same farm might not be independent.

Spa-types of human and animal MRSA samples were compared for each farm, including the results of the samples taken from the sample collectors pre- and post-farm visit. Antibiotic resistance patterns of human MRSA strains were compared for each *spa*-type by chi-square analysis.

Results

Altogether 106 farms were contacted until 50 farmers (47%) agreed to participate in the research. The response rate was similar in different regions and types of pig farms. The most common reasons for non-participation were no interest or time, or retirement from farming. A total of 232 people were sampled on 50 farms: 50 farmers, 171 family members and 11 co-workers. The individual participation on the selected farms was high: we collected swabs and questionnaires from 221/231 reported household members (96%).

MRSA status per farm

On 28/50 (56%) of the farms pig or dust samples tested positive (hereafter referred to as 'MRSA- positive farms') and on 15/50 (30%) of the farms one or more MRSA-positive persons were found. MRSA in humans was only found on MRSA-positive farms (Figure 2.1.1). On the 15 farms with MRSA-positive people, pigs were also positive with the exception of one farm; on this farm, however, dust samples were positive. The prevalence of MRSA in farm residents was the highest on farms with both sows and finisher pigs. Finishing farms were more often MRSA positive than farms with sows only, but only part of the positive finishing farms (38%) housed MRSA-positive people while on all MRSA-positive farms where sows were present, MRSA was also found in farm residents (see Figure 2.1.2).

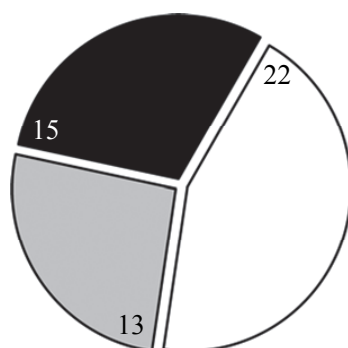


Figure 2.1.1 Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) per farm. Number of farms (total 50) indicated in segments. Farms with one or more MRSA-positive persons and MRSA in pigs or dust (black area); farms with MRSA in pigs or dust only (grey); farms completely MRSA negative (white).

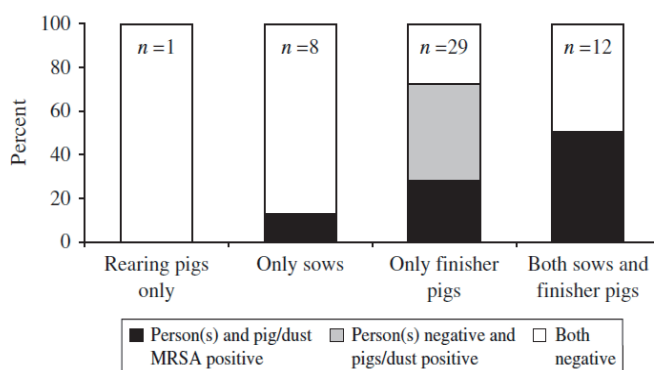


Figure 2.1.2 Proportion of farms with methicillin-resistant *Staphylococcus aureus* (MRSA)-positive people, pigs and dust compared to farms with or without sows and finisher pigs. Number of farms (total 50) indicated in bars.

MRSA status per person – univariate analysis

MRSA was identified in 33/232 people (14%). **Table 2.1.1** shows the relationship between MRSA positivity and the main potential risk factors (univariate analysis). At the individual level, living on a MRSA-positive farm was the most important risk factor. The higher the proportion of positive samples from pigs or dust per farm, the higher the positivity rate in people. The intensity of contact with pigs was another important determinant. In persons working with pigs on a regular basis, 29% (95% CI 20-38) carried MRSA whereas 12% (95% CI 3-29) of persons who did not work with pigs but entered the pighouse(s) at least once per week were positive. Only 2% (95% CI 0-6) of those who reported no contact with pigs were positive. On MRSA-positive farms, 49% of the people working with pigs harboured MRSA (95% CI 36-62).

At the farm level, the presence of sows related to a higher rate of MRSA in people. Several other factors were associated with MRSA positivity in humans, i.e. age, gender, sharing of towels, type of farm and number of pigs on farm. The presence of finisher pigs, number of new pig-batches recently received, and cleaning and disinfection measures in the pighouse did not influence MRSA rates.

No indication for other potential causes of MRSA was found. Four persons (on two farms) who were diagnosed with MRSA previously, proved negative at this sampling. Twelve family members worked in a hospital or nursing home but none of them carried MRSA. We did not find a relationship with recent hospitalization, team sports or presence of horses, cattle or other animals on the farm. MRSA appeared to be more frequent in people with skin problems ($P=0.06$), but these were only reported by a small number ($n=17$).

MRSA per person on positive farms – multivariate analysis

On the 28 positive farms 139 people were sampled and the incidence of MRSA was 24%. Multivariate regression analysis of the results from the positive farms determined two significant risk factors for MRSA carriage out of 12 factors identified as potential risk factors in the univariate analysis. These were 'intensity of contact with pigs on the farm' and 'presence of sows and finishing pigs' (**Table 2.1.2**). The random cluster effect of 'farm' was not significant.

Table 2.1.1 Human MRSA carriage of persons living on pig farms in relation to individual and farm-related characteristics on 50 pig farms in the Netherlands (left) and the on subgroup of 28 MRSA-positive farms (right), January-October 2007; univariate logistic regression analysis.

	Persons from all farms		Persons from MRSA-positive farms	
	Total	% MRSA+	Total	% MRSA+
Total	232	14.2	139	23.7
<i>Individual factors</i>				
Gender				
Male	125	20.8	74	35.1
Female	107	6.5	65	10.8
Age (yr)				
0–18	80	3.8	48	6.3
19–65	138	19.6	84	32.1
66–86	14	21.4	7	42.9
Contact with pigs				
Intensive	98	28.6	57	49.1
Minimal	25	12.0	14	21.4
No	109	1.8	68	2.9
Contact with cattle*	74	20.3	56	26.8
Skin problems reported*	17	29.4	11	45.5
Shared use of towels*	78	21.8	47	36.2
<i>Farm-related factors</i>				
MRSA-positive pigs on farm*	122	26.2	122	26.2
MRSA-positive dust on farm*	115	25.2	115	25.2
Percentage positive pool samples#				
No MRSA-positive pigs on farm	110	0.9	17	5.9
10–50%	30	13.3	30	13.3
60–100%	92	30.4	92	30.4
Percentage positive dust samples#				
No MRSA-positive dust found	117	3.4	24	16.7
20–60%	51	11.8	51	11.8
80–100%	64	35.9	64	35.9
Type of pigs present				
Both sows and finishing pigs	58	25.9	32	46.9
Only sows	40	12.5	9	55.6
Only finishing pigs	129	10.1	98	13.3
Only rearing pigs	5	0.0	0	0.0
Size of farm#				
Small (<400 sows+finisher pigs)	76	10.5	30	26.7
Medium (400–1000 pigs)	74	8.1	47	12.8
Large (>1000 pigs)	82	23.2	62	30.6
Personal hygiene in stable#				
Low	56	7.1	35	11.4
Intermediate	77	13.0	47	21.3
High	99	19.2	57	33.3

Percentages in bold are considered significant at $P < 0.05$ level. * MRSA rate where factor present (shown) compared to factor absent (not shown). # Divided into equal groups for optimal comparison.

Table 2.1.2 Risk factors for human MRSA identified by unilevel multivariate analysis, based on persons sampled at the 28 farms where pigs or dust samples were MRSA positive (n=139).

	OR (95% CI)	P value multivariate
Contact with pigs		
Intensive	39.9 (7.6-208.8)	<0.0001
Minimal	9.4 (1.2-73.5)	0.03
No	1.0	
Type of pigs present		
Both sows and finishing pigs	8.8 (2.6-29.9)	0.001
Only sows	3.5 (0.8-16.6)	0.11
Only finishing pigs	1.0	

OR, Odds ratio, CI, Confidence interval. P values in bold are considered significant at $P < 0.05$ level.

Genotyping MRSA

All MRSA isolates from human samples were Panton–Valentine leukocidin (PVL) negative. Seven different but related *spa*-types were found, most commonly t011 (45%), t567 (21%) and t108 (15%) which are all known NT-MRSA strains. Furthermore, two cases of t899 (known NT-MRSA) were found and also two t2330, one t2741 and one t588. The latter three *spa*-types were consequently submitted for PFGE analyses and also identified as NT-MRSA. In the pig and dust samples, t011 and t108 *spa*-types were most prevalent, as well as t567, t899 and t2330. Two *spa*-types, t588 and t2741, were found in people but not in pigs. Some *spa*-types found in pigs were not present in people.

On all 15 farms with MRSA-positive people, the *spa*-types in human samples matched with the types found in pigs on the same farm (30/33 persons). These were of *spa*-types t011 (eight farms), t108 (three farms), t567 (two farms), t2330 (one farm) and t899 (one farm). On 13/15 farms the type found in the pigs was the only *spa*-type found in people. However, on two farms persons were carrying another *spa*-type than found in the pigs. On one farm two co-workers harboured t108 and t2741 while the farmer and pigs carried t011; this farm also cared for other animals (sheep and horses). On the other farm, a household member yielded t588 while two other household members and the pigs had t108; on this farm a child had been previously diagnosed with MRSA.

Antimicrobial resistance patterns

All human MRSA isolates were resistant to tetracycline and all isolates were fully susceptible to vancomycin, teicoplanin, nitrofurantoin, fusidic acid and rifampin. Other antimicrobials showed variable resistance (**Table 2.1.3**).

Table 2.1.3 Susceptibility to antibiotics of human MRSA isolates (farm residents and collectors), by *spa*-type.

	Susceptible strains (%)				
	t011 (n=28)	t108 (n=16)	t567 (n=8)	Other (n=8)	Total (n=60)
Nitrofurantoin	100	100	100	100	100
Vancomycin	100	100	100	100	100
Rifampicin	100	100	100	100	100
Linezolid	100	100	100	100	100
Mupirocin	100	100	100	100	100
Fusidic acid	100	94	87	100	97
Ciprofloxacin	79	87	25	75	74
Gentamicin	71	100	100	87	67
Cotrimoxazole	36	81	25	50	48
Erythromycin	50	37	75	62	48
Clindamycin	50	37	75	62	48

The levels of resistance to ciprofloxacin, fosfomycin, tobramycin and cotrimoxazole were dependent on *spa*-types (χ^2 , $P < 0.05$). Of the predominant *spa*-types, t011 showed higher levels of resistance to tobramycin and cotrimoxazole than the other *spa*-types, while t567 was more frequently resistant to fosfomycin and cotrimoxazole and less sensitive to ciprofloxacin.

Effect of short-term exposure of human and animal sample collectors

The research assistant who collected the human samples on 50 farms remained negative for MRSA throughout the research period, whereas 13/32 veterinary assistants collecting samples from pigs and stable dust had MRSA-positive samples on one or more occasions. On 13 farm visits (10 assistants), the collector was MRSA positive directly after sampling but negative by the next day. Two collectors were still positive the day after the visit; a sample from one of them 1 month later was negative, no repeat sample was available from the other. The *spa*-types identified from 11/12 collectors corresponded with the types found in the pighouses. One collector was already carrying MRSA before his first farm visit: he visited three farms and remained MRSA positive with *spa*-types not corresponding to the MRSA types on these farms.

Discussion

This study shows that 30% of farms have MRSA-positive farm residents and 56% have MRSA present in pighouses. In all farm residents the incidence of MRSA was 14% and this doubled (29%) in persons working with pigs. The farms investigated were a representative sample of the Netherlands by region and type of farm, although the response rate (47%) may have caused a bias for farms cooperating because of previous

knowledge on MRSA (and an interest in participating) or no knowledge (and no fear of participating). Nasal sampling is known to identify the majority of MRSA carriers [27]. Visits were spaced and samples carefully handled to avoid cross-contamination from one farm to another.

The transmission route of MRSA is probably from pigs to people. People were found to be positive only on farms with MRSA in pigs or dust, increasingly so with higher positivity rates in pigs. *Spa*-typing showed that 91% of people were colonized with similar strain(s) as the pigs on their farm. All MRSA strains were of NT-MRSA type and closely related. Most strains were of *spa*-types identified previously as NT-MRSA and of the animal-related MLST type ST398 [11,28] and additional PFGE typing confirmed that all other *spa*-types were NT-MRSA. Two *spa*-types, t588 and t2741, were recovered from people but not pigs; more *spa*-types were found in pigs than in human samples.

Intensive and repeated exposure to positive pigs appears to be an important factor in MRSA colonization. MRSA positivity was common in persons working with pigs and also persons with less intensive but regular contact (weekly) with the animals. The MRSA transmission from pigs to humans was higher on farms with sows than on finisher farms, while MRSA in pigs circulated more frequently on finisher farms. Management of (breeding) sows (with regular deliveries, care of piglets) requires closer contact with pigs, especially with piglets, as well as longer working hours in the pighouse than management of finisher pigs and this may lead to a higher rate of MRSA transmission from pigs to humans. The high prevalence of NT-MRSA in dust samples from pighouses implies that MRSA could be spread by inanimate material as well, as has been postulated for hospital ICUs [29,30].

The persistence of MRSA carriage is not determined in our point-prevalence survey. Assuming a continuous exposure, farm residents can be expected to remain MRSA positive, however, distinguishing persistent carriers would need repeated sampling [31, 32]. The results from repeated samples of our research teams imply that the risk of acquiring MRSA during short farm visits is limited to transient carriage, even when exposure to pigs is intense. It would be interesting to follow NT-MRSA carriage of pig farmers when they are no longer in contact with pigs (i.e. when on holiday or retiring).

The low MRSA rate (2%) in persons with no contact with pigs suggests a low level of human-to-human transmission of NT-MRSA. Hospital screening activities after detection of a MRSA carrier showed that animal-related ST398 MRSA led to fewer secondary cases (three from 24 index patients) than other MRSA genotypes (62 cases from 56 index patients [32]).

Impact of NT-MRSA in Dutch health-care system

The 14% MRSA carriage of pig-farm residents is much higher than the 0.12% prevalence in the open population and at primary care (studied in 2005–2006 [33]) and 0.03% at routine hospital admission (data from 1999–2000 [4]). Countrywide, there are about 9700 pig farms (National database CBS, 2005). Extrapolation of our results indicates that nearly 6000 MRSA carriers (range 4000–9500) may be expected in pig-farm residents. This justifies the hospital strategy to screen people having contact with pigs in Dutch hospitals since 2006 and agrees with a high proportion of NT-MRSA cases in the national MRSA surveillance database. Moreover, a recent report from hospitals showed that 30% of index patients carried animal-related MRSA [34].

The increase in MRSA-positive patients, identified by hospital screening, attributable to farm-animal MRSA will cause increased costs due to care in isolation, longer stay in hospital, specific diagnostics and medication [14]. However, the clinical relevance for the people concerned still needs further investigation. Transmissibility from human to human appears to be low and few symptomatic cases of NT-MRSA infections have been found [35].

Use of antimicrobials in pig farming and consequences for MRSA in humans

Antimicrobial selection pressure in general is one of the probable factors that have facilitated the emergence and spread of veterinary MRSA [28]. Antimicrobial consumption in pig farming in the Netherlands is substantial compared to other livestock farming [36]. Tetracyclines and trimethoprim–sulfonamide (trimisulfa) are most widely used; we found levels of resistance to these antibiotics of 100% and 52%, respectively. In other recent Dutch studies resistance to trimethoprim-sulfamethoxazole was not or hardly ever found in MRSA in pigs and humans [11, 12], hence this resistance might be currently emerging. The pattern of resistance of the MRSA samples in our study was otherwise comparable to that in other recent studies [11–13].

Implications for health care and future research

Since 2007, adjusted guidelines for hospitals in the Netherlands require screening for MRSA and care in isolation for all people professionally in contact with live pigs or veal calves. Our results, however, have shown that the frequency and intensity of contact with pigs, whether professional or not, are a determinant for MRSA risk and hence the terminology ‘contact’ should be refined further. The persistency of NT-MRSA carriage in humans after different types of exposure should also be studied further.

NT-MRSA appears to be frequently transmitted from pigs to people, but less so from person to person (the present study [15,33]). The need for strict management of

patients (isolated care) might be reviewed if human-to-human transmission of NT-MRSA is indeed as limited as shown.

Although the antimicrobial resistance pattern found here has no consequences yet for current treatment options of MRSA, further spread of NT-MRSA and selection of resistant strains by the high use of antimicrobials in pig farming, may impede the usefulness of antimicrobials in the future or necessitate differential treatment of MRSA and NT-MRSA (and thus T/NT-typing before treatment). The use of antimicrobials in pig farming should be studied in relation to MRSA prevalence and possible alternative treatment strategies investigated.

We found no association between personal hygiene and MRSA carriage, possibly because personal hygiene at the level that we investigated was not an important factor in pig-to-human MRSA transmission, and transmission of MRSA between humans did not seem to play an important role. Other factors, such as intensity of contact with animals and actual use of cleaning and protection methods may be more important. Protection methods might need to be adjusted to the type of pigs and activities in the stables. The role of hygienic measures in transmission reduction also requires further study.

NT-MRSA will be no doubt studied more extensively in other countries in Europe in the future. The pig-farming sector involves a wide European network of farms and the accompanying meat industry. Pig farms in other countries are probably facing a similar problem as the Dutch farms, although this has as yet not been reported as extensively. Action is needed at a European level to assess the situation and design appropriate measures to prevent further spread of NT-MRSA.

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Chapter 2.2

High prevalence of nasal MRSA carriage in slaughterhouse workers in contact with live pigs in the Netherlands

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Summary

Livestock-associated MRSA has been found in various animals, livestock farmers and retail meat. This study aimed to determine the prevalence and determinants of nasal MRSA carriage in pig slaughterhouse workers. Three large pig slaughterhouses in the Netherlands were studied in 2008 using human and environmental samples. The overall prevalence of nasal MRSA carriage in employees of pig slaughterhouses was 5.6% (14/249) (95% CI 3.4–9.2) and working with live pigs was the single most important factor for being MRSA positive (OR 38.2, $P < 0.0001$). At the start of the day MRSA was only found in environmental samples from the lairages (10/12), whereas at the end of the day MRSA was found in the lairages (11/12), the dirty (5/12) and clean (3/12) areas and green offal (1/3). The MRSA status of the environmental samples correlated well with the MRSA status of humans working in these sections ($r = 0.75$). In conclusion, a high prevalence of nasal MRSA carriage was found in pig-slaughterhouse workers, and working with live pigs is the most important risk factor. Exact transmission routes from animals to humans remain to be elucidated in order to enable application of targeted preventive measures.

Introduction

Since 2003, a distinct clone of methicillin-resistant *Staphylococcus aureus* (MRSA), related to the livestock reservoir has emerged in the human population [1]. As this clone was found to be non-typable (NT) by pulsed-field gel electrophoresis using the *Sma*I restriction enzyme, it was originally called NT-MRSA [2,3]. Multi-locus sequence typing revealed that all strains belonged to the clonal complex 398 (CC398) [4]. At present, it is clear that people who have frequent contact with pigs or veal calves have extremely high MRSA CC398 carriage rates compared to national community prevalences (25–35% vs. 0.1% in the Netherlands) [5–8].

As a result of the elevated prevalences in this specific population, the ‘search and destroy’ policy in the Netherlands was adapted; persons in contact with live pigs and veal calves are added to the high-risk group and should be screened for MRSA upon hospital admission [9]. As a consequence, the number of MRSA CC398-carrying patients found in the Netherlands increased dramatically to nearly 30% of all newly detected MRSA strains in 2007 [10], and 42% in 2008 [11]. The proportion of MRSA in *S. aureus* nosocomial infections remained very low (<2%), compared to other countries [12].

In a recent survey by the Food and Consumer Product Safety Authority in the Netherlands (VWA) MRSA was found in 11% of retail meat (with a minimum MRSA prevalence of 2% in game and a maximum of 35% in turkey) [13]. Other studies also found MRSA in retail meat, in varying percentages (2.5% [14], 19% [15], 0.7% [16], 5% [17], 0% [18] and 17%, R. de Jonge, J. E. Verdier and A. H. Havelaar, unpublished observations).

In animal husbandry-dense areas, the majority of newly identified human MRSA carriers concerns this livestock-associated MRSA [19], and recently, the first hospital outbreaks of CC398 have been reported [20,21]. Meanwhile, serious invasive infections due to CC398 have been observed [22–27]. Therefore, the emergence of this new livestock-associated clone poses a potential public health risk that warrants close monitoring.

The high prevalence of MRSA in meat products and in people working with livestock raises the question whether slaughterhouse workers, who are in contact with pigs (dead or alive) and meat products, are also at risk. Therefore, we performed a cross-sectional survey on nasal MRSA CC398 carriage in employees of pig slaughterhouses, and on the occurrence of MRSA in different slaughterhouse sections.

Methods

Study population, questionnaires and human sampling

Three pig slaughterhouses were enrolled in the study on the basis of voluntary participation, from a complete list of 10 large pig slaughterhouses in the Netherlands. All were located in the south and the east of the country, in areas with a high pig density. By using a structured questionnaire, slaughterhouse-specific information was collected, e.g. number of employees, slaughterhouse capacity, specifics on lairages and the production process, information on microbiological contamination of the carcasses and working benches and hygiene measures.

Slaughterhouse workers were enrolled in the survey based on voluntary participation. A written consent was obtained from each participant. The survey contained questions on age, gender, country of birth, recent antibiotic use, job description, working in more than one section of the slaughterhouse (rotation), wearing plastic gloves, living on a livestock farm, and contact with family members working in healthcare or in livestock farming. Slaughterhouse workers were divided in three different categories according to their activities: contact with live pigs, dead pigs or other. When subjects indicated that they worked in more than one section, they were included in the category with the most intense contact with live animals.

Nasal swabs (Venturi Transystem, Copan Innovation, Italy) were taken from workers in order to determine the presence of MRSA. This study was approved by the Medical Ethical Committee of the University Hospital Utrecht (file no. 08/050).

Environmental sampling

To determine the MRSA status of the different slaughterhouse sections, environmental wipe samples were taken from surfaces in each section (**Figure 2.2.1**) at the beginning and at the end of the working day using Sodibox wipes (Raisio Diagnostics B.V. Nieuwerkerk aan den IJssel, The Netherlands). Sections of the slaughterhouse were divided in two different categories according to the cleanliness of the animal/carcass: dirty or clean areas. In the dirty area, the carcass surface is cleaned by scalding, depilation and singeing. In the clean area, the carcass is eviscerated and processed into meat products.

Microbiological methods

Nasal swabs were incubated in Mueller-Hinton enrichment broth (Becton Dickinson, USA) with 6.5% NaCl, for 18-48 h at 35°C. Then 10 µl of the broth was plated onto a MRSA-ID culture plate (bioMérieux, France), and incubated overnight at 35°C. Suspect

(green) colonies were identified as *S. aureus* by a latex agglutination test (Staphaurex Plus; Murex Diagnostics Ltd, UK) and tested for ceftoxitin sensitivity by the disc diffusion method [28]. The obtained MRSA isolates were subsequently stored at -80°C. Environmental sample wipes were soaked in 100 ml Mueller-Hinton enrichment broth with 6.5% NaCl and incubated for 18 h at 37°C. Next, 1 ml of the broth was transferred into 9 ml Phenol Red mannitol broth with 5 mg/ml ceftizoxime and 75 mg/ml aztreonam (bioMérieux) and incubated for 18 h at 37°C. Subsequently, 10 µl of the suspension was transferred onto a Columbia agar plate with 5% sheep blood. In parallel, Brilliance MRSA culture plates (Oxoid, UK) were inoculated with 10 µl suspension and incubated for 18 h at 37°C. Colonies were subcultured until pure.

Confirmation of the isolates was done by a multiplex PCR specific for *S. aureus* [29], the *mecA* gene [30], and the Panton-Valentine leucocidin (PVL) toxin genes [31]. Isolates were defined as MRSA on the basis of their *mecA* gene presence. Staphylococcal protein A (*spa*) typing was conducted according to Harmsen *et al.* [32]. On all MRSA-positive environmental and human samples, antimicrobial susceptibility was tested using the Vitek system (bioMérieux SA, France) according to the manufacturer’s instructions.

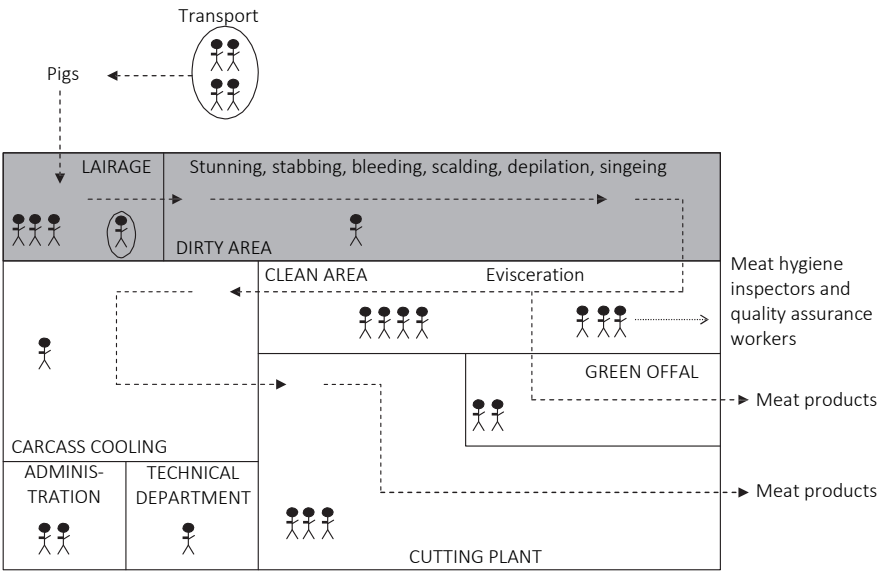


Figure 2.2.1 Schematic representation of the sections of the production chain (dotted lines) in a pig slaughterhouse. The shaded area represents sections where live pigs are located (dirty area). Each human figure represents about 10 persons, circled persons are not actual slaughterhouse employees (livestock transport workers and official veterinarians and auxiliaries).

Sample size and statistical analysis

The prevalence of MRSA nasal carriage in the general population in the Netherlands was assumed to be <0.5%. A nasal carriage rate of $\geq 2\%$ in slaughterhouse workers was considered as a significant increase. The required sample size was calculated as 450 subjects ($\alpha=0.05$, $\beta=0.10$).

Prevalence of MRSA in slaughterhouse workers was calculated as a percentage of the total amount of samples in general and specified per category and job description. Wilson confidence intervals (CI) were calculated. Univariable exact logistic regression was performed using SAS, version 9.1 [33]. Odds ratios (OR) were determined by comparing different categories and job descriptions within those categories. In order to calculate the association between the human and environmental samples and because of the skewed distributions of the percentages of positive persons and environmental samples per section, Spearman's rank correlation was used.

Results

Slaughterhouse characteristics

In the three selected slaughterhouses, the total number of employees varied between 80 and 260. The total number of slaughtered pigs per day varied between 3800 and 5000, all pigs originated from farms in the Netherlands. In one slaughterhouse, cattle were slaughtered as well, but in separate rooms in the same building.

Humans

Of the total of 497 slaughterhouse workers 195 (39.2%) agreed to participate. An additional 41 livestock transport workers and 13 official veterinarians and auxiliaries (i.e. persons from the VWA, who monitor and assist the meat hygiene inspectors) were included, yielding a total of 249 study subjects, including 16 female participants. Mean age was 43 years (range 19-73 years), and the mean working week was 41 h (range 7-80 h).

We found an overall nasal MRSA prevalence of 5.6% in slaughterhouse workers (14/249, **Table 2.2.1**). MRSA carriage was found exclusively in persons having contact with live pigs (15.1%), compared to subjects not working with live pigs (0.0%, OR 38.2, **Table 2.2.2**).

Table 2.2.1 Prevalence of nasal MRSA carriage in slaughterhouse workers

Contact with pigs	Function	Total	MRSA	Percentage	95% CI
Live pigs	Livestock transport worker	41	9	22.0	12.0 – 36.7
	Official veterinarian+auxiliary	13	2	15.4	4.3 – 42.2
	Lairage worker	32	2	6.3	1.7 – 20.1
	Dirty area worker	7	1	14.3	2.6 – 51.3
Dead pigs*		127	0	0.0	0.0 – 2.9
Other#		29	0	0.0	0.0 – 11.7
Total		249	14	5.6	3.4 – 9.2

CI, Confidence interval (data from three slaughterhouses combined). * Clean area worker, carcass cooling and cutting plant worker, green offal worker, meat hygiene inspector, quality assurance worker. # Administrative and technical personnel.

Table 2.2.2 Univariable exact logistic regression analysis

Characteristic	Total	MRSA	Percentage	OR	95% CI	P value
Gender						
Female	16	0	0.0	Ref.		
Male	233	14	6.0	1.4	0.2 – ∞	0.77
Born abroad	60	1	1.7	0.2	0.0 – 1.6	0.22
Living on livestock farm	24	3	12.5	2.8	0.5 – 11.7	0.28
Recent antibiotic use	28	3	10.7	2.3	0.4 – 9.5	0.40
Contact with family members in healthcare or livestock farming	47	3	6.4	1.2	0.2 – 4.7	1.00
Working with live pigs	93	14	15.1	38.2	6.3 – ∞	<0.0001
Rotation	59	3	5.1	0.9	0.3 – 3.5	1.00
Wearing plastic gloves						
Always	53	2	3.8	Ref.		
Sometimes	76	6	7.9	2.2	0.4 – 22.9	0.57
Never	113	6	5.3	1.4	0.2 – 14.9	1.00

OR, Odds ratio ; CI, confidence interval ; ref. reference category. Boldface values belong to characteristics that are significantly related to MRSA, when comparing the presence of the relevant factor vs. the absence of it.

Nine of the 41 (22%) livestock transport workers were MRSA positive, as well as 2/13 (15%) veterinarians and auxiliaries. In total, 3/195 (1.5%, 95% CI 0.5-4.4%) employees of slaughterhouses (excluding livestock transport workers and official veterinarians and auxiliaries) were MRSA positive; these were all working in the dirty area of the slaughterhouse. No specific slaughterhouse function proved to be a significant risk factor, when comparing different activities within the clean and the dirty areas. Twenty-three persons indicated working in both dirty and clean areas and only one of these was found MRSA-positive.

Regarding potential determinants and confounders, no significant difference in persons with and without MRSA was found (**Table 2.2.2**). Furthermore, no significant differences in MRSA prevalence in humans between slaughterhouses were found.

Environment

At the start of the day MRSA was only found in environmental samples from the lairages (10/12) (**Table 2.2.3, Figure 2.2.1**). At the end of the day MRSA was found in the lairages (11/12), the dirty (5/12) and clean (3/12) areas and green offal (1/3). Spearman's correlation coefficient, a measure for the correlation between MRSA status of the environmental samples and the humans working in these areas, is 0.75 ($P=0.002$). The squared correlation ($0.75 \times 0.75 = 0.56$) gives the coefficient of determination; 56% of variance in percentage of positive persons can be explained by environmental contamination.

Table 2.2.3 MRSA in environmental samples taken at start and end of working day

Pigs	Department	Start of the day			End of the day		
		Total	MRSA	Percentage	Total	MRSA	Percentage
Live	Lairage	12	10	83.3	12	11	91.7
	Dirty area	12	0	0.0	12	5	41.7
Dead	Clean area	12	0	0.0	12	3	25.0
	Carcass cooling	12	0	0.0	12	0	0.0
	Cutting plant	8	0	0.0	8	0	0.0
	Green offal	3	0	0.0	3	1	30.0

Data from three slaughterhouses combined.

Spa typing and antimicrobial susceptibility testing

In total, 14 human and 32 environmental MRSA strains were collected. The predominant *spa* type was t011 in both human subjects (11/14) and environmental samples (21/32). *Spa* type t108 was only found once in a human nasal sample, and also once in an environmental sample from the corresponding slaughterhouse. An additional 10 environmental isolates from the other slaughterhouses were typed as t108. *Spa* type t571 was only found once in environmental samples, and t034 and t1451 were found only once in humans, not in environmental samples of the corresponding slaughterhouse. From two environmental samples two different *spa* types were isolated, in both cases t011 and t108. PVL-positive strains were not found.

Antimicrobial susceptibility testing revealed that all MRSA isolates from humans and the environment are resistant against tetracycline (**Table 2.2.4**), and 19/46 isolates show combined erythromycin and clindamycin resistance. Furthermore, all isolates are sensitive for mupirocin and vancomycin (only human isolates tested). *Spa* type t108 appears to have less combined erythromycin + clindamycin resistance ($0/11=0.0\%$) than t011 ($17/32=53.1\%$, $P=0.002$). No clear difference in resistance pattern between the human and environmental isolates was determined.

Table 2.2.4 Antimicrobial susceptibility profiles of all human and environmental MRSA isolates

Antimicrobial	Humans (n=14)		Environmental (n=32)	
	Resistant	Percentage	Resistant	Percentage
Tetracycline	14	100.0	32	100.0
Erythromycin	8	57.1	12	37.5
Clindamycin	8	57.1	12	37.5
Gentamicin	1	7.1	11	34.4
Ciprofloxacin	0	0.0	6	18.8
Trimethoprim-sulfamethoxazole	3	21.4	1	3.1
Rifampicin	0	0.0	0	0.0
Fusidic acid	0	0.0	0	0.0
Linezolid	0	0.0	0	0.0
Mupirocin	0	0.0	0	0.0
Tobramycin	1	7.1	n.t.	
Vancomycin	0	0.0	n.t.	
Nitrofurantoin	0	0.0	n.t.	
Neomycin	n.t.		1	3.1
Amikacin	n.t.		0	0.0

n.t., Not tested.

Discussion

To our knowledge, this is the first study on the prevalence of nasal MRSA in pig slaughterhouse workers. Working with live pigs is the most important determinant for nasal CC398 carriage, justifying the present hospital infection control guidelines in the Netherlands, which indicate that contact with live pigs is a risk factor for MRSA carriage. Working with dead pigs does not seem to be a risk factor for MRSA carriage.

The prevalence of 15.1% in persons working with live pigs is comparable to data found elsewhere, e.g. 26% and 14% in pig farmers and 12.5% in veterinarians attending an international pig health convention [1,5,34]. A low prevalence was found in Danish veterinarians (3.9%) [35], but higher nasal prevalences were found in German pig farmers on MRSA-positive farms (86%), German pig veterinarians (45%) and USA pig farmers (45%) [36,37].

The overall MRSA prevalence in all subjects in the current study is 5.6%, which is significantly higher than the general population prevalence reported in the Netherlands (0.1%) [7,8,38]. The higher prevalence in livestock transport workers compared to lairage workers might be explained by the less intense physical contact with pigs by lairage workers, who often use sticks to herd the animals. Transport workers earmark all animals at pick up and often herd the animals with their bare hands. Second, high-pressure spray cleaning of the truck may result in formation of MRSA aerosols, which can be inhaled by the transport worker. Insight into these mechanisms may give more information on the transmission route of MRSA.

During the day MRSA accumulates, particularly in the first stages of the production process, which predominantly deals with live pigs. Since pigs were loaded into the lairages at night, the lairages were not clean at the time of sample collection at the beginning of the day. Moreover, the lairages are cleaned every day, but not disinfected. There is a significant association between the presence of MRSA in different sections, and the percentage of MRSA-positive persons working in these relevant sections. It is possible that acquisition of MRSA occurs through contaminated surfaces [39]. However, presence of MRSA on different surfaces does not necessarily imply that there is an increased risk of human MRSA acquisition via the environment: where the lairages have a high percentage of MRSA-positive samples at the end of the day (92%), a relatively low percentage of lairage workers had acquired the bacterium (6.3%). It is plausible that animals spread MRSA to both humans and the environment, and human acquisition of MRSA seems to be more likely by contact with MRSA-positive animals than through environments with MRSA in dust or aerosols.

All *spa* types found in our study were previously confirmed as belonging to the CC398 livestock-associated MRSA clone [40]. The most predominant *spa* types in both human and environmental isolates were t011 and t108, which is in accord with previous studies in pigs and pig farmers [1,4,5,22,41,42]. The subject with t034 was an official veterinarian and the *spa* type t1451 came from a livestock transport worker, these persons often have more animal contacts than in the slaughterhouse alone. Antimicrobial susceptibility, in particular tetracycline resistance was comparable to profiles found in other studies for livestock-associated MRSA [2,5,22].

The prevalence of MRSA found in retail meat in other studies is considerable, the prevalence of MRSA found in employees of pig slaughterhouses in this study is low. The role of slaughterhouse employees in transmitting MRSA to the meat products thus does not seem to be large. Especially as persons working with meat products were all negative in this study. This finding is in accord with an unpublished study (R. de Jonge, J. E. Verdier and A. H. Havelaar, unpublished observations), where none of 101 employees from the cold-meat processing industry and institutional kitchens carried MRSA. It is probable that another transmission route to retail meat is involved here. Contamination of meat with MRSA by the environment (surfaces) and/or equipment, or from animals to carcasses/meat products is more likely to occur. This kind of cross-contamination has already been demonstrated for *Salmonella* spp. in pig slaughterhouses [43].

Our study has a few limitations. As with every questionnaire, survey recall bias, selection bias, and language bias may have occurred. Next, the low number of slaughterhouses visited (n=3) yields little power to find significant differences between slaughterhouses. Nevertheless, we assume that these results are representative for all Dutch pig slaughterhouses, because the working conditions in all pig slaughterhouses in the Netherlands are comparable due to automation and the strict legislation on hygiene

and animal handling. Despite a smaller sample size than calculated beforehand, the number of subjects is still sufficient to confirm previous findings on the risk of acquiring MRSA for people in contact with live pigs. Possibly more risk factors could be found if the number of slaughterhouse workers was larger, e.g. country of birth, recent antibiotic use, amount of hours worked per week, and contact with healthcare. Furthermore, no pigs were sampled in our study, but in a previous study on MRSA at Dutch slaughterhouses MRSA was detected in 81% of the Dutch slaughter batches and 39% of the individual pigs [2]. Environmental samples are considered to be a good proxy for animal MRSA carriage, concerning the association found between environmental and animal samples in other studies (OR 27.5, $\kappa=0.68$) [44]. Longitudinal information on duration of MRSA carriage and the possibility of transient colonization is not yet available; this will be our group's next study subject.

In conclusion, nasal MRSA CC398 is found in pig slaughterhouse workers in significantly higher percentages than the general population prevalence in the Netherlands. It is found exclusively in persons working with live pigs. In addition to contact with live pigs, environmental contamination might also play a role in the acquisition of MRSA, but exact transmission routes from animals to humans remain to be elucidated in order to enable application of targeted preventive measures.

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Chapter 2.3

Prevalence of livestock-associated MRSA in communities with high pig- densities in the Netherlands

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Abstract

Background

Recently, livestock-associated methicillin-resistant *Staphylococcus aureus* CC398 has been discovered in animals, livestock farmers and retail meat. This cross-sectional study aimed to determine the spread to persons not in direct contact with livestock in areas with a high density of pig farms.

Methodology/Principal Findings

With a random mailing in 3 selected municipalities in the Netherlands, adult persons were asked to fill in a questionnaire and to take a nose swab. In total, complete information was obtained on 583 persons. Of the 534 persons without livestock-contact, one was positive for MRSA (0.2%; 95% confidence interval, <0.01–1.2). Of the 49 persons who did indicate to be working at or living on a livestock farm, 13 were positive for MRSA (26.5%; 95% confidence interval, 16.1–40.4). All *spa*-types belonged to CC398.

Conclusions/Significance

Livestock-associated MRSA has a high prevalence in people with direct contact with animals. At this moment it has not spread from the farms into the community.

Introduction

Traditionally, methicillin-resistant *Staphylococcus aureus* (MRSA) has been considered a hospital-associated pathogen. Recently, the epidemiology of MRSA has changed from the confined settings of the hospital to the general population. Community-associated MRSA has been shown to cause severe infections in previously healthy persons [1].

A new development is the emergence of a distinct clone of MRSA that is related to an extensive reservoir in pigs and cattle. It was first recognized in the Netherlands in 2003 [2]. As this clone was found to be non-typable by pulsed-field gel electrophoresis (PFGE) with *Sma*I, it was originally called NT-MRSA [3]. Further research revealed that all of these strains belonged to multilocus sequence type clonal complex (CC) 398 [4]. A subsequent case-control study confirmed that people in contact with pigs and veal calves were more prone to carry MRSA CC398 [5]. At present it is clear that people who have frequent contact with live pigs and veal calves have extremely high carriage rates (prevalence 25-35%) [6]. By the end of 2008, 42% of all newly detected MRSA strains in the Netherlands were CC398, up from 30% by the end of 2007 (www.rivm.nl/mrsa).

A recent survey by the Food and Consumer Product Safety Authority in the Netherlands (VWA) found MRSA on 11% of the meat samples in retail (with a minimum MRSA prevalence of 3% in game and a maximum of 31% in turkey) [7]. Other studies confirmed the contamination of meat with MRSA, although the prevalence varied (2.5% [8], 17% [9], 0.7% [10], 5% [11], 0% [12] and 17% R. de Jonge, J.E. Verdier and A.H. Havelaar, submitted). So far, a relation between eating meat and MRSA-carriage is not found, but it is of concern that this type of MRSA has entered in the food chain and handling of meat could thus become a mode of acquisition of MRSA.

Meanwhile, serious invasive infections from Europe, Asia and America due to MRSA CC398 have been reported [5,13-18]. In hospitals in husbandry-dense areas in the Netherlands, the majority of newly identified MRSA carriers are CC398 [19], and the first outbreak with MRSA CC398 in hospitals has been reported [20]. This means that MRSA is not only a human pathogen, but also a zoonotic pathogen, particularly affecting people working in animal husbandry.

In order to get an idea of the magnitude of the problem, knowledge on the exact spread of this specific clone in the general community is desired. The current study aimed and succeeded to determine if MRSA CC398 has spread from the farms into the rest of the community in areas with an extremely high density of pig farms.

Material and methods

Ethics statement

The medical ethical committee of the St. Elisabeth Hospital in Tilburg approved the study.

Enrollment

This cross-sectional study was conducted between July 2008 and January 2009 in three municipalities from the area with the highest density of pigs in the Netherlands, i.e. Venray, St. Anthonis and Meijel. They are located in the southeast of the Netherlands with a relatively low human population-density and a pig-density of approximately 3,000 pigs per square kilometer [21] (**Figure 2.3.1**). A random sample of adult persons (≥ 18 years of age) from the local registry of inhabitants was taken. The sample was stratified for age and gender according to the characteristics of the general population of the Netherlands. Stratification to livestock-contact was not performed in order to prevent response bias.

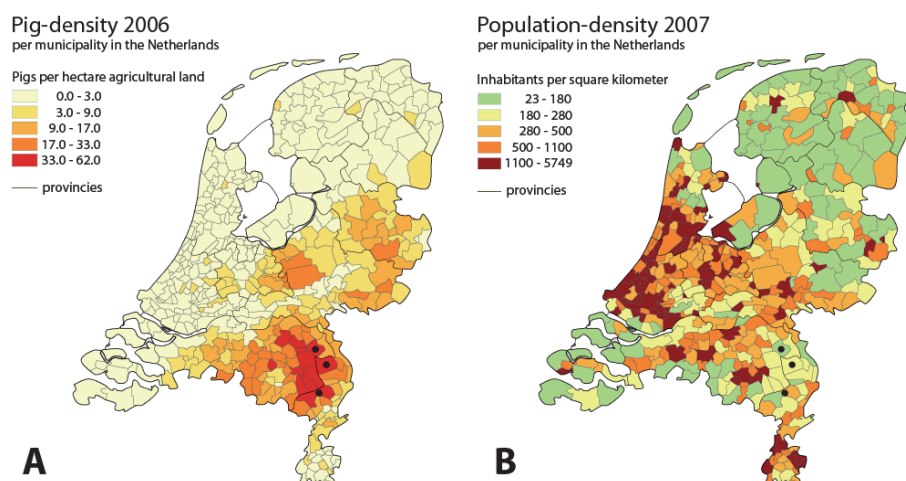


Figure 2.3.1 The pig-density and population-density in the Netherlands. Pig-density is depicted in panel A, population-density is depicted in panel B. The participating municipalities of St. Anthonis, Venray and Meijel are indicated with “●”. Source: CBS Statistics Netherlands (www.cbs.nl).

Sample size

The sample size was calculated, based on the following assumptions. The background prevalence of MRSA was assumed to be less than 0.5% [22-24]. To confirm that the prevalence of MRSA in persons living in pig-dense areas without livestock-contact is 2% or more with an alpha-error of 0.05 and a beta-error of 0.10, the estimated sample size was 450 persons who had no contact with livestock. After correction for livestock-contact (25%) and non-response (75%), a questionnaire was mailed to 2703 people. The following questions had to be answered: age, gender, living at a livestock farm, contact with livestock, working in healthcare, past history of MRSA, contact with known MRSA positive persons in the last year and hospitalization abroad in the last six months. Participants were asked to supply a written informed consent.

Samples and microbiological procedures

Subsequently, appropriate transport medium and instructions for sampling were supplied by mail to the participants. A nasal swab was taken by the subjects themselves and sent by mail to one of the participating microbiology laboratories to determine the presence of MRSA. Nasal swabs were inoculated on Columbia blood agar plates with 5% sheep blood to check for adequate sampling and subsequently enriched in Mueller-Hinton broth containing 6.5% NaCl. Both media were incubated for 24 h at 35°C. From the overnight Mueller-Hinton broth, 10 µl was streaked onto MRSA ID (bioMérieux, La Balme Les Grottes, France) agar plates with a sterile loop using a three-streak dilution method. The results were read after 20 h of incubation at 35°C. Growth of colonies showing green coloration was considered to be indicative for MRSA. Colonies with colors other than green, or no growth at all were considered negative. The procedure was performed as recommended by the manufacturer. Green colonies were confirmed to be MRSA by latex agglutination [25], cefoxitin disk diffusion [26] and duplex PCR (*mecA* gene and the *S. aureus* specific target Martineau-sequence). In addition, staphylococcal protein A (*spa*) typing was conducted according to Harmsen et al. [27]. Resistance profiles to 21 antimicrobial agents of all confirmed MRSA strains were determined with the VITEK system (bioMérieux SA, Craponne, France) according to the manufacturer's instructions.

Statistical analyses

MRSA prevalence rates with Wilson's 95% confidence intervals (CI) were reported separately for persons with and without livestock-contact, based on information from the questionnaire. Contacted persons were compared to responders with Wilson signed rank and chi-square tests for age and gender categories. Possible determinants for

MRSA carriage – apart from livestock-contact – were calculated with crude univariate and adjusted multivariate odds ratios with logistic regression.

Results

The flow chart of the study procedure is depicted in **Figure 2.3.2**. Of the 2703 persons contacted for participation, 644 persons (23.8%) returned their informed consent form and questionnaire. From these persons, 583 (90.5%) returned the nasal swab to the microbiological laboratory. All nasal swabs grew micro-organisms on the Columbia blood agar plates, indicative for adequate sampling.

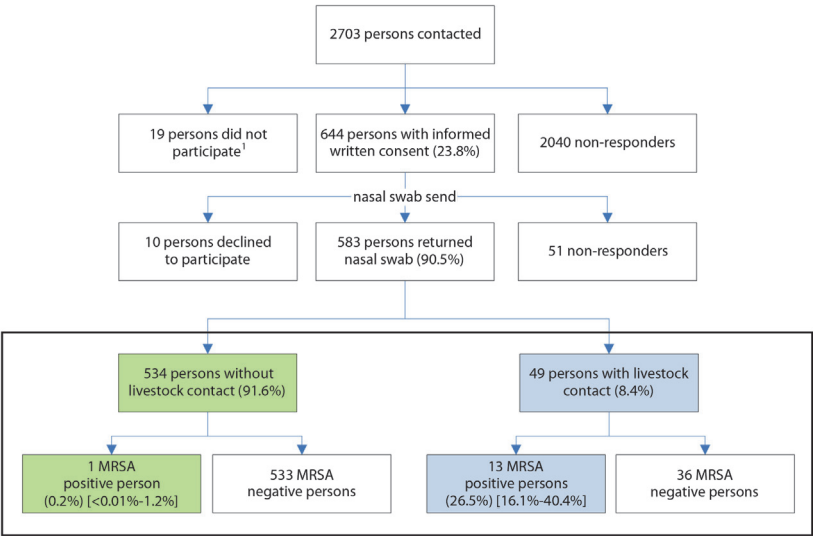


Figure 2.3.2 Flow chart of the study procedure and major results. Major study results are depicted in the box.

¹ Nineteen persons with incomplete response: 9 persons returned the questionnaire but not the informed written consent, 5 persons declined to participate, 2 persons died and 3 persons returned the informed consent after the deadline.

The median age of the 583 participants was 50 years (interquartile range (IQR) 21 years, total range 18–91 years), significantly higher than that of the contacted persons (n=2703, median 46 years, IQR 26 years, $P<0.001$). The percentage of men in the 583 participants was 42.7%, which is significantly ($P=0.006$) lower compared with 49.0% in the contacted group. Specifically, men of 18–40 years of age enrolled to a lesser extent in the study (data not shown).

Of the 534 persons without livestock-contact only one person (0.2%; 95% CI < 0.01–1.2) tested positive for MRSA (**Figure 2.3.2**). In contrast, thirteen (26.5%; 95% CI 16.1–40.4) of the 49 persons with livestock-contact (either work at or live on a livestock farm) tested positive for MRSA. Eleven of the 13 MRSA positive persons reported contact with pigs, one with veal calves and one with poultry. Four had been tested positive for MRSA previously, and 7 out of 13 had reported recent contact with MRSA positive persons. None of the other factors asked for in the questionnaire (working in healthcare, hospitalization abroad) was a significant risk factor for carriage of MRSA, in both the univariate and multivariate analysis.

All recovered MRSA strains have *spa*-types that belong to the known livestock-associated clone CC398 [28]. Antibiotic resistance patterns also grossly correspond with MRSA CC398, being uniformly resistant to tetracycline (**Table 2.3.1**).

Table 2.3.1 *Spa*-types and antibiotic resistance patterns of the recovered MRSA strains

Contact with	<i>Spa</i> -type	te	tr	er	cl	ge	to	ci	ni	va	ri	fu	li	mu
Veal calves	t011	R	R	S	S	R	I	S	S	S	S	S	S	S
Pigs	t011	R	S	R	R	S	S	S	S	S	S	S	S	S
Pigs	t011	R	R	R	R	R	I	S	S	S	S	S	S	S
Pigs	t011	R	S	R	R	S	S	S	S	S	S	S	S	S
Pigs	t011	R	R	R	R	I	I	S	S	S	S	S	S	S
Pigs	t011	R	R	R	R	R	S	S	S	S	S	S	S	S
Pigs	t011	R	S	S	S	S	S	S	S	S	S	S	S	S
Pigs	t108	R	S	S	S	S	S	S	S	S	S	S	S	S
No livestock	t108	R	S	S	S	S	S	S	S	S	S	S	S	S
Poultry	t108	R	R	R	R	R	I	R	S	S	S	S	S	S
Pigs	t571	R	R	S	S	S	S	S	S	S	S	S	S	S
Pigs	t2330	R	R	R	R	S	S	S	S	S	S	S	S	S
Pigs	t2330	R	R	S	S	S	S	S	S	S	S	S	S	S
Pigs	t2330	R	R	R	R	S	S	S	S	S	S	S	S	S

All *spa*-types belong to CC398 [28]. S=sensitive, R=resistant, I=intermediate sensitivity, te=tetracyclin, tr=trimethoprim/sulfamethoxazole, er=erythromycin, cl=clindamycin, ge=gentamicin, to=tobramycin, ci=ciprofloxacin, ni=nitrofurantoin, va=vancomycin, ri=rifampicin, fu=fusidic acid, li=linezolid, mu=mupirocin.

Discussion

The 0.2% (95% CI <0.01–1.2) prevalence of carriage of MRSA among persons not reporting contact with livestock was low and comparable to that in the general population (<0.01–0.13%) [22-24]. The one *spa*-type found belonged to CC398, indicating an initial source in livestock. Since this person reported no direct contact with livestock, the route of transmission remains unclear. It could be indirect contact with a MRSA CC398 carrier or by possible environmental contamination. A recent study

sampled 422 pupils from a secondary school in Germany not living on pig farms, and did not find any MRSA, which is comparable to this study [29].

Of the persons who reported contact with livestock, 26.5% were positive for MRSA. This is comparable to data found elsewhere, i.e. 26% and 14% in pig farmers and 12.5% in veterinarians attending an international pig health convention [2,6,30], but lower than found in a German study in pig farms (45%) and veterinarians (45%) [29]. This supports the present national guidelines in the Netherlands, which state that persons in regular contact with live pigs or veal calves should be screened for MRSA upon hospital admission. All MRSA strains in the present study had antibiotic susceptibility profiles comparable with other MRSA CC398 strains e.g. tetracycline-resistant and mupirocin-susceptible.

The main purpose of the present study was to investigate the potential spread of MRSA CC398 into the community. This can occur either through person to person spread or by contamination of the environment and it would be detected first in these areas with an extremely high pig-density. The current low prevalence in these communities is therefore reassuring.

Another potential route of transmission is through contaminated meat. MRSA has been found at a relatively high prevalence in retail meat samples (up to 17%). However, the amount of MRSA per sample was low (<10 colony forming units per gram meat) [7]. The risk that contaminated meat will cause spread of MRSA into the community is considered to be low [31]. In this study, we did not find any spread of livestock-associated MRSA in persons not having contact with livestock. Although we have no information on the dietary habits of the participants we assume that in a random sample most people will regularly eat meat. This indicates that the high prevalence of MRSA in retail meat does not contribute significantly to transmission of MRSA into the community at this time. Similar results were also found in other studies, that showed only high MRSA-carriage rates in persons in direct contact with livestock [5].

There are two limitations of this study. First, the chance for selection bias. The response on the first invitation letter was 23.8%, being grossly comparable to the response to other random mailing studies in the Netherlands (32%, 44% and 28% [32-34]). The response of persons invited to send a nasal swab was 90.5%, which is considered adequate. However, there were significant differences in gender and age between contacted persons and the subjects who participated. Earlier random mailing studies in the Netherlands dealing with unrelated topics reported the same deviations in response percentages; namely fewer men of 18–40 years of age [32-34]. Therefore, we consider the response in line with studies on unrelated topics and the chances for selection bias as negligible. In addition, this selection bias would only be of concern when one would expect that men of 18–40 years of age are at a higher risk for colonization with MRSA, compared to other gender and age groups. We currently have no reason to assume this.

Another possible limitation is nasal self-swabbing; since subjects have to swab their own nostrils, this may affect the quality of sampling. We checked for sampling adequacy by looking for the presence of micro-organisms in general. In addition, a recent study comparing samples taken by professional samplers and by individuals themselves showed excellent concordance of the results [35]. These results were confirmed in a short validation study performed by our own group (B. van Cleef, unpublished results). Therefore, the quality of the samples taken in the present study can be considered to be adequate. Nevertheless, checking for the carriage rate of *S. aureus* (approximately 30% in the general population) might have lessened this limitation of nasal self-swabbing [36].

The outcome of this survey is reassuring, considering the potential impact of MRSA CC398 on public health, as there was very limited spread to persons without livestock-contact in areas with an extremely high pig-density. This lower transmissibility of MRSA CC398 compared to other MRSA strains was also found in hospital-based studies [19,37]. These findings indicate that strains from CC398 are primarily adapted to animals and do not easily spread among humans. This would limit the impact of this recently emerged clone on public health.

In conclusion, MRSA CC398 has an extremely high prevalence in people who are in contact with livestock, but has not spread into the rest of the community at this time. Therefore, preventive measures should primarily be aimed at persons who work with animals or live on farms.

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Chapter 2.4

Persistence of livestock-associated methicillin-resistant *Staphylococcus aureus* in field workers after short-term occupational exposure to pigs and veal calves

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Abstract

The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage in pig and veal calf farmers in the Netherlands is estimated at 25 to 35%. However, no information is available about MRSA carriage in humans after short-term occupational exposure to pigs or veal calves. This study examines the prevalence and duration of MRSA acquisition after short-term intensive exposure to pigs or veal calves for persons not exposed to livestock on a daily basis. The study was performed with field workers who took samples from the animals or the animal houses in studies on MRSA prevalence in pig and veal farms. They were tested for MRSA by taking nasal samples before, directly after, and 24 h after they visited the farms. There were 199 sampling moments from visits to 118 MRSA-positive farms. Thirty-four of these visits (17%) resulted in the acquisition of MRSA. Thirty-one persons (94%) appeared negative again after 24 h. There were 62 visits to 34 MRSA-negative farms; none of the field workers acquired MRSA during these visits. Except for that from one person, all *spa*-types found in the field workers were identical to those found in the animals or in the dust in animal houses and belonged to the livestock-associated clone. In conclusion, MRSA is frequently present after short-term occupational exposure, but in most cases the strain is lost again after 24 h.

Introduction

Beginning in 2003, a specific clone of methicillin-resistant *Staphylococcus aureus* (MRSA) associated with animal husbandry has emerged [1]. This livestock-associated MRSA (LA-MRSA) clone belongs to multilocus sequence typing (MLST) clonal complex (CC) 398 [2], and humans in close contact with pigs are often colonized. Humans in contact with other animals, such as veal calves and poultry, may also have a significantly higher prevalence of MRSA carriage than the general population [3-8].

So far, the prevalence of LA-MRSA carriage is known only for persons with long-term exposure to livestock, such as persons living or working on pig or veal calf farms or livestock veterinarians [4,7,9]. In the Netherlands, a vigorous "search-and-destroy policy" is maintained, successfully controlling MRSA in health care settings by screening persons at risk for MRSA presence [10] (www.wip.nl). As part of this policy all persons with livestock contact are screened for the presence of MRSA upon admission to a hospital. Since it is not clear whether persons with short-term exposure to livestock acquire MRSA, the necessity of screening these persons is questionable. This study examines the prevalence and duration of MRSA acquisition after intensive short-term exposure to pigs or veal calves in persons not exposed to livestock on a daily basis.

Methods

Study design and study population

During two cross-sectional studies investigating the prevalence of LA-MRSA on randomly selected pig and veal farms in the Netherlands [4,7], dust samples from the animal houses and nasal swabs from pigs and veal calves were taken by field workers on the same day. These field workers (n=40) all had short (up to a maximum of 3 h per day) but intensive contact with animals and dust on the farms and were therefore at risk of acquiring MRSA on MRSA-positive farms. Intensive contact was defined as direct physical contact with the animals during the farm visit. Acquisition was defined as a MRSA-negative initial swab, followed by a MRSA-positive swab. Standard personal protective equipment included boots and overalls provided by the farm, gloves, mouth masks, and hair nets. Hygienic procedures, including hand washing and showering, were mandatory when the field workers left the animal houses. Nasal swabs were taken from these field workers before, directly after, and 24 h after their farm visits and were tested for MRSA presence. Field workers who had livestock exposure other than on the farms concerned were excluded from the analysis. In addition, data on farm characteristics (i.e. farm type, number of animals, other animals present, and hygiene measures) were collected by questionnaire and have been described previously [4,7].

The study protocols of both cross-sectional studies were approved by the medical ethics committees of the institutes involved as required by the law of the Netherlands [4,7]. All farms were visited by more than one field worker. For the veal calf farms, more than one farm could be visited on one field day. One sampling moment refers to a set of three individual nasal swabs (taken before, directly after, and 24 h after the field day) pertaining to a field day on which one or more farms were visited by a field worker. Therefore, the number of field days could be different from the number of sampling moments and the number of farms visited. A farm was considered to be MRSA positive when MRSA was found in one or more animals or in dust samples taken on that particular farm. When only MRSA-negative farms were visited, the field day was considered to be MRSA negative. If one or more MRSA-positive farms were visited, the field day was considered to be MRSA positive. A schematic overview of the study design is given in **Figure 2.4.1**.

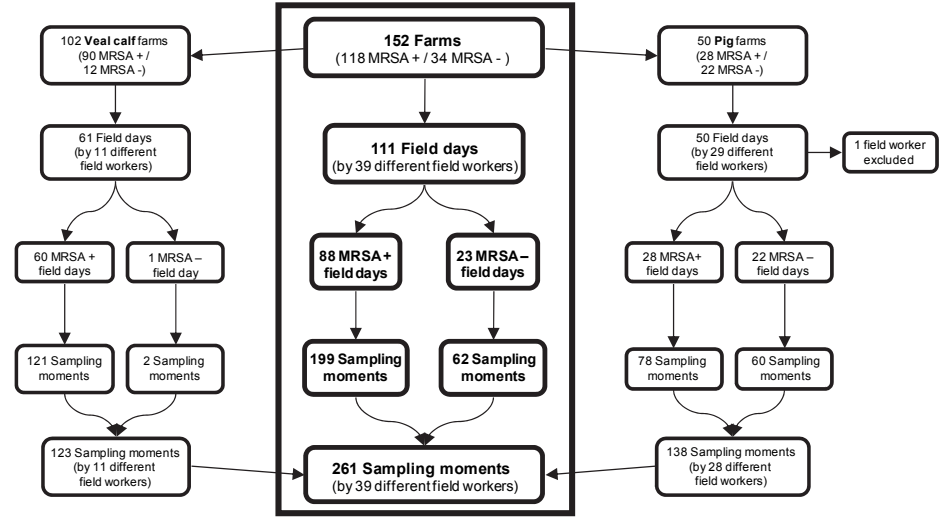


Figure 2.4.1 Schematic overview of study design.

Laboratory analysis

Nasal swabs from the veal calf field workers were analysed individually as published previously [11]. Briefly, swabs were inoculated in a pre-enrichment medium containing Mueller-Hinton broth with 6.5% NaCl. After overnight aerobic incubation at 37°C, selective enrichment in phenol red mannitol broth (BioMérieux, France) with 75 mg/l aztreonam and 5 mg/l ceftizoxime was performed. Ten microliters of the selective

enrichment broth was inoculated onto sheep blood agar (Biotrading, Netherlands) and Brilliance MRSA agar (Oxoid, Netherlands). The nasal swabs of the pig farm field workers were analysed similarly; however, the selective enrichment step was excluded because of different protocols in the laboratories analyzing these samples. All suspected colonies were identified as *S. aureus* using standard techniques: colony morphology and coagulase assays. The presence of the *mecA* gene was confirmed by PCR. The strains were *spa* typed by sequencing of the repetitive region of the protein A gene *spa* [12]. The strains of all positive dust and pooled pig samples and a random selection of samples from three MRSA-positive veal calves per farm were *spa* typed. Data were analyzed by using Ridom Staphtype software, version 1.4.

Data analysis

Statistical analysis of complete data sets was performed using SAS software, version 9.1 [13]. Descriptive analyses were undertaken, followed by logistic multivariate multilevel regression analysis (GLIMMIX and LOGISTIC procedures) to identify determinants of MRSA carriage. A P value of <0.05 was considered statistically significant.

Results

In total, 152 farms (50 pig and 102 veal calf farms) were visited by 40 different field workers. One field worker was excluded because he was continuously exposed to livestock at home, and analyses were performed on data related to the remaining 39 field workers. None of them was MRSA positive on the initial swab. The 152 farms were visited in 111 field days. In total, 261 individual sampling moments were obtained. A total of 118 (78%; 28 pig and 90 veal calf farms) MRSA-positive farms were visited in 88 field days, and 34 (22%; 22 pig and 12 veal calf farms) MRSA-negative farms were visited in 23 field days. **Figure 2.4.1** and **Table 2.4.1** summarize the farm and field day characteristics for the pig and veal calf farms. More-extensive farm characteristics have been published in previous studies [4,7].

Table 2.4.1 Overview of characteristics for pig and veal calf farms

Source	No. of farms visited	Avg. no. of animals on farm (range)	Avg. MRSA prevalence on farm [% (range)]:	
			In animals	In dust samples
Pigs	50	932 (0-3200) ^a	33% (0-100) ^b	34% (0-100) ^d
Veal calves	102	565 (25-2200)	28% (0-100) ^c	47% (0-100)

^a Only sows and finisher pigs were counted. ^b Data were obtained from pooled pig samples (10 pools of 6 pigs per farm). ^c Data were obtained from individual veal calf samples (10 to 43 samples per farm). ^d Five dust samples were taken per farm.

In total, 199 individual sampling moments were present for 34 different field workers visiting the MRSA-positive farms (**Table 2.4.2**). These 34 different field workers acquired MRSA on 34 out of 199 visits (17%; 95% confidence interval [CI], 13 to 22%). Overall, 16 field workers (48%; 95% CI, 33 to 65%) acquired MRSA at least once. Five of them acquired MRSA twice (field workers 3, 7, 25, 26, and 27), and three acquired MRSA on more than two visits (field workers 4, 23, and 24). The field workers who acquired MRSA more than once visited, on average, more positive farms than field workers who acquired MRSA once (median number of positive farms visited per field worker, 10 and 1, respectively). Although the correlation between the number of sampling moments and the number of MRSA acquisitions was high, its statistical significance was only borderline (Spearman's rho, 0.87; P, 0.09).

Table 2.4.2 Overview of sampling moments pertaining to positive field days and MRSA acquisition before, directly after, and 24 h after sampling

Farm type	Field worker(s) ^a	No. of field days (no. of farms visited)	No. of positive field days	No. of positive samples ^b ;		
				Before visit	Directly after visit	24h after visit
Pig	1	9 (9)	2	0	1 (t011)	0
	2	1 (1)	1	0	1 (t011)	0
	3	15 (15)	11	0	2 (t011 [1], t108 [1])	0
	4	18 (18)	13	0	3 (t011 [1], t108 [2])	0
	5	1 (1)	1	0	1 (t108) ^c	0
	6	1 (1)	1	0	0	1 ^d (t108)
	7	7 (7)	6	0	2 (t108[1], t567 [1])	0
	8	3 (3)	1	0	1 (t108)	0
	9	1 (1)	1	0	1 (t011)	1 ^e (t011)
	10	1 (1)	1	0	1 (t011)	0
	11	1 (1)	1	0	1 (t108)	0
	12-23	74 (74)	39	0	0	0
Veal calf	24	55 (82)	54	0	8 (t011)	0
	25	39 (77)	39	0	5 (t011)	0
	26	2 (5)	2	0	2 (t011)	1 ^{df}
	27	5 (10)	5	0	2 (t011)	0
	28	7 (10)	7	0	2 (t011)	0
	29-34	15 (14)	14	0	0	0
	Total	34	261	199	0	33 ^g

^a Field workers whose visits to positive farms did not result in MRSA acquisition are grouped together.

^b Designation in parentheses are *spa* types. Where different *spa* types are present for one individual, the number of samples with a particular *spa* type is given in brackets. ^c This *spa*-type is not identical to the *spa* type on the farm visited. ^d The individual tested MRSA negative following subsequent visits to negative farms.

^e The individual was not tested again. ^f The sample was not *spa* typed. ^g Excluding field worker 6, who acquired MRSA after 24 h.

After 24 h, 31 of the 33 field workers who had acquired MRSA (94% [95% CI, 83 to 98%]) were negative again. Only one field worker who was negative directly after exposure was found to be positive after 24 h; he tested negative after subsequent farm

visits. The *spa* types found in field workers were t011 (n=25), t108 (n=8), and t567 (n=1), all of which belong to CC398. All MRSA isolates except for that from one field worker (field worker 5) had *spa* types identical to those isolated either from animals or from dust on the same farms on the same visit. Persons who acquired MRSA more than once were positive for different *spa* types at different moments, depending on the farm visited.

The 34 MRSA-negative farms were visited by 19 different field workers. None of them acquired MRSA on the 62 field days.

Further statistical analysis showed that field workers acquired MRSA more often when they had visited farms where more MRSA-positive animals were present. Similar associations were found for pig and veal calf field workers (Table 2.4.3). No significant associations were found with other farm characteristics.

Table 2.4.3 MRSA acquisition in field workers in relation to MRSA prevalence among farm animals

Source ^a	Odds ratio (per 10% increase in prevalence) ^b	95% CI ^c
Pigs	2.04	1.24-3.34
Veal calves	1.28	1.06-1.53

^a Pig samples were pooled (10 pools of 6 pigs per farm). Samples were taken from individual veal calves. The number of veal calves sampled per farm (ranging from 10 to 43 samples per farm) was equal to the square root of the total number of veal calves on that farm. ^b Adjusted for the number of MRSA-positive dust samples on the farms. ^c P<0.05.

Discussion

This study indicates that short-term occupational exposure to pigs or veal calves on MRSA-positive farms frequently results in the acquisition of MRSA. However, within 24 h after exposure, 94% of those who had acquired MRSA tested negative again; the majority of people who acquire LA-MRSA during short-term occupational exposure lose the strain within 24 h. Possibly, the high prevalence of MRSA carriage in livestock farmers and livestock veterinarians found in cross-sectional surveys is partly the result of repeated contamination instead of real colonization [4,7,9]. Further longitudinal studies are needed to clarify these and other possible types of carriage and to determine the true dynamics and determinants of LA-MRSA carriage in humans. It is questionable whether the nasal presence of MRSA should be considered true colonization or whether it is better described as contamination. We presume that in the animal houses on MRSA-positive farms, high concentrations of MRSA are present in the dust, and it is well known that *S. aureus* can survive in dust for long periods [14]. People who work in these animal houses inhale MRSA-contaminated dust particles that may persist in the nares for hours to days without truly colonizing the epithelial cells [15].

Therefore, there is a risk of overestimating colonization rates, and other cross-sectional studies could overestimate colonization for the same reason. For *S. aureus*, it is confirmed that persistent colonization occurs only in 20% of persons; 60% are intermittent carriers, and 20% are noncarriers [16].

In this study, some persons acquired MRSA more frequently than others; 52% of the field workers never acquired MRSA despite their visits to MRSA-positive farms (17/33), while 24% of the field workers acquired MRSA once and another 24% acquired MRSA more than once. This could not be attributed to the number of sampling days or to sampling on specific farms, possibly due to a lack of statistical power. An explanation for this difference in acquisition may be differences in susceptibility to MRSA. Many different studies have been performed to reveal host susceptibility patterns for both *S. aureus* and MRSA, indicating that this could somehow play a role in MRSA acquisition [17]. In this specific setting, hygienic behaviour during work and the use of personal protective equipment may have influenced the potential for acquiring MRSA. This was not evaluated in this study and needs further investigation.

The *spa* type of one field worker (field worker 5) was different from that found on the particular farm visited (t108 versus t011); he did not report any other contact with livestock. The most plausible explanation is that more than one *spa* type was present on the farm, as found in another study [18]. Due to the analytical method applied, this was not detected in the dust samples.

Part of the “search-and-destroy policy” is to screen health care workers who have been exposed to MRSA-positive patients without taking transmission-based precautions. Those who are persistently colonized are temporarily suspended from work. Samples are taken not during the work shift on which the health care worker has been exposed but during the next work shift [10] (www.wip.nl). This is done to limit the number of false-positive results due to contamination. This is consistent with our study results, which show that presence of MRSA after short-term occupational exposure to livestock rarely persists for more than 24 h.

The small sample size was the main limitation of our study, however, significant associations between MRSA acquisition and positive animal and dust samples were found. Another limitation of this study is the difference between the analytical methods applied for the examination of the swabs from the pig and veal farms [4,7]. Studies on hospital-acquired MRSA strains in human samples suggest that selective enrichment broth with large amounts of antimicrobials can inhibit the growth of *S. aureus* in general [19]. However, the detection of LA-MRSA using additional enrichment, as in this study, does not affect MRSA growth [11]. Since we found only LA-MRSA strains in the nasal swabs of both pig and veal calf field workers, and all suspected strains were confirmed by *mecA* gene PCR in both protocols, it is not likely that this difference has influenced the study results.

In conclusion, LA-MRSA is frequently acquired after short-term occupational exposure. However, the majority of people who acquire LA-MRSA during occupational exposure test negative for MRSA again within 24 h. This calls into question whether these individuals are colonized or contaminated. Screening of individuals upon hospital admission within 24 h after exposure to livestock does not seem reasonable.

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Chapter 2.5

Livestock-associated methicillin-resistant *Staphylococcus aureus* in humans, Europe

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Abstract

To estimate the proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from humans that were sequence type (ST)398, we surveyed 24 laboratories in 17 countries in Europe in 2007. Livestock-associated MRSA ST398 accounted for only a small proportion of MRSA isolates from humans; most were from the Netherlands, Belgium, Denmark and Austria.

Introduction

Livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) was first associated with human disease in 2003, when a MRSA clone associated with a reservoir in pigs and cattle was isolated from a human. This clone was not typable by pulsed-field gel electrophoresis with *Sma*I macrorestriction digestion and belonged to multilocus sequence type (ST) 398 [1]. Since then, rates of MRSA ST398 carriage have been high (25%-35%) for persons in the Netherlands who have frequent contact with pigs and veal calves, but associated illness is rare [2]. However, in Europe, Asia and the United States, invasive infections and a hospital outbreak of MRSA ST398 have been reported [3]. We estimated the proportion of MRSA isolates from humans in Europe in 2007 that were ST398.

The study

Questionnaires were mailed to 43 laboratories in 23 European countries, selected on the basis of expertise and publications about MRSA. Questions asked for level of laboratory and typing methods used, number of MRSA isolates identified in 2007, number of these isolates that were typed, and number of typed isolates that were MRSA ST398. MRSA isolates were considered to be ST398 if they 1) belonged to multilocus ST398; 2) were *spa* types t011, t034, t108, t567, t571, t588, t753, t898, t899, t1184, t1254, t1255, t1451, t1456, t1457, t2123, t2330, t2383, t2582, or t3013 ([4], National Institute for Public Health and the Environment, unpub. data); or 3) were not typable by pulsed-field gel electrophoresis with *Sma*I macrorestriction digestion. Laboratories were asked to report data on clinical isolates only (as opposed to screening isolates) and to provide the distribution by body site.

For each laboratory, the proportion of MRSA ST398 among all typed MRSA isolates from humans and the 95% Wilson confidence interval (CI) were calculated. For laboratories that typed all MRSA isolates, χ^2 testing compared proportions of isolates from various body sites for MRSA ST398 isolates and for other MRSA isolates.

For each country, we compared the proportions of MRSA ST398 among human MRSA isolates with number of pigs per km², number of cattle <1 year of age (a surrogate for veal calves) per km², and 2 indices multiplying these animal densities with human population densities. Criteria were that a laboratory had to report ≥ 100 MRSA isolates and type $>25\%$ of those isolates, thus leaving 14 national or regional laboratories from 12 countries. For Austria, data from 2 laboratories were pooled because these laboratories did not report duplicate isolates. For Denmark, only data on MRSA clinical isolates were used. Data for 2007 on midyear human population, pig production, and

production of cattle <1 year of age were obtained from Eurostat (<http://epp.eurostat.ec.europa.eu>) except for pig production in Switzerland (Swiss Statistics, www.bfs.admin.ch) and Turkey (Turkstat, www.turkstat.gov.tr). Land area was obtained from The World Factbook (www.cia.gov/library/publications/the-world-factbook). For Germany, 1 region with high pig density was considered separately from the rest of the country. Data for this region (Eurostat regional Nomenclature of Territorial Units for Statistics code DEA3, corresponding to laboratory 8) were obtained from Eurostat, the Chamber for Agriculture Nordrhein-Westfalen: "Zahlen zur Landwirtschaft 2008" (www.landwirtschaftskammer.de/wir/pdf/zahlen-landwirtschaft-2008.pdf), and the statistical office of Nordrhein-Westfalen in Germany.

Questionnaires were received from 24 laboratories (response rate 56%) in 17 countries. Data from Malta and Slovenia and from 1 laboratory in Italy were not analyzed because these laboratories did not type MRSA isolates. Among the remaining 15 countries, 8 countries reported a combined total of 8,262 MRSA isolates with typing results, of which 142 (1.7%, 95% CI 1.5–2.0%) were MRSA ST398 (Table 2.5.1).

Table 2.5.1 Characteristics of laboratories that reported MRSA and livestock-associated MRSA ST398 isolates from human samples, Europe, 2007^a.

Laboratory no.	Country	Type of laboratory	Source of MRSA isolates	No. MRSA isolates recieved	No. MRSA isolates typed	MRSA ST398 isolates	
						No. (%)	95% CI
1	Austria	National ref	All	523	523	0	0-0.7
2	Austria	National ref	All	586	586	16 (2.7)	1.7-4.4
3	Belgium	National ref	All	329	149	7 (4.7)	2.3-9.4
4	Czech Republic	National ref	Blood	37	10	0	0-27.8
5	Denmark	National ref	All	659	659	14 (2.1)	1.3-3.5
	Denmark	National ref	Clinical	370	370	6 (1.6)	0.7-3.5
6	Finland	National ref	All	1,323	1,323	1 (0.1)	0-0.4
7	Germany	National ref	Clinical	1,293	1,293	9 (0.7)	0.4-1.3
8	Germany	Regional ref	Clinical	866	866	37 (4.3)	3.1-5.8
9	Greece	National ref	Clinical	336	336	0	0-1.1
10	Hungary	National ref	All	365	63	0 ^b	NA
11	Iceland	National ref	Clinical	21	21	0	0-15.5
12	Italy	National ref	Clinical	108	108	1 (0.9)	0.2-5.1
13	Ireland	National ref	Clinical	832	696	0	0-0.5
14	The Netherlands	National ref	Clinical	478	478	57 (11.9)	9.3-15.1
15	The Netherlands	Local	Clinical	12	12	3 (25.0)	8.9-53.2
16	Sweden	National ref	All	1,127	1,127	8 (0.7)	0.4-1.4
17	Switzerland	Local	Clinical	587	65	0 ^b	NA
18	Switzerland	Regional ref	All	182	182	0	0-2.1
19	Switzerland	Regional ref	Clinical	64	64	0	0-5.7
20	Switzerland	Local	All	80	78	0	0-4.7
21	Turkey	Local	Clinical	198	60	0	0-6.0

^a MRSA, methicillin-resistant *Staphylococcus aureus*; ST398, sequence type 398; CI, confidence interval; ref, reference laboratory; NA, not applicable. ^b Not reported because laboratory typed <25% of MRSA isolates.

The proportions of MRSA ST398 per country were 0–11.9%; the countries with the highest proportion were the Netherlands (11.9%), Belgium (4.7%), Denmark (1.6%), and Austria (1.4%, pooled data). The proportion of isolates from blood was significantly lower for MRSA ST398 than for other MRSA clinical isolates. No difference was observed for other body sites (Table 2.5.2).

Table 2.5.2 Distribution of typed MRSA ST398 and other MRSA clinical isolates, by body site, 7 European countries, 2007^a.

Sample source	No. (%) typed clinical isolates		P value ^b
	MRSA ST398, n=113	Other MRSA, n=3,435	
Blood	2 (1.8)	343 (10.0)	0.004
Respiratory tract	20 (17.7)	451 (13.1)	0.16
Skin and wound	76 (67.3)	2,312 (67.3)	0.99
Urinary tract	6 (5.3)	173 (5.0)	0.90
Other	9 (8.0)	156 (4.5)	0.09

^a Only data from 9 national or regional laboratories in 7 countries that reported clinical isolates and typed all these isolates were included. Boldface indicates statistical significance (p<0.05). MRSA, methicillin-resistant *Staphylococcus aureus*; ST398, sequence type 398. ^b χ^2 test.

The proportion of MRSA ST398 among human MRSA isolates correlated with pig density (Spearman ρ =0.79, p =0.001) and with the index combining this density with human population density (Spearman ρ =0.76, p =0.002). The proportion of MRSA ST398 among human MRSA isolates also correlated, although less significantly, with the density of cattle <1 year of age (Spearman ρ =0.61, p =0.05) and with the index combining this density with human population density (Spearman ρ =0.74, p =0.01).

Conclusions

Livestock-associated MRSA ST398 was reported from 8 of 15 European countries. Except for 4 countries and 1 region in Germany, the proportion of MRSA ST398 among MRSA isolates from humans was <2%, suggesting that in 2007 this livestock-associated clone contributed to only a small fraction of all MRSA in humans. A recent study of laboratories in 26 European countries during September 2006–February 2007 found no MRSA ST398 among *S. aureus* isolates from persons with invasive infections [5]. MRSA ST398 has been isolated from human samples from Austria [5], the Netherlands [2], Belgium [6], Italy [7], Spain [8], Germany [9], Portugal [10], Denmark [11], the Czech Republic [12], Sweden [13], and France [14]. This study demonstrated MRSA ST398 in human samples in Switzerland and Finland. Although few data have been published on the proportion of MRSA ST398 in Europe, Springer et al. [15] reported that during 2006

through mid-2008, among 1,043 human MRSA isolates in Austria, 21 (2.0%) were MRSA ST398, which is similar to the proportion (1.4%, pooled data) found in our study.

Among isolates from blood, a significantly lower proportion were MRSA ST398 than other MRSA. This finding suggests that MRSA ST398 is associated with less severe disease, as indicated [5].

The proportion of MRSA ST398 among human MRSA isolates in European countries correlated with pig and veal calf densities and with an index combining pig or veal calf density and human population density. In addition to the well-documented risk factor of occupational exposure to pigs and veal calves, proximity of humans to pigs and veal calves may contribute to transmission of MRSA ST398 from animals to humans. However, the fact that farms are not equally distributed throughout a country may explain the higher proportion of MRSA ST398 among MRSA isolates from humans in certain European countries and regions.

Use of readily available data bears some limitations. Laboratories were not randomly selected, which could result in selection bias. However, bias was limited because most laboratories were national reference laboratories that routinely collect MRSA isolates countrywide. Also, countries may have active national or local screening policies, may select which isolates to type, and may use typing techniques that are not always fully comparable. To minimize these variations, when possible we reported on data from clinical isolates only and excluded data from laboratories that reported few isolates and did not type most MRSA isolates. We also provided a list of MRSA *spa* types that at the time of the study had been identified as corresponding to MRSA ST398. Other *spa* types and multilocus sequence types belonging to the livestock-associated MRSA clones have been recently reported [4] and were not included in our study.

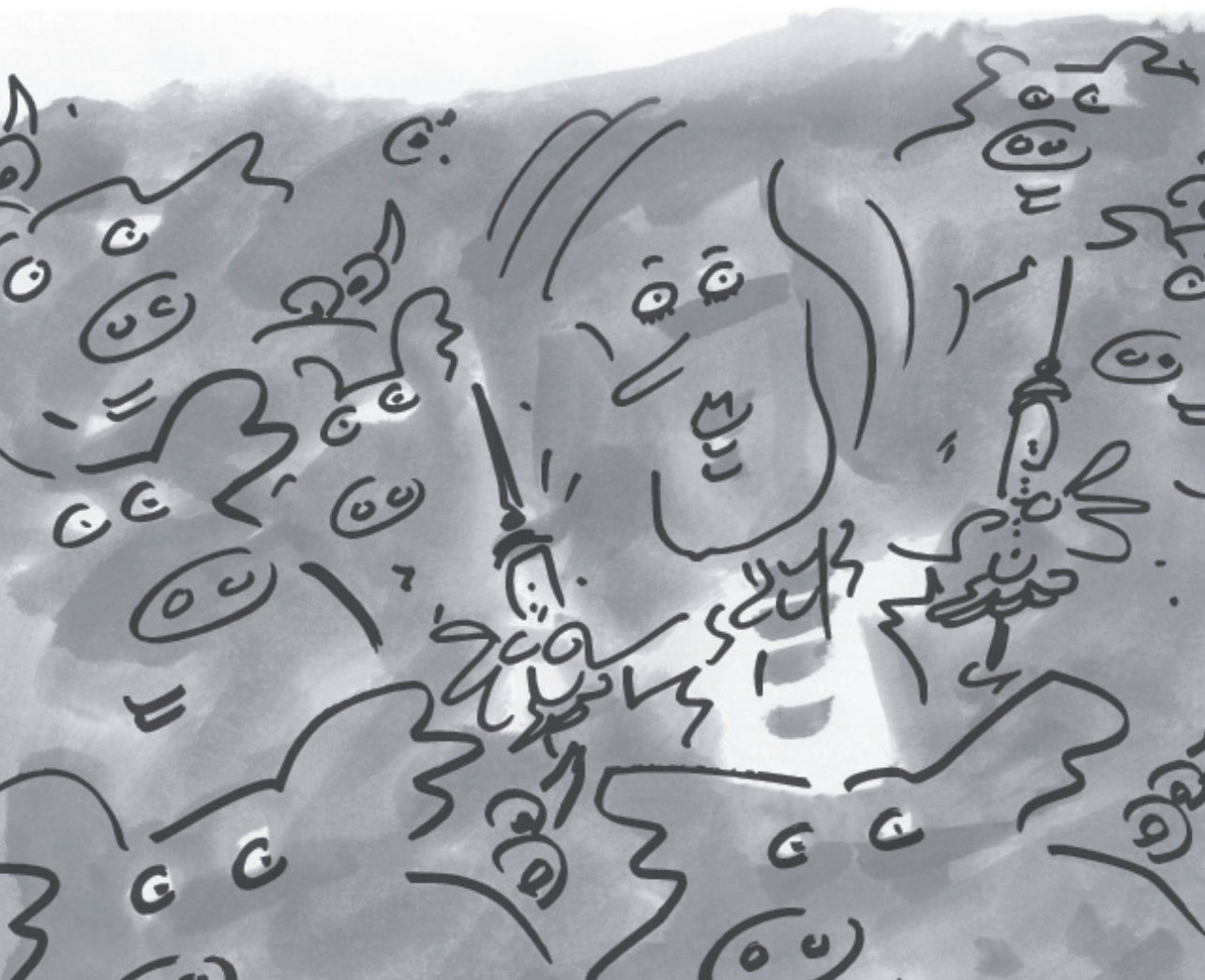
This cross-national prevalence study found livestock-associated MRSA ST398 in human samples in several European countries. However, the relatively low proportion of MRSA ST398 among MRSA isolates from humans in most countries suggests that MRSA ST398 contributes to only a small fraction of all MRSA in humans.

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Chapter 3

Clinical impact of (livestock-associated) MRSA



Chapter 3.1

Cross border comparison of MRSA bacteraemia between the Netherlands and North Rhine- Westphalia (Germany): a cross- sectional study

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Abstract

Background

We describe the impact of methicillin-resistant *Staphylococcus aureus* (MRSA) in two neighbouring regions in Europe with a comparable population size, North Rhine-Westphalia (NRW) in Germany and the Netherlands.

Methodology/principal findings

We compared the occurrence of MRSA in blood cultures from surveillance systems. In the Netherlands in 2009, 14 of 1,510 (0.9%) *Staphylococcus aureus* bacteraemia episodes under surveillance were MRSA. Extrapolation using the number of clinical admissions results in a total of 29 MRSA bacteraemia episodes in the Netherlands or 1.8 episodes per 1,000,000 inhabitants. In 2010 in NRW, 1,029 MRSA bacteraemias were reported, resulting in 57.6 episodes of MRSA bacteraemia per 1,000,000 inhabitants: a 32-fold higher incidence than in the Netherlands.

Conclusion/significance

Based on an estimated attributable mortality of 15%, the Dutch approach would save approximately 150 lives per year by the prevention of bacteraemia only.

Introduction

Staphylococcus aureus (*S. aureus*) is a member of the commensal flora of the skin and mucous membranes. It is also known to cause serious infections, mostly in hospitalized patients, especially those undergoing dialysis or surgical procedures [1]. Methicillin-resistant *S. aureus* (MRSA) is resistant to beta-lactam antibiotics (penicillins, cephalosporins and carbapenems), and is a public health concern in many countries all over the world.

At first, MRSA emerged in hospitals (hospital-associated: HA-MRSA), but more recently community-associated MRSA (CA-MRSA) emerged and caused illnesses of varying severity outside the hospitals. In 2005, MRSA was first linked to livestock and a genetically distinct group of strains was identified as livestock-associated MRSA (LA-MRSA) [2]. Nowadays, the boundaries between HA-MRSA and CA-MRSA have become less clear and LA-MRSA has entered the arena. MRSA infections are superimposed on the existing infections caused by methicillin-susceptible *S. aureus* (MSSA) and thereby add significantly to the total burden of disease [3].

Within Europe marked inter-country variations exist for the prevalence of MRSA, which can be due to differences in control measures, usage of antimicrobials and numerous other factors [4,5]. With this study, we describe the large impact of MRSA in two neighbouring and almost equally large populated regions, North Rhine-Westphalia (NRW) in Germany and the Netherlands, by comparing the occurrence of MRSA in blood culture isolates.

Methods

Ethics statement

This study made use of available data, retrieved by national or regional surveillance programs. Therefore no additional ethical approval for this study was needed.

The Netherlands

The study was based on data from the national antibiotic resistance surveillance system in the Netherlands (ISIS-AR) [6]. The 22 participating laboratories serve approximately 50% of all hospital beds. Data from 2009 on *S. aureus* isolates were extracted and evaluated for material of origin; blood isolates were used, as these are objective markers of invasive infections. Only the first blood isolate per patient was included.

In order to compare data from the Netherlands with NRW, extrapolation of MRSA blood cultures from ISIS-AR to the whole of the Netherlands was performed using the

proportion of clinical admissions, which were extracted from mandatory annexes from annual reports on 2009 (Available: www.jaarverslagenzorg.nl. Accessed 2012 Feb 6.), excluding one-day and psychiatric admissions. The annexes were only mandatory in 2009, therefore this year was used to compare data from the Netherlands with NRW. Clinical admissions were added up and compared to the national number of clinical admissions in 2009 (Statistics Netherlands. Available: www.cbs.nl. Accessed 2012 Feb 6.), excluding the same two categories.

In order to show the number of MRSA bacteraemia episodes per 1,000,000 inhabitants, population numbers of the Netherlands were derived from Statistics Netherlands (Available: www.cbs.nl. Accessed 2012 Feb 6.). Extrapolation was done under the assumption that the relation between clinical admissions and population density is the same for laboratories participating in the study and for those who do not.

North Rhine-Westphalia (Germany)

Data from NRW in 2010 were collected from the mandatory reporting of MRSA blood culture isolates from laboratories (State Institute for Health and Work of the state North Rhine-Westphalia. Available: www.liga.nrw.de. Accessed 2011 Nov 9.), which comprises data from all 404 hospitals in NRW. The year 2010 was the first complete year, and this year was used to compare data from NRW with the Netherlands.

The population number of NRW in 2010 was derived from North Rhine-Westphalian company for Information and Technics (Available: www.it.nrw.de. Accessed 2011 Nov 9.).

Results

The Netherlands

Data from 2009 from the 22 participating labs in ISIS-AR resulted in 1,512 episodes of *S. aureus* bacteraemia. Of the 1,510 bacteraemia episodes with resistance information, 14 were methicillin resistant (0.9%). Of the patients with MRSA bacteraemias, 79% were male and 71% were older than 65 or younger than two years of age. The median age was 69 years (interquartile range 56-74).

The sum of clinical admissions from hospitals belonging to the participating laboratories in ISIS-AR in 2009 was 903,623, which is 48% of the total of 1,899,000 admissions in the Netherlands. Extrapolating the 14 episodes of MRSA bacteraemia in 2009 to the whole of the Netherlands using the proportion of clinical admissions, there would have been $14 \times (1,899,000 / 903,623) = 29.4$ episodes of MRSA bacteraemia in 2009 in the

Netherlands in total. This can be recalculated into an incidence of $29.4/16,485,787=1.8$ MRSA bacteraemic episodes per 1,000,000 inhabitants in 2009.

North Rhine-Westphalia (Germany)

Data from the mandatory reporting of MRSA blood culture isolates from 1 January 2010 to 31 December 2010 comprising all 404 hospitals in NRW resulted in 1,029 episodes of MRSA bacteraemia. This can be recalculated into an incidence of $1,029/17,872,763=57.6$ MRSA bacteraemias per 1,000,000 inhabitants of NRW, which is 32-fold higher than in the Netherlands ($57.6/1.8=32.0$, Fisher exact $p<0.0001$, **Figure 3.1.1**). Of the patients with MRSA bacteraemia, 61% were male and 74% were older than 65 or younger than two years of age. The median age was 73 years (interquartile range 65-80).

3.1

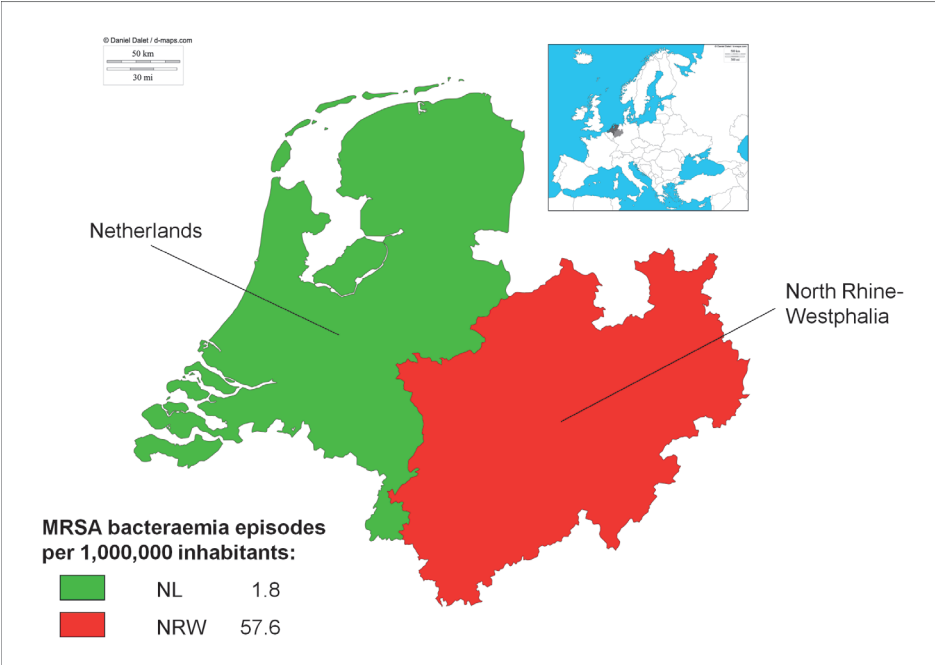


Figure 3.1.1 Map of the Netherlands (NL) and North Rhine-Westphalia (NRW). MRSA bacteraemia episodes per 1,000,000 inhabitants for the years 2009 (NL) and 2010 (NRW). A large difference in MRSA bacteraemia episodes was found in this study. Inlay: position of NL and NRW in Europe. Copyright d-maps.com (used maps: paybas22, rhenanienord38, europemax09).

Discussion

This study found a large difference in the incidence of MRSA bacteraemias between the region of North Rhine-Westphalia in Germany and the Netherlands. NRW had approximately 1,000 episodes of MRSA bacteraemia more in 2010 than the Netherlands in 2009 (1,029-29.4=999), which resulted in a 32-fold higher incidence of MRSA per 1,000,000 inhabitants. The best explanations for this difference in incidence of MRSA bacteraemia in these two adjoining regions with comparable inhabitant numbers are differences in healthcare structure and differences in the implementation of an MRSA control strategy.

Healthcare structure differences

The differences in healthcare structure can be illustrated with several examples. First, there are grossly 3 times more hospitals in NRW (413 compared to 115 in 2009 in NRW and the Netherlands, respectively), and about twice as much hospital beds (6.8 and 3.3 per 1,000 inhabitants in 2009 in NRW and the Netherlands, respectively) (Statistics Netherlands. Available: www.cbs.nl. Accessed 2012 Feb 6, State Information and Techniques North Rhine-Westphalia. Available: www.it.nrw.de. Accessed 2011 Nov 9, EUROSTAT. Available: <http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home/>. Accessed 2012 Feb 6.).

Second, in NRW 2.3-fold more patients are admitted to the hospital compared to the Netherlands, while the length of stay is comparable (EUROSTAT. Available: <http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home/>. Accessed 2012 Feb 6.). More frequent (re)admissions in combination with a higher MRSA proportion among *S. aureus* isolates (26.6% for NRW [7] versus 1.6% for the Netherlands, this study) may lead to a higher introduction rate of MRSA in hospitals in NRW, however this does probably not fully explain the large 32-fold difference in MRSA bacteraemia episodes in this study.

Third, in Germany there are mainly large commercial laboratories that serve large areas, few hospitals have clinical microbiologists available on site. In the Netherlands clinical microbiology laboratories including microbiologists are present in almost every hospital. They are actively involved in the infection control strategy. Recently, in Germany a new law was accepted that made the presence of microbiologists in hospitals mandatory, which might lead to improvements in infection control. It seems that the implementation of an MRSA control policy is more effective in a healthcare structure like the Netherlands.

MRSA control strategy differences

The 'search and destroy' (S&D) strategy in the Netherlands consists of active screening and pre-emptive isolation of persons admitted to foreign hospitals in the previous two months, persons previously positive for MRSA, and persons in contact with live pigs or veal calves (for complete national guidelines see www.wip.nl). Individuals that carry MRSA are actively decolonized with the exception of those who have livestock contact [8]. When unexpected cases are found, active contact tracing is performed.

In Germany there are recommendations actually more strict than the Dutch strategy, with an extra risk group of persons admitted to local hospitals in the last 12 months (for complete German guidelines, see www.rki.de). However, up to now a complete implementation in Germany has only been shown in several regional MRSA-networks (www.eursafety.eu, www.mre-net.org), other regions (among which parts of NRW) have not fully implemented the screening and isolation strategy. This is in contrast to the Netherlands, where the S&D strategy is fully implemented in all hospitals and controlled by the independent national health inspectorate.

The effectiveness of screening regimens depends on various factors, such as the number of patients admitted and re-admitted, the patient-health-care worker ratio [9], the existence of and adherence to hygiene protocols to prevent transmission [10], and host susceptibility [4]. Several articles have studied the effect of individual screening interventions (see [11] for an overview), but so far the effectiveness of the total bundle of the S&D strategy has not been tested. The presence of fully implemented proper national guidelines in all healthcare institutions might be the key to MRSA infection control.

MRSA prevalence, antimicrobial use and population characteristics

Other factors that may contribute to a higher incidence of MRSA bacteraemia are the prevalence of MRSA in the general population, antimicrobial use and population characteristics. Germany has on average 2.5 MRSA cases per 100 inpatients, in contrast to 0.2 MRSA cases per 100 inpatients in the Netherlands [12,13,14,15]. Moreover, as previously stated, of all *S. aureus* blood isolates in NRW in 2009, 26.6% were MRSA [7], compared to 1.6% in the Netherlands (this study).

Additionally, antimicrobial consumption in ambulatory care in NRW is 16.2 defined daily doses (DDD) per 1,000 inhabitants per day, as opposed to 11 DDD in the Netherlands (ESAC country sheets 2008. Available: www.esac.ua.ac.be. Accessed 2012 Feb 6, Germap 2008. Available: www.bvl.bund.de. Accessed 2011 Nov 9.).

Lastly, when looking at population characteristics or cultural factors [4,5], the gender and age of patients did not differ between the regions (79% and 61% males, Fisher

exact $p=0.29$, and 71% and 74% persons older than 65 or younger than two years of age, Fisher exact $p=1.00$, in the Netherlands and NRW, respectively).

Study limitations

This study made use of available data, which bears some limitations. First, selection bias may count for part of the results. In the region of NRW all MRSA blood cultures are supplied, as for the Netherlands only about 50% of admissions are covered, leading to the need to extrapolate these data. The missing hospitals are relatively often located in the larger cities. If hospitals in large cities would have more MRSA bacteraemia episodes, for example because of more foreign patients, patients that travel more often or more complicated patients, the results of this study would underestimate the true MRSA bacteraemia prevalence, and thereby overestimate the difference between the regions studied. On the other hand, the participating laboratories are located more often in pig dense areas where MRSA carriage rates are higher than elsewhere in the Netherlands, hypothetically leading to an overestimation of MRSA bacteraemia prevalence and an underestimation of the difference between the studied regions. However, LA-MRSA carriage does not often result in bacteraemias. Regardless of the above, other studies do not indicate that there are large differences in MRSA prevalences in hospitals within the Netherlands [4,15]. Therefore we assume that our extrapolation is legitimate.

A second limitation of this study is the difference in time periods that were studied (2009 in the Netherlands and 2010 in NRW, for argumentation see Methods). No major legislative changes have been described for both countries, and MRSA prevalences are estimated to be roughly stable over these years [7,16]. For these reasons we feel that comparison of these two different years is justifiable.

Third, the effects found in this study could be an underestimation since only bacteraemias were counted. If all MRSA-infections were included, the morbidity, mortality and costs saved will probably be more than demonstrated hereunder. So these assessments can be considered a minimal estimate of the benefits.

Possible effects

The possible impact of the observed differences can be estimated based on several assumptions. MRSA bacteraemia increases the burden of disease as it does not appear to replace the susceptible strains, but comes on top of it [17,18]. This would mean that the Dutch approach prevents approximately 1,000 MRSA bacteraemia episodes per year, not only possibly resulting in less morbidity, but also in less mortality and less costs. Several studies show that the attributable one-year mortality of MRSA bacteraemia has a lower limit of 15% (Duin Van D, Fraser T, Jain A, Gordon S, Shresta N.

Attributable mortality after *S. aureus* bacteraemia. Oral presentation at the annual scientific meeting of the Society of Healthcare Epidemiology of America 2011, abstract 299. Available: <http://shea.confex.com/shea/1/webprogram/Paper4403.html>. Accessed 2012 Feb 6.) [18,19,20]. Extrapolating these numbers results in a minimum estimate of $1,000 \times 15\% = 150$ deaths that would not occur in the Netherlands because of MRSA bacteraemia. According to a recent mathematical model it will also save money as MRSA bacteraemia is associated with substantial costs [21].

Conclusions

In conclusion, we observed a huge difference in the incidence of MRSA bacteraemia episodes in two comparable and neighbouring regions, North Rhine-Westphalia in Germany and the Netherlands. The estimated annual savings for the Netherlands are at least 150 lives. Next to differences in healthcare structure, the active 'search and destroy' strategy in the Netherlands appears to be an efficient way to prevent many MRSA infections.

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Chapter 3.2

Low incidence of livestock-associated methicillin-resistant *Staphylococcus aureus* bacteraemia in the Netherlands in 2009

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a worldwide problem in both hospitals and communities all over the world. In 2003, a new MRSA clade emerged with a reservoir in pigs and veal calves: livestock-associated MRSA (LA-MRSA). We wanted to estimate the incidence of bacteraemias due to LA-MRSA using national surveillance data from 2009 in the Netherlands. We found a low incidence of LA-MRSA and MRSA bacteraemia episodes, compared to bacteraemias caused by all *S. aureus* (0.04, 0.18 and 19.3 episodes of bacteraemia per 100,000 inhabitants per year, respectively). LA-MRSA and MRSA were uncommon compared to numbers from other countries as well. MRSA in general and LA-MRSA in specific does not appear to be a public health problem in the Netherlands now. The low incidence of LA-MRSA bacteraemia episodes may best be explained by differences in the populations affected by LA-MRSA versus other MRSA. However, reduced virulence of the strain involved, and the effectiveness of the search and destroy policy might play a role as well.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a worldwide problem in both hospitals and communities all over the world. *Staphylococcus aureus* (*S. aureus*) is typically a resident of the skin and mucous membranes, but also known for serious infections as wound infections, necrotizing pneumonia, endocarditis, osteomyelitis and sepsis [1].

In 2003, a new MRSA clade with a reservoir in pigs and veal calves emerged: so called livestock-associated MRSA (LA-MRSA) [2]. Until now, high prevalences of LA-MRSA carriage are found in persons in close contact with pigs and veal calves (around 30%) [3,4]. Severe infections have been described occasionally [5] and a few outbreaks have been reported [6-8].

Studies find less virulence genes in LA-MRSA strains [9-12], but the possible effect of this finding has not been studied yet in clinical samples. We wanted to estimate the current incidence of severe infections due to LA-MRSA using a national surveillance database in the Netherlands.

Methods

This observational study was based on data from the national antibiotic resistance surveillance system (ISIS-AR, www.rivm.nl/cib/themas/isis-ar) which included data from participating microbiological laboratories, and the national MRSA surveillance program (<http://mrsa.rivm.nl>), where index strains are collected from newly recognized MRSA carriers and/or MRSA infections all over the Netherlands [13].

The 22 participating laboratories in ISIS-AR covered approximately 50% of all hospital beds. Data from 1st January 2009 to 31st December 2009 on *S. aureus* blood isolates with their susceptibility profile were extracted, only the first blood culture isolate per patient per year was included. Since only the material of origin was known, and not the severity of infection, bacteraemias were used as a measure of severe infections. For skin and soft tissue, *S. aureus* can cause a range of infections, from harmless skin lesions to severe deep tissue infections. However, in this database it is impossible to distinguish between these.

ISIS-AR included antibiotic susceptibility profiles, but no information on *spa*-types. The national MRSA surveillance program did have data on *spa*-types and multiple-locus variable number of tandem repeat analysis (MLVA), but did not contain methicillin-sensitive *S. aureus* (MSSA) isolates, and did not specifically contain blood isolates, as every first MRSA positive sample per patient was submitted. Since livestock farmers and other known MRSA risk groups are screened upon admission (for complete national

guidelines, see www.wip.nl), it might be possible that a MRSA nasal isolate was submitted to the national MRSA surveillance program, instead of a blood isolate that was found later during hospitalization, resulting in an underestimation for MRSA and LA-MRSA bacteraemias. Therefore, bacteraemia episodes from ISIS-AR were matched to *spa*-types from the national MRSA surveillance database, and missing *spa*-types were retrieved by contacting the individual laboratories. These *spa*-types were used to identify livestock origin, using the criteria defined by Huijsdens et al. [14], and expert opinion from the national reference laboratory. In addition, MLVA was used to determine genetic relatedness between the MRSA strains coming from blood isolates from 2008-2010.

Gender and age from non-LA-MRSA and LA-MRSA bacteraemia episodes were compared with Fisher's exact test and Wilcoxon-Mann-Whitney test for independent samples with non-normal distributions. For proportions, Wilson confidence intervals (CI) were calculated.

National incidences of all *S. aureus*, MRSA and LA-MRSA in blood cultures were calculated by multiplying the ISIS-AR counts by the proportion of ISIS-admissions to the total number of clinical admissions. The number of clinical admissions of hospitals belonging to the participating laboratories in ISIS-AR was extracted from mandatory annual reports in 2009 (publicly available on www.jaarverslagenzorg.nl), excluding one-day admissions and psychiatric admissions. The total national number of clinical admissions in 2009 was available from Statistics Netherlands (www.cbs.nl), excluding the same two categories. Extrapolation was performed under the assumption that the relation between clinical admissions and population density is the same for laboratories participating in ISIS-AR as those who do not.

The number of *S. aureus* and MRSA carriers in the Netherlands in 2009 were calculated by multiplying the total inhabitant number of the Netherlands (Statistics Netherlands) by 27% [15] or by 0.11% [16], respectively. The number of LA-MRSA carriers in 2009 was calculated by multiplying the total number of persons working in veal calf farming (Statistics Netherlands) by 38% [17], plus the results of multiplying the number of persons working in pig farming (Statistics Netherlands) by 63% (preliminary results from own study group). Chances of a bacteraemia per carrier were calculated by dividing the number of carriers by the number of bacteraemias.

Results

Data from the year 2009 from the 22 participating labs in ISIS-AR resulted in 1,512 episodes of *S. aureus* bacteraemia. Of the 1,510 episodes with resistance information, 14 were MRSA ($14/1,510=0.9\%$, CI 0.6-1.6%). Of the 13 MRSA bacteraemia episodes

with known *spa*-types, three were LA-MRSA (3/13=23%, CI 8-50%, **Table 3.2.1**). MLVA results are shown in **Figure 3.2.1**. The *spa*-type of isolate 14 could not be traced back in the national MRSA surveillance database or the original laboratory database, and was reported missing. Four isolates (#10-13) showed the same relative rare *spa*-type (t3848), and originated from the same medical centre. These patients are probably part of a hospital outbreak. Gender and age distributions in the LA- and non-LA-MRSA groups were not significantly different ($p=1.00$ and $p=0.46$, respectively).

Table 3.2.1 *Spa*-types of 14 MRSA blood culture isolates from the Netherlands in 2009

Isolate no.	Gender	Age (year)	<i>Spa</i> -type	Livestock-associated ^a
1	Female	74	t002	No
2	Male	79	t002	No
3	Male	70	t011	Yes
4	Female	55	t011	Yes
5	Male	8	t015	No
6	Male	60	t038	No
7	Male	53	t108	Yes
8	Male	0	t740	No
9	Male	76	t3365	No
10	Male	73	t3848	No
11	Male	68	t3848	No
12	Male	69	t3848	No
13	Female	67	t3848	No
14	Male	89	Unknown	Unknown

^a Livestock association is determined using Huijsdens *et al.* [14].

The figure represents as a minimum spanning tree based on MLVA types (MT). Each MT is displayed as a circle with the *spa*-type of the isolate next to it in text, the size denotes the number of isolates, and the color represents the MLVA complex (MC), which are indicated in the legend as well. MC398 stands for MLVA complex 398, which represents the livestock-associated strains.

The sum of clinical admissions from hospitals belonging to the participating laboratories in ISIS-AR in 2009 was 903,623, which is 48% of the total of 1,899,000 admissions in the Netherlands. The total inhabitant number for the Netherlands in 2009 was 16,485,787. For *S. aureus*, MRSA and LA-MRSA bacteraemia episodes, incidences are shown in **Table 3.2.2**.

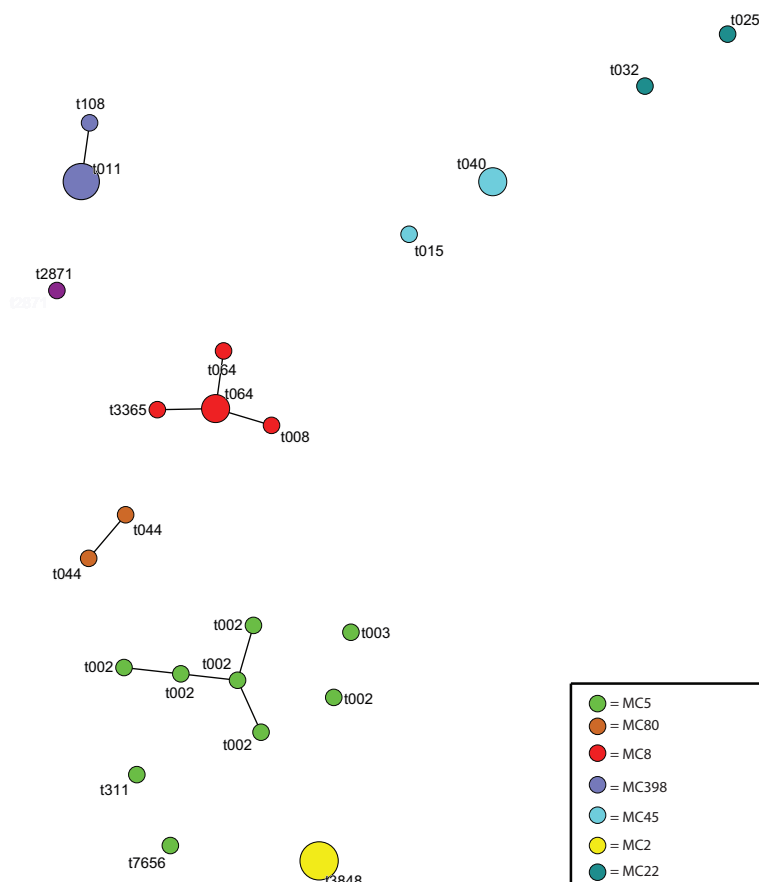


Figure 3.2.1. Genetic relatedness of 30 MRSA blood isolates from ISIS-AR from 2008-2010 in the Netherlands.

Table 3.2.2 Incidence of bacteraemia episodes in the Netherlands in 2009.

Type of <i>S. aureus</i>	Episode counts from ISIS-AR	Extrapolation to the Netherlands ^a	Incidence per 100,000 inhabitants ^b
All <i>S. aureus</i>	1,512	3,177.5	19.3
MRSA	14	29.4	0.18
LA-MRSA	3	6.3	0.04

^a Extrapolation performed by multiplying the ISIS-AR counts by the proportion of ISIS-admissions to the total number of clinical admissions (1,899,000/903,623). ^b Incidence per 100,000 inhabitants calculated by dividing the total number for the Netherlands by the total number of inhabitants*100,000 (16,485,787*100,000).

The number of *S. aureus* and MRSA carriers in the Netherlands was 4,451,162.5 (16,485,787*0.27), and 18,134.4 (16,485,787*0.0011), respectively. The numbers of persons working in veal calf or pig farming in 2009 were 5620 and 7682.5, respectively,

resulting in 6,975.6 LA-MRSA carriers ((5620*0.38)+(7682.5*0.63)). For *S. aureus*, MRSA and LA-MRSA bacteraemia episodes, chances per carrier are shown in **Figure 3.2.2**.

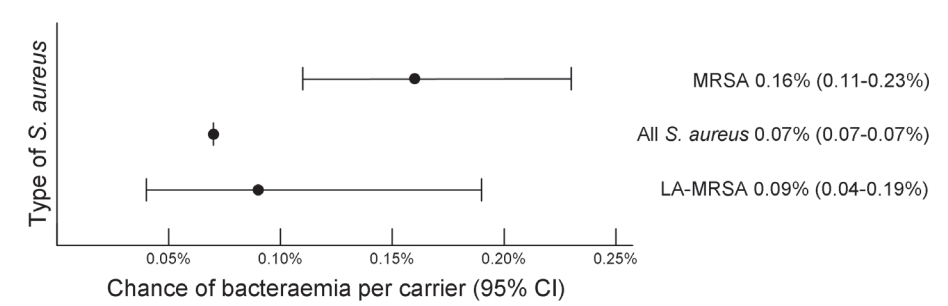


Figure 3.2.2 Bacteraemia chances per carrier in the Netherlands in 2009

Discussion

Incidences of *S. aureus* bacteraemias

This study based on surveillance data shows that the incidences of LA-MRSA bacteraemias (0.04/100,000 inhabitants) and MRSA bacteraemias (0.18/100,000 inhabitants) are negligible compared to that of *S. aureus* (19.3/100,000 inhabitants). The number of LA-MRSA bacteraemias appears to have been stable over the last years (3 LA-MRSA bacteraemias from 22 laboratories in ISIS-AR in 2009 versus 6 tetracyclin/doxycylin-resistant MRSA bacteraemias from 28 laboratories in 2012, data not shown). Methicillin-sensitive *S. aureus* appears to be livestock-associated as well in a substantial number of cases, Verkade and colleagues have recently studied this thus far unknown phenomenon [18].

In a study from Laupland et al., incidences of 2.4 and 26.3 MRSA and *S. aureus* bacteraemias per 100,000 inhabitants were reported for Finland, Australia, Sweden, Canada and Denmark from 2000 to 2008 [19]. Both *S. aureus* and MRSA bacteraemias appear to be less prevalent in the Netherlands, compared to these numbers. Since MRSA-prevalences in the Netherlands are best comparable to those from Northern Europe (Ears-Net, <http://ecdc.europa.eu>), data from only Finland, Sweden and Denmark were averaged, resulting in 0.4 and 27.1 MRSA and *S. aureus* bacteraemias per 100,000 inhabitants, which is more comparable to our results. Unfortunately, data on LA-MRSA were not available in the study of Laupland et al.

Data from ISIS-AR are very well comparable to data from the national MRSA surveillance program in the Netherlands, where 20 MRSA bacteraemias were counted in 2009, of

which 4 were LA-MRSA [20]. Contrary to expectations expressed in the Methods section, there does not seem to be an underestimation of LA-MRSA bacteraemias in this surveillance program.

Chance of bacteraemia per carrier

The chance for an MRSA carrier to develop a bacteraemia appears to be significantly higher compared to carriers of *S. aureus*, since both confidence intervals do not overlap. For LA-MRSA, due to low numbers confidence intervals are wide, resulting in no significant differences with either other MRSA or *S. aureus* in general. Nevertheless, there appears to be a trend towards a lower chance for a bacteraemia in LA-MRSA carriers, compared to carriers with other MRSA.

Livestock-associated MRSA

The low incidence of LA-MRSA bacteraemia, as well as the trend for a lower bacteraemia chance per LA-MRSA carrier could be explained in different ways. First, LA-MRSA strains may be less virulent for humans than other MRSA, as less virulence genes have been reported in these strains [9-12,21]. However, experts worry that the rapid evolution of this specific clade may result in gaining new virulence genes in the near future [22].

Second, the Dutch search and destroy strategy includes an active and effective screening regimen that identifies most patients with LA-MRSA at hospital admission (for complete guidelines see www.wip.nl). Decolonization is a part of this strategy, and may prevent the development of bacteraemia [16].

Third and probably most important, differences in patient characteristics may be responsible for the low incidence of LA-MRSA bacteraemias. Persons carrying LA-MRSA are usually working in the livestock industry, and are healthy persons. Persons carrying other (hospital or community associated) MRSA are in general admitted to a hospital, less healthy and probably more likely to develop invasive disease. In addition, an intact skin, which is considered to be the key determinant for protection against *S. aureus* infections, is more likely in livestock farmers than in inpatients, owing to venous lines, operations etc. [1,23].

Study limitations

This study made use of available data, which bares some limitations: we chose not to include skin and soft tissue infections, and only looked at bacteraemias, since this is the only direct and unambiguous measure for severe infections that can be derived from the available data. We realize that this might imply a possible underestimation of

infections. We advise to include other conditions like severe skin and soft tissue infection and pneumonia in future studies.

In addition, selection bias may count for part of the results. Only about 50% of admissions were covered by ISIS-AR, missing some hospitals in larger cities, with the participating laboratories located more often in pig dense areas. If hospitals in large cities would have more non-LA-MRSA bacteraemia episodes, for example because of more foreign patients, patients that travel or complicated patients, the results of this study would underestimate the true MRSA bacteraemia prevalence, and overestimate the LA-MRSA bacteraemias. We consider this effect to be minimal, as other studies do not indicate that there are large differences in MRSA prevalences in hospitals within the Netherlands [24].

Another potential cause of overestimation of the chance of LA-MRSA bacteraemias is the fact that we might have underestimated the number of LA-MRSA carriers, which we calculated from the number of persons working in veal or pig farms only, excluding professional groups as livestock transporters, slaughterhouse workers and veterinarians. In contrast, a possible underestimation of the chance of LA-MRSA bacteraemias might result from a proportion of 'carriers' being only contaminated with LA-MRSA, thus not truly colonized. This is supported by a previous study from this group, that reported that MRSA is frequently present after short-term occupational exposure, but in most cases the strain is lost again after 24 hours [25]. Altogether, we believe that possible variations in the chance of a LA-MRSA bacteraemia are of equal size as the confidence interval that is displayed in **Figure 3.2.2**.

Lastly, international comparison of LA-MRSA bacteraemias is difficult, since the Netherlands have a unique position with both an extremely low MRSA prevalence and a high number of people working in livestock farming. It appears nevertheless that LA-MRSA currently has a low rate of infections in European countries [26].

Conclusion

This study found a low incidence of LA-MRSA bacteraemia episodes, which may best be explained by differences in the populations affected by LA-MRSA versus other MRSA. However, reduced virulence of the strain involved, and the effectiveness of the search and destroy policy might play a role as well. At present the impact of MRSA in general and LA-MRSA in particular on bacteraemias for the Dutch population appears to be very limited.

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Chapter 3.3

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

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Abstract

There is limited knowledge about the effect of livestock-associated (LA-) methicillin-resistant *Staphylococcus aureus* (MRSA) carriage on health-related quality of life (QoL). With this study, we explored whether LA-MRSA causes infections or affects health-related QoL in pig farmers. This prospective cohort study surveyed persons working on 49 farrowing pig farms in the Netherlands for 1 year (2010-2011). On six sampling moments, nasal swabs, environmental samples and questionnaires on activities and infections were collected. At the end of the study year, persons were asked about their QoL using the validated SF36 and EQ-5D questionnaires. Of the 120 persons, 44 (37%) were persistent MRSA carriers. MRSA carriage was not associated with infections, use of antimicrobials, healthcare contact and health-related QoL items in univariate or multivariate analysis, most likely due to the 'healthy worker effect'. Despite high carriage rates the impact of LA-MRSA carriage in this population of relatively healthy pig farmers on health and health-related QoL appears limited, more research is needed for confirmation.

Introduction

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

In the Netherlands, a low carriage rate of methicillin-resistant SA (MRSA) was found in the community (0.1%) [6]. Therefore, new MRSA clades, such as livestock-associated MRSA (LA-MRSA), were easily recognized. The prevalence of LA-MRSA carriage ranged from 20–60% in people working with pigs [8–13]. Close contact with livestock was shown to be the major risk factor for LA-MRSA acquisition [11,13].

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

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

Methods

Study design and selection of farms

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

Sampling moments

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

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

The Short Form 36 (SF-36) [33,34] measured 8 dimensions of health: physical functioning, physical role functioning (i.e. limitations due to physical difficulties), bodily pain, social functioning, emotional role functioning, mental health, vitality and current general health perception. Each dimension scored 0 (least favourable health state) to 100 (most favourable health state). An additional question on health change, compared to a year earlier, was added, which could be scored as much better (score 0), a bit better (score 25), the same (score 50), a bit worse (score 75), or much worse (score 100) [34]. Two summary scales (physical and mental) were calculated and standardized to a mean of 50 with a standard deviation of 10 in the general population for easy comparison.

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

Definitions

Pig farmers were defined as individuals who worked in the stables for at least 20 hours per week at the start of the study, regardless of whether they lived on the pig farm premises or not. Persistent MRSA carriers were defined as persons with all cultures positive for MRSA, regardless of typing results, non-carriers had no positive MRSA-cultures, and intermittent carriers were the remaining persons. Nasal and

oropharyngeal samples were combined, if one of two was positive, the concerning sampling moment was considered positive.

Laboratory analysis

The extended laboratory procedure is described elsewhere [13]. In short, cultures were performed by incubating swabs on chromID *S. aureus* and chromID MRSA agar plates (BioMérieux, La Balme Les Grottes, France). Wet wipe samples (Sodibox, Nevez, France) were double-enriched and subsequently cultured on blood and *Brilliance*TM MRSA agar plates (Oxoid, Basingstoke, UK).

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

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

Statistical analysis

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

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

The effect of MRSA on acquisition of infections and use of antimicrobials was studied in sets of two consecutive sampling moments with PROC GENMOD, a generalized estimated equations model (logistic regression), with adjustment for correlations between repeated observations from the same individual and for potential confounders (age, gender, MRSA in home and stable environment, working in the stables, giving birth assistance to sows, wearing facemasks). All determinants with univariate Chi-square p-values of ≤ 0.20 were eligible for multivariate analysis. When Spearman's rho for two determinants was ≥ 0.70 , collinearity was assumed, and the determinant with

the highest prevalence ratio (PR) [41] or lowest p-value was selected for the multivariate analysis. Association of MRSA and healthcare contact, which was not asked on all sampling moments and therefore did not vary over the 1-year study period, was studied with Chi-square tests, or Fisher's exact tests when expected cell counts were below five.

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

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

Ethical considerations

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

Results

Study population

Of the 281 persons from 49 farms who entered the study (pig farmers, employees and household members), 198 worked with sows (70%), mostly men of working age. Of these persons, 62 did not fill in the QoL questionnaires and another 16 were persistent MSSA carriers, leaving 120 persons for analysis (**Table 3.3.1**).

Table 3.3.1 General characteristics of the study population

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

Persistent: samples from all sampling moments positive; Non-carrier: all samples negative; Intermittent: the remaining persons; MRSA: methicillin-resistant *Staphylococcus aureus*; IQR: interquartile range (i.e. p25-p75). Only persons working with sows, who have filled in the Quality of Life questionnaire and were not persistent methicillin-susceptible *Staphylococcus aureus* carriers were depicted (n=120). ^a Median amount of hours worked per week were shown for all study subjects, being both pig farmers (persons who work in the stables, n=88) and household members (n=32).

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

Infections, use of antimicrobials and healthcare contact

Of the 198 persons working with sows, 398 sets of consecutive sampling moments were available for analysis, of which 198 were initially without MSSA. Frequencies and univariate logistic regression results are shown in **Table 3.3.2**. In total, 23 infections were present on 198 sampling moments (12%, Wilson 95% CI 8-17%, one person had both eczema and an open wound on one sampling moment). Skin infections were found on 21 of 198 (11%, 95% CI 7-16%) sampling moments, divided into 10% (10/100, 95% CI 6-17%) of MRSA-positive sampling moments, and 11% (11/98, 95% CI 6-19%) of MRSA-negative sampling moments in the 1 year study period. These skin infections occurred in 19 different persons (19/116=16%, 95% CI 11-24%). Presence of infections or use of antimicrobials was not significantly associated with MRSA in a previous sampling moment in univariate analysis. Additional multivariate analysis did not reveal associations with MRSA carriage either.

Table 3.3.3 shows the absence of an association of healthcare contact and MRSA carriage groups.

Table 3.3.2 Acquisition of infections and use of antimicrobials associated with MRSA, in consecutive sets of sampling moments.

Current sampling moment	Total (n=198)	Previous sampling moment		
		MRSA (n=100)	no MRSA (n=98)	P-value ^a
Abscess – n (%)	0 (0%)	0 (0%)	0 (0%)	n/a
Bacteraemia – n (%)	0 (0%)	0 (0%)	0 (0%)	n/a
Osteomyelitis – n (%)	2 (1%)	1 (1%)	1 (1%)	0,58
Impetigo – n (%)	0 (0%)	0 (0%)	0 (0%)	n/a
Pneumonia – n (%)	0 (0%)	0 (0%)	0 (0%)	n/a
Open wounds – n (%)	10 (5%)	4 (4%)	6 (6%)	0,29
Boils – n (%)	1 (1%)	0 (0%)	1 (1%)	n/a
Diabetes mellitus – n (%)	0 (0%)	0 (0%)	0 (0%)	n/a
Eczema – n (%)	11 (6%)	7 (7%)	4 (4%)	0,65
Psoriasis – n (%)	0 (0%)	0 (0%)	0 (0%)	n/a
Use antimicrobials – n (%)	13 (7%)	7 (7%)	6 (6%)	0,25

MRSA: methicillin-resistant *Staphylococcus aureus*; n/a: not applicable. ^aUnivariate p-values for association with logistic regression, adjusted for correlated observations from the same person.

Table 3.3.3 SF-36 questionnaire, EQ-VAS and healthcare contact, in relation to different types of MRSA carriage.

Dimension	MRSA carriage – mean score (SD)			P-value ^a
	Persistent	Intermittent	Non	
Physical functioning	93 (11)	95 (9)	95 (11)	0,74
Role functioning, physical	93 (20)	92 (22)	90 (26)	0,87
Bodily pain	91 (15)	87 (17)	85 (17)	0,42
General health perception	79 (14)	77 (16)	79 (15)	0,70
Vitality	77 (15)	72 (14)	75 (10)	0,15
Social functioning	92 (12)	91 (18)	91 (13)	0,91
Role functioning, emotional	97 (10)	94 (21)	93 (25)	0,62
Mental health	84 (12)	85 (9)	81 (8)	0,21
Physical summary scale	54 (6)	53 (6)	54 (7)	0,84
Mental summary scale	55 (6)	55 (6)	54 (5)	0,50
Health change last year	47 (12)	51 (8)	50 (0)	0,07
EQ-VAS current health status	85 (11)	86 (9)	83 (9)	0,45
Admitted to hospital last year – no/total (%)	2/44 (5)	1/53 (2)	3/22 (14)	0,13 ^b
Outpatient clinic last year – no/total (%)	9/44 (20)	8/53 (15)	5/22 (23)	0,68 ^c

SF-36: short form 36; EQ-VAS: EuroQol visual analogue scale; Persistent: samples from all sampling moments positive; Non-carrier: all samples negative; Intermittent: the remaining persons; MRSA: methicillin-resistant *Staphylococcus aureus*; SD: standard deviation. ^aOne-way ANOVA. ^bFisher's exact test. ^cChi-square test.

Short Form 36

Table 3.3.3 shows SF-36 data, where different MRSA-carriage types (persistent, intermittent, non) did not differ in mean scores.

When comparing persistent MRSA carriers with those who did not carry MRSA persistently, no relation was found with physical or mental summary scales, as depicted in **Table 3.3.4**. Only a higher age was associated with a lower physical summary scale

(-1.32 points per 10 years, 95% CI -2.30- -0.35, p 0.03) in univariate analysis. Additional multivariate analysis did not reveal associations with persistent MRSA carriage either.

Table 3.3.4 Univariate regression on summary scales of SF-36 questionnaire

Determinant	Mental summary scale			Physical summary scale		
	Estimate	95% CI	Type 3 p ^a	Estimate	95% CI	Type 3 p ^a
Persistent MRSA	0,78	-1,40-2,97	0,48	0,52	-1,46-2,50	0,61
MRSA in home samples	1,27	-2,18-4,72	0,48	2,51	-2,02-7,03	0,36

SF-36: Short Form 36; CI: confidence interval; Persistent: samples from all sampling moments positive; MRSA: methicillin-resistant *Staphylococcus aureus*. Univariate results from linear regression, adjusted for correlated observations from the same farm. Estimates (beta) with 95% CI are shown, reflecting the magnitude of outcome (MCS or PCS) change with change of the determinant value. ^a Type 3 p-values are calculated using likelihood-ratio tests.

EuroQol 5D

Domain scores per MRSA carriage type are illustrated in **Figure 3.3.1**. Since no persons scored severe problems (score 3) on any domain, the results were dichotomised into no problems (score 1) and some problems (score 2). No differences were found between different carrier groups. The mean EQ-VAS score is shown in **Table 3.3.3**, where no differences were found between groups of MRSA carriers.

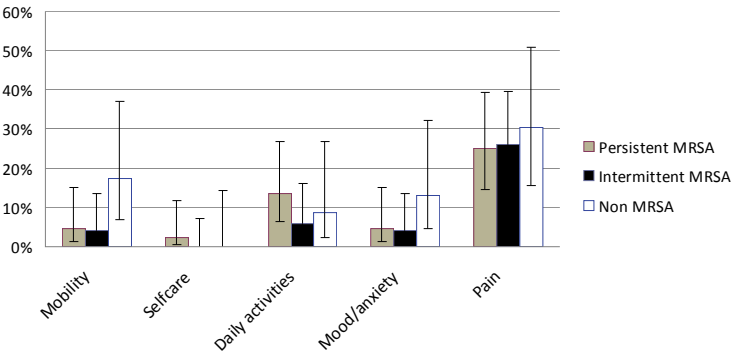


Figure 3.3.1 Persons with problems on EQ-5D domains. EQ-5D: EuroQol 5 dimensions; Persistent: samples from all sampling moments positive; Non-carrier: all samples negative; Intermittent: the remaining persons; MRSA: methicillin-resistant *Staphylococcus aureus*. Prevalence of problems per dimensions were shown, with 95% Wilson confidence interval bars. No significant differences between different groups were found with Fisher’s exact tests.

Univariate logistic (domains) and linear (EQ-VAS) regression is shown in **Table 3.3.5**. Self-care was not analysed since only a single person reported problems in self-care. Persistent MRSA carriage was not associated with any EQ-5D domain or VAS-score.

Table 3.3.5 Univariate regression EQ-5D questionnaire^a.

Determinant	Mobility			Daily activities			Mood/anxiety			Pain			EQ-VAS		
	PR	95% CI	Chi ² p	PR	95% CI	Chi ² p	PR	95% CI	Chi ² p	PR	95% CI	Chi ² p	Esti-mate	95% CI	Type 3 p ^b
Persistent	0,58	0,13-2,67	0,48	2,03	0,75-5,49	0,16	0,67	0,12-3,70	0,65	0,91	0,51-1,63	0,75	-0,11	-3,96-3,72	0,95
MRSA															
MRSA in	0,17	0,05-0,57	0,00	1,01	0,22-4,58	0,99	0,70	0,30-1,67	0,42	0,70	0,30-1,67	0,42	-0,80	-6,20-4,59	0,77
home samples															

EQ-5D: EuroQol 5 dimensions; EQ-VAS: EuroQol visual analogue scale; CI: confidence interval; Persistent: samples from all sampling moments positive; MRSA: methicillin-resistant *Staphylococcus aureus*; PR: prevalence ratio; Ref: reference category. Univariate results from logistic regression, adjusted for correlated observations from the same farm. Prevalence ratio's with 95% confidence intervals are shown. The EQ-VAS score is analysed linearly, estimates are shown. ^a Self-care was not analysed, since only one person reported problems in self-care. ^b Type 3 p-values are calculated using likelihood-ratio tests.

The presence of MRSA in home samples was significantly associated with a lower score for mobility problems. Male gender and more hours working at the farm was significantly associated with a higher VAS-score (4.88 points higher, 95% CI 1.69-8.08, p 0.01; 1.01 points per 10 hours, 95% CI 0.19-1.83, p 0.03, respectively). Higher age and wearing a facemask both had a non-significant trend with problems in all domains (except for mood/anxiety), including a lower VAS-score. Additional multivariate analysis did not reveal associations as well.

Discussion

Infections, antimicrobial use and healthcare contact

S. aureus is a known pathogen for skin infections [1], these infections subsequently may result in prolonged carriage since the infection provides a persisting source. In the case of LA-MRSA, the repeated and extremely high exposure rates in the stables may result in prolonged carriage as well. In 11% (95% CI 7-16%) of the sampling moments in this study skin infections were present (10% of MRSA-positive and 11% of MRSA-negative sampling moments). The incidence of bacterial skin infections in the general Dutch population is estimated to be 3.2% per year [42]. This is lower than the numbers reported from our study population, which consisted of livestock farmers who have an unusual high exposure to SA and high MRSA and MSSA carriage rates, compared to the general population [19]. Moreover, due to the nature of working in the stables, pig farmers are expected to have more skin abrasions than an average person. Therefore it is likely that the study population is associated with a higher chance of skin infections [3]. Wardyn et al. reported an incidence rate of 6.6 skin infections per 1000 person-months (equal to $6.6 \times 12 = 7.9\%$ per year) in a combined group of persons with livestock contact and community-based controls in the USA [18]. A study among Dutch livestock veterinarians found a prevalence of skin infections of 25% in persistent SA carriers, compared to 0% in non-carriers [24]. These numbers are more comparable to the results from our study.

Regarding serious infections, osteomyelitis was acquired on two moments ($2/198 = 1\%$, 95% CI 0.3-3.6%), one with and one without previous MRSA carriage. During routine care, no cultures were performed to find the causative organism. Serious infections with LA-MRSA have been reported before in 0.8 to 14% of serious MRSA-infections [17-23].

Development of infections, antimicrobial use and healthcare contact (admittance to hospital and visiting outpatient clinic) were not associated with MRSA carriage in the current study in univariate and multivariate analysis. In the literature, several studies

show that LA-MRSA has a low amount of virulence genes compared to other MRSA strains [19,25–29]. The most likely explanation for the absence of an association of infections with MRSA carriage is the limited effect of SA carriage in general among healthy individuals.

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

Short Form 36

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

EuroQol 5D

No participant scored severe problems on the health-related QoL domains, and no significant associations could be found between EQ-5D domains or EQ-VAS scores and (persistent) MRSA carriage. Again, the relatively healthy working population, a true low impact of LA-MRSA on QoL or a low distinctive power might be possible explanations for these findings.

The presence of MRSA in home samples was associated with fewer mobility problems, which may best be explained by the healthy worker effect as well: persons with MRSA more often work in the stables, and more often contaminate their home environment than persons without MRSA. These MRSA-positive persons are in general more healthy than the not-working population, and therefore have fewer mobility problems. The

same explanation can be given on the association between male gender and more hours working at the farm and a higher VAS-score (higher=better).

Study limitations

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

Furthermore, the culture method is limited in detecting MSSA and MRSA in one sample as different entities [13]. So the MRSA samples may have had undetected MSSA as well. We excluded persistent MSSA carriers from our analysis, but due to this limitation the number of persistent MSSA carriers with MRSA might have been underestimated, leading to an incomplete exclusion and bias. Since MSSA can lead to infections, and the presence of MSSA is shown to be negatively associated with presence of MRSA [13,31], this bias is expected to result in an underestimation of the pathogenicity of MRSA. However, since MSSA carriage numbers are comparable to literature we do not expect this bias to be of significant relevance.

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

Conclusions

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

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Chapter 4

Dynamics of carriage of livestock-associated MRSA



Chapter 4.1

Dynamics of livestock-associated methicillin-resistant *Staphylococcus aureus* CC398 in pig farm households: a pilot study

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Abstract

The aim of this study was to determine the long-term carriage rates and transmission dynamics of methicillin-resistant *Staphylococcus aureus* (MRSA) in pig farmers and their household members. During a 6-month period in 2009-2010, 4 pig farms in Denmark, Belgium, and the Netherlands, respectively, were studied for the presence of MRSA. The proportion of persistent carriers was significantly higher among farmers than among household members (87% vs. 11%) and significantly higher in household members from Belgium compared to those from Denmark and the Netherlands (29% vs. 0% vs. 6%). Determinant analysis of MRSA carriage revealed that pig contact was the most important determinant for MRSA carriage among household members and that the increased MRSA carriage rate observed among household members from Belgium is linked to country-specific differences in pig exposure. These findings demonstrated that even in pig farms with very high carriage rates of MRSA both in livestock and farmers, the risk for household members to acquire MRSA is limited and still depends strongly on pig exposure. By restricting access to the stables and exposure to pigs, MRSA acquisition by household members could be greatly reduced.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a threat to public health worldwide. Next to the well-known hospital-associated and community-associated clones, another specific clone unrelated to the aforementioned has been discovered, which originates from an extensive reservoir in food-producing animals: livestock-associated (LA-) MRSA. This clone belongs typically to multi-locus sequence type (ST) 398 and closely related STs within clonal complex (CC) 398, lacks Pantone-Valentine leukocidin (PVL), and is resistant to tetracycline. The presence of LA-MRSA in pigs and pig farmers was first described in France [1] and evidence of pig-to-farmer transmission of this clone was subsequently observed in the Netherlands [2]. Several surveys in livestock in Europe confirmed a high prevalence of LA-MRSA in pigs [3,4] and in livestock farmers (up to 30%) [5,6,7], but lower carriage rates in people living on farms but with limited direct contact to food-producing animals (2% to 16%) [5,8,9].

Moreover, previous studies have shown that LA-MRSA carriage was directly related to the intensity of livestock exposure [7,8,9]. Nasal carriage seems to be persistent for farmers continuously exposed to colonized livestock [10] or transient for people with sporadic or indirect contact [11]. In addition, secondary transmission and spread amongst humans has so far been studied in healthcare settings only and seems to be limited compared to other MRSA clones [12,13]. To date, little is known about the potential transmission routes amongst humans living in farm settings, the original environment of LA-MRSA. Some studies have suggested that carriage in household members could depend on the presence of positive livestock farmers [8] and that the contaminated farm environment could contribute to the transmission as well [10,14]. However, these studies were limited to a short period of time and the in-house farm environment was not studied.

Therefore, the aim of this longitudinal epidemiological study was to determine the long-term carriage rates and transmission dynamics of LA-MRSA in pig farmers and their household members.

Methods

Study design

We conducted a 6-month longitudinal study (8 sampling moments) of MRSA carriage among farmers and their household members living on pig farms with LA-MRSA in Belgium, Denmark, and the Netherlands during 2009-2010. This study was developed as a tool for potential larger future investigations and therefore included only a small sample size per country. In each country, 4 pig farms were selected based on the

following criteria: presence of LA-MRSA in the pig herd, which was detected in previous national screening studies in which ten randomly selected animals per age group were nasally swabbed to determine the presence of MRSA CC398; farrow-to-finish production system; and ≥ 3 members of the farmer's household. All household members were asked to participate in the study after receiving full information from the investigators. The study protocol was approved by the Ethics Committee of the Erasme Hospital-Université Libre de Bruxelles in Belgium (protocol no. P2009/203), the Danish National Committee on Biomedical Research Ethics (protocol no. H-4-2009-112) and the Danish Data Protection Agency (protocol no. 2009-54-0821), and the Medical Ethical Committee of the St. Elisabeth hospital in the Netherlands (protocol no. 0933). The volunteers signed an informed consent form and were asked to agree to nasal swabbing and to answer standard questionnaires at each sampling moment. In cases where children were under the age of 18 years, written consent was obtained from their parents. Swabs were taken before and during pig exposure by the investigators (during exposure: midday samples) and by the volunteers themselves (before exposure: morning samples) using the Venturi Transystem (Copan Innovation, Brescia, Italy). Samples from the house environment (farmer's favorite dog or cat, farmer's favorite chair, outside door handles, and TV remote control) were taken by the investigators using Sodibox wet wipe cloths (Sodibox, Nevez, France). The rate of sample collection was calculated considering available human and environmental samples at the time of the visit, some samples were missed for several reasons, among them: absence of households in the moment of the visit or impossibility to access into the farmer's house.

Microbiological analysis

MRSA from individual samples was detected using standard methods [6]. Briefly, swabs were inoculated in a brain heart infusion enrichment broth containing 7.5% NaCl and incubated for 24 h at 35°C. Subsequently, 10 μ l of a 0.5 \times McFarland suspension was inoculated onto chromID MRSA agar plates (bioMérieux, Marcy l'Etoile, France) and incubated for 24 h at 35°C. One suspected staphylococcal colony was selected from each plate and purified twice on Columbia agar with 5% sheep blood (Bio-Rad, Belgium) for further identification.

Species identification, presence of the *lukF-lukS* genes encoding PVL, and resistance to methicillin were confirmed by a triplex PCR assay [15]. Isolates (n=100) from the first and last sampling moment at which MRSA was isolated from each volunteer/environmental site, respectively, was characterized by *spa* typing using the Ridom Staph Type standard protocol (<http://www.ridom.com>) and the Ridom *SpaServer* (<http://spa.ridom.de/index.shtml>), *SCCmec* typing [16], and antimicrobial susceptibility testing (spectinomycin, gentamicin, kanamycin, tobramycin, rifampin, trimethoprim-

sulfamethoxazole, clindamycin, erythromycin, linezolid, chloramphenicol, mupirocin, ciprofloxacin, minocycline, tetracycline, and fusidic acid) using Neo-Sensitabs (Rosco, Taastrup, Denmark) in accordance with the Clinical Laboratory Standards Institute guidelines (CLSI 2011), as described elsewhere [17]. Multidrug resistance (MDR) was defined as resistance to ≥ 4 non- β -lactam antimicrobial classes. MLST [18] was performed on MRSA isolates representing each *spa* type.

The genetic relatedness of a subset of these isolates ($n=36$) representing one farm per country was further assessed by pulsed-field gel electrophoresis (PFGE) using *Cfr9I* (Fermentas GmbH, Germany) as previously described [19]. PFGE patterns were analysed using Bionumerics version 6.5 (Applied Maths, Kortrijk, Belgium) according to previously described criteria [20].

Epidemiological data

Demographic data (gender, age, occupation, status in the family), farm- and animal-related variables (exposure to pigs, cattle, poultry, horses, and pets, handling antimicrobial drugs to pigs, use of hygiene/protective measures, and occupational activities), life style determinants (eating preferences, exposure to raw meat, smoking, contact sports, travel), and medical history (exposure to health care facilities, antibiotic usage) were collected for each volunteer at each sampling moment.

Definitions

Volunteers were categorized into individuals exposed to pigs >30 hours per week and individuals exposed to pigs ≤ 30 hours per week, on average (termed farmers and household members, respectively), and were assigned to 1 of 3 groups with regard to MRSA carriage: persistent carriers (100% of the cultures were positive for MRSA), non-carriers (100% of the cultures were negative for MRSA), and intermittent carriers (all other volunteers).

Statistical analysis

The data were analyzed using SAS software version 9.2 (SAS Institute Inc., Cary, North Carolina, USA). Comparison of proportions was done with Chi-square tests, or Fisher's exact tests when expected cell counts were below 5. Determinants for MRSA carriage in household members were stratified per country. All tests were 2-sided, and the significance level was set at $p=0.05$.

Results

MRSA carriage in the study population

A total of 60 persons (20 per country) participated in the study, including 15 farmers and 45 household members (**Table 4.1.1**). Altogether, 453 midday samples (both farmers and household members, sample collection rate 95% [453/480]), 69 morning samples (farmers only, sample collection rate 71% [69/96]), and 357 environmental samples (sample collection rate 93% [357/384]) were analyzed. The proportion of persistent carriers was significantly higher among farmers than among household members (87% vs. 11%; Fisher's exact $p < 0.0001$, **Table 4.1.1**) and did not vary between countries (farmers from Belgium, 100%; Denmark, 80%; the Netherlands, 75%; Fisher's exact $p = 0.49$). The majority (87% [60/69]) of morning samples from farmers were positive for MRSA.

Table 4.1.1 Methicillin-resistant *Staphylococcus aureus* (MRSA) carriage among 15 farmers and 45 household members

Category, country	No. of non-carriers (%)	No. of intermittent MRSA carriers (%)	No. of persistent MRSA carriers (%)
Farmers			
Belgium	0 (0)	0 (0)	6 (100)
Denmark	0 (0)	1 (20)	4 (80)
The Netherlands	0 (0)	1 (25)	3 (75)
Total	0 (0)	2 (13)	13 (87)
Household members			
Belgium	2 (14)	8 (57)	4 (29)
Denmark	14 (93)	1 (7)	0 (0)
The Netherlands	13 (81)	2 (13)	1 (6)
Total	29 (64)	11 (24)	5 (11)

The proportions of both intermittent and persistent MRSA carriers were significantly higher in household members from Belgium compared to Denmark and the Netherlands (intermittent: 57% vs. 7% vs. 13% [Fisher's exact $p = 0.004$] and persistent: 29% vs. 0% vs. 6% [Fisher's exact $p = 0.03$]).

Environmental samples

The isolation rates of MRSA in the environmental samples are shown in **Table 4.1.2**. The overall isolation rate from environmental samples was 22% (78/357), with important geographic variations (Belgium 42%; Denmark 22%; the Netherlands, 5%; BE vs. DK $p = 0.0004$; BE vs. NL $p < 0.0001$; NL vs. DK $p = 0.0001$).

Table 4.1.2 Isolation rates of methicillin-resistant *Staphylococcus aureus* in 357 environmental samples

Origin	No. of positive samples (%)			
	Belgium	Denmark	The Netherlands	Total
Dog or cat	11 (48)	9 (29)	2 (6)	22 (26)
Chair	9 (29)	10 (31)	5 (16)	24 (25)
Outside door handle	11 (46)	2 (6)	0 (0)	13 (15)
Television remote control	12 (50)	7 (22)	0 (0)	19 (22)
Total	43 (42)	28 (22)	7 (5)	78 (22)

Our study did not show a positive association between environmental samples and MRSA carriage in household members (100% (9/9) of Belgian household members with MRSA in environmental samples were MRSA positive during the study, compared to 60% (3/5) of Belgian household members from MRSA-negative environments, $p=0.11$; for Danish household members these numbers are 10% (1/10), 0% (0/5), $p=1.00$; and for Dutch household members 23% (3/13), 0% (0/3), $p=1.00$).

Molecular and phenotypic characterization

All 100 isolates subjected to molecular genotyping and antimicrobial susceptibility testing had characteristics that were compatible with LA-MRSA CC398: they displayed closely related *spa* types t011, t034, t108, t1451, t2370, and t6017 and belonged to ST398 within CC398; they lacked the *lukF-lukS* genes encoding PVL; they carried SCCmec type V (92%) or IV (8%); and they were resistant to tetracycline (100%). In addition, 19% were MDR. Isolates recovered from farmers, household members, and environmental samples from each farm were highly homogeneous in terms of *spa* typing, SCCmec typing, and antimicrobial susceptibility patterns (data not shown). Furthermore, isolates originating from the same farm had indistinguishable PFGE patterns (**Figure 4.1.1**).

Determinants of MRSA carriage among household members

Pig contact rate (hours per week), exposure to pigs within last 7 days, contact to sows, and handling antimicrobial drugs to pigs were significantly associated with MRSA carriage among household members in at least country (**Table 4.1.3**), whereas no associations were found for gender, age, status in the family, other occupations, eating preferences, exposure to raw meat, smoking, contact sports, travel, medical history, exposure to other animals (cattle, poultry, horses, and pets), use of hygiene/protective measures, presence of a farmer with MRSA in the household, and presence of MRSA in the household environment.

Dice (Opt 0.50%) (Tol 0.8%) (H>0.0% S>0.0%) [11.0%-91.0%]

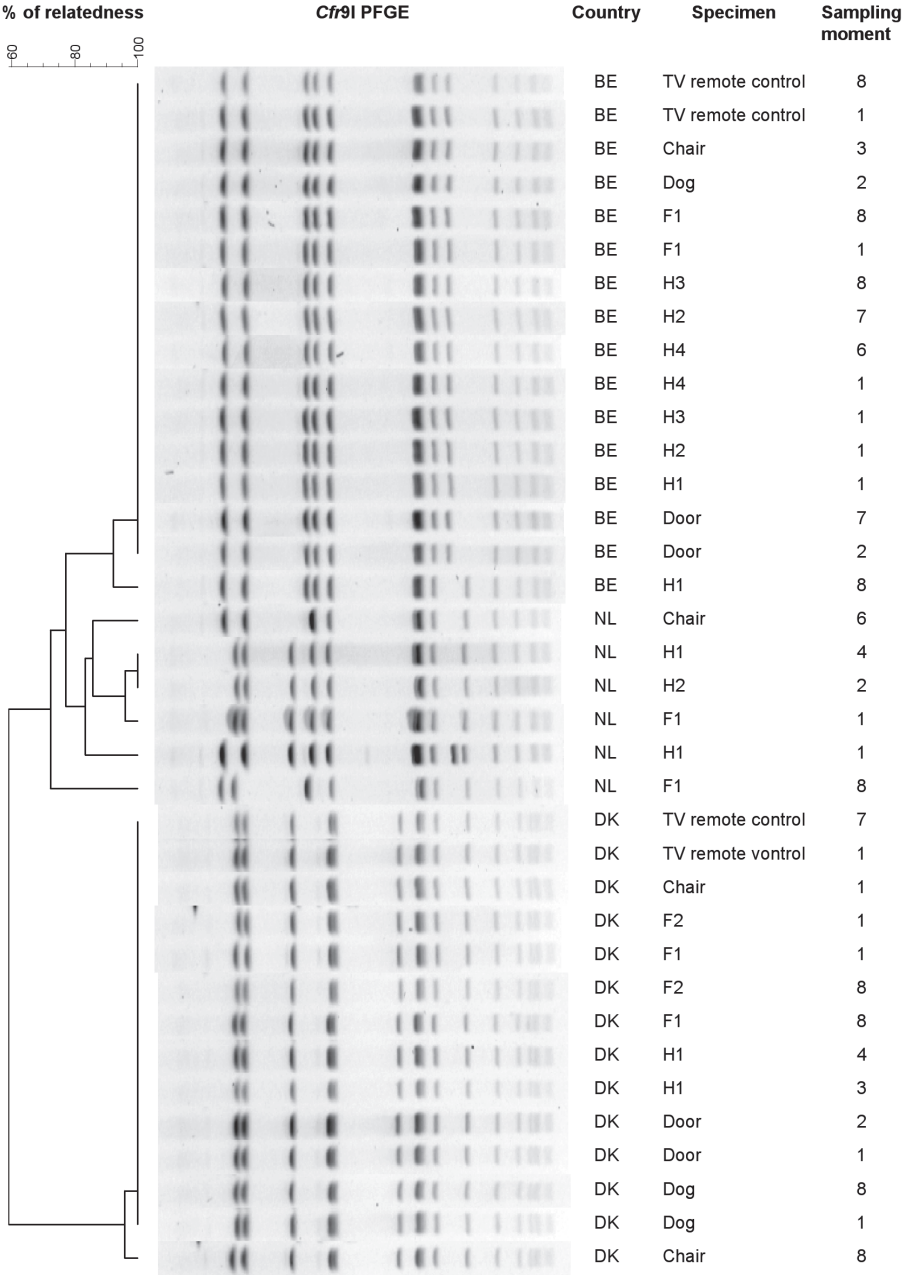


Figure 4.1.1 PFGE patterns of MRSA isolates (n=36) from a single farm per country (Belgium n=16, Denmark n=14, Netherlands n=6)

Table 4.1.3 Determinants for persistent and intermittent methicillin-resistant *Staphylococcus aureus* (MRSA) carriage among household members

Determinant	Belgium			Denmark			The Netherlands		
	Total no.	No. of carriers (%)	p	Total no.	No. of carriers (%)	p	Total no.	No. of carriers (%)	p
Total	14	12 (86)		15	1 (7)		16	3 (19)	
Pig exposure time									
10-30 hours per week	4	4 (100)	1,00	1	0 (0)	1,00	2	2 (100)	0,03
<10 hours per week	10	8 (80)		14	1 (7)		14	1 (7)	
Exposure to pigs within last 7 days									
Yes	11	11 (100)	0,03	3	0 (0)	1,00	6	3 (50)	0,04
No	3	1 (33)		12	1 (8)		10	0 (0)	
Contact to sows									
Yes	9	9 (100)	0,11	3	0 (0)	1,00	6	3 (50)	0,04
No	5	3 (60)		12	1 (8)		10	0 (0)	
Handling antimicrobial drugs to pigs									
Yes	1	1 (100)	1,00	1	0 (0)	1,00	2	2 (100)	0,03
No	13	11 (85)		13	1 (8)		14	1 (7)	

Notes: p, Fisher's Exact p value. p values in bold indicate significant differences.

The association between country, age, and average pig exposure time among household members in each country is illustrated in **Figure 4.1.2**. In general, household members from Belgium were more exposed to pigs and at an earlier age compared to household members from the Netherlands and Denmark. These findings suggest that the increased MRSA carriage rate observed among household members from Belgium is linked to country-specific differences in pig exposure.

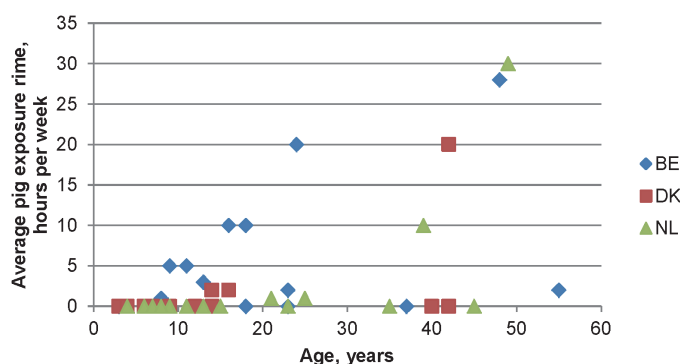


Figure 4.1.2 Association between country, age, and average pig exposure time among household members in each country. BE, Belgium; DK, Denmark; NL, the Netherlands

Discussion

In this study, we found that 87% of pig farmers were persistent LA-MRSA nasal carriers for a period of at least 6 months. Moreover, the majority of the pig farmers tested MRSA positive before exposure to pigs, which is consistent with persistent carriage rather than re-acquisition and loss on a daily basis. This finding supports that farmers can be a source of household transmission. However, presence of a farmer with MRSA could not be associated with household transmission, since all farmers were MRSA positive during the study.

The carriage rate found in this study is much higher than previously reported on positive pig farms (49% of farmers and 6% of household members) [5], veal farms (positive and negative farms combined: 38% of farmers and 16% of household members) [8], and in field workers visiting MRSA positive pig and veal farms (48% of field workers) [11] in the Netherlands. In addition, lower rates were found in Belgium as well (37.8% of farmers, co-workers and households in positive and negative farms combined) [6]. This could be the result either of a rising MRSA prevalence in people over time, or, more likely, due to the limited number of farms per country included in this study. A very remarkable finding was the large difference in LA-MRSA carriage rate among household members of the different countries. In Denmark and the Netherlands, the carriage rate, defined as intermittent and persistent carriers together, ranged between 7-19%, which is comparable to the MRSA nasal carriage rates found in family members of Dutch veal calf farmers (16%) [8], but much higher than the 0.2% reported in the Netherlands in people without any livestock contact [21]. In Belgium, a dramatically high carriage rate was found among household members (86%), which was comparable to that of the farmers. This can be explained by our finding that household members in Belgium were more exposed to pigs and at an earlier age compared to household members from the Netherlands and Denmark where exposure to pigs was largely restricted to farmers. As expected, all MRSA isolates shared typical characteristics of LA-MRSA in terms of *spa* typing and MLST, lack of the *lukF-lukS* genes encoding PVL, *SCCmec* typing, and antimicrobial susceptibility patterns as previously reported [6,8,9,17]. A novel finding was the frequent isolation of LA-MRSA in Belgian farm house environments (42%), which can be a reflection of the higher LA-MRSA carriage rate among Belgian household members. Although it has been suggested that the environment might play a role in LA-MRSA transmission amongst family members, our study did not show a positive association between environmental samples and MRSA carriage in household members (100% (9/9) of Belgian household members with MRSA in environmental samples were MRSA positive during the study, compared to 60% (3/5) of Belgian household members from MRSA-negative environments, Fisher's exact $p=0.11$; for Danish household members these numbers were 10% (1/10), 0% (0/5), $p=1.00$; and for Dutch household

members 23% (3/13), 0% (0/3), $p=1.00$). However, the high rates found in companion animals, particularly in Belgium, have to be interpreted with caution since the role of pets as potential vectors and/or reservoirs of LA-MRSA is still not clear and needs future research. Notably, the finding that exposure to a persistent carrier (farmer) did not imply a risk for spread to household members confirms that human-to-human transmission of this clone seems to be very limited, as previously reported [12,13,22].

Our results are of interest when developing strategies for preventing spread of LA-MRSA to household members of pig farmers. By restricting access to the stables and exposure to pigs, the risk of LA-MRSA acquisition by household members could be greatly reduced. However, this needs further investigation and confirmation by future studies.

In conclusion, we have demonstrated that even in pig farms with a very high carriage rate of MRSA in both livestock and pig farmers, the risk for household members to acquire MRSA is limited and depends strongly on pig exposure.

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Chapter 4.2

Self-sampling is appropriate for detection of *Staphylococcus aureus*: a validation study

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Abstract

Background

Studies frequently use nasal swabs to determine *Staphylococcus aureus* carriage. Self-sampling would be extremely useful in an outhospital research situation, but has not been studied in a healthy population. We studied the similarity of self-samples and investigator-samples in nares and pharynxes of healthy study subjects (hospital staff) in the Netherlands.

Methods

One hundred and five nursing personnel members were sampled 4 times in random order after viewing an instruction paper: 1) nasal self-sample, 2) pharyngeal self-sample, 3) nasal investigator-sample, and 4) pharyngeal investigator-sample.

Results

For nasal samples, agreement is 93% with a kappa coefficient of 0.85 (95% CI 0.74-0.96), indicating excellent agreement, for pharyngeal samples agreement is 83% and the kappa coefficient is 0.60 (95% CI 0.43-0.76), indicating good agreement. In both sampling sites self-samples even detected more *S. aureus* than investigator-samples.

Conclusions

This means that self-samples are appropriate for detection of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*.

Introduction

Staphylococcus aureus (*S. aureus*) is a well-known human commensal on skin and mucous membranes, with as most important ecological niche the nares [1,2]. In addition to colonization, *S. aureus* can cause skin and soft tissue infections, and more severe infections like necrotizing pneumonia, osteomyelitis and sepsis, which are regularly seen in hospitals in patients with other comorbidities.

Studies frequently use nasal swabs to determine *S. aureus* carriage, sometimes supplemented by pharyngeal swabs to increase the validity of the sampling method [3-5]. Especially in research settings in populations outside hospitals, self-sampling is often performed to enhance response and lower travel costs and time for both the studied subject and the investigator [6,7].

The only study examining self-sampling for methicillin-resistant *S. aureus* (MRSA) is Lautenbach et al. [8]. They sampled 56 MRSA-positive inpatients in the US and compared different anatomic sites and patient-collected versus provider-collected samples. Agreement percentages of 82% (nose) and 91% (throat) were reported, demonstrating excellent agreement. However, the study population (US inpatients) is not comparable to European citizens considering health status and MRSA-prevalence; the latter is several times higher in the US compared to the Netherlands and Northern Europe [9,10]. Moreover, sampling order always was a sample collected by a provider, followed by a patient-collected sample. Lastly, instructions for self-sampling were not clear: were photographs available, was clearly stated which area to sample and with which technique?

We therefore studied the similarity of self-samples and investigator-samples in testing the presence of *S. aureus* in nares and pharynxes of healthy study subjects in the Netherlands, using random sample order and clear instructions.

Methods

This crossover screening validation took place in April 2009. From nursing and technical personnel working in different wards in the Amphia hospital in Breda, the Netherlands, 4 samples were taken in random order: 1) nasal self-sample, 2) pharyngeal self-sample, 3) nasal investigator-sample, and 4) pharyngeal investigator-sample. A convenience sample size of around 100 subjects (400 samples) was determined.

Before sampling, a one page instruction was shown with photographs of both samplings (see **Additional Figure 4.2.1**), no spoken instructions were given in order to represent a true self-sample. The subject was advised to sample both inner nares, especially in the tip of the nose, and both tonsils or tonsillar arches with a different swab, both in a

turning movement. Venturi Transystem swabs with Stuart medium were used (Copan Innovation, Italy). Before or after these two self-samples, the investigator sampled the nares and pharynx using the same technique as described above.

Swabs were inoculated on SA ID agar plates (bioMérieux, La Balme Les Grottes, France) and Columbia blood agar plates with 5% sheep blood, and enriched in Mueller-Hinton broth containing 6.5% NaCl. From the overnight Mueller-Hinton broth, 10 µl was streaked onto an SA ID agar plate. The results were read after overnight incubation at 35°C. Colonies showing green coloration were considered indicative for *S. aureus*, and were confirmed by standard techniques: colony morphology, coagulase slide test, DNase test, and a coagulase tube when discrepancies arose. Colonies with colours other than green, or no growth at all were considered negative. The procedure was performed as recommended by the manufacturer.

From 2x2 tables, percentage agreement was calculated. Cohen's simple kappa coefficients with 95% Confidence Intervals (CI) were calculated with SAS, version 9.3 (SAS Institute Inc., Cary, NC, USA). A kappa coefficient of >0.6 was considered to indicate good agreement, a coefficient of >0.8 was considered to indicate excellent agreement, and a coefficient of 1 indicated perfect agreement. Gold standards were created combining self-samples and investigator-samples per sampling site; if either one was positive, that person was considered *S. aureus* positive on that site. Sensitivities were calculated with OpenEpi [11].

Results

A total of 105 nursing and technical personnel members were sampled. Thirty percent (31/105) of nasal investigator-samples were *S. aureus* positive, compared to 34% (36/105) of self-samples. For pharyngeal samples, these numbers were 27% (28/105) and 34% (36/105), respectively. **Table 4.2.1** shows that nasal self-samples and investigator-samples have an agreement of 93% and a kappa coefficient of 0.85 (CI 0.74-0.96). For pharyngeal samples an agreement of 83% and a kappa coefficient of 0.60 (CI 0.43-0.76) was obtained. In both sampling sites self-samples even detected more *S. aureus* than investigator-samples.

Table 4.2.1 Self-samples versus investigator-samples per sampling site

Nasal samples		Investigator-samples		
		SApos	SAneg	Total
Self-samples	SApos	30	6	36
	SAneg	1	68	69
	Total	31	74	105
Agreement		93%		
Kappa coefficient		0.85 (CI 0.74-0.96)		

A. Nasal samples. SApos: *S. aureus* positive; SAneg: *S. aureus* negative; CI: confidence interval

Pharyngeal samples		Investigator-samples		
		SApos	SAneg	Total
Self-samples	SApos	23	13	36
	SAneg	5	64	69
	Total	28	77	105
Agreement		83%		
Kappa coefficient		0.60 (CI 0.43-0.76)		

B. Pharyngeal samples. SApos: *S. aureus* positive; SAneg: *S. aureus* negative; CI: confidence interval.

Table 4.4.2 shows that, compared to the site-specific gold standard, nasal self-samples have a sensitivity of 97% (CI 86-100%), and pharyngeal self-samples have a sensitivity of 88% (CI 75-95%).

Table 4.4.2 Self-samples versus gold standard per sampling site

Nasal samples		Gold standard		
		SApos	SAneg	Total
Self-samples	SApos	36	0	36
	SAneg	1	68	69
	Total	37	68	105
Sensitivity		97% (CI 86-100%)		

A. Nasal samples. SApos: *S. aureus* positive; SAneg: *S. aureus* negative; CI: confidence interval

Pharyngeal samples		Gold standard		
		SApos	SAneg	Total
Self-samples	SApos	36	0	36
	SAneg	5	64	69
	Total	41	64	105
Sensitivity		88% (CI 75-95%)		

B. Pharyngeal samples. SApos: *S. aureus* positive; SAneg: *S. aureus* negative; CI: confidence interval.

Discussion

This experimental study shows that self-samples and investigator-samples are very similar in testing the presence of *S. aureus* in nares and pharynxes. For nasal samples, agreement is 93% with a kappa coefficient of 0.85, indicating excellent agreement, for pharyngeal samples agreement is 83% and the kappa coefficient is 0.60, indicating good agreement. This means that self-samples are appropriate for detection of *S. aureus* and MRSA.

Furthermore, when looking at the discordant pairs, nasal self-samples tend to yield more *S. aureus* compared to investigator-samples. A rational explanation might be that swabbing is more thoroughly done by persons themselves compared to swabbing by investigators, who press less hard, resulting in fewer microorganisms picked up by the swab and a lower prevalence of *S. aureus*. For pharyngeal swabs the same can be stated, but results are less clear. This is probably due to the fact that taking swabs from tonsils or tonsillar arches is more complex, compared to swabs from the nares. In addition, the investigators observed that the instructions for pharyngeal samples were less well adhered to than the instructions for nasal samples.

This strengthens the conclusions from Lautenbach et al., although agreement percentages are slightly different (82% for nares and 91% for throat) [8]. Moreover, using the information given in this reference, kappa coefficients can be calculated, which are different from the coefficients found in this study (kappa coefficients of 0.28, CI -0.05-0.60 for nares and 0.80, CI 0.64-0.97 for throat). Possible explanations for these discrepancies can be the different populations studied (US inpatients versus healthy subjects from the Netherlands) or different methods used (strict or random sampling order, type of instructions). Hanselman et al. have used nasal self-sampling, and found *S. aureus* percentages in US teachers consistent with literature, indicating appropriateness of self-sampling [12]. More studies demonstrate the usefulness of self-samples, albeit from other anatomic sites and for other microorganisms, as human papilloma virus, group-B streptococci, respiratory viruses, and sexually transmitted diseases [7,13-15].

The validity of nasal and pharyngeal self-sampling cannot be established entirely correctly, as there is no true gold standard. However, when comparing to combined self-samples and investigator-samples per site, nasal self-samples have a sensitivity of 97% (CI 86-100%), and pharyngeal self-samples have a sensitivity of 88% (CI 75-95%). Lautenbach et al. have calculated sensitivities based upon a gold standard of combined samples from nares, throat, axillae, groin and perineum, and found sensitivities of 91% (CI 80-97%) for nasal self-samples, and 67% (CI 53-79%) for throat self-samples [8].

This study has three limitations. The nursing personnel members are expected to have more prior knowledge of sampling methods compared to the standard outhospital

person. This might lead to an overestimation of the adequacy and validity of self-sampling. Moreover, false sampling was not checked for by, for example, placing the swab on a standard sheep blood agar, which detects whether any viable microorganisms are present on the swab. However, the investigators were standing next to the subject when sampling took place, making false sampling unlikely. In addition, *S. aureus* prevalence corresponds to literature, confirming adequate sampling [1]. Checking for false sampling with blood agar probably is a useful suggestion when using self-samples in a research situation, however. Lastly, three investigators were involved in taking the investigator-samples, which might lead to slightly different sampling techniques. As these investigators are all well trained on sample taking and sampling techniques were discussed beforehand, we believe the effect of this variation is negligible. Data on who took which samples was unfortunately not recorded, giving no opportunity to verify this statement.

Conclusions

In conclusion, self-sampling the nares and pharynx for the presence of *S. aureus* has an excellent and good agreement with investigator-samples, respectively. Self-sampling saves both time and costs, and enhances response rate, which can be extremely useful in outhospital study situations of e.g. community-acquired or livestock-associated MRSA.

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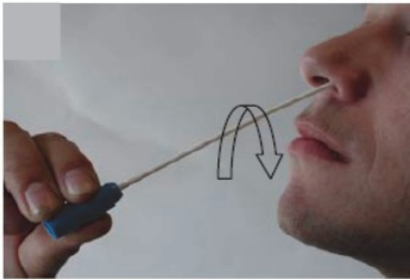
Instruction for *S. aureus* sampling

In case of *S. aureus* the following sites are sampled:

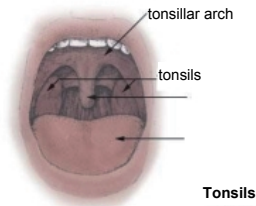
Nose and Throat.

Sampling method for *S. aureus*

Nose The inner side of both nostrils, especially in the tip of the nose. Swab in a turning movement with a sterile swab



Throat Both tonsils or, if tonsils are removed, tonsillar arches Swab in a turning movement with a sterile swab.



Every sample is taken with a different swab.

If there are questions, feel free to contact the Department of Infection Prevention telephone xxx-xxxxxxx

Department of Infection Prevention Amphia Hospital 2009

Additional Figure 4.2.1 Instruction for *S. aureus* sampling

Chapter 4.3

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

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Abstract

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

Introduction

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

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

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

Methods

Study design and selection of farms

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

Sampling occasions

During the 1-year study period, there were six sampling occasions: day 0, day 4, day 7, month 4, month 8, and month 12. Quantitative nasal and oropharyngeal swabs, extensive questionnaires, and wet wipe samples of four defined surfaces in house (backdoor handle, remote control of television, favourite chair of pig farmer, and dog neck/back) and four surfaces in the stables (farrowing and weaning stables, both sampled twice) were collected on day 0. Nasal swabs were introduced in the nostril and rotated once. Oropharyngeal swabs sampled the area of the inner cheek including the

tonsils. Refrigerated swabs were transported to the laboratory, and cultured within 24 h. In addition, dry electrostatic dust collector cloths (EDCs) [14] were placed in the farrowing and weaning stables (two per stable) and on the highest cupboard in the living room of the house, and were left in place for 2 weeks before quantitative analysis. On the remaining sampling occasions, subjects semi-quantitatively sampled their own nose and filled in a short questionnaire. Swab-instructions were sent with the swabs. EDCs were placed on the same five locations on months 4, 8, and 12. An extra semi-quantitative sample of the throat was taken by the subjects themselves on month 12. Results of the individual cultures were disclosed at the end of the study.

Definitions

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

Questionnaires

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

Laboratory analysis: cultures

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

Semi-quantitative cultures were performed by inoculating dry swabs (Copan) directly onto *S. aureus*, MRSA, and Columbia agar plates with 5% sheep blood, and Müller–Hinton enrichment broth. Wet wipe samples (Sodibox, Nevez, France) were double-enriched in Müller–Hinton broth, followed by phenol mannitol broth with ceftizoxime

(BioMérieux), which was subsequently cultured on blood and Brilliance™ MRSA agar plates (Oxoid, Basingstoke, UK), whether color change occurred in the phenol mannitol broth or not.

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

Laboratory analysis: EDC PCR

The EDC was suspended in FE-buffer (150 mM NaCl, 1 mM EDTA), mixed in a Stomacher blender (Seward Limited, London, UK), and stored at -20°C until further processing. DNA was isolated and purified with a Versant Molecular kPCR molecular system (Siemens Healthcare Diagnostics, The Hague, the Netherlands). For each sample, 5 µL was used to detect four targets with a LightCycler 480-II (Roche Diagnostics, Almere, the Netherlands): (i) *mecA* for methicillin-resistance [17], (ii) C01 for livestock-association [18], (iii) *femA* [17], and (iv) *nuc* [19] for detection of *S. aureus*.

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

Molecular typing

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

Statistical analysis

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

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

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

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

Ethical considerations

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

Results

MRSA carriage

This 1-year prospective cohort study included 110 pig farmers (88 men), with a median age of 45 (range 17-67 years), equally divided into farmers ($n=55$) and employees ($n=55$). The median number of working hours per week in the stables at the start of the study was 44 (range 20-80 h per week). **Table 4.3.1** demonstrates that 38% were persistent MRSA nasal carriers. Variation in MRSA prevalence and cross-sectional prevalence per sampling occasion are shown in **Figure 4.3.1**, the average MRSA prevalence was 63% (range 59–71%).

Table 4.3.1 Carriage patterns of *Staphylococcus aureus* in 110 pig farmers.

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

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

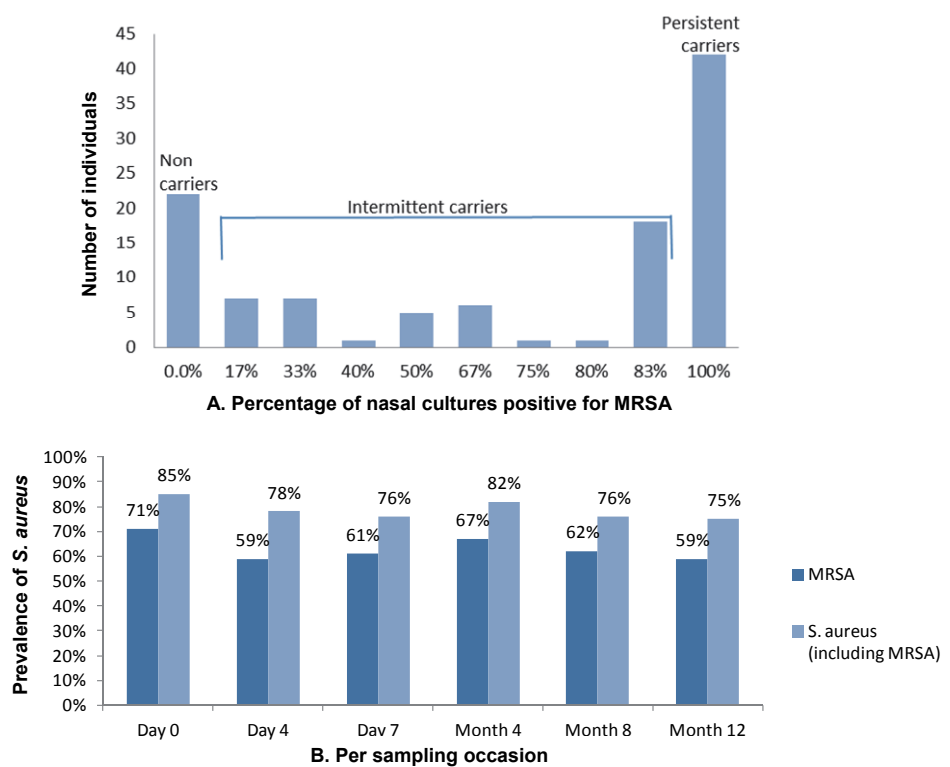


Figure 4.3.1 Variation in methicillin-resistant *Staphylococcus aureus* (MRSA) nasal prevalence, resulting in persistent, intermittent and non- carriers (A) and mean cross-sectional nasal *S. aureus* prevalences per sampling occasion (B).

The median number of MRSA in nasal samples at the start of the study was 3.2 log-transformed CFU (log CFU, interquartile range (IQR: p25–p75) (IQR) 1.8–3.8), regarding positive samples only. For oropharyngeal samples a median of 1.0 log CFU (IQR 0.0–1.6) was found. Within this group of MRSA carriers at the start of the study, pig farmers with a higher number of CFU were significantly more often persistent nasal carriers, as shown in **Figure 4.3.2** (PR per log CFU=1.31, 95% CI 1.12–1.54, $p<0.0001$ for nasal samples and 1.08, 95% CI 0.91–1.28, $p=0.40$ for oropharyngeal samples). In addition, the presence of MRSA in oropharyngeal swabs at the start of the study (0/1) was significantly associated with persistent MRSA nasal carriage (PR=2.89, 95% CI 1.65–5.10, $p<0.0001$).

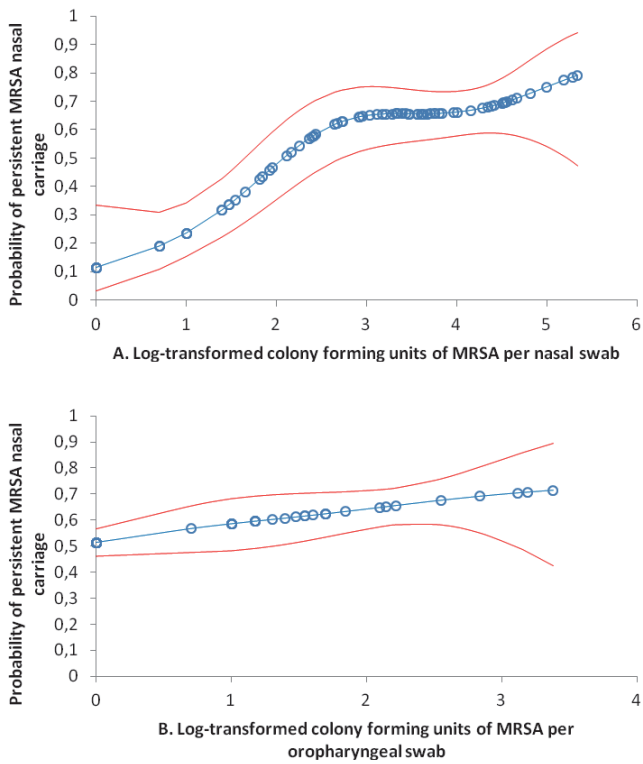


Figure 4.3.2 Amount of methicillin-resistant *Staphylococcus aureus* (MRSA) in nasal (A) and oropharyngeal (B) swabs from start of study and probability of persistent MRSA carriage in pig farmers who were MRSA positive at start of the study. Non-parametric regression model (smoothing) with 95% confidence bands.

MSSA carriage

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

Environmental samples

At least one wet wipe sample was MRSA-positive in 63% of houses and 84% of stables at the start of the study. In addition, at least one dry EDC was positive in 98% of stables, but in only 6% of houses (Table 4.3.2).

Table 4.3.2 Environmental samples positive for methicillin-resistant *Staphylococcus aureus* in 49 pig farms.

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

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

Table 4.3.2 also shows stable-EDC LA-MRSA eqCFU counts per farm during the study. Median eqCFU counts per sampling occasion did not vary significantly in time (data not shown). The median pig farm had 164 eqCFU (IQR 84–298) of LA-MRSA on its stable-EDC during the study. Farms with persistent and intermittent MRSA carriers had median stable-EDC eqCFU of 169 and 159, respectively, whereas those with non-carriers had 40 eqCFU (p<0.0001).

Determinants for persistent nasal MRSA carriage

Supporting Table S4.3.1 describes determinants for persistent nasal MRSA carriage in pig farmers with a univariate p≤0.20 and a prevalence in pig farmers ≥0.10. No collinearity

between determinants was observed. Presence of MRSA in the oropharynx at the start of the study, an age of 40–49 years, a working week of ≥ 40 h, giving birth assistance to sows, and moving piglets were univariately significantly associated with persistent MRSA nasal carriage. Determinants such as having a body part pierced in the last 12 months (n=3), using corticosteroid medication (n=2), and having psoriasis (n=3) were significantly associated with persistent MRSA carriage, but were observed sporadically and therefore not included in the multivariate analysis.

In a multivariate backwards stepwise analysis, an age of 40–49 years, a working week of ≥ 40 h, giving birth assistance to sows, and to a lesser extent, washing hands when leaving the stables were significantly related with persistent MRSA nasal carriage (**Table 4.3.3**). Lower persistent MRSA carriage rates were found in farmers who removed manure of finisher pigs, continuously wore a facemask when working in the stables, and to a lesser extent, had contact with cats.

Table 4.4.3 Determinants for persistent methicillin-resistant *Staphylococcus aureus* nasal carriage in pig farmers after multivariate analysis.

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

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

Interactions between wearing a facemask and giving birth assistance to sows or removing manure of finisher pigs was not observed. Pig farmers continuously wearing a facemask did not work in stables with more eqCFU LA-MRSA, compared with pig farmers not always wearing a facemask (165 and 117 eqCFU, respectively, Wilcoxon Rank Sum test p=0.65). According to **Table 4.3.2**, there were ten farms with farmers who did not carry MRSA during the study year, all had LA-MRSA present in the environmental samples at the start of the study. Of the 12 persons working on these farms, three (20%) wore facemasks continuously. This was more compared with persons from farms with persistent carriers (3/64=4%, Fisher’s Exact p=0.07), but not when compared with farms with intermittent carriers (5/20=20%, p=1.00).

Molecular typing

In total, all 495 MRSA strains from 102 pig farmers were MLVA-typed and *spa*-typed, and depicted in **Supporting Figure S4.3.1**. All MRSA isolates from nose, oropharynx and wet wipe samples had *spa*-types and MLVA-types (MTs) corresponding to clonal complex (CC) 398, also known as the livestock-associated clade [23]. Of the MRSA isolates, 92% were MT398 (n=248 isolates), MT572 (n=186), and MT574 (n=19). In addition, 94% of MRSA isolates belonged to *spa*-types t011 (n=252), t108 (n=187), and t034 (n=27), where t011 generally matched with MT398 and t108 with MT572.

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

Discussion

MRSA carriage: persistence and prevalence

The persistent MRSA carriage rate found in pig farmers in this study (38%) was higher compared with the only other longitudinal study for MRSA in livestock farmers (18% in veal farmers) [7]. The persistent total *S. aureus* carriage rate from pig farmers (52%) was also very high compared with the general population (20%) [1,24].

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

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

argue that true colonization might be a plausible explanation for the majority of the persistent carriage observed.

MRSA carriage is associated with environmental contamination

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

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

Facemasks protect for persistent MRSA carriage

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

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

MSSA is negatively associated with MRSA carriage

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

interval between the sampling occasions, other reasons for the absence of MRSA cannot be excluded (antibiotic use, nature and amount of work in the stables, status of innate immunity, etc.).

Other determinants for persistent MRSA carriage

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

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

Study limitations

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

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

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

Conclusions

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

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Table S4.3.1 Determinants for persistent methicillin-resistant *Staphylococcus aureus* (MRSA) nasal carriage in pig farmers^a.

Determinant	Farmers n (total n=110)	Persistent carriers n (%) (total n=42)	p-value ^b
MRSA-positive throat sample at day 0	52	30 (58)	<0.0001
Work in the stables >40 h/week	55	28 (51)	<0.0001
Give birth assistance to sows in the last 7 days	74	34 (46)	0.02
Move piglets in the last 7 days	84	38 (45)	0.03
Visit nursing home in the last 12 months	14	8 (57)	0.06
Contact with goats in the last 12 months	14	8 (57)	0.09
Continuously wear facemask when working in stables	11	1 (9)	0.10
Eat beef weekly	39	19 (49)	0.10
Clean finisher pig stables in the last 7 days	33	9 (27)	0.11
Wet wipe samples positive in stables of weaned piglets day 0	80	34 (43)	0.13
Wash hands when leaving stables	99	40 (40)	0.14
Remove manure of finisher pigs in the last 7 days	24	6 (25)	0.15
Contact with veal calves in the last 12 months	12	2 (17)	0.19
Contact with cats in the last 12 months	37	11 (30)	0.20
Remove manure of piglets in the last 7 days	46	21 (46)	0.20
Age 17–39 years	35	9 (26)	Ref
Age 40–49 years	43	23 (53)	0.01
Age 50–67 years	32	10 (31)	0.44

^a All determinants with univariate Chi-square p-values ≤ 0.20 and prevalence $>10\%$ were shown. ^b Univariate generalized estimated equations model with persistent nasal MRSA carriage as dependent variable. MRSA, methicillin-resistant *Staphylococcus aureus*; Ref, reference category.

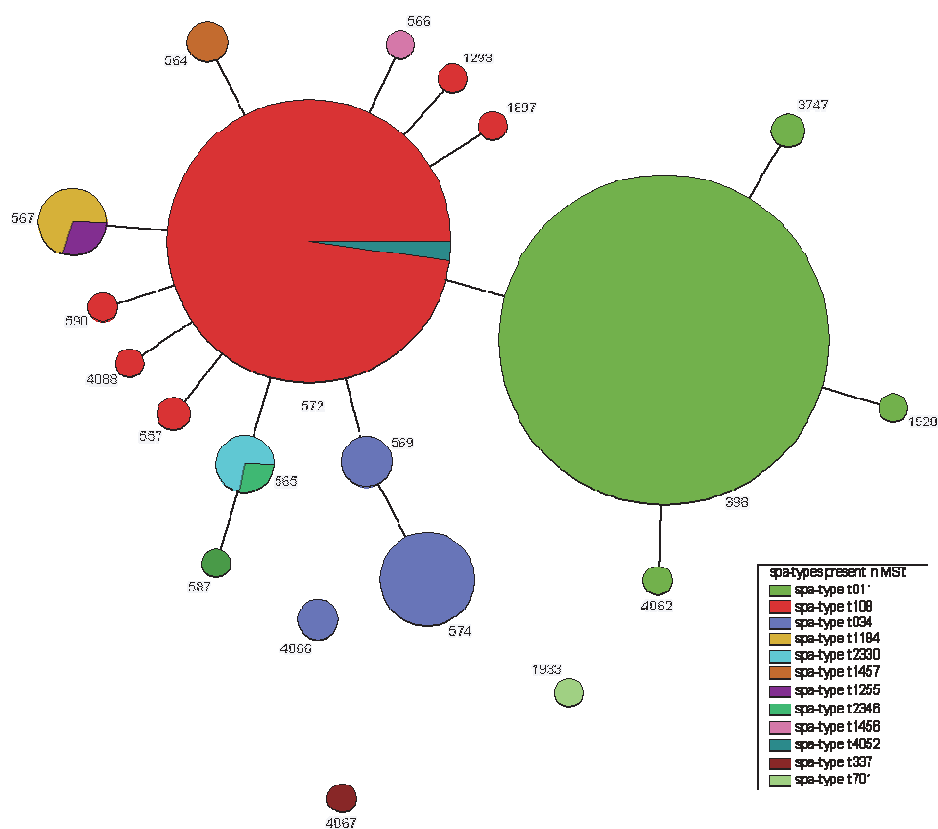


Figure S4.3.1 Minimum spanning tree (MST) of multiple-locus variable number of tandem repeat analysis (MLVA) results and *spa*-types of methicillin-resistant *Staphylococcus aureus* isolates from pig farmers working in 49 pig farms.

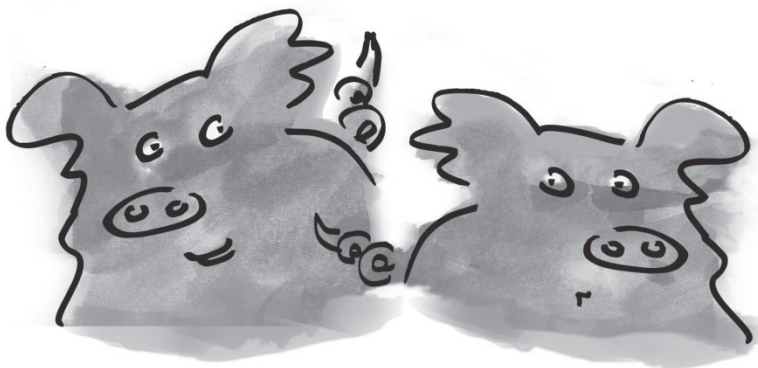
Each circle represents a MLVA-type with the name printed next to the circle, the size of the circle symbolises the amount of isolates belonging to this type, colors represent *spa*-type.

Chapter 4.4

Livestock-associated MRSA in household members of pig farmers: transmission and dynamics of carriage, a prospective cohort study

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Abstract

This prospective cohort study describes carriage of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in household members from 49 farrowing pig farms in the Netherlands (2010–2011). Of 171 household members, 4% were persistent MRSA nasal carriers, the MRSA prevalence on any given sampling moment was 10% (range 7–11%). Working in the stables (of which 98% was MRSA-positive, prevalence ratio (PR)=2.11 per 10 hours), working with sows (PR=1.97), and living with an MRSA-positive pig farmer (PR=4.63) were significant determinants for MRSA carriage. Significant protective factors were carriage of methicillin-susceptible *Staphylococcus aureus* (MSSA) (PR=0.50), and wearing a facemask when working in the stables (37% decreased prevalence). All MRSA strains during the study period were known livestock-associated types, the bacteriophage $\phi 3$ was not found in household members. Transmission from pigs and the environment appeared to be important determinants, human-to-human transmission could not sufficiently be differentiated. Wearing a facemask when working in the stables and carriage of MSSA are potential interventional targets.

Introduction

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA, multi-locus sequence type 398) is a relatively new MRSA clade (i.e. subtype coming from one ancestor), first described in 2005 [1]. The prevalence of LA-MRSA carriage in people working with livestock animals ranges from 20 to 63% [2–8], the prevalence in pigs and veal calves rises up to 75% [9]. Direct contact with livestock was shown to be a major risk factor for carriage of LA-MRSA [4,5,8].

In household members of livestock farmers, the prevalence varies from 4 to 16% [4,10,11]. Their determinants of carriage, as well as the exact roles of human-to-human transmission and the home environment are yet undetermined. Therefore, this study aims to describe the dynamics of carriage of LA-MRSA in household members of pig farmers. The results of this study provide targets for limiting the acquisition and spread of LA-MRSA.

Methods

Study design and selection of farms

This prospective cohort study surveyed persons living on 49 farrowing pig farms in the Netherlands for 1 year (2010–2011). Pig farms were randomly selected among participants from a previous study [9], which contained randomly selected farrowing pig farms from all Dutch pig farms. A detailed analysis of determinants of MRSA and methicillin-susceptible *S. aureus* (MSSA) carriage in the pig farmers of this study has been described elsewhere [8].

Sampling moments

During the 1-year study period, there were six sampling moments: day 0, day 4, day 7, month 4, month 8, and month 12. On day 0, quantitative nasal and oropharyngeal Eswabs (Copan, Brescia, Italy), and extensive questionnaires, regarding exact activities on the pig farm, contact with animals, hospital contact, personal use of antimicrobials or immunosuppressive drugs, underlying disorders (e.g. eczema or other skin diseases) and presence of indwelling catheters and/or open wounds, were collected. Wet wipe samples (Sodibox, Nevez, France) of four defined surfaces in house (backdoor handle, television remote control, chair of pig farmer, and dog neck/back) and four surfaces in the stables (farrowing and weaning stables, in duplicate) were collected as well. Nasal swabs were introduced in the nostril and rotated once. Oropharyngeal swabs sampled the area of the inner cheek including the tonsils. Refrigerated swabs were transported

to the laboratory, and cultured within 24 hours. In addition, dry electrostatic dust collector cloths (EDCs) [12] were placed in the farrowing and weaning stables (in duplicate) and on the highest cupboard in the living room of the house, and were left in place for 2 weeks before quantitative analysis.

On the remaining sampling moments, nose dry swab (Copan) self-samples were analysed semi-quantitatively and short questionnaires were filled in. Swab instructions were sent with the swabs. EDCs were placed on the same five locations in months 4, 8 and 12. An additional throat self-sample was analysed semi-quantitatively in month 12. Results of the individual cultures were disclosed at the end of the study.

Definitions

Household members were defined as individuals who lived on the pig farm premises, and worked in the stables for less than 20 hours per week at the start of the study. Pig farmers were defined as individuals who lived on the pig farm premises, and worked in the stables for at least 20 hours per week at the start of the study.

Persistent carriers were defined as persons with all nasal cultures positive for MRSA, regardless of typing results, non-carriers had no positive cultures, and intermittent carriers were the remaining persons.

Laboratory analysis: cultures

The extended laboratory procedure is described elsewhere [8]. In short, quantitative cultures were performed by serially diluting ESwab–medium and incubating on chromID *S. aureus* and chromID MRSA agar plates (BioMérieux, La Balme Les Grottes, France). The number of CFU was counted on both plates. Semi-quantitative cultures were performed with dry swabs on the same media, directly plated and after enrichment. Wet wipe samples were enriched in two consecutive media and subsequently cultured on blood and Brilliance MRSA agar plates (Oxoid, Basingstoke, UK).

All *S. aureus* strains were defined by green colonies on selective *S. aureus* agar in combination with a positive coagulase slide and DNase test. Methicillin susceptibility was tested using the cefoxitin disk diffusion method according to EUCAST standards [13], followed by a duplex PCR for the *nuc* and *mecA* genes as described previously [14].

Laboratory analysis: EDC PCR

For each EDC sample, four targets were detected with a LightCycler 480-II real-time quantitative PCR (Roche Diagnostics, Almere, the Netherlands): (i) *mecA* for methicillin-resistance [15], (ii) C01 for sequence type 398 [16], (iii) *femA* [15] and (iv) *nuc* [17] for detection of *S. aureus* [8].

The numbers of CFU-equivalents (eqCFU) per PCR and per EDC were calculated. The *S. aureus* concentration was the maximum of either *femA* or *nuc*; the concentration of MRSA was the minimum of *mecA* and *S. aureus*; the concentration of LA-MRSA was the minimum of MRSA and C01.

Molecular typing

MRSA isolates from nares, oropharynx and wet wipes were genotyped with staphylococcal protein A (*spa*) typing and multiple-locus variable number of tandem repeat analysis (MLVA), as described previously [18,19]. All MRSA strains from pig farmers, household members, employees and surface samples were tested for the presence of the bacteriophage $\phi 3$, a possible marker of increased human-to-human transmissibility [20,21], with an in-house PCR method.

Statistical analysis

An extended description of statistical analysis can be found elsewhere [8]. In short, data were analysed with SAS, version 9.3 (SAS Institute Inc., Cary, NC, USA). For each person, the proportion of nasal cultures positive for MRSA, methicillin-susceptible *S. aureus* (MSSA) and *S. aureus* in general was calculated, resulting in persistent, intermittent and non-carriers. The effect of exclusive MSSA nasal carriage on MRSA carriage was studied in sets of two consecutive sampling moments in persons initially without MRSA.

Univariate and multivariate calculations used PROC GENMOD, a generalized estimated equations model, with nasal MRSA carriage in household members as dependent variable (persistent and intermittent carriage combined versus non-carriage). All determinants with univariate Chi-square p-values of ≤ 0.20 , a prevalence in household members of $> 5\%$, and a number of missing observations $\leq 20\%$ were eligible for multivariate analysis. When Spearman's rho for two determinants was > 0.70 , collinearity was assumed, and the determinant with the highest prevalence ratio (PR) [22] or lowest p-value was selected for the multivariate analysis.

Ethical considerations

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

Results

MRSA carriage

This 1-year prospective cohort study included 171 household members from 45 pig farms (68 men), with a median age of 16 years (range 0–70). Four farms, in which there were no household members apart from the farmer, were excluded from the analysis. The median number of working hours per week in the stables at the start of the study was 1 (range 0–18). **Figure 4.4.1a** demonstrates that 4% of household members were persistent MRSA nasal carriers. MRSA was found at least once in 23% of the partners of farmers (6/26), 19% of the children (20/105) and both parents of farmers (2/2) during the study period. Variation in MRSA prevalence and cross-sectional prevalence per sampling moment are shown in **Figure 4.4.1b**, the average MRSA prevalence was 10% (range 7–11%). Inclusion of oropharyngeal samples in the definition of persistence resulted in 67% non-carriage, 29% intermittent carriage and 4% persistent carriage (74% – 22% – 4% without oropharyngeal samples, respectively, **Figure 4.4.1a**). Out of 1021 samples, 49 were missing (5%). Sensitivity analysis, defining the missing samples as either MRSA positive or negative, did not reveal much variance in carriage rates.



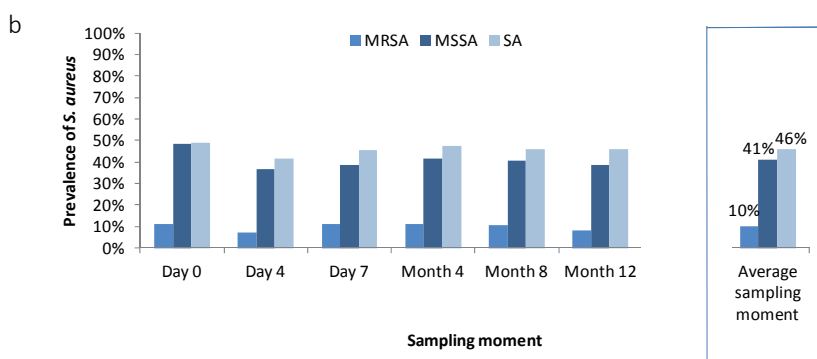


Figure 4.4.1 Carriage^a of MRSA, MSSA and *S. aureus*^b in household members of pig farmers (a) and mean cross-sectional nasal MRSA, MSSA and *S. aureus*^b prevalences per sampling moment (b). ^a A persistent carrier was a person with all nasal cultures positive, non-carriers had no positive cultures, intermittent carriers were the remaining persons. ^b Since MRSA and MSSA could co-exist in one sample, and *S. aureus* carriage could be a combination of MRSA and/or MSSA, the numbers do not add up. For example, a person carried MRSA on four out of six sampling moments, and MSSA on the remaining two sampling moments. This person was an intermittent MRSA carrier, an intermittent MSSA carrier, but a persistent *S. aureus* carrier.

The median number of MRSA in nasal samples at the start of the study was 2.4 log-transformed CFU, interquartile range (IQR: p25-p75)=1.2–3.6), regarding positive samples only. For oropharyngeal samples a median of 0.7 log CFU (IQR=0.0–1.3) was found. The presence of MRSA in oropharyngeal swabs at the start of the study was statistically significantly associated with MRSA nasal carriage (PR=2.47, 95% confidence interval (CI) 1.44–4.24, $p=0.00$). Within the group of MRSA carriers at the start of the study, household members with a higher number of CFU showed a trend of being more often a persistent nasal carrier, as shown in **Figure 4.4.2**.

Pig farmers carrying MRSA persistently were present in 22 pig farms, in which 31% of the household members carried MRSA during the study (persistently or intermittently, 29/94). Pig farmers carrying MRSA intermittently originated from 3 farms, in which 30% of household members were MRSA positive (14/46). Non-carrying pig farmers came from 10 farms, in which 6% of household members were MRSA positive (2/31). MRSA positive pig farmers and household members (persistent or intermittent) often coincided (PR in multivariate analysis 4.63, $p=0.02$, **Table 4.4.1**).

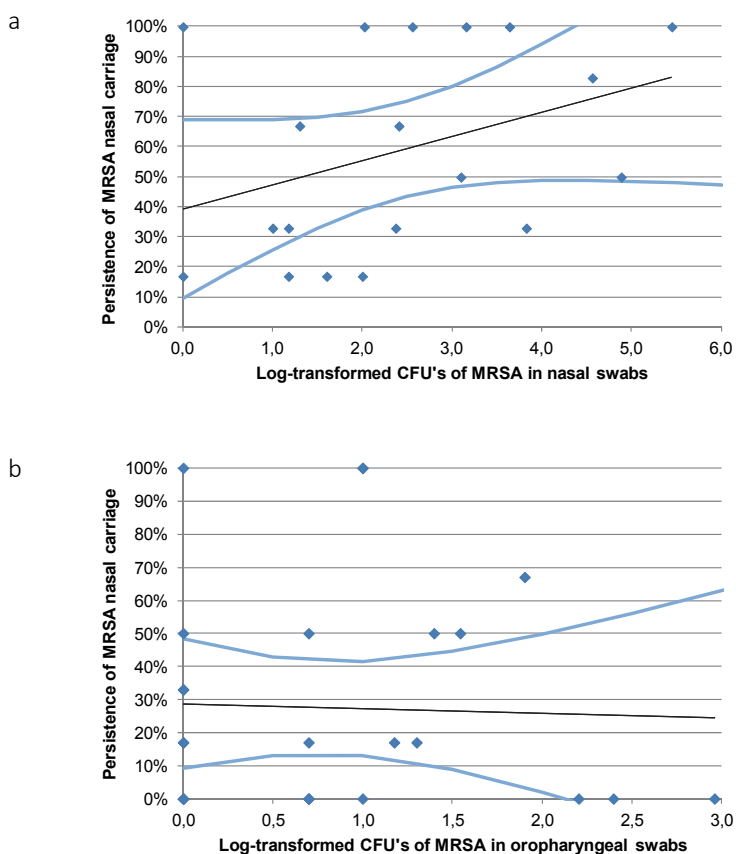


Figure 4.4.2 Linear regression model and 95% confidence bands between log-transformed colony forming units of methicillin-resistant *Staphylococcus aureus* in nasal (A) and oropharyngeal (B) swabs from start of study (x-axis) and persistence of MRSA nasal carriage during one year (y-axis) in household members who were MRSA-positive at start of the study. Prevalence ratio (PR) per log CFU=1.23, 95% confidence interval 0.95–1.60, $p=0.11$ for nasal samples. PR=0.78, 95% CI 0.33–1.88, $p=0.58$ for oropharyngeal samples.

Table 4.4.1 Determinants of MRSA nasal carriage in household members of pig farmers after multivariate analysis.

Determinant	PR (95% CI)	p-value
Working in stables - per 10 hours/week	2.11 (1.56-2.85)	<0.0001
Taking care of sows in the last 7 days	1.97 (1.26-3.08)	<0.0001
Exclusive MSSA at start study	0.50 (0.28-0.88)	0.02
Member of family with MRSA-positive pig farmer	4.63 (1.23-17.38)	0.02

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; PR (95% CI), Prevalence ratio with 95% confidence intervals. Bold-typed p-values were statistically significant (i.e. <0.05).

S. aureus versus MRSA

As shown in **Figure 4.4.1**, the prevalence of persistent nasal *S. aureus* carriage (MRSA and MSSA combined) in household members was 23%, and the prevalence of *S. aureus* carriage at the different time points was on average 46%. Exclusive MSSA nasal carriage (without MRSA) was negatively associated with the acquisition of MRSA as measured on the next sampling moment, although only when those moments were months apart (day 0, month 4, month 8, month 12), as shown in **Table 4.4.2**. When considering sampling moments that were days apart, no significant association was seen.

Table 4.4.2 Effect of exclusive MSSA nasal carriage on MRSA carriage in the next sampling moment.

	Previous MSSA carriage - no. (%) ^a		PR (95% CI)	p-value
	Yes	No		
MRSA carriers, high frequency sampling ^b	5 (4)	8 (4)	0.96 (0.32-2.84)	0.94
MRSA carriers, low frequency sampling ^c	2 (1)	17 (7)	0.17 (0.04-0.74)	0.02
MRSA carriers, all sampling moments ^d	10 (4)	25 (6)	0.63 (0.31-1.26)	0.19

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; PR (95% CI), Prevalence ratio with 95% confidence intervals. Bold-typed p-values were statistically significant (i.e. <0.05). Since only MRSA carriers were considered in this table, numbers were low. ^a Only persons at risk for MRSA acquisition were considered, i.e. MRSA negative on the previous sampling moment. ^b High frequency sampling moments: day 0, day 4, day 7. ^c Low frequency sampling moments: day 0, month 4, month 8, month 12. ^d All sampling moments: day 0, day 4, day 7, month 4, month 8, month 12. Numbers do not add up, since day 0 was included in both subgroups, resulting in different sets of samples.

Environmental samples

At the start of the study, 82% of wet wipe samples and 98% of dry EDCs in the stables were MRSA-positive (**Table 4.4.3**). MRSA carriage was significantly associated with the presence of MRSA in dog wipe samples, for other wet wipe samples no association was found. MRSA in dry EDC samples from the home environment was univariately associated with MRSA in household members; in multivariate analysis no significant association was seen (**Supplemental Table S4.4.1**). Pig farms with MRSA-positive household members had significantly higher median stable-EDC LA-MRSA concentrations, compared to pig farms with no MRSA-positive household members (**Table 4.4.3**).

Table 4.4.3 Environmental samples positive for MRSA.

	All farms ^a (n=45)	Farms with household members who were ^b		p-value ^c
		MRSA carriers ^b (21 farms)	MRSA negative ^b (24 farms)	
<i>Wet wipe samples at start of study - no. positive farms/total no. farms (%)</i>				
Stables	37/45 (82)	19/21 (90)	18/24 (75)	0.25
House	28/44 (64)	15/21 (71)	13/23 (57)	0.31
Door handle	12/44 (27)	8/21 (38)	4/23 (17)	0.12
Remote control	11/44 (25)	6/21 (29)	5/23 (22)	0.60
Chair	26/43 (60)	13/20 (65)	13/23 (57)	0.57
Dog	11/33 (33)	8/16 (50)	3/17 (18)	0.05
<i>EDCs at start of study - no. positive farms/total no. farms (%)</i>				
Stables	44/45 (98)	20/21 (95)	24/24 (100)	0.47
House	3/44 (7)	3/21 (14)	0/23 (0)	0.10
<i>EDCs during study - median LA-MRSA eqCFU per cloth (IQR)</i>				
Stables		160 (56–460)	92 (39–233)	0.03
House		0 (0–0)	0 (0–0)	0.13

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; PR (95% CI), Prevalence ratio with 95% confidence intervals; IQR, interquartile range (p25–p75); eqCFU, colony forming units equivalents. ^a Four farms did not have household members. ^b MRSA carriers were carrying MRSA nasally persistently or intermittently, MRSA negative persons did not have any MRSA during the study. ^c P-values between proportions were calculated with Chi-square tests or Fisher's Exact tests when 50% of the expected cell values were <5, EDC eqCFU counts were compared using Wilcoxon-Rank-Sum tests. Bold typed numbers indicate significance.

Determinants for MRSA carriage

Univariate determinants of MRSA carriage in household members (persistent and intermittent carriage combined) are depicted in **Supplemental Table S4.4.1**. Age, number of hours per week working in the stables, exclusive MSSA carriage at start of the study, contact with sheep, various tasks in the stables (among which giving antimicrobials to pigs, working with sows, piglets, weaned piglets, and finisher pigs), and presence of MRSA in wet wipe samples of remote control and the farmers chair were significantly associated with MRSA carriage in univariate analysis.

Continuously wearing a facemask when working in the stables and less contact with pigs in the last year were determinants with >20% of missing observations, but significantly negatively associated with MRSA carriage (**Supplemental Table S4.4.1**). Household members continuously wearing a facemask when working in the stables had a 37% decreased prevalence of MRSA carriage during the study (PR for non-carriage 1.37, 95% CI 1.11-1.70, p<0.0001). Sensitivity analysis, i.e. placing the missing samples in either category of wearing facemasks, did not reveal a different PR or significance level. In addition, wearing a facemask was not associated with specific farm tasks, or farm management (e.g. hygiene regulations, size of farm).

The number of hours per week working in the stables, taking care of sows, and being a household member of an MRSA-positive pig farmer was significantly associated with the

risk of MRSA carriage during the study in multivariate analysis (**Table 4.4.1**). Exclusive carriage of MSSA (without MRSA) at the start of the study was significantly associated with a 50% decreased risk of MRSA carriage on any later sampling moment. Excluding the number of hours per week working in the stables from the analysis did not result in a different model.

Molecular typing

In total, 110 MRSA strains from 47 household members were typed using MLVA (**Supplemental Figure S4.4.1**). All MRSA isolates from nose, oropharynx and wet wipe samples had *spa*-types and MLVA-types (MTs) corresponding to MLVA complex (MC) 398, also known as the livestock-associated clade [23]. Ninety percent of MRSA isolates from household members carried MLVA-types MT572 (n=40 isolates), MT398 (n=38), or MT567 (n=21). *Spa*-typing showed that 89% of MRSA isolates belonged to *spa*-types t108 (n=40), t011 (n=36), t899 (MT567, n=12), or t034 (MT569 and MT556, n=10), where *spa*-type t011 generally matched with MT398 and *spa*-type t108 with MT572. The remaining *spa*-types found were t1255 (MT567, n=8), t3479 (MT398, n=2), t1184 (MT567, n=1), and t4852 (MT572, n=1).

Five out of six household members (83%) who were carrying MRSA persistently carried the same MT or a single-locus variant (SLV) throughout the study. This group of persons consisted of three persons that carried MT398 consistently, one person carried MT572 and one person carried a combination of MT398 and MT572. In addition, MRSA strains from all eight persons who were positive in both nose and oropharynx at the same sampling moment yielded the same MT or a SLV. In 21 farms, both pig farmers and their household members carried MRSA during the study. Within 6 of these 21 farms (29%) all persons had LA-MRSA with similar MT or a SLV.

The bacteriophage $\phi 3$ was found in 3% (20/794) of all MRSA isolates in this study. These 20 isolates were obtained from three pig farmers, three employees (both groups sometimes carrying the bacteriophage $\phi 3$ on multiple moments) and four surface samples, originating from six farms. All MRSA isolates with the bacteriophage $\phi 3$ were of MT398 and *spa*-type t011. None of the household members carried MRSA isolates with the bacteriophage $\phi 3$.

Discussion

Dynamics of *S. aureus* carriage in household members of pig farmers

MRSA was present in 26% of household members (4% persistent carriage, 22% intermittent carriage) during the study, with a mean prevalence of 10% on any given

sampling moment. The first sampling moment showed a higher prevalence, which can probably be explained by the additional quantitative measurements performed at that moment only. The mean prevalence is somewhat higher than generally reported in household members of pig or veal farmers in the literature, being 4-8% [4, 5, 10]. However, Graveland et al. recently described MRSA in 53% of 97 household members of veal farmers (2% persistent carriage, 51% intermittent carriage), with a mean prevalence of 16% [11]. The more intense sampling in the latter study (on average 10 swabs in two months) might have resulted in a lower number of persistent carriers (the more samples, the higher chance for a negative one, resulting in less persistence), and a higher number of intermittent carriers, compared to our study.

S. aureus (MRSA and MSSA combined) carriage rates from this study (23% persistent carriage, 52% intermittent carriage, 25% non-carriage) were comparable to those found in literature [24, 25]. Non-carriers were more prevalent in literature, which is possibly a result of the extensive exposure to *S. aureus* in the stables, homes and pig farmers of the specific population studied here. MRSA carriage does not seem to change *S. aureus* numbers much (**Figure 4.4.1**), only non-carriage falls from 31% (MSSA) to 25% (SA), a relative difference of 19%. Whether this is because of addition or replacement cannot be concluded from this study. The detection of MSSA in the presence of MRSA in the same sample may be affected as well (see Study limitations).

Contact with pigs

Primary exposure to MRSA-positive pigs appeared to be an important risk factor for nasal MRSA carriage of household members during the study. This was demonstrated by the finding that working in stables and working with sows were significant determinants in the multivariate analysis, the observed association with the frequency of pig contact in univariate analysis, and the extreme exposure likelihood in the stables (82% of wet wipe samples and 98% of EDCs were MRSA-positive). The finding that pig contact is an important determinant of nasal MRSA carriage is confirmed by the literature [4,10,11,26,27].

In addition, household members continuously wearing facemasks when working in the stables had 37% less MRSA carriage, compared to household members who sometimes or never wore a facemask when working in the stables, confirming that the risk is coming from the presence of MRSA in the stables. The use of facemasks to prevent acquisition of microbes is common practice in healthcare. In the Netherlands, it is recommended to wear a facemask to prevent acquisition of MRSA by healthcare workers when caring for MRSA-positive patients [28]. However, there are few studies regarding the effectiveness of facemasks for prevention of acquisition of MRSA or other micro-organisms [29]. Pig farmers are generally advised to wear them because of the

heavy environmental dust load and bacterial exposure in the stables [30]. This observational study showed a protective effect in both household members and pig farmers [8], which should be confirmed in a prospective trial as it offers a simple, cheap and effective measure to prevent occupational acquisition of MRSA.

Contact with MRSA-positive pig farmers and MRSA in the environment

The presence of an MRSA-positive pig farmer was found to be significantly associated with MRSA carriage in household members in multivariate analysis. However, it is not possible to distinguish between human-to-human transmission and transmission via the stables or home environment in this study. Almost all household members visit the stables at least occasionally, resulting in exposure to MRSA-positive pigs and/or dust. Alternatively, the pig farmer will very likely contaminate home surfaces directly with MRSA from the stables, or with own colonizing MRSA strains, and MRSA can transfer to household members from these surfaces.

A higher MRSA load present in the environment was associated with MRSA carriage in household members, suggesting a large role for environmental contamination, as described before [31,32]. Only 29% of farms with MRSA-positive persons had MLVA types comparable between all persons in a farm. Lastly, the bacteriophage $\phi 3$, carrying the virulence genes *chp* and *scn*, and thought to be the best genetic marker for human-to-human transmission of LA-MRSA since it is specifically found in human-associated clades [20,21], was not present in LA-MRSA isolates of household members from this study. Therefore, the environment appears to be a very important determinant for human carriage, and true human-to-human transmission rates are better studied in other settings. In a study where household members of pig veterinarians did not have contact with livestock, transmissibility of LA-MRSA appeared to be reduced, compared to non-LA-MRSA [33].

MSSA is negatively associated with MRSA

In multivariate analysis, MSSA nasal carriage in household members was associated with absence of MRSA during the study. This association is confirmed regarding consecutive sets of samples, where exclusive MSSA nasal carriage (without MRSA) was associated with a 83% decreased risk of MRSA acquisition on the next sampling moment, but only when those moments are months apart. The nasal presence or absence of MRSA appears to vary with a frequency of months, instead of days. More studies have demonstrated the existence of bacterial competition, not only for *Staphylococcal* spp. among themselves, but also between genera [34,35]. The present study in pig farmers [8], and a recent study in veal calf farmers found a negative association between MSSA and MRSA carriage as well [11]. This may offer an opportunity for interventions to

reduce the rates of MRSA in livestock. Specific protecting MSSA-types can be further studied in future as well, since in this study only MRSA strains were typed.

Study limitations

The samples from this study were largely based on self-sampling techniques, which might not have been sufficient, resulting in a possible underestimation of MRSA carriage. However, a pilot study showed excellent agreement for the nose (agreement=93%, kappa=0.85, 95% CI 0.74–0.96) and good agreement for oropharyngeal samples (agreement=83%, kappa=0.60, 95% CI 0.43–0.76) [36]. Moreover, the high MRSA carriage rates found in this study, compared to other studies, do not indicate an underestimation.

Furthermore, the culture method is limited in detecting MSSA and MRSA in one sample as different entities. When a sample was MRSA-positive, the potential presence of MSSA in the same sample might have been missed, because only when two morphologically different colonies existed on the blood agar plate, both were determined. In that case, the number of MSSA CFU was the number of SA CFU minus the number of MRSA CFU. The reported numbers of MRSA, isolated MSSA, or *S. aureus* carriage in general are correct, because only samples with simultaneous presence of MRSA and MSSA are affected. In addition, the negative association found between MRSA acquisition and exclusive MSSA carriage is correct, since this was studied in MRSA-negative samples where underestimation of MSSA was not an issue. Therefore, we believe that this effect is of negligible impact on our results.

Third, some potential risk factors had >20% missing observations, leading to exclusion from multivariate analysis. This limits the number of variables that could be studied but not the reliability of the variables that were included in the analysis.

Conclusions and recommendations

We conclude that pig contact and transmission from the environment are important determinants of MRSA carriage in household members of pig farmers. Human-to-human transmission needs more research. MSSA carriage and wearing facemasks when working in the stables may offer possible interventions for the acquisition of MRSA in this highly endemic setting.

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Supplemental materials

Table S4.4.1 Determinants of MRSA nasal carriage in household members of pig farmers, univariate analysis^a.

Determinant	Value	Total - no. (n=171)	MRSA - no. (%) (n=45)	p-value
<i>General determinants</i>				
Age - per 10 years				0.01
Gender	Male	68	22 (32)	0.20
	Female	102	23 (23)	Ref
	Missing	1	0 (0)	
Exclusive MSSA at the start of the study	Yes	67	8 (12)	<0.0001
	No	102	36 (35)	Ref
	Missing	2	0 (0)	
<i>Contact with animals</i>				
Contact with sheep in the last 12 months	Yes	16	6 (38)	0.08
	No	152	38 (25)	Ref
	Missing	3	0 (0)	
Contact with poultry in the last 12 months	Yes	17	6 (35)	0.14
	No	146	37 (25)	Ref
	Missing	8	0 (0)	
<i>Working in the stables</i>				
Worked in stables - per 10 hours/week				<0.0001
Gave antimicrobials to pigs in the last 7 days	Yes	15	9 (60)	<0.0001
	No	150	34 (23)	Ref
	Missing	6	0 (0)	
Health care of sows in the last 7 days	Yes	20	12 (60)	<0.0001
	No	148	32 (22)	Ref
	Missing	3	0 (0)	
Birth assistance of sows in the last 7 days	Yes	18	11 (61)	<0.0001
	No	150	33 (22)	Ref
	Missing	3	0 (0)	
Removed manure of sows in the last 7 days	Yes	28	12 (43)	0.02
	No	140	32 (23)	Ref
	Missing	3	0 (0)	
Other activities with sows in the last 7 days	Yes	19	8 (42)	0.15
	No	149	36 (24)	Ref
	Missing	3	0 (0)	
Health care of piglets in the last 7 days	Yes	31	16 (52)	<0.0001
	No	137	28 (20)	Ref
	Missing	3	0 (0)	
Removed manure of piglets in the last 7 days	Yes	12	5 (42)	0.13
	No	156	39 (25)	Ref
	Missing	0	0 (0)	
Moved weaned piglets in the last 7 days	Yes	22	11 (50)	<0.0001
	No	146	33 (23)	Ref
	Missing	3	0 (0)	
Feeded weaned piglets in the last 7 days	Yes	15	6 (40)	0.10
	No	153	38 (25)	Ref
	Missing	3	0 (0)	
Healthcare of weaned piglets in the last 7 days	Yes	14	8 (57)	<0.0001
	No	154	36 (23)	Ref
	Missing	3	0 (0)	

Table S4.4.1 (continued)

Determinant	Value	Total - no. (n=171)	MRSA - no. (%) (n=45)	p-value
Feeded finisher pigs in the last 7 days	Yes	11	6 (55)	<0.0001
	No	157	38 (24)	Ref
	Missing	3	0 (0)	
<i>Environment / surroundings</i>				
Lives in family with MRSA-positive pig farmer	Yes	140	43 (31)	0.09
	No	31	2 (6)	Ref
Wet wipe samples of remote control positive for MRSA on day 0	Yes	43	19 (44)	0.04
	No	125	26 (21)	Ref
	Missing	3	0 (0)	
Wet wipe samples of back door positive for MRSA on day 0	Yes	49	20 (41)	0.07
	No	119	25 (21)	Ref
	Missing	3	0 (0)	
Wet wipe samples of pet positive for MRSA on day 0	Yes	119	36 (30)	0.16
	No	39	5 (13)	Ref
	Missing	13	0 (0)	
Wet wipe samples of farmers chair pos on day 0	Yes	94	33 (35)	0.04
	No	69	10 (14)	Ref
	Missing	8	0 (0)	
<i>Determinants not used for multivariate analysis (too many missings)</i>				
Pig contact in the last 12 months	Daily	6	6 (100)	<0.0001
	Weekly	45	20 (44)	0.03
	Monthly	37	8 (22)	0.46
	<Monthly	34	5 (15)	Ref
	Missing	49	6 (12)	0.83
Wear facemask in stables	Continuously	7	0 (0)	0.01
	Sometimes	20	8 (40)	Ref
	Never	95	31 (33)	Ref
	Missing	49	6 (12)	

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; Ref, reference category. Bold-typed p-values were statistically significant (i.e. <0.05). ^a All determinants with univariate Chi-square p-values ≤0.20, prevalence >5%, number of missing observations ≤20%, and highest PR or lowest p-values when collinear (i.e. Spearman's rho >0.70) were shown.

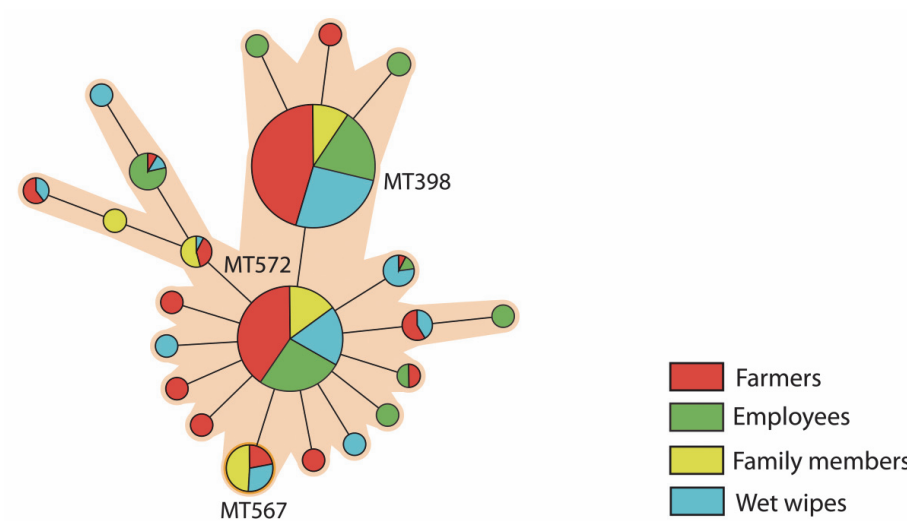
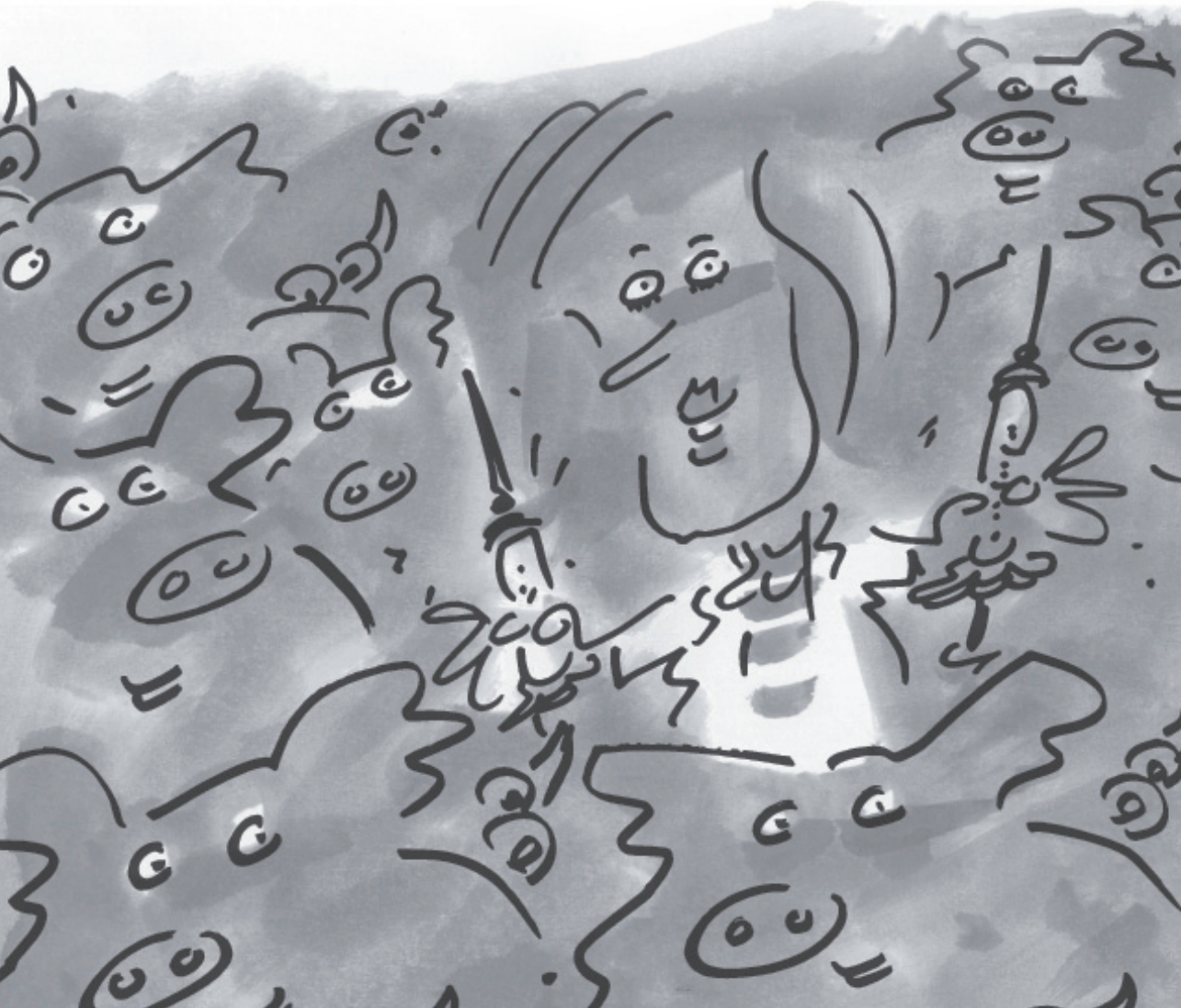


Figure S4.4.1 Minimum spanning tree of MLVA-results of methicillin-resistant *Staphylococcus aureus* isolates of household members, pig farmers, employees and wet wipe samples. Each circle represents a MLVA-type with the name of the larger groups printed next to the circle, and the size of the circle symbolizes the amount of isolates belonging to this type.

Chapter 5

General discussion



Introduction

In general, soon after the introduction of a new antimicrobial product, the first resistant micro-organisms are described. Methicillin was introduced as an innovative drug to treat penicillin-resistant *Staphylococcus aureus* (*S. aureus*) in 1959. Methicillin-resistant *S. aureus* (MRSA) was first reported in 1961, only 2 years after introduction of the drug [1]. Since then, MRSA has spread, resulting in a worldwide public health threat. In 2007 in European Union member states plus Iceland and Norway, 171,200 MRSA infections occurred and resulted in more than one million extra days spent in the hospital [(2). In the United States, an estimated 94,000 MRSA infections caused more than 18,000 deaths in 2005 [3]. Public health consequences have gained further importance since MRSA has changed its epidemiology from being primarily hospital related to an infection prevalent in the community, affecting previously healthy persons.

Only a few countries have been able to control the spread of MRSA thus far. The Netherlands, together with the Scandinavian countries, have the lowest proportions of MRSA (**Figure 5.1**), probably due to restricted antimicrobial use in humans (**Figure 5.2**), their strict infection control strategy in health care, also called ‘Search and Destroy’ (S&D), and their hospital structure (low number of beds per room and high healthcare worker to patient ratio).

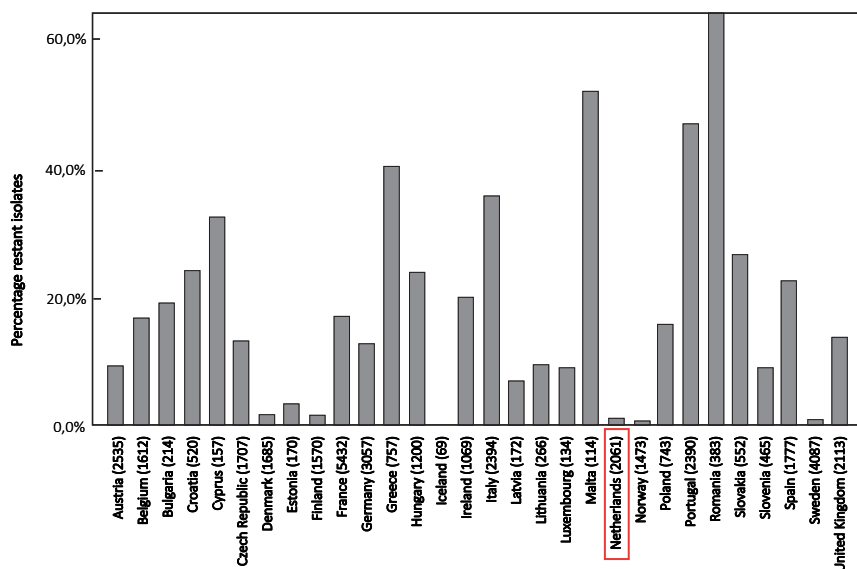


Figure 5.1 Proportion of MRSA in *S. aureus* blood cultures in Europe in 2013 (number of isolates in brackets after country name). Source: EARS-Net interactive database, <http://ecdc.europa.eu>, accessed January 2015.

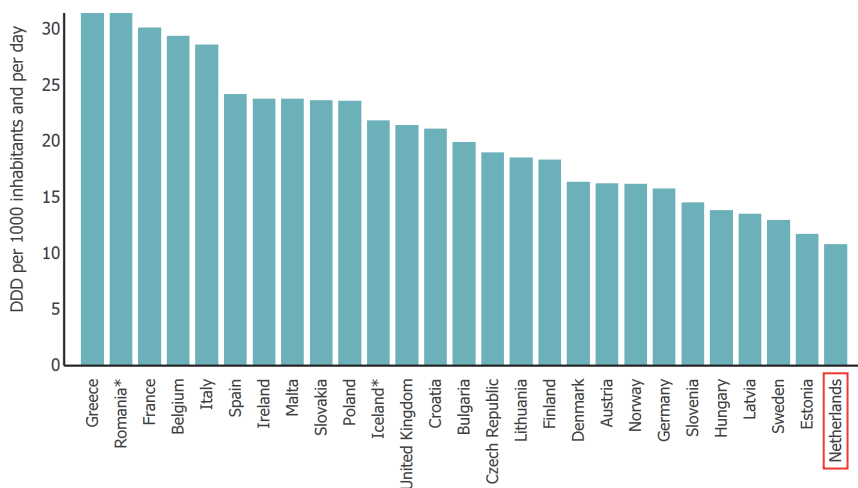


Figure 5.2 Consumption of antimicrobials for systemic use in the community in Europe, reporting year 2013.
 * Country provided only total care data. Source: ESAC-Net interactive database, <http://ecdc.europa.eu>, accessed June 2015.

Soon after the turn of the century a new variant of MRSA emerged that was related to a reservoir in food production animals. The emergence of this so-called ‘livestock-associated MRSA’ (LA-MRSA) posed a threat to the successful control strategy in the Netherlands. To determine the potential implications of LA-MRSA it is essential to know the epidemiology, characteristics and dynamics of this highly clonal subtype of MRSA. The research described in this thesis aimed to gain more insight into LA-MRSA carriage in persons in contact with pigs. The resulting targets for interventions and recommendations for the future, aimed at controlling the public health threat of LA-MRSA, are presented at the end of this chapter, together with the main conclusions of this thesis.

Colonization or contamination?

Nasal MRSA carriage in persons in contact with live pigs was described in **Chapters 2.1, 2.2, 2.4, 4.1, 4.3, and 4.4**, with a prevalence up to 63%, rising well above the normal *S. aureus* carriage rate of 30% [4,5]. Environmental contamination levels were extremely high, with MRSA present in stable dust in 98% of farms, often in very high numbers (**Chapter 4.3**) [6–8]. Additionally, in **Chapter 2.4** we showed that LA-MRSA did not persist for more than 24 hours in 94% of persons after short but intensive livestock animal contact, and Graveland et al. found reduced MRSA carriage rates in veal farmers in

absence of veal calf contact [9]. These observations gave rise to the question whether a pig farmer with LA-MRSA in his nose was truly colonized. Alternatively, the nose may be contaminated by the high amounts of MRSA that were inhaled during work without resulting in true colonization. This is an important distinction, because nasal contamination with LA-MRSA is considered less likely to cause infection, and it disappears rapidly when the subject is not exposed to the source anymore.

Our assumption was that contaminated persons would not carry MRSA for prolonged periods. However, we found a high percentage (38%) of pig farmers that were persistently colonized with LA-MRSA, i.e. they were positive on all 6 sampling moments during the one year follow up (**Chapter 4.2**). This is more or less double the percentage of persistent *S. aureus* carriers in the population [4,5]. Other findings that support the hypothesis that many pig farmers were truly colonized are: 1) the vast majority (88%) of the persistent carriers, carried the same type of strain during the one year follow up period (**Chapter 4.2**), 2) persistent carriers of LA-MRSA had on average higher colony counts than intermittent carriers which is reported to be associated with true colonization [4,10], and 3) throat carriage was significantly associated with persistent MRSA carriage in pig farmers (prevalence ratio (PR)=2.89, $p<0.001$, **Chapter 4.2**). The latter finding is more supporting the colonization theory, since inhalation of MRSA-contaminated (i.e. contamination) dust is expected to be found primarily in the nose and not in the more distant throat.

We conclude that the high exposure to LA-MRSA in the stables does not only cause temporary contamination of the nasal cavity but also frequently causes true colonization. This hypothesis is supported by an experimental study that showed that healthy volunteers that were exposed to a mixture of *S. aureus* strains often were persistently colonized with livestock-associated *S. aureus* [11]. True colonization of pig farmers, their employees or family members may therefore result in an increased risk of infection and poses a threat to public health.

Addition or replacement?

The emergence of MRSA may add to the existing burden of *S. aureus* infections (addition). The size of this effect is disputed since there are also indications of bacterial interference, also known as competition for colonization space (the nares), between *S. aureus* strains. If bacterial interference would lead to replacement of MSSA by MRSA, the total infection burden would be unaffected (replacement) [12]. The debate currently is in favour of addition [13]. For LA-MRSA a similar discussion can be held, since such information is critical for estimating the threat to public health from LA-

MRSA, assessing the consequences for health-economics, and demonstrating the results of infection control strategies.

MSSA-carrying pig farmers and their household members are less likely to acquire MRSA, as shown in **Chapters 4.3** and **4.4** (PR=0.37 $p=0.01$, and PR=0.17 $p=0.02$, respectively). Bacterial interference might play a role here, which is supporting the replacement-theory [14]. A negative association between MRSA and MSSA carriage was demonstrated for veal farmers as well (9). On the other hand, the combined MSSA and MRSA carriage rates in pig farmers (**Chapter 4.3**) are much higher than those from normal *S. aureus* (see before) [4,5], which is an argument in favour of the addition-theory.

We conclude that probably both addition and replacement play a role in the epidemiology of *S. aureus* and in particular for LA-MRSA. The extremely high *S. aureus* carriage rate in pig farmers poses a potential threat to their health. Since this high carriage rate is determined almost entirely by exposition in the stables, LA-MRSA should be considered a potential occupational health risk. This risk should be further quantified and control measures are needed.

Human-to-human transmission

Human-to-human transmission is the main determinant for the spread of LA-MRSA through the population. The magnitude of the public health threat depends mainly on this characteristic, in combination with strain virulence. Families living on or working at a pig farm are a suitable study population, especially when they are prospectively studied, as was done in **Chapters 4.3** and **4.4**.

The problem with household members of pig farmers was, however, that they were in contact with the farmer (human-to-human transmission), the home environment (house-to-human transmission), and the stable environment (stable-to-human transmission), since they very often assisted the pig farmer in the stables. The effects of these three transmission routes could not clearly be separated in this study population, and the extent of the role of human-to-human transmission can be investigated better in populations not exposed to livestock, for example household members of livestock-veterinarians [15].

Evolution of LA-MRSA in pig farms in time

The prevalence of MRSA in pig farmers found in the latest studies was higher compared to older studies on pig farmers in this thesis: from 29% in 2007 (**Chapter 2.1**) to 63% in

2010 (**Chapter 4.3**). Similarly, Broens et al. have shown that pig herd prevalence rose from 30% in the beginning of 2007 to 75% in the end of 2008 [6]. The extremely high MRSA prevalence in livestock probably results in very high carriage in humans in contact with these animals. The carriage rate of MRSA in pig farmers as reported in **Chapter 4.3** is higher than any other population that has been described to date (MRSA prevalence in pig or veal farmers vary from 20% to 45% in various international studies [9,16–21]). In the general population in the Netherlands, the MRSA prevalence is estimated at 0.1% [22].

Potential for public health threat

The previous paragraphs have shown that a public health threat may arise from LA-MRSA, which needs to be controlled. At present, in healthcare settings LA-MRSA appears to be under control, but this does not seem to be the case in animal husbandry. Currently, both LA-MRSA and MRSA in general do not appear to have much impact on public health in the Netherlands, illustrated by the minimal additive effect on the burden of *S. aureus* bacteraemias, as shown in **Chapters 3.1** and **3.2**, and the absence of an association between LA-MRSA and infections and health-related quality of life in **Chapter 3.3**. While prevalences in livestock farming probably have increased close to a saturation point, LA-MRSA seldomly seems to cause serious problems in farms or hospitals. Nonetheless, experts worry that the rapid evolution of LA-MRSA may result in acquisition of new characteristics in the near future [23], since LA-MRSA has proven to be able to exchange many mobile genetic elements between strains, suggesting that this clade can rapidly adapt to changes [24,25]. Examples are the acquisition of resistance genes for methicillin and tetracycline, Panton-Valentine leukocidin, an important virulence factor in CA-MRSA, and the $\phi 3$ -bacteriophage encoding human-specific immune evasion factors, also referred to in **Chapter 4.4** [23,26]. Also, a study based on whole genome sequencing provided evidence that the clade of LA-MRSA originated in humans, and lost some immune evasion genes when it entered the livestock population, while acquiring resistance genes [26]. Potentially this genetic event could happen in a reverse manner (acquisition of virulence factors while maintaining resistance traits), as *S. aureus* has the capacity to adapt to and switch into novel hosts [27]. The immense reservoir that has been created in livestock could cause serious consequences for public health [28].

MRSA in livestock animals is a different story. Enormous amounts of antimicrobials are sold to the livestock industries yearly, equalling 5-10 times the amounts used in human medicine, and thereby strongly selecting for antibiotic resistant bacteria in the animals (**Figure 5.3**). This huge reservoir is a potential risk for public health. Wageningen

University published on its MARAN-website that antibiotic sales have declined with 51% in the period 2009-2012, from 495 to 244 tonnes (www.maran.wur.nl) [29]. This is an important step in the right direction, and declining MRSA-prevalence in pigs are described [30]. However, this intervention alone is most likely far from sufficient to turn the vast amount of resistance that is already present.

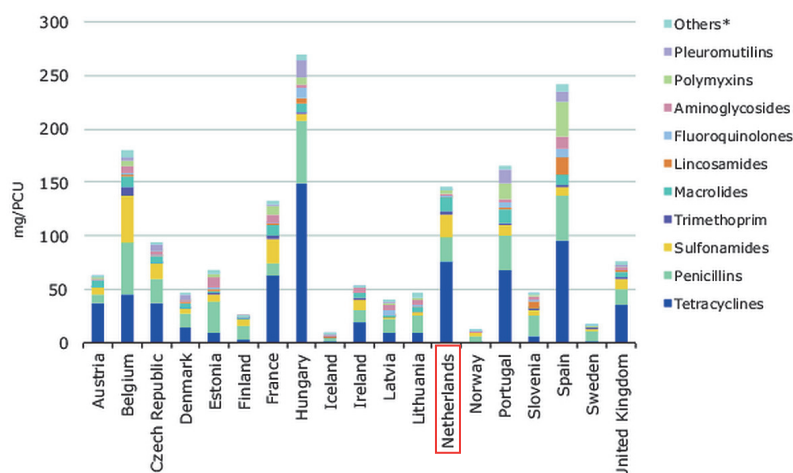


Figure 5.3 Sales for food-producing species, including horses, in mg/PCU¹, of the various veterinary antimicrobial classes, by country², for 2010. Reprinted from [31]. ¹The population correction unit (PCU) is used as the term for the estimated weight. 1 PCU = 1 kg of different categories of livestock and slaughtered animals. ² Differences between countries can partly be explained by differences in animal demographics, in the selection of antimicrobial agents and in dosage regimes, among other factors. * Amphenicols, cephalosporins, other quinolones and other antibacterials (classified as such in the ATCvet system).

Possible interventions from this thesis

The studies presented in this thesis provide possible intervention methods, in order to cope with the imminent public health threat from LA-MRSA in the animal reservoir. As shown in **Chapters 2.1, 2.2, 2.3, 4.1, 4.3 and 4.4**, the most important determinant for humans to acquire LA-MRSA is contact with pigs. Next to the abovementioned need for reduction in antimicrobial use in the livestock industry, an important preventive advice is to avoid contact with LA-MRSA positive animals. This applies specially to household members, since their presence in the stables is sometimes superfluous. For pig farmers absence of animal contact is not an option.

Another possible intervention resulting from **Chapters 4.3** and **4.4** that may be effective in both pig farmers and household members is the use of mouth masks, which has not been observed before in persons in contact with livestock. In our observational study it was associated with a strong reduction of the likelihood to carry MRSA. This should be confirmed in an interventional study. Also, the observation that carriage of MSSA was associated with a lower risk of acquiring MRSA indicates that bacterial interference plays a role. The usefulness of inoculating with a non-pathogenic MSSA or other less virulent microorganisms to possibly prevent LA-MRSA from attachment to the nasal mucosa, needs to be investigated [14,32].

Recommendations for further studies

Each study provides some answers but generates many more questions, so do the studies described in this thesis. The cohort study described in **Chapters 3.3, 4.3** and **4.4** collected many MSSA isolates from humans and the environment. Further characterization with sophisticated typing methods can provide more insight into the role for LA-MSSA in the dynamics of carriage of *S. aureus* in persons involved in livestock farming, and in the burden of livestock-associated infections, as recent publications suggest a larger role as previously thought [26,33–35].

In addition to using available data, this cohort is extremely valuable in monitoring the evolution and epidemiology of LA-MRSA in pig farms. A sampling frequency of once a year should be enough to keep track of presumed changes. Together with the other existing cohorts of pig veterinarians and veal farmers and their household members, it provides a usable inside view of LA-MRSA.

The most important issue in order to prevent a possible LA-MRSA public health threat is to examine how to decrease the reservoir present in the stables. A large reduction in antimicrobial use in animal husbandry in the Netherlands has already been achieved, and the first promising effects on the MRSA prevalence in pigs are described [30]. Furthermore, thorough studies should be planned to examine the best way to clean the stables in order to reduce contamination pressure for humans, for example with water, or with probiotics. It might nevertheless not be possible to eradicate MRSA from the livestock stables considering the extent of the current reservoir and many parts of the pig production chain that are contaminated. Therefore, studies exploring and validating other (abovementioned) intervention methods are useful.

Main conclusions from this thesis

The research in this thesis gained more insight into the prevalence, determinants, and spread of LA-MRSA in persons in contact with pigs, and contributed to a better understanding of dynamics of carriage and possible public health threat. The following main conclusions can be drawn from this thesis:

- LA-MRSA has an extremely high prevalence in persons in contact with live pigs and their family members. Other persons at the farm are at a considerably lower risk, albeit much higher than the general population.
- The public health threat of LA-MRSA in the Netherlands at the moment appears to be low. Nevertheless the fast genetic evolution of this specific strain can cause problems in the (near) future and warrants careful monitoring and further research.
- The best way to minimize a possible public health threat is to challenge LA-MRSA at the primary source: the animal reservoir. Eradication of LA-MRSA in pig farms is however unlikely to be achieved at short notice. A further decrease in antimicrobial use in animal husbandry is possible though, as is research in using probiotics and bacterial competition, and selection of MRSA-negative pig production chains.

Follow up of the initiated cohorts is useful to monitor changes and test interventions, in order to gain control of the situation.

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Summary



Introduction

Staphylococcus aureus (*S. aureus*) is a commensal bacterium for humans, and generally resides on skin and mucous membranes, like nares, pharynx, and perineum. About one in three persons carry *S. aureus* in their nose. Next to being a commensal bacterium, *S. aureus* is also known for its pathogenic potential, with a range of harmless skin infections like impetigo and furuncles to severe infections like sepsis, osteomyelitis and pneumonia.

Shortly after the introduction of methicillin for the treatment of *S. aureus* infections, the first methicillin-resistant *S. aureus* (MRSA) was reported. At first the problems centred in hospitals (hospital-associated MRSA: HA-MRSA), however, in the last decades MRSA causes infections in the community in previously healthy persons as well (community-associated MRSA: CA-MRSA). Due to a strict search and destroy strategy in the Netherlands, prevalence of MRSA in hospitals has remained low, compared to other countries.

In 2003 the association of MRSA with livestock was first reported in the Netherlands. Subsequent investigations revealed a huge reservoir of MRSA in livestock, which consisted of a highly clonal population of MRSA, designated livestock-associated MRSA (LA-MRSA). Pig farmers and pig veterinarians were frequently carrying these strains, with an initially reported prevalence of approximately 25%.

Aim of this thesis

The initial studies described in this thesis aim to estimate prevalence of and find determinants for LA-MRSA carriage in multiple populations: pig farmers (**Chapter 2.1**), their household members (**Chapter 2.1**), slaughterhouse workers (**Chapter 2.2**), persons living in pig-dense municipalities (**Chapter 2.3**), and field workers with intense pig contact for a short duration (**Chapter 2.4**). In addition, the presence of LA-MRSA in European countries was described in **Chapter 2.5**. The public health threat of MRSA in general and LA-MRSA in specific was studied in **Chapters 3.1, 3.2 and 3.3**. Lastly, dynamics of carriage in pig farmers and household members are presented in **Chapters 4.1, 4.3 and 4.4**.

Prevalence of LA-MRSA carriage

Persons in direct contact with pigs or veal calves had the highest risk of nasal LA-MRSA carriage. **Chapter 2.1** shows that in 2007 29% of pig farmers were LA-MRSA positive, by 2010 this cross-sectional prevalence rose to 63%. During follow up it was found that 38% of the pig farmers carried LA-MRSA persistently (**Chapter 4.3**).

Studies in other professional groups with pig contact show comparable results. In pig slaughterhouse personnel in contact with live pigs LA-MRSA prevalence was 15% (**Chapter 2.2**). Field workers temporarily taking samples from pigs and stables acquired LA-MRSA at rates as high as 48%. Of these persons, 94% were LA-MRSA negative after 24 hours, indicating that short and intense pig contact can result in LA-MRSA acquisition but carriage usually lasts for very short periods. (**Chapter 2.4**).

Four percent of household members of pig farmers were LA-MRSA positive in 2007 (**Chapter 2.1**), in 2010 the cross-sectional prevalence was 10%, and 4% of household members were persistently carrying LA-MRSA in their nose (**Chapter 4.4**). Household members of pig farmers had LA-MRSA carriage percentages much lower than those of the livestock farmers themselves, but were still more often MRSA positive than community dwelling individuals in the Netherlands.

Persons living in the most pig-dense municipalities in the Netherlands, but without pig contact were MRSA positive in 0.2% (**Chapter 2.3**), which is in accordance with the general Dutch MRSA carriage percentage, estimated at 0.11%. These findings result in the conclusion that LA-MRSA has not yet spread from the farms into the community on a measurable scale.

LA-MRSA internationally

Chapter 2.5 illustrates that in several European countries LA-MRSA could be found, but mostly in low proportions of MRSA clinical isolates. Countries with higher densities of pigs and veal calves, and higher human population densities had higher proportions of LA-MRSA. Apparently, both livestock animals and humans need to be in close relation to each other in order to spread the bacterium.

Potential for public health threat

The Dutch rate of MRSA bacteraemia (including those caused by LA-MRSA) was lower than in other countries (**Chapters 3.1** and **3.2**), and an association between LA-MRSA and infections or quality of life could not be proven (**Chapter 3.3**). Next to differences in

healthcare structure and human antimicrobial use, the active search and destroy strategy in the Netherlands appeared to be effective in preventing many serious MRSA infections (**Chapter 3.1**). In addition, the population affected by LA-MRSA is healthier compared to the average MRSA infected patient, contributing to a lower infection rate for LA-MRSA as well. Finally, reduced virulence of the livestock-associated strains for humans may have played a role (**Chapter 3.2**). At the moment, in humans both MRSA at large and LA-MRSA specifically in the Netherlands appears to be under control.

Dynamics of LA-MRSA carriage

Nasal carriage of a methicillin-sensitive *S. aureus* (MSSA) was associated with less LA-MRSA carriage for both pig farmers and their household members, as described in **Chapters 4.3** and **4.4**. This might have been the result of a phenomenon known as bacterial interference, or competition for colonization space. Whether LA-MRSA replaced MSSA in previous MSSA carriers, or came on top of MSSA carriage numbers, is not completely clear.

The high prevalence found in pig farmers was likely the result of true colonization; repeated contamination of the nose by inhalation of LA-MRSA contaminated dust appeared to be less obvious (**Chapter 4.3**). Indeed, contamination of the environment in the stables was extreme, with high amounts of LA-MRSA found. On the contrary, persistently LA-MRSA carrying pig farmers had higher nasal loads of LA-MRSA, and were more often throat carriers, compared with pig farmers who intermittently or never carried MRSA.

Like for the farmers, pig contact was the most important determinant for LA-MRSA carriage in household members (**Chapter 4.4**). Human-to-human transmission from pig farmers to household members seemed to play a role as well, but needs to be studied further in other settings.

Possible interventions

Chapters 4.3 and **4.4** report that continuously wearing a mouth mask while working in the stables was associated with a lower risk for LA-MRSA carriage for both pig farmers and their household members. This and other infection control measures can be verified in future intervention studies, where the existing cohort can be utilized and further studied.

The huge reservoir of resistance in livestock is potential treat for the future. Follow up of the existing cohorts results in a useful inside view of LA-MRSA. Molecular evolution,

human-to-human transmission, illnesses, prevalence and possible interventions can be studied in these fully recorded groups of pig and veal farmers, livestock veterinarians, and their household members.

Livestock animals remain the primary source of LA-MRSA. Attempts are already made to eliminate LA-MRSA from pig farms by minimizing antimicrobial use in animals and by exploring cleaning methods of stables. Since elimination of this bacterial strain from the animals and stable environment will probably not be feasible, following up the created cohorts, and testing interventions is needed in order to gain control of the situation.

Samenvatting



Introductie

Staphylococcus aureus (*S. aureus*) is een bacterie die op de huid en slijmvliezen (onder andere keel en neus) van mensen leeft. Ongeveer 1 op de 3 mensen draagt deze bacterie in zijn neus. Soms kan *S. aureus* ook ziekte veroorzaken, zoals krentenbaard en steenpuisten, maar ook bloedvergiftiging, botontsteking en longontsteking.

MRSA staat voor methicilline-resistente *S. aureus*; deze bacteriën zijn ongevoelig (resistent) voor de meest gebruikte antibiotica (bijvoorbeeld methicilline). In de beginjaren gaf MRSA vooral problemen in ziekenhuizen, de laatste jaren worden echter steeds meer problemen in de bevolking gezien. In Nederland geldt er een 'search and destroy' beleid, waarbij er in ziekenhuizen actief naar MRSA gezocht wordt, en er daarna een passende behandeling gestart wordt. Mede hierdoor is er niet veel MRSA in Nederland te vinden, vergeleken met andere landen.

In 2003 is voor het eerst MRSA gevonden bij een veehouder. Daarna werd duidelijk dat er in vee heel veel MRSA voorkomt. Deze MRSA-bacteriën lijken erg op elkaar, en worden veegerelateerde MRSA (in het Engels livestock-associated MRSA: LA-MRSA) genoemd. In de eerste onderzoeken droeg 25% van de varkenshouders en varkensdierenartsen deze LA-MRSA bij zich.

Doel van dit proefschrift

Dit proefschrift heeft als doel meer inzicht te geven in LA-MRSA. De eerste onderzoeken van dit proefschrift beschrijven hoe vaak LA-MRSA voorkomt, en wat de risicofactoren zijn voor LA-MRSA. **Hoofdstuk 2.1** kijkt naar varkenshouders en gezinsleden, **hoofdstuk 2.2** naar slachthuismedewerkers, **hoofdstuk 2.3** naar inwoners van varkensrijke gemeenten, en **hoofdstuk 2.4** naar mensen die monsters namen, en die voor een korte duur intensief contact hadden met varkens. In **hoofdstuk 2.5** wordt het vóórkomen van LA-MRSA in verschillende Europese landen beschreven. Of LA-MRSA een gevaar voor de volksgezondheid is, wordt beschreven in **hoofdstukken 3.1, 3.2 en 3.3**. Als laatste wordt de ontwikkeling van LA-MRSA in de tijd bij varkenshouders en hun gezinsleden beschreven in **hoofdstukken 4.1, 4.3 en 4.4**.

Vóórkomen van LA-MRSA

Mensen met direct contact met varkens of vleeskalveren, hadden de grootste kans om LA-MRSA te dragen in hun neus. In **hoofdstuk 2.1** staat dat in 2007 29% van de varkenshouders LA-MRSA had, in 2010 was dit percentage 63%. Als we meerdere monsters nemen, blijkt dat 38% van de varkenshouder altijd LA-MRSA bij zich draagt (**hoofdstuk 4.3**).

Onderzoeken bij andere groepen mensen die voor hun beroep in aanraking komen met varkens laten vergelijkbare resultaten zien. In varkensslachthuizen droeg 15% van de medewerkers, die in aanraking kwam met levende varkens, LA-MRSA bij zich (**hoofdstuk 2.2**). Mensen die de monsters namen, en die slechts kortdurend, maar intensief contact met varkens hadden, hadden in 48% van de gevallen LA-MRSA, en 94% hiervan was 24 uur later weer LA-MRSA-vrij. Na kortdurend contact lijkt men LA-MRSA dus niet lang bij zich te dragen (**hoofdstuk 2.4**).

Van de gezinsleden van varkenshouders had 4% LA-MRSA in 2007 (**hoofdstuk 2.1**). Dit was in 2010 gestegen tot 10%, en 4% droeg de LA-MRSA bacterie op elk meetmoment in de neus (**hoofdstuk 4.4**). Gezinsleden hadden minder LA-MRSA dan varkenshouders, maar meer dan de gemiddelde Nederlander.

Mensen die in varkensrijke gemeenten in Nederland wonen, maar die geen contact met varkens hebben, droegen in 0,2% van de gevallen MRSA (**hoofdstuk 2.3**). Dit is gelijk aan het gemiddelde Nederlandse percentage van 0,11%. LA-MRSA lijkt zich nog niet verspreid te hebben van de varkenshouderijen naar de omwonenden.

LA-MRSA internationaal

In **hoofdstuk 2.5** staat dat LA-MRSA te vinden is in verschillende Europese landen, meestal in lage percentages. Hoe meer varkens en vleeskalveren in een land zijn, en hoe meer mensen, des te meer LA-MRSA er gevonden is. Dieren en mensen moeten kennelijk dicht bij elkaar zijn om deze bacterie te verspreiden.

Gevaar voor de volksgezondheid

Bloedvergiftingen door MRSA en LA-MRSA kwamen in Nederland minder vaak voor dan in andere landen (**hoofdstukken 3.1** en **3.2**). Bovendien kon er geen verband worden aangetoond tussen LA-MRSA en ontstekingen, of tussen LA-MRSA en een verminderde kwaliteit van leven (**hoofdstuk 3.3**). Naast het 'search and destroy' beleid, lijkt de structuur van de gezondheidszorg in Nederland goed geschikt om MRSA-

ontstekingen te vóórkomen (**hoofdstuk 3.1**). Bovendien is de gemiddelde LA-MRSA drager gezonder dan de drager van andere MRSA-soorten. Dit draagt ook bij aan het lage aantal infecties door LA-MRSA. Als laatste lijkt LA-MRSA minder ziekmakend vermogen te hebben dan de andere MRSA-soorten (**hoofdstuk 3.2**). Het MRSA-probleem in Nederland lijkt onder controle.

Ontwikkeling van LA-MRSA in de tijd

Neusdragerschap met methicilline-gevoelige *S. aureus* (MSSA) bleek beschermend te zijn voor LA-MRSA voor zowel varkenshouders als gezinsleden (**hoofdstukken 4.3 en 4.4**). Dit kan komen door iets wat bacteriële interferentie, of competitie, genoemd wordt. Of LA-MRSA in plaats van MSSA komt in de neus, of samen met MSSA aanwezig is, is niet duidelijk.

Het feit dat LA-MRSA in varkenshouders heel veel vóórkomt, komt waarschijnlijk doordat de bacterie echt groeit in de neus (kolonisatie). Herhaalde besmetting van het neusslijmvlies door inademen van stof wat met LA-MRSA besmet is, lijkt minder waarschijnlijk (**hoofdstuk 4.3**). We vonden wel grote hoeveelheden LA-MRSA in omgevingskweken.

Net als voor de varkenshouders was contact met varkens de belangrijkste risicofactor om LA-MRSA te dragen voor gezinsleden (**hoofdstuk 4.4**). Mens-op-mens overdracht binnen een gezin lijkt een rol te spelen, maar moet verder onderzocht worden.

Mogelijke maatregelen

Hoofdstukken 4.3 en 4.4 melden dat het dragen van een mondkapje minder LA-MRSA-dragers oplevert, zowel bij varkenshouders als bij gezinsleden. Deze en andere mogelijke maatregelen moet getest worden in toekomstig onderzoek.

De grote aanwezigheid van LA-MRSA in vee is een gevaar voor de toekomst. Als we de onderzochte groepen varkenshouders, varkensdierenartsen en hun gezinsleden in de toekomst vaker testen, kunnen we de ontwikkeling van LA-MRSA in de gaten houden.

Vee blijft de belangrijkste bron van LA-MRSA. Daarom is het belangrijk om deze bacterie zo veel mogelijk uit de veestapel te krijgen. Dit gebeurt al door antibioticagebruik in de veehouderij te minimaliseren. Ondanks dit gaat LA-MRSA waarschijnlijk niet meer uit de stallen verdwijnen, hierdoor wordt het onderzoeken van maatregelen belangrijk om de situatie onder controle te krijgen.

Dankwoord



Dankwoord

En dan nu: het laatste hoofdstuk. Zou het ook het moeilijkste zijn? Ik vertel het u aan het eind.

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Mark. Wat moet ik hier nu schrijven wat mijn gevoel goed uit gaat drukken? Bedankt? Dat zou tekort schieten. Je hebt me enorm gesteund in het proces van dit boekje de afgelopen jaren, soms ten koste van jezelf. En ik denk dat we alletwee in deze tijd veel geleerd hebben. Ik voel me vol, vol van liefde en geluk, blij dat we gekozen hebben om onze toekomst samen te delen. Onze toekomst met onze twee prachtige dochters, Vera en Carmen, die het hopelijk maar wat cool vinden dat hun naam in een boek staat (al is het woord 'cool' vast niet cool meer tegen die tijd). Ik hou van jullie. Onvoorwaardelijk.

En nee, dit was niet het moeilijkste hoofdstuk om te schrijven. Wel het belangrijkste.

Brigitte

Curriculum Vitae



Curriculum Vitae

Brigitte Antonia Geertruida Lucia Megens - van Cleef werd geboren op 28 januari 1980 te Oss. In 1998 haalde zij haar VWO diploma Atheneum aan het Mondriaancollege te Oss. Daarna is zij bij de toenmalige Katholieke Universiteit Nijmegen (thans Radboud Universiteit Nijmegen Medical Center) Biomedische Gezondheidswetenschappen gaan studeren, met als hoofdvak Pathobiologie en bijvakken Epidemiologie en Antropogenetica. Na afronding van deze studie in 2002 heeft zij aan dezelfde universiteit de studie Geneeskunde met goed gevolg afgerond in 2006, met als keuzecoschap Klinische Genetica en als afsluitend coschap Kindergeneeskunde. Zij heeft van 2006 tot 2007 gewerkt als arts-assistent niet in opleiding tot specialist (ANIOS) bij de afdeling Kindergeneeskunde van het St. Elisabeth ziekenhuis te Tilburg. Daarna is zij in 2007 als promovendus begonnen bij de afdeling Medische Microbiologie en Infectiepreventie van het VU medisch centrum te Amsterdam, onder begeleiding van promotores Prof.dr. Jan Kluytmans en Prof.dr. Andreas Voss, en co-promotor dr. Birgit van Benthem. Het project wat geresulteerd heeft in dit boekje. Van 2007 tot 2012 was zij fulltime gedetacheerd naar de afdeling Epidemiologie en Surveillance van het Centrum Infectieziektebestrijding van het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) te Bilthoven, in samenwerking met het Laboratorium voor Microbiologie en Infectiepreventie van het Amphia ziekenhuis te Breda. In 2012 begon zij haar opleiding tot arts-microbioloog in het Laboratorium voor Medische Microbiologie en Immunologie van het St. Elisabeth ziekenhuis te Tilburg en het Laboratorium voor Medische Microbiologie en Infectiepreventie van het Amphia ziekenhuis te Breda, onder begeleiding van opleider Prof.dr. Jan Kluytmans. In 2015 heeft zij besloten haar opleiding niet te vervolgen en is gaan werken als arts infectieziektebestrijding bij de afdeling Infectieziekten van de Gemeentelijke Gezondheidsdienst (GGD) te Amsterdam. Brigitte is getrouwd met Mark Megens, samen hebben ze 2 dochters: Vera van 5 jaar en Carmen van 6 maanden.

List of publications



List of publications

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Staphylococcus aureus (*S. aureus*) is a commensal bacterium in about one in three humans. Methicillin-resistant *S. aureus* (MRSA) can be carried by persons in contact with livestock animals, as these are a large reservoir of MRSA (livestock-associated MRSA: LA-MRSA).

The research described in this thesis aimed to gain more insight into the prevalence, determinants, spread, dynamics and public health threat of LA-MRSA carriage in persons in contact with pigs. Its main conclusions are:

- LA-MRSA has an extremely high prevalence in persons in contact with live pigs and their family members; it was found in up to 63% of pig farmers, 10% of household members, 15% of pig slaughterhouse personnel, and 46% of field workers. However, LA-MRSA has not yet spread from the farms into the community; only 0,2% of persons living in the most pig-dense municipalities in the Netherlands carried MRSA.
- Pig contact was the most important determinant for LA-MRSA carriage. Carriage of a methicillin-sensitive *S. aureus* (MSSA) and continuously wearing a mouth mask were associated with less LA-MRSA carriage.
- The public health threat of LA-MRSA in the Netherlands at the moment appears to be low; the Dutch rate of MRSA bacteraemia was lower than in other countries, and an association between LA-MRSA and infections or quality of life could not be proven.
- Nevertheless, the fast genetic evolution of this specific strain can cause problems in the (near) future. Follow up of the initiated cohorts is useful to monitor changes and test interventions, in order to gain control of the situation.

