Characterization of livestock-associated MRSA CC398 detection, transmission & virulence

Erwin Verkade

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Characterization of livestock-associated MRSA CC398: detection, transmission and virulence

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Erwin Johan Mario Verkade

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promotor: prof.dr. J.A.J.W. Kluytmans
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CHAPTER 1

General introduction

Erwin Verkade
Jan Kluytmans

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Over the past 15 years the epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) has changed significantly. Being initially a nosocomial pathogen, other clones have been detected in the community, leading to infections in relatively young and healthy individuals lacking contact with healthcare. More recently, a specific clone of MRSA CC398 emerged, which has spread extensively in livestock animals and is also found in retail meat. People in contact with food production animals are at high risk of colonization. The ways in which MRSA CC398 can be transmitted to humans are direct contact with animals, environmental contamination, and eating or handling contaminated meat. The role of MRSA CC398 as a food pathogen needs further research. Recently, whole genome sequencing and other genetic analyses have shown that livestock-associated strains are distinct from human-derived strains. However, there is also an exchange of strains between the reservoirs. Livestock-associated and human-associated strains of CC398 share some virulence factors, but there are also distinct virulence factors that appear to be important in host adaptation. Exchange of genes encoding these virulence factors between strains may expand the host range and thereby threaten public health. Since the emergence of MRSA CC398 in humans, approximately 10 years ago, this clone has shown a remarkable evolution, which is described in this review.

**Introduction**

*Staphylococcus aureus* is a well-known commensal and pathogen of a large number of animal species, including humans. A wide variety of infections can be caused by *S. aureus*, from superficial skin and soft tissue infections to life-threatening endocarditis, toxic-shock syndrome, and necrotizing pneumonia [1]. The bacterium is widespread, colonizing approximately a third of the population [2, 3], with the most common carriage site being the anterior nares [4]. Shortly after the introduction of penicillin in the 1960s, methicillin-resistant *S. aureus* (MRSA) was identified in the UK [5], and was then recognized as a hospital-associated pathogen worldwide [6]. Recent studies have shown that MRSA represents a major infectious disease burden in the US, causing an estimated 94,000 infections and 18,650 deaths in 2005 [7], which is more than the 16,268 deaths caused by HIV/AIDS in that same period [8]. Since approximately 10–15 years, an increased number of infections due to community-acquired MRSA (CA-MRSA) have been reported in Europe and the US [9, 10]. For the most part, CA-MRSA strains belong to clonal lineages not associated with hospital-associated MRSA (HA-MRSA). In addition, a specific clone of MRSA associated with animal husbandry emerged. It was first reported in humans in the Netherlands in 2003 [11, 12]. This so-called livestock-associated MRSA (LA-MRSA) belongs to multilocus
sequence type clonal complex 398 (MRSA CC398) [13]. By the end of 2008, 42% of all newly identified MRSA strains in humans in the Netherlands belonged to this clone (www.rivm.nl/mrsa). The main risk groups for MRSA CC398 carriage are humans with professional exposure to pigs and veal calves [14, 15]. MRSA CC398 is rarely found outside these risk groups [16]. These findings have initiated research examining its implications. In the present article, we reviewed the literature concerning (1) the prevalence of MRSA in companion animals, livestock and humans and (2) the transmission between animals and humans, and human-to-human in order to assess its impact on public health.

**Review methodology**

A search in PubMed was performed using the major subheadings ‘drug resistance, bacterial’, ‘methicillin resistance’, ‘Staphylococcus aureus’, ‘evolution’, ‘epidemiology’, ‘animals’, ‘MRSA’, ‘CC398’, ‘animals’, ‘transmission’ and ‘infections’ searched in free text. Additional articles were obtained by searching the citations within the retrieved papers. Internet searches were done to obtain data from the European Union, World Organisation for Animal Health (OIE), Centre for Disease Control and Prevention (CDC) and (inter)national surveillance programmes on antimicrobial use and resistance. Furthermore, the topic was discussed with colleagues and information on ongoing studies was incorporated.

**MRSA CC398 in animals**

The isolation of MRSA from animals was first reported in 1972 following its detection in milk from mastitic cows [17]. Since then, MRSA has been isolated from many different animal species. 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. Generally, MRSA strains of companion animals differ from those in livestock and meat production animals. This probably is because in companion animals, MRSA acquisition is primarily a humanosis, the strains carried by human owners being passed onto their animals [18,19].

MRSA in pigs was first reported in France and subsequently demonstrated to belong to CC398 [20]. Later, MRSA CC398 was discovered to be widespread in pig farms in the Netherlands [11], and has been identified in several countries in Europe, North America, and Asia [21]. MRSA CC398 strains have important genotypic and phenotypic characteristics: (1) they are non-typeable by PFGE using SmaI [22]; (2) many of them belong to ST398 or closely related STs associated with CC398 [23]; (3) they carry SCCmec type IVa or V and are different from those carried by other MRSA genotypes commonly found in the community.
and healthcare settings [24]; (4) they often exhibit co-resistance to many non-β-lactam antimicrobials (e.g. macrolide (70%), trimethoprim (65%), gentamicin (14%), ciprofloxacin (8%), and trimethoprim-sulfamethoxazole (4%)), including those commonly used in animal production [25]; and (5) the majority of isolates lack toxins such as Panton-Valentine leukocidin (PVL) and other enterotoxins [26]. In pigs, MRSA CC398 has 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% [12, 27, 28]. In veal production, high prevalence of MRSA CC398 were found as well: 88% of the farms and 28% of the calves tested positive [15].

In addition to livestock, Richter and colleagues have reported MRSA CC398 in fattening turkeys and people living on farms that house fattening turkeys. Eighteen (90%) of 20 investigated flocks were positive for MRSA, and 22 (37%) of 59 persons sampled were positive for MRSA. In most flocks MRSA that could be assigned to CC398 were detected. In five flocks MRSA of spa-type t002 that is not related to CC398 were identified [29].

Mulders et al. have recently shown a high MRSA carriage rate in poultry and slaughterhouse personnel [30]. Forty Dutch broiler flocks, in six slaughterhouses as well as 466 employees were sampled. Of the employees, 26 were positive (5.6%). A total of 405 broilers were sampled upon their arrival at the slaughterhouse, of which 6.9% were positive. These broilers originated from 40 Dutch slaughter flocks of which 35% were positive. Among the 119 MRSA isolates, predominantly ST398 was found, although 28% belonged to ST9 (spa-type t1430).

A recent study investigated nasal MRSA carriage among horses. In a sample of 110 (Belgian, French, Dutch and from Luxemburg) horses presented at a Belgian equine clinic, 12 (10.9%) horses carried MRSA [31]. They all harbored SCCmec type IVa or V and belonged to spa-type t011 or t1451 of the CC398 lineage. All isolates were tetracycline resistant and sulfonamide and enrofloxacin susceptible. These results show that CC398 is present in West European horses.

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 [27, 28, 32]. 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 CC398 between animals within a farm by air [28, 33, 34].
S. aureus does not typically colonize dogs or cats but may have a transient association and can occasionally cause severe infections [35]. As in humans, MRSA infections in animals are predominantly skin and soft tissue infections (especially post-surgical). Significant risk factors for MRSA infection among companion animals are previous antibiotic treatment, number of antimicrobial courses, number of hospitalization days, implant devices, surgical interventions and contact with humans who have been previously hospitalized [36, 37]. These factors are more or less similar to those defining HA-MRSA infections in humans [6].

The first outbreak of MRSA among horses was reported in 1993, with 11 horses infected post-operatively in a veterinary teaching hospital in Michigan [38]. To date, several investigators have detected the presence of MRSA among horses in Europe, America, and Asia, with slight differences in prevalence being found [39]. During 2006 and 2007 small clusters of MRSA infections in horses were recorded in different clinical departments of a veterinary university. The infections were caused by different MRSA clones (ST1, ST254, and ST398). In the same time, nasal colonization of veterinarians, veterinary personnel, and students was observed indicating transmission to humans [40].

Typically, S. aureus has not caused much illness in pigs. MRSA may not have been detected until recently because many veterinary diagnostic laboratories will only report the presence or absence of coagulase-positive Staphylococci, and may not provide the full species nor follow-up with a full panel of resistance testing. Further, skin infections on pig farms are more commonly caused by Staphylococcus hyicus than S. aureus. However, skin infections due to S. aureus have occasionally been documented in pigs [41]. Also an outbreak of exudative epidermitis among piglets was attributed to MRSA CC398 in the Netherlands. In addition, isolates from pig infections with S. aureus were included as a comparison group to colonized pig farmers and bank or insurance workers in 2006 [20]. Four of the 14 (29%) swine isolates causing infections were CC398.

The first report of MRSA causing infections in cattle was published in the early 1970s, when the bacterium was isolated from the milk of dairy cows with mastitis in Belgium [17]. In the past few years, MRSA has also been isolated from cows (or their milk) in Korea, Hungary, Mexico, the Netherlands, and the UK [42–44]. Subsequently, several reports have described bovine udder infections caused by MRSA CC398 [45]. Recently, another group of livestock-associated MRSA strains was discovered in humans and dairy cattle in Europe. This group includes a number of S. aureus lineages (ST130, ST425, and ST1943) that were hitherto thought to be bovine-specific, but are now also found as carriage or clinical isolates in humans [46]. Moreover, a new mec determinant, named mecA_{LGA257}, which is sharing 70% homology with mecA, was recently identified among MRSA strains recovered from cattle and humans [47]. The mecA_{LGA257} allele is associated with a unique SCC mec element designated
SCCmecXI, and is predicted to be present in about 1.4 % of bovine *S. aureus* isolates in up to 2.8 % of herds. This finding confirms the continuous evolution of *S. aureus* strains and their resistance elements in the animal and human domains.

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**Transmission of MRSA CC398 between animals and humans**

Most reports have shown that MRSA strains from horses and humans in close contact differ from those spread in the human population. In Canada, a CA-MRSA clone has been isolated from horses (CC8), and has become well adapted to the animal setting [48]. In central Europe, MRSA CC398 has emerged since 2006 in equines, and horse-to-human transmission has also been reported in the Netherlands [49, 50]. In the latter study, the percentage of MRSA isolates found in equine clinical samples increased from 0% in 2002 to 37% in 2008. MRSA of *spa*-type t064, belonging to MLST ST8 and *spa*-types t011 and t2123, both belonging to the livestock-associated MLST ST398, predominated. During an outbreak of post-surgical MRSA infections in horses at a veterinary teaching hospital in 2006/2007, MRSA isolates of *spa*-type t2123 were cultured from 7 horses and 4/61 personnel which indicated zoonotic transmission. After intervention the outbreak stopped. However, another outbreak occurred in 2008, where 17 equine MRSA isolates of *spa*-type t011 (*n* = 12), t2123 (*n* = 4), and t064 (*n* = 1) were found. This time, 16/170 personnel were positive for MRSA with *spa*-type t011 (*n* = 11) and t2123 (*n* = 5). Personnel in close contact with horses were more often MRSA-positive (15/106) than those without (1/64). Screening of horses upon admission showed that 9.3% were MRSA-positive predominantly with *spa*-type t011. Weekly cross-sectional sampling of all hospitalized horses for 5 weeks showed that 42% of the horses were MRSA-positive at least once, again predominantly with *spa*-type t011, which suggests that nosocomial transmission took place. Fifty-three percent of the environmental samples were MRSA-positive, including samples from students’ and staff members’ rooms, and all were *spa*-type t011. This indicates that humans contribute to spreading the organism. Our results show that nosocomial transmission occurs in equine clinics and suggests that personnel play a role in the transmission.

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 [51].

The ways in which MRSA CC398 can be transmitted to humans are direct contact with the animals, environmental contamination, and eating or handling contaminated meat. Hospital surveys that investigated the association of MRSA CC398 in human patients with a reservoir in animals identified cattle as source for human carriage, next to pigs. Carriers of MRSA
CC398 were more often people in contact with pigs or cattle than carriers of other strains of MRSA [23, 52]. 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%) [14]. The association between intensity of human-animal contact and MRSA prevalence of humans was confirmed on veal farms [33].

In another study it was found that the density of MRSA CC398 in a particular area corresponds to the density of pig farming, whereas the density of other MRSA strains corresponds to the density of the human population [23]. Subsequently, Feingold et al. performed a study using the existing dataset of persons with MRSA CC398 carriage and controls who carried other types of MRSA using geospatial information [53]. Their findings indicated that regional density of livestock is a risk factor for nasal carriage of MRSA CC398 for persons with and without direct contact with livestock. This finding has been emphasized in recent research that found MRSA CC398 carriage in persons without connections to the farm environment [54]. In addition, a recent study indicated that proximity to farms is a potential risk factor, even in absence of direct contact between humans and animals [55].

MRSA in meat products

Studies on MRSA in meat products demonstrated that MRSA has entered the food chain [56, 57]. MRSA was found frequently in meat products originating from animals. A recent survey of the Dutch Food and Safety Authority in almost 1300 retail meat products 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. Spa-typing was carried out on most (n = 138) of the isolated MRSA strains in which 22 different spa-types were found and 116 (84%) isolates proved to be CC398. A substantial percentage of MRSA isolates from poultry (10% [4/41]), pig products (10% [2/20]), and veal (23% [9/39]) belonged to spa-types which until now did not seem to be animal-related, and may be of human origin [57]. In contrast, only 5 of 318 (1.6%) raw food samples of food-producing animals (148 chicken, 55 pork, 46 veal, 19 lamb, 10 turkey, 8 rabbit and 12 minced-meat samples) and of wild animals (8 game bird, 4 wild boar, 4 deer and 4 hare samples) were found to harbor MRSA in Spain [58]. Two MRSA strains from pork and veal corresponded to ST398-SCCmecV (spa-types t011 and t1197, respectively), two strains from chicken and rabbit were typed as ST125-SCCmecV-t067, and the strain from a wild boar was ST217-SCCmecV-t032. All MRSA were PVL negative. Another Canadian survey found that 31 of 402 (7.7%) retail meat samples harbored MRSA. Three major types were obtained: CC398 (30%), CC8 (40%) and CC5 (30%) [59]. Another study performed by O’Brien et al.
obtained similar results [60]. Three hundred ninety-five pork samples were collected from a total of 36 stores in Iowa, Minnesota, and New Jersey. Twenty-six (6.6%) MRSA positive samples were identified in this study and spa-types associated with CC398 (t034, t011) were found in 26.9% of the MRSA isolates, while 46.2% had spa-types t002 and t008, which are common human types of MRSA that also have been found in live swine. So far, the risk for public health of eating MRSA-positive meat is assumed to be of minor importance [56, 61]. However, a recent case–control study by van Rijen et al. demonstrated that humans colonized with MRSA CC398 had eaten significantly more frequently chicken in comparison with controls, indicating that contaminated meat may be a source of MRSA in humans [62]. Further studies are needed to confirm this source of MRSA.

### MRSA CC398 in humans

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 [63]. The five leading types predominately harbor SCCmec type I, II or III and are often multidrug-resistant [64]. The proportion of methicillin-resistance in invasive *S. aureus* 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. The prevalence of MRSA in clinical isolates in these low-prevalence countries remained relatively stable over time. However, from 1999 to 2010 small increases were found in the Netherlands (0.34–1.21%), Denmark (0.28–1.32%) and Finland (0.95–2.29%) (data available from http://www.ecdc.europa.eu).

In the last two decades, several reports appeared about MRSA infections in healthy individuals in the community without healthcare-associated risk factors [65, 66]. Such infections are distinct from HA-MRSA infections in terms of genetic background, epidemiology, clinical spectrum and antibacterial resistance. CA-MRSA isolates frequently carry SCCmec IV or V, are susceptible to a limited number of antimicrobials and may contain additional virulence factors [65]. 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 [63]. 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.

CC398 was originally reported in swine farmers in France, in a study examining carriage of *S. aureus* in farmers and controls [20]. Farmers had a higher *S. aureus* carriage rate than controls (44.6% vs 24.1%). Most isolates were methicillin-susceptible. Nineteen different clonal complexes were identified, including MRSA CC398. Many of the subsequent studies on MRSA CC398 were carried out in the Netherlands. In the initial report from this country, the
presence of MRSA in screening cultures from a 6-month-old girl admitted to a hospital was described [11]. Subsequently, the girl’s parents, who lived on a pig farm, were found to be colonized with MRSA as well. This case was remarkable since MRSA is still uncommon in the Netherlands: 0.11% of patients were found MRSA-positive upon hospital admission in one study [67]. Therefore, an investigation was initiated to determine the source of colonization, surveying MRSA in regional pigs and pig farmers. The study revealed that 6/23 farmers (23%) and one pig were colonized with MRSA, and all isolates belonged to CC398 [11]. Some years later, an international study among pig veterinarians revealed an overall prevalence of 12.5%. MRSA carriers originated from nine different countries [68]. Investigators have also begun sampling farms in North America. The first study was carried out in Canada, including 285 pigs and 25 workers on 20 farms in Ontario. Twenty-five percent of pigs and 20% of humans were found to carry MRSA, with the majority of these coming from spa-types associated with CC398 [69].

Little is known about the dynamics of MRSA CC398 carriage in persons with regular contact to livestock. Cuny et al. found an extreme high MRSA CC398 nasal colonization of 45% among livestock veterinarians [16]. Carriage may be prolonged, as shown in a recent report from Germany, where the majority of pig farmers (59%) did not lose their MRSA CC398 carriage after the holidays [70]. Furthermore, when volunteers were actively colonized with methicillin-susceptible S. aureus (MSSA) CC398, they often carried it for prolonged periods [71]. Finally, a two-year prospective cohort study with 137 veterinarians that mainly work with pigs and veal calves showed a mean MRSA CC398 prevalence of 44% (range 42–46%). In total, 88 veterinarians (65%) carried MRSA CC398 at least once [72]. Thirty-two veterinarians (23%) had MRSA-positive test results throughout the entire study period and 18 of those (56%, 13% of all veterinarians) had five identical MLVA types and can therefore be considered as true persistent MRSA CC398 carriers. On the other hand, there are several studies that stated that MRSA CC398 is not a good colonizer in humans. A recent study among field workers with short-term occupational exposure to pigs and veal calves suggested a high rate of transient contamination, without substantial persistent colonization [73]. Another study showed that MRSA prevalence among veal calf farmers was strongly reduced (58%) after absence of animal contact [33]. The reasons for these discordant findings are unclear.

...... Molecular epidemiology of S. aureus CC398

Apart from the occurrence of livestock-associated MRSA CC398 among humans, there is a second epidemiological event (i.e. the emergence of MSSA CC398 infections) for which an epidemiological link to livestock is lacking. In order to study the origin and evolution of S. aureus CC398 Price et al. applied whole-genome sequence typing (WGST) to a diverse
collection of *S. aureus* CC398 isolates (*n* = 89), including MRSA and MSSA from animals and humans spanning 19 countries and four continents [74]. The WGST-based phylogeny found in that study strongly suggests that the CC398 lineage originated in humans as MSSA and then spread to livestock, where it subsequently acquired the SCC* mec* cassette and methicillin-resistance. The isolates that formed the most basal clades on the WGST-based phylogenetic trees were almost all human-associated MSSA strains, suggesting that these isolates were the most ancestral of those tested in the study. All of the human-associated MSSA strains carried a φSa3 prophage in association with human innate immunomodulatory genes, whereas the φSa3 prophage was identified in only one livestock-associated isolate.

The human innate immunomodulatory genes carried by φSa3 prophages play crucial roles in human niche adaptation [75]. The loss of human-niche-specific genes in livestock-associated isolates may be a result of adaptation to non-human hosts. The data presented by Price et al. suggest that MRSA CC398 lost the human innate immunomodulatory genes and acquired resistance to methicillin and tetracycline after the introduction to livestock [74]. The tetracycline resistance gene *tet*(M) was nearly universal among livestock-associated MRSA CC398 and MSSA isolates and completely missing from human-associated strains. The MRSA CC398 isolates found in humans who have direct livestock contact exhibit the same molecular patterns as the livestock-associated strains (i.e. φSa3 prophage negative, *tet*(M)-positive), indicating that human re-adaptation (re-acquiring the φSa3 prophage and loss of *mecA*) is rare. These findings were confirmed in a recent study by McCarthy et al. [76] where genetic analysis of isolates epidemiologically associated with human-to-human transmission in multiple countries and continents identified a common clade that was negative for *tet*(M) and positive for bacteriophage 3. Additionally, the widespread use of tetracycline in food animal production is likely to select for livestock-associated *S. aureus* CC398 without differentially selecting for MRSA strains. Likewise, zinc and other metals are frequently used in animal feed formulations and may coselect for MRSA CC398 strains that carry the czr*C* zinc resistance gene, as suggested previously [77]. This hypothesis is supported by the fact that the vast majority of MRSA CC398 strains carry SCC*mec* type Vc, which contains the czr*C* gene. It is clear that there are many genetic changes involved on the cutting edge of exchange between humans and animals. Further research is required to characterize the full scope of the genetic changes associated with the shift from humans to livestock and back.

**Human-to-human transmission of MRSA CC398**

Although many studies have been carried out examining colonization with MRSA CC398 on farms and in people who have animal contact, an increasing number of reports have shown MRSA CC398 isolated from patients in hospitals, and found in persons with no known contact with live animals. Transmission within the healthcare setting has been observed in several studies. The first documented hospital-associated outbreak of MRSA CC398 occurred...
The authors found five patients with MRSA CC398 colonization and/or infection. In addition, 5 of 238 (2.1%) healthcare workers were found to be colonized. All strains belonged to CC398 and were spa-type t567. Fanoy et al. [79] have reported an outbreak in a Dutch residential care facility for visually and intellectually disabled people. In addition to the index case, two residents and three staff members were colonized with MRSA CC398. Furthermore, an outbreak of MRSA CC398 in a nursing home in the Netherlands was described by Verkade et al. [80]. Seven residents and two healthcare workers with MRSA CC398 were identified. The most likely source had been living on a pig farm until recently, before he moved to the nursing home. He reported regular visits to his son at the pig farm. The MRSA strain responsible for this outbreak was spa-type t011, which belong to CC398.

Considering the extensive reservoir in livestock and people who work with these animals, there are relatively few cases of MRSA CC398 in people who are not directly involved in farming. Recent surveys have showed that MRSA CC398 was 4 to 6-fold less transmissible compared with HA-MRSA strains in a hospital-setting [52, 81, 82]. One of the first studies that examined the role of living in a livestock-dense region as a risk factor did not find it to be a risk factor [83]. This study used a random mailing in the three most pig-dense municipalities in the Netherlands. Of the 534 adult respondents without livestock contact, 1 person was positive for MRSA (0.2%), compared with 13 of 49 persons who worked or lived on a livestock farm (26.5%). Conversely, there are a few recent studies indicating that MRSA CC398 might have spread into the general population. A recent study by Lekkerkerk et al. [84] found that MRSA with no link to established Dutch risk factors for acquisition, so-called MRSA of unknown origin (MUO), has now emerged. National MRSA surveillance data (2008-2009) were analyzed for epidemiological determinants and genotypic characteristics. A quarter (24%) of the 5545 MRSA isolates registered were MUO, i.e. not from defined risk groups. Two distinct genotypic MUO groups were distinguished: MUO CC398 (352; 26%) and MUO non-CC398 (998; 74%), which suggests spread of MUO CC398, not by direct contact with livestock (pigs, veal calves), but through the community. Furthermore, Wulf et al. [85] performed a retrospective analysis of all MRSA isolates in the laboratory database from 2002 till 2008 including typing results and clinical data from infection control archives and patient charts. The implementation of the screening of people in contact with pigs and veal calves for MRSA led to an increase in the average number of newly identified carriers from 16 per year between July 2002 and July 2006 to 148 between July 2006 and December 2008. This is a 925% increase of which 82% (108/132) was due to CC398. The majority (74%) came from targeted screening but 7% was due to unexpected findings. A wide range of infections with CC398 occurred in patients with and without contact with livestock varying from post-operative wound infections to sepsis and post-trauma osteomyelitis with an overrepresentation of spa-type t567 among the clinical isolates.

Noteworthy, MSSA CC398 has been found to be commonly present in pigs but not in other livestock animals [86]. It is not clear whether is this is generally true. However, MSSA CC398 is not limited to pork, and has also been identified among S. aureus isolates obtained from...
humans. It has been identified both in patients without contact with livestock [87] and in healthy humans [88]. In contrast to MRSA CC398, where the spread of the clone is limited to persons with contact to livestock, the MSSA CC398 clone found frequently in northern Manhattan is readily transmitted among humans independent of animal contact [89]. Furthermore, they found that human-associated MSSA CC398 isolates bound more avidly to human keratinocytes and keratin derivatives than livestock-associated CC398 isolates. It is therefore suggested that this differential binding ability may translate directly into decreased transmission of livestock-associated CC398 among humans lacking close animal contact. Binding of livestock-associated CC398 isolates to human keratinocytes was diminished, but it was not abolished. In summary, Uhlemann et al. [89] presented epidemiological, molecular, and comparative genomic evidence that MSSA isolates of the CC398 lineage are easily transmissible among humans and have a genome that is well adapted to the human host. The MSSA CC398 clone is encountered among humans in geographically dispersed areas [90–93].

**MRSA CC398 infections in humans** ......................................................................................................................................

A spectrum of infections with MRSA CC398 have been documented, ranging from relatively minor or localized infections including abscesses [79, 91, 94] and various skin and soft tissue infections [52, 95], urinary tract infections [91], wound infections [49, 91], mastitis [13], and conjunctivitis [96], as well as more serious or invasive infections, including bacteraemia [91, 93, 97], pneumonia [49, 52] (including necrotizing pneumonia [98]), osteomyelitis [52, 96], pyomyositis [94], and postoperative infections [95]. Although most of these have been documented in the past 5 years, one MSSA CC398 bacteraemia isolate from Denmark dates back to 1992 [99]. Nevertheless, in a survey of invasive *S. aureus* infections conducted among 26 European countries, during a 6-month period, a total of 2890 isolates were studied. CC398 constituted only 0.4% of the sample, and all were MSSA, reinforcing the idea that strains of this lineage are not frequent causes of systematic infections [100].

While SCC*mec* acquisition seems to be fairly common in MRSA CC398 [91, 94], the transfer of staphylococcal toxins genes appears to be rarer. Several studies have found that CC398 typically lacks many previously identified toxin genes [27, 101-103], including the PVL gene. Only a handful of studies have found PVL-positive CC398 strains [90, 102-104]. However, as toxin genes may be present on mobile elements such as phage or plasmids, it is possible that isolates of CC398 may acquire such genes.

Despite the diverse array of infection types reported, it has been suggested that MRSA CC398 is less virulent than other human MRSA strains. On the other hand, there are many case reports of severe invasive infections due to MRSA CC398, such as endocarditis [105].
Besides, a strong increase over time in the prevalence of *S. aureus* CC398 in bloodstream infection was observed in several studies [93, 97]. Seventeen of the 18 bloodstream infection isolates were methicillin-susceptible and none had the common pig-borne *spa*-types t011, t034 or t108 [97]. The mode of acquisition of the *S. aureus* CC398 isolates by the patients remains unclear. Moreover, all *S. aureus* CC398 bloodstream infections were diagnosed in patients lacking livestock exposure. These invasive infections are in general associated with MSSA CC398 *spa*-type t571 [23, 97]. This human-adapted CC398 subclone is now increasingly identified in hospitals [106]. It appears to be highly receptive for horizontal gene transfer. A possible explanation is that MSSA CC398 *spa*-type t571 have a deficiency in the *Sau*I type I restriction system, resulting in hyper-susceptibility to acquisition of foreign genes [107]. The recent observation of the genome content of MSSA CC398 bloodstream infection isolates suggest that the emergence could have arisen as a consequence of recent horizontal transfers in diverse pig-borne isolates [97]. Horizontal transfer of phages allows bacteria to adapt to specific niches, and may lead to the emergence of new clones. The acquisition of the phage likely plays a role in the increasing ability of the MSSA CC398 *spa*-type t571 isolates to colonize humans. Interestingly, MRSA CC398 *spa*-type t571 have also been found in persons with direct contact with livestock [23, 72]. The presence of MSSA CC398 (mainly *spa*-type t571) as a source of infections in geographically dispersed regions, including China, many European countries, North America, the Caribbean, and Colombia, supports the notion that specific MSSA CC398 strains constitute potentially pandemic pathogens. Our global knowledge about the molecular epidemiology of MSSA CC398 is limited, since much of the surveillance work on *S. aureus* is centered on MRSA CC398 strains. These findings highlight the importance of expanding our knowledge of the molecular epidemiology of MSSA CC398 strains globally. In addition, further investigations are needed to better understand the emergence and spread of different CC398 subclones into the general population.

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**Summary of conclusions and recommendations**

The epidemiology of MRSA has changed significantly over the past decades. MRSA was traditionally regarded to be mainly a nosocomial pathogen, but since two decades it has entered the community causing serious infections. Moreover, MRSA infection and colonization has been documented in several animal species. Several reports have presented information suggesting that animals may act as a source for zoonotic staphylococcal infections in humans. Recently, MRSA CC398, 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 CC398 can be introduced into hospitals and serious infections and outbreaks have been reported, although the spread and virulence may be less than other variants of *S. aureus*. 

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Considering the huge spread of MRSA CC398 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. The most probable transmission route seems to be by direct contact, but the role of MRSA as a food pathogen needs more research.

Only recently, whole genome sequence-based provided evidence that CC398 originated in humans, transferred to livestock, and acquired resistance to methicillin and tetracycline after the introduction to livestock. The present situation is a widespread resistant bacterium with an enormous reservoir in livestock. The impact of MRSA CC398 appears to be low at the moment. However, when MRSA CC398 acquires genetic elements harboring virulence factors and antibiotic resistance it may pose a significant public health problem in the future. Therefore, careful monitoring of the evolution and epidemiology of MRSA CC398 is important.

\textbf{Outline of the thesis} ........................................................................................................

This thesis focuses on MRSA CC398 in livestock veterinarians. The main aim of this thesis was to gain more insight in the persistence and dynamics of MRSA CC398 carriage, the humans-to-human transmission and the morbidity associated with MRSA CC398 carriage in healthy individuals. For these purposes, data were collected in cross-sectional and longitudinal prospective cohort studies.

MRSA CC398 is relatively susceptible for most non-beta-lactam antibiotics, with the exception of tetracycline, for which they are almost always resistant. Because tigecycline is a derivative of tetracycline, the activity of this new drug for MRSA CC398 was determined. \textbf{Chapter 2} describes a study that investigated \textit{in vitro} activity of tigecycline against a collection of MRSA strains collected in the Netherlands, including livestock-associated strains. For the treatment and control of MRSA it is essential to have reliable diagnostic tools. Since the emergence of MRSA CC398 in livestock and humans, screening of individuals for the detection of MRSA is widely practiced. \textbf{Chapter 3, 4} and \textbf{5} present clinical evaluations of three commercial available chromogenic agars for the detecting of MRSA in nasal and oropharyngeal swabs originating from livestock veterinarians and pig farmers.
In chapter 6 the results are presented of a cross-sectional study in which the spread of MRSA CC398 from the farms into the rest of the community in areas with an extremely high density of pig farms was determined. Chapter 7 describes a marked increase in prevalence of *S. aureus* CC398 among bloodstream infections isolated in an area with a relative high density of pigs between 2010–2011 compared to collections that were isolated between 1996–1998 and 2002–2005 using a CC398 specific PCR.

Chapter 8, 9 and 12 are devoted to the VET study, a longitudinal prospective cohort study in which the prevalence, dynamics, transmission and morbidity of MRSA CC398 among livestock veterinarians were investigated. The dynamics and determinants of *S. aureus* carriage in livestock veterinarians in a two-year prospective cohort study are described in chapter 8. Human-to-human transmission is the main determinant for the spread through a human population. The results of the study that investigated human-to-human transmission of MRSA CC398 from livestock veterinarians and their household members are documented in chapter 9. Furthermore, the transmissibility of MRSA CC398 and MRSA non-CC398 was compared in a cross-sectional study. Chapter 10 describes an outbreak of MRSA CC398 in a Dutch nursing home among seven residents and two healthcare workers indicating that MRSA CC398 can indeed lead to human-to-human transmission.

MRSA CC398 isolates are hard to discriminate when using current molecular typing techniques, such as spa-typing, MLST and MLVA. Chapter 11 presents the results of the study in which whole genome mapping was performed to assess whether this technique was suitable to identify transmission events of MRSA CC398 in a community setting. Chapter 12 focuses on the morbidity associated with MRSA CC398 carriage in livestock veterinarians in a two-year prospective cohort study.

Finally, the major findings of this thesis are summarized and discussed in chapter 13. Public health aspects of MRSA CC398 and the implications for the search and destroy policy in the Netherlands are discussed in greater detail. In addition, recommendations for further research and continuous surveillance of the virulence potential, antimicrobial resistance profiles and human colonization capacity of MRSA CC398 are being made.


General introduction


64. Enright MC. The evolution of a resistant pathogen - the case of MRSA. Current Opinion in Pharmacology 2003; 3:474–479


28 General introduction


Detection and susceptibility testing of LA-MRSA
In vitro activity of tigecycline against methicillin-resistant *Staphylococcus aureus*, including livestock-associated strains

Erwin Verkade
Carlo Verhulst
Xander Huijsdens
Jan Kluitymans

*Eur J Clin Microbiol Infect Dis* 2009; 60: 345-350
Abstract

The in vitro activity of tigecycline was determined using a well-defined collection of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates (n = 202), including 33 livestock-associated strains. Susceptibility testing was performed using the Etest system. Among the 202 MRSA strains, three (1.5%) had a minimum inhibitory concentration (MIC) value for tigecycline greater than 0.5 mg/l, which are considered to be resistant. When these strains were tested using Iso-Sensitest medium, the MICs were substantially lower and no resistance was found. This discrepancy warrants further investigations into the preferred test conditions for tigecycline. In conclusion, tigecycline showed good activity against MRSA strains in vitro.

Introduction

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have traditionally been a problem in health-care settings [1]. According to a report from the National Nosocomial Infections Surveillance (NNIS) System, approximately 60% of all *S. aureus* isolated from patients in intensive care units in US hospitals were methicillin-resistant in 2003 [2]. For the last approximately 10 years, MRSA has expanded its territory to the community, causing severe infections in previously healthy persons all over the world [3, 4]. In 2003, a new clone of MRSA was observed in The Netherlands that is related to an extensive reservoir in pigs and cattle [5]. The livestock-associated clone is characterized by being non-typable by *Smal* pulsed-field gel electrophoresis (PFGE). By the end of 2007, nearly 30% of all MRSA observed in The Netherlands were of this type [6]. There are important differences between livestock-associated MRSA (LA-MRSA), healthcare-associated MRSA (HA-MRSA) and, community-associated MRSA (CA-MRSA) regarding the susceptibility against antimicrobial agents. HA-MRSA isolates are frequently multidrug-resistant, while CA-MRSA and LA-MRSA are relatively susceptible for most non-beta-lactam antibiotics, with the exception of tetracycline for LA-MRSA, for which they are almost always resistant. This is most likely due to the extensive use of this antimicrobial agent in animal husbandry. Because tigecycline is related to tetracycline, it is important to determine the activity of this new drug for LA-MRSA.

The treatment of serious MRSA infections has been based, for many years, upon the use of glycopeptides, i.e., vancomycin and teicoplanin. However, concerns over increasing rates of heteroresistance and tolerance to glycopeptides [7] has urged the development of newer agents. Tigecycline is the first commercially available member of the glycyclines, a new class of antimicrobial agents. The glycyclines are derivatives of the tetracycline antibiotics,
with structural modifications that result in activity against gram-positive, gram-negative, and anaerobic micro-organisms, including multidrug-resistant strains. It exhibits generally bacteriostatic action by reversibly binding to the 30S ribosomal subunit and inhibiting protein translation [8].

The purpose of the present study was to assess the in vitro activity of tigecycline against MRSA isolates collected in The Netherlands using a well-defined collection of strains that included a representative sample of LA-MRSA strains.

--- Materials and methods ---

A total of 202 MRSA isolates were tested in this study. All MRSA isolates are part of the MRSA strain collection of the National Institute of Public Health and Environmental Protection (RIVM), Bilthoven, The Netherlands. The collection consisted of three subsets. The first set of isolates used in this study contained 76 MRSA isolates that were collected between 1990 and 1998 in The Netherlands (old MRSA). The second set was 93 MRSA isolates collected between 2003 and 2005 (recent MRSA). These MRSA strains all had a unique PFGE typing result. The third set of isolates tested consisted of 33 LA-MRSA strains and were collected between 2003 and 2005. They had been collected in a previous study and the strains in our evaluation are the index cases of the previous survey [9]. All 202 isolates have been confirmed as S. aureus and methicillin-resistant using a duplex polymerase chain reaction (PCR) for the mecA gene and coagulase gene as described previously [10, 11].

The minimum inhibitory concentration (MIC) for tigecycline was determined by using the Etest system (AB Biodisk, Solna, Sweden) with a concentration range of 0.016 to 256 μg/ml. Etest strips contained a concentration gradient of the antimicrobial agent with a standard amount of calcium throughout the strip. Etest strips were applied to the surface of 150-mm Mueller–Hinton agar plates. Plates were incubated at 35°C in ambient air for 24 h prior to reading the MIC results. In addition, the MICs of the following antimicrobial agents were determined simultaneously: oxacillin, gentamicin, cotrimoxazole, ciprofloxacin, erythromycin, clindamycin, rifampin, daptomycin, tetracycline, linezolid, vancomycin, and teicoplanin. All MICs were determined using the Etest system. For vancomycin and teicoplanin, the Etest strips were placed on brain heart infusion agar, using a high inoculum (2.0 McFarland) and an extended incubation time (48 h) to be able to detect hGISA isolates. Isolates were categorized as susceptible or resistant to an antimicrobial agent according to the breakpoints published by the Clinical and Laboratory Standards Institute (CLSI) [12]. The proposed breakpoint for tigecycline is greater than 0.5 mg/l for S. aureus (both methicillin-resistant and methicillin-susceptible strains). The 11 MRSA strains with the highest MIC for tigecycline on Mueller–Hinton agar plates were subsequently applied on 90-mm Iso-Sensitest agar plates
The observed MIC range for tigecycline was 0.05 to 1.0 μg/ml, with MIC values at which 50 and 90% of the isolates tested are inhibited (MIC\textsubscript{50} and MIC\textsubscript{90}) of 0.19 and 0.38 μg/ml, respectively. The MIC\textsubscript{50} and MIC\textsubscript{90} of LA-MRSA, old MRSA, and recent MRSA isolates for tigecycline and other antibiotics are outlined in Table 1. No significant difference was found in the portion of tigecycline resistance between recent MRSA and old MRSA. None of the LA-MRSA isolates were resistant for tigecycline. Three (2%) of the 169 tested MRSA isolates were resistant for tigecycline. Of the 76 old MRSA isolates, two (3%) isolates had MICs for tigecycline greater than 0.5 μg/ml and are, therefore, considered to be resistant for tigecycline. In addition, one isolate (1%) of the 93 recent MRSA strains had an MIC value for tigecycline greater than 0.5 μg/ml. The 11 MRSA strains with the highest MIC for tigecycline on Mueller–Hinton agar plates were retested using Etest strips that were applied on Iso-Sensitest agar plates (Oxoid Ltd.) and simultaneously on Mueller–Hinton agar plates. The MIC values for tigecycline of the 11 MRSA strains applied on Mueller-Hinton agar plates were comparable with the previously obtained MIC values. On Iso-Sensitest medium, these 11 MRSA strains had significantly 2-fold lower MIC values for tigecycline using linear regression analysis (p < 0.001). Figure 1 shows the shift towards lower MIC values for tigecycline when MRSA strains were applied on Iso-Sensitest medium. The MICs for tigecycline showed a significant correlation with those of tetracycline (r = 0.518; p < 0.001) and teicoplanin (r = 0.325; p < 0.001).

LA-MRSA was the most susceptible group of strains. They were only significantly more often resistant to tetracycline (Table 1). Old MRSA strains were more often resistant to most groups of antimicrobial agents, compared to recent MRSA. The only agent that was significantly (p < 0.001) more resistant in recent strains in comparison with old strains was vancomycin.
Table 1  MIC$_{50}$ and MIC$_{90}$ values of antimicrobial agents against 76 MRSA collected between 1990 and 1998 (old MRSA), 93 MRSA collected between 2003 and 2005 (recent MRSA), and 33 livestock-associated MRSA (LA-MRSA) strains collected in The Netherlands between 2003 and 2005

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Old MRSA</th>
<th>Recent MRSA</th>
<th>LA-MRSA</th>
<th>Old vs Recent</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC$_{50}$</td>
<td>MIC$_{90}$</td>
<td>MIC$_{50}$</td>
<td>MIC$_{90}$</td>
<td></td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.19</td>
<td>0.42</td>
<td>0.19</td>
<td>0.38</td>
<td>0.25</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>12</td>
<td>256</td>
<td>32.0</td>
<td>256</td>
<td>12.0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.13</td>
<td>32</td>
<td>0.025</td>
<td>0.525</td>
<td>0.38</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>32</td>
<td>32</td>
<td>24.0</td>
<td>32.0</td>
<td>0.38</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>256</td>
<td>256</td>
<td>0.38</td>
<td>256</td>
<td>0.25</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.19</td>
<td>256</td>
<td>0.09</td>
<td>256</td>
<td>0.06</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.012</td>
<td>32</td>
<td>0.006</td>
<td>0.60</td>
<td>0.004</td>
</tr>
<tr>
<td>Rifampin</td>
<td>0.38</td>
<td>0.75</td>
<td>0.38</td>
<td>0.75</td>
<td>0.13</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>12</td>
<td>32</td>
<td>0.38</td>
<td>29.6</td>
<td>32</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1.0</td>
<td>1.5</td>
<td>1.0</td>
<td>1.5</td>
<td>0.75</td>
</tr>
<tr>
<td>Linezolid</td>
<td>3.0</td>
<td>4.0</td>
<td>4.0</td>
<td>8.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>3.5</td>
<td>12</td>
<td>4.0</td>
<td>12.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

$^a$ A p-value of <0.01 is considered to be statistically significant

$^b$ The Etest system with a high inoculum and 48 h of incubation was used

Figure 1  Linear regression analysis of MIC values for tigecycline using Mueller–Hinton medium and Iso-Sensitest medium
In a well-defined collection of MRSA, we found that a minority (1.5%) was resistant for tigecycline. All strains were isolated before tigecycline had been used in patients. The results of this study slightly differed from other data on European and North American antibiotic-resistant clinical isolates that were phenotypically characterized [13, 14, 15]. In a recent study, the in vitro activity of tigecycline against 38 MRSA and the correlation of this activity with their resistance gene content were determined [16]. Tigecycline demonstrated good activity against MRSA, with MIC\textsubscript{50} and MIC\textsubscript{90} values of 0.12 and 0.25 μg/ml, respectively. Overall, tigecycline showed an MIC range of 0.06 to 0.25 μg/ml. The tigecycline MICs determined in our study were slightly higher.

In another study, Fluit et al. [17] found the MIC range for tigecycline to be 0.06 to 2.0 μg/ml. For the 106 *S. aureus* isolates tested, two (2%) isolates had MIC values for tigecycline greater than 0.5 μg/ml and are, therefore, considered to be resistant. These findings are identical to our results. In our study, the MICs for tigecycline showed a significant correlation with the MICs for tetracycline. Fluit et al. found no relation between the presence of tetracycline resistance determinants *tet*(K) or *tet*(M) and the MICs for tigecycline observed for *S. aureus*, although tetracycline-susceptible isolates were more often susceptible to tigecycline.

The possible correlation of the in vitro susceptibility of tigecycline and tetracycline prompted us to include LA-MRSA in the evaluation. These strains are known to have high levels of tetracycline resistance. Also, the current evaluation showed that 28 out of the 33 (85%) LA-MRSA strains were resistant against tetracycline. However, none of the LA-MRSA isolates tested was resistant against tigecycline. Conversely, we found tigecycline resistance in three of the HA-MRSA strains when incubated on Mueller–Hinton agar plates. Because of the recently reported influence of the test conditions on the in vitro susceptibility of tigecycline, we also tested a subset of the strains on Iso-Sensitest base medium [18]. Eleven MRSA strains with the highest MICs for tigecycline were selected and retested on Mueller–Hinton agar and on Iso-Sensitest medium. On Mueller–Hinton agar, the results were identical to the initial result, but on Iso-Sensitest medium, the MICs for tigecycline were much lower, and all strains were considered to be susceptible. The results for tigecycline are influenced by the concentration of manganese in the medium [18]. As Mueller–Hinton agar is a biological medium, the concentration of manganese may vary. Iso-Sensitest is a biochemical medium, which is well-defined. However, the CLSI standard recommends the use of Mueller–Hinton medium for the susceptibility testing of tigecycline using the Etest system [19]. This discrepancy requires further investigations into the underlying mechanisms.
An interesting aspect of this study is the remarkable difference in resistance against various classes of antibiotics between old and more recent strains of MRSA. The older strains were, in general, much more resistant than the more recent strains (Table 1). This may reflect the emergence of CA-MRSA in recent years, which are, in general, more susceptible [4]. The only antimicrobial agent with significantly higher MICs in recent MRSA was vancomycin. This has recently been reported by other groups and may reflect the increased use of this agent in hospitals all over the world [20, 21]. As vancomycin is considered to be the cornerstone of therapy for serious MRSA infections, the increasing MICs are a worrying finding. It stresses the need for alternative therapeutic agents. The LA-MRSA strains were also relatively susceptible to many classes of antibiotics, with the exception of tetracycline. MICs for tigecycline were comparable in all three groups of strains.

In conclusion, tigecycline exhibited broad *in vitro* activity against a collection of MRSA strains collected in The Netherlands, including livestock-associated strains. Using the recommended methodology, we found three strains to be resistant. However, these strains were considered to be susceptible when Iso-Sensitest medium was used. This discrepancy warrants further investigations into the preferred test conditions because the interpretation of the in vitro susceptibility of tigecycline is affected significantly.

**Acknowledgments**

This study was financially supported by Wyeth Pharmaceuticals.
Reference List


Performance of Oxoid Brilliance™ MRSA medium for the detection of methicillin-resistant Staphylococcus aureus: an in vitro study
Abstract

Oxoid Brilliance™ MRSA was evaluated for its ability to identify methicillin-resistant *Staphylococcus aureus*. A well-defined collection of staphylococci was used (n = 788). After 20 h incubation, the sensitivity was 99.6% and the specificity was 97.3%. This new medium is a highly sensitive method for the screening of MRSA.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged worldwide as a nosocomial pathogen of major importance, and the incidence of infections caused by MRSA continues to increase [1, 2]. Recently, MRSA has emerged in the community [3]. Also a new clone has been identified that is related to an extensive reservoir in animals. Persons who are in direct contact with pigs frequently carry this animal-related MRSA [4, 5]. Laboratory-based screening for MRSA colonization of patients and health care workers remains a cornerstone of infection control measures to limit the spread of this organism [6]. The extension of MRSA beyond its known boundaries poses an additional challenge for microbiological laboratories to improve their screening strategies. Methods of detecting MRSA in clinical samples ideally should have a high sensitivity and specificity combined with a short time to reporting of the results. To identify *S. aureus* from contaminated samples more easily and more reliably, selective media have been developed [7].

The purpose of this study is to evaluate the in vitro sensitivity and specificity of a new selective medium, called Oxoid Brilliance™ MRSA for the identification of MRSA, using a well-defined collection of strains.

Materials and methods

A collection consisting of 266 MRSA strains, 257 methicillin-susceptible *S. aureus* (MSSA) strains, and 265 coagulase-negative staphylococci (CNS) was used. The collection was described previously [8–10]. In short, the MRSA isolates were collected in the Netherlands between 1989 and 1998 and were obtained from the MRSA strain collection of the National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands. Identification of strains as *S. aureus* and as being methicillin resistant was performed by duplex PCR for the meca gene and the coagulase gene [11]. Bacteriophage typing was
performed as described previously [12, 13], by using the international set of phages at 1 and 100 times the routine test dilution concentrations, an additional set of Dutch phages, and a set of experimental MRSA phages. The 266 MRSA isolates included in the study comprised 247 different phage types. Three isolates were not typable. All clonal lineages were represented with the exception of the recently emerged animal-related strains, ST398 [4].

CNS strains were confirmed as coagulase-negative using both DNase test and Remel Staphaurex® Plus. The MSSA and CNS strains were isolated from cultures of blood from patients who were admitted to the following hospitals between January 1996 and May 1999: St. Elisabeth Hospital and Tweesteden Hospital, Tilburg, the Netherlands; Pasteur Hospital, Oosterhout, the Netherlands; Tweesteden Hospital, Waalwijk, the Netherlands; and Amphia Hospital, Breda, the Netherlands. Only one isolate per patient was included. Isolates were identified by a latex agglutination test (Staphaurex Plus; Thermo Fisher Scientific, Dartford, UK), by the detection of free coagulase using a tube coagulase test with rabbit plasma, and by the detection of DNase (Oxoid DNase agar; Thermo Fisher Scientific, Basingstoke, UK). If the results of these tests were discordant, an AccuProbe culture identification test (Gen-Probe, San Diego, CA, USA) was performed according to the manufacturer’s instructions, and this was considered the “gold standard”. The \textit{S. aureus} blood culture isolates were classified as methicillin-susceptible at the time of collection by broth microdilution susceptibility testing performed according to CLSI (formerly NCCLS) standards [14]. The isolates were stored at -70 °C until they were tested.

\textit{Oxoid Brilliance™ MRSA} is a new chromogenic medium for identification of MRSA in human specimens and was supplied as prepoured culture plates from Thermo Fisher Scientific (Basingstoke, UK). The composition of the chromogenic and selective mix is proprietary. On \textit{Oxoid Brilliance™ MRSA}, MRSA strains form distinctive denit blue colonies (Figure 1). The selective mixture inhibits MSSA strains, most bacteria not belonging to the genus \textit{Staphylococcus}, and yeasts. Results can be read after 18 h, according to the manufacturer. The isolates were inoculated onto Columbia agar plates with 5% sheep blood and incubated for 24 h at 35°C. From the resulting cultures, a suspension of 0.5 McFarland was made, and subsequently, 10 µl was streaked onto an \textit{Oxoid Brilliance™ MRSA} plate 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 blue coloration was considered to be indicative of MRSA. No growth or colonies with colors other than blue was considered negative. The procedure was performed as recommended by the manufacturer. Discordant results of MRSA strains (no growth on \textit{Oxoid Brilliance™ MRSA}) were confirmed by \textit{mecA} gene and \textit{S. aureus} PCR as described previously [11]. If the \textit{mecA} PCR was negative the strain was removed from the analysis.
Results

The results obtained with Oxoid Brilliance™ MRSA are shown in Table 1. In the current evaluation, 29 of the 266 MRSA strains gave discordant results (10.9%). Subsequently, a PCR for the meca gene was performed on these isolates and 28 strains had a negative result. These 28 MRSA strains were removed from the analysis, according to the protocol. After 20 h incubation the sensitivity of Oxoid Brilliance™ MRSA was 237 out of 238 (99.6% [95% CI 98.2%–99.9%]) and the specificity was 508 out of 522 (97.3% [95% CI 96.4%–98.2%]). The one MRSA strain that did not grow on the Oxoid Brilliance™ MRSA agar plate, was tested for its cefoxitin susceptibility using disk diffusion. The cefoxitin zone diameter of this strain was 28 mm, which is considered susceptible according to the CLSI criteria [14]. Five of the MSSA strains and nine of the CNS strains also grew blue colonies ranging from light blue through the typical denim blue color and were reported as positive on Oxoid Brilliance™ MRSA agar. The five MSSA strains had cefoxitin zone diameters ranging from 24 to 30 mm, which is considered susceptible and falls within the susceptible range of the wild type strains [15]. The false-positivity rate of Oxoid Brilliance™ MRSA due to MSSA strains was lower than for CNS (Table 1), but these results were not statistically significant ($p = 0.42$).
Table 1  Results for Oxoid Brilliance™ MRSA medium after 20 h of incubation

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Number of strains with a positive test result/total number of strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSSA</td>
<td>5/257 (1.9)</td>
</tr>
<tr>
<td>CNS</td>
<td>9/265 (3.4)</td>
</tr>
<tr>
<td>Total (CNS + MSSA)</td>
<td>14/522 (2.7)</td>
</tr>
<tr>
<td>MRSA</td>
<td>237/238 (99.6)</td>
</tr>
</tbody>
</table>

Discussion

Oxoid Brilliance™ MRSA was a highly reliable screening tool for the detection of MRSA. All but one MRSA strain included in the analysis was detected after 20 h of incubation. In a recent study, the in vitro sensitivity and specificity of MRSA ID (bioMérieux, La-Balme-Les-Grottes, France) was evaluated using the same collection of strains [9]. A total of 251 MRSA strains, 249 MSSA strains and 478 CNS strains were tested. The sensitivity of Oxoid Brilliance™ MRSA used in the present evaluation was significantly higher than MRSA ID in the previous evaluation (99.6% [237 out of 238] vs 96.4% [242 out of 251] respectively, \( p = 0.021 \)). The specificity of Oxoid Brilliance™ MRSA was lower than that of MRSA ID (97.3% [508 out of 522] and 98.2% [714 out of 727] respectively), but this difference was not significant \( (p = 0.33) \). In another study, Diederen et al. evaluated the in vitro sensitivity and specificity of CHROMagar MRSA (CHROMagar Microbiology, Paris, France), using the same collection of strains [8]. A total of 216 MRSA strains and 241 MSSA strains were tested. Oxoid Brilliance™ MRSA used in the present evaluation has a higher sensitivity at 24 h of incubation than CHROMagar MRSA medium (99.6% [237 out of 238] and 95.4% [206 out of 216] respectively, \( p = 0.004 \)). After 48 h of incubation the sensitivity of CHROMagar MRSA raised to 100%. The specificity of Oxoid Brilliance™ MRSA medium, considering MSSA, was lower at 24 h of incubation than that of CHROMagar MRSA medium (98.1% [252 out of 257] and 100% [241 out of 241] respectively), but this difference was not statistically significant \( (p = 0.06) \).

A remarkable finding is the absence of the mecA gene in 28 MRSA strains. One might speculate that these strains were not the original strains in the collection. However, we have shown in a previous evaluation that this was due to loss of the mecA gene in the freezer. In that study, 36 (14.4%) of 250 methicillin-resistant *Staphylococcus aureus* isolates had lost the mecA gene [16]. In the current evaluation, 28 (10.5%) of 266 previously confirmed MRSA strains no longer harbored the mecA gene. These findings have important implications for the management of strain collections as well as the use of strain collections for an in vitro
evaluation. In order to avoid an underestimation of the sensitivity of the MRSA screening test under evaluation, discordant test results must be checked for the presence of the mecA gene at that moment in time.

The strength of this study is that we used a well-defined collection of strains with many different strains. Therefore, we can conclude that this medium is able to detect almost all MRSA strains that can be found in clinical samples. There are also several limitations to this study. First, a relatively high inoculum was used of isolates in pure culture. In clinical samples there will usually be a relatively low amount of MRSA and there are other species present as well. This could lead to more false-negative results in a clinical evaluation, compared with our results. Also, the growth of species other than CNS was not evaluated. This may lead to more false-positive results when Oxoid Brilliance™ MRSA medium is used on clinical samples. The performance on patient samples has to be determined in a prospective clinical evaluation. Kilgour et al. evaluated Oxoid Brilliance™ MRSA, CHROMagar MRSA and MRSA ID for the detection of MRSA [17]. A total of 300 freshly collected swabs from a total of 99 patients were analyzed in this study. When combined results for all media were analyzed, 269 of the 300 clinical samples were identified as negative for MRSA. Oxoid Brilliance™ MRSA medium correctly identified the highest number of samples positive for MRSA (28 out of 31) within 24 h, yielding a sensitivity of 90.3%. CHROMagar and MRSA ID required 48 hours incubation and both media yielded sensitivities of 83.9% (26 out of 31). The highest specificity was also achieved by Oxoid Brilliance™ MRSA (97.4%), which yielded only 7 false-positive results. CHROMagar and MRSA ID yielded 30 and 33 false-positives, providing specificities of 88.8% and 87.7% respectively. This small scale clinical study confirms the results of our study.

In conclusion, Oxoid Brilliance™ MRSA is a sensitive and a specific tool for differentiation between MSSA and CNS/MRSA in vitro. Oxoid Brilliance™ MRSA is able to detect a large number of different MRSA strains after only 20 h of incubation. Therefore, with Oxoid Brilliance™ MRSA, optimal results can be obtained within a day. In addition, further clinical studies will be performed to determine the utility of Oxoid Brilliance™ MRSA for the detection of MRSA directly from clinical samples.
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15. Online document
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Clinical evaluation of Oxoid Brilliance™ MRSA agar in comparison with MRSA ID medium for detection of livestock-associated methicillin-resistant Staphylococcus aureus

Erwin Verkade
Marianne Ferket
Jan Kluytmans

Abstract

Oxoid Brilliance™ MRSA agar and MRSA ID medium were evaluated for their abilities to identify methicillin-resistant Staphylococcus aureus in clinical samples. Nasal and throat samples (n = 629) were taken from veterinarians and their household members. The sensitivities of Oxoid Brilliance™ MRSA agar and MRSA ID medium after 20 h of incubation were 63.6 and 64.5%, and the specificities were 94.1 and 99.4%, respectively. After an enrichment step the sensitivities increased to 96.3 and 97.2%, but the specificity decreased to 88.7 and 98.5%, respectively. Oxoid Brilliance™ MRSA agar as well as MRSA ID medium are a sensitive method for the screening of MRSA in combination with broth enrichment, but positive results require confirmation.

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) has emerged worldwide as a nosocomial pathogen of major importance, and the incidence of infections caused by MRSA continues to increase [1-2]. Recently, MRSA has emerged in the community [3-4]. Also a new clone has been identified which is related to an extensive reservoir in animals. Persons who are in direct contact with pigs frequently carry livestock-associated MRSA. They belong mainly to Multi Locus Sequence Type 398 (ST398) [5-6]. The extension of MRSA beyond its known boundaries poses an additional challenge for microbiological laboratories to improve their screening strategies. Screening for MRSA among various human populations with increased risk for carriage is an essential component of MRSA control strategies. This includes both patients and health care workers [7]. In human healthcare settings, different procedures employed for the detection of MRSA from clinical samples have variable results [8]. Methods to detect MRSA in clinical samples ideally should have a high sensitivity and specificity combined with a short time to reporting of the results. To identify S. aureus from contaminated samples more easily and reliably, selective media have been developed [9].

The purpose of this study is to evaluate the in vivo sensitivity and specificity of a selective medium, called Oxoid Brilliance™ MRSA agar, for the identification of MRSA, using nose and throat samples taken from veterinarians and their household members in an ongoing study.
Oxoid *Brillance™* MRSA agar is a chromogenic medium for identification of MRSA in human specimens and was supplied as prepared culture plates from Thermo Fisher Scientific, Basingstoke, UK. The composition of the chromogenic and selective mix is proprietary. On Oxoid *Brillance™* MRSA agar, MRSA isolates form distinctive denim blue colonies. The selective mixture inhibits methicillin-susceptible *Staphylococcus aureus* (MSSA) strains, most bacteria not belonging to the genus *Staphylococcus*, and yeasts. Results can be read after 18 hours, according to the manufacturer.

Nose and throat samples were taken from veterinarians who work mainly with pigs. Also the household members were sampled and subsequently the samples were sent by mail in a transport container with amies medium (Transwab®, Medical Wire & Equipment, UK) to the Laboratory for Microbiology and Infection Control in the Amphia Hospital.

A total of 629 freshly collected swabs from a total of 318 patients were analyzed in this study. Samples were alternately inoculated onto both Oxoid *Brillance™* MRSA agar and MRSA ID (bioMérieux), and diluted with a sterile loop using a three-streak dilution method. In addition, broth enrichment containing Mueller – Hinton broth supplemented with 6.5% NaCl was inoculated using the same swabs. From the overnight broth enrichment, subcultures were inoculated onto both Oxoid *Brillance™* MRSA agar and MRSA ID. Both directly as overnight enriched inoculated plates were read separately after 18-24 h incubation at 35-37°C by different laboratory technicians on different benches. The procedure was performed as recommended by the manufacturer. Growth of colonies on the Oxoid *Brillance™* MRSA agar plates showing blue coloration or green coloration on the MRSA ID agar plates were considered to be indicative for MRSA. No growth, or colonies with colors other than blue or green, were considered negative. Presumptive *S. aureus* colonies were initially identified by a latex agglutination test (Staphaurex™ Plus; Thermo Fisher Scientific, Dartford, UK), and by the detection of DNase (Oxoid DNase agar; Thermo Fisher Scientific, Basingstoke, UK). If the results of these tests were discordant, isolates were identified by detection of free coagulase using a tube coagulase test with rabbit plasma. When isolates were identified as *S. aureus* the methicillin susceptibility was determined using the cefoxitin disk diffusion test according to CLSI standards [10]. All cefoxitin resistant isolates were confirmed to be MRSA using a duplex PCR for the *mecA* gene and coagulase gene, as described previously [3,11], and this was considered the “gold standard”. When MRSA was recovered from one or more of the media, the sample was considered to be positive.
A total of 629 freshly collected nasal and throat samples were analyzed in this study. In total, there were 318 nose samples and 311 throat samples. When combined results for all media were analyzed, 107 of the 629 (17.0%) samples harbored MRSA. The presence of MRSA in nose samples was comparable with that of throat samples (18.9% [60/318] versus 15.1% [47/311] respectively, \( p = 0.243 \)). The prevalence of MRSA for persons with direct contact with pigs was significantly higher compared with persons that do not have contact with pigs (60.5% [52/86] versus 8.4% [18/232], \( p < 0.001 \)). At the same time, 83 of the 168 (49.4%) samples obtained from veterinarians harbored MRSA. In contrast, only 24 of the 461 (5.2%) samples from the household members were positive for MRSA. There were no significantly differences in performance of both chromogenic agar plates in regard to specimen types. The results obtained with directly inoculated media and after broth enrichment are shown in Table 1. The sensitivity of directly inoculated Oxoid Brilliance™ MRSA agar was comparable with that of directly inoculated MRSA ID medium (63.6% [68/107] versus 64.5% [69/107] respectively, \( p = 1.0 \)). However, the specificity of directly inoculated Oxoid Brilliance™ MRSA agar was significantly lower than MRSA ID (94.1% [491/522] versus 99.4% [519/522] respectively, \( p < 0.001 \)). The negative predictive value (NPV) of directly inoculated Oxoid Brilliance™ MRSA and MRSA ID were comparable (92.6% [491/530] versus 93.2% [519/557] respectively, \( p = 1.0 \)). The positive predictive value (PPV) of directly inoculated Oxoid Brilliance™ MRSA agar was significantly lower than MRSA ID (68.7% [68/99] versus 95.8% [69/72] respectively, \( p < 0.001 \)). Addition of a broth enrichment resulted in a substantial increase of the yield of MRSA for both agar plates. Oxoid Brilliance™ MRSA agar recovered 103 MRSA strains, resulting in a sensitivity of 96.3%. This was comparable to the sensitivity of MRSA ID after broth enrichment (97.2% [104/107], \( p = 1.0 \)). The highest specificity was achieved by MRSA ID (98.5%), which yielded only 8 false positive results. This was much more for Oxoid Brilliance™ MRSA agar which had 59 false positives, resulting in a specificity of 88.7% (\( p < 0.001 \)). The colors of the colonies ranged from light blue to the specific denim blue coloration considered typical for Oxoid Brilliance™ MRSA agar. In combination with broth enrichment, the NPV of Oxoid Brilliance™ MRSA agar was comparable with that of MRSA ID (99.1% [463/467] versus 99.4% [514/517] respectively, \( p = 1.0 \)). However, the PPV of Oxoid Brilliance™ MRSA agar was significantly lower than MRSA ID (63.6% [103/162] versus 92.9% [104/112] respectively, \( p < 0.001 \)).
Table 1 Analytical performance of Oxoid **Brilliance™** MRSA agar and MRSA ID medium in a prospective clinical study of nasal and throat samples (n = 629, including n = 107 MRSA-positive samples)

<table>
<thead>
<tr>
<th>Medium</th>
<th>Method</th>
<th>TP</th>
<th>FP</th>
<th>TN</th>
<th>FN</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>PPV %</th>
<th>NPV %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brilliance™</strong> MRSA</td>
<td>Direct only</td>
<td>68</td>
<td>31</td>
<td>491</td>
<td>39</td>
<td>63.6</td>
<td>94.1</td>
<td>68.7</td>
<td>92.6</td>
</tr>
<tr>
<td></td>
<td>Direct+ enrichment</td>
<td>103</td>
<td>59</td>
<td>463</td>
<td>4</td>
<td>96.3</td>
<td>88.7</td>
<td>63.6</td>
<td>99.1</td>
</tr>
<tr>
<td>MRSA ID</td>
<td>Direct only</td>
<td>69</td>
<td>3</td>
<td>519</td>
<td>38</td>
<td>64.5</td>
<td>99.4</td>
<td>95.8</td>
<td>93.2</td>
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<tr>
<td></td>
<td>Direct+ enrichment</td>
<td>104</td>
<td>8</td>
<td>514</td>
<td>3</td>
<td>97.2</td>
<td>98.5</td>
<td>92.9</td>
<td>99.4</td>
</tr>
</tbody>
</table>

TP, true positive; FP, false-positive; TN, true negative; FN, false-negative; PPV, positive predictive value; NPV, negative predictive value

*False-positive = all colonies showing blue coloration on the Oxoid Brilliance™ MRSA agar or colonies showing green coloration on the MRSA ID medium which were not confirmed to be methicillin-resistant Staphylococcus aureus

Discussion

Several studies have recently evaluated various novel chromogenic selective media for the detection of MRSA from clinical samples [12–14]. Comparison of the results of these studies is not easy because of differences in the study design: swab type, body site sampled, direct inoculation versus prior homogenization in saline or broth, incubation time and the use of broth enrichment. In the present study, we evaluated the in vivo performance of Oxoid **Brilliance™** MRSA agar with and without broth enrichment in a population with a high prevalence of MRSA carriage. Oxoid **Brilliance™** MRSA agar had a high sensitivity when used in combination with broth enrichment. However, without broth enrichment the sensitivity was significantly lower. This confirms the importance of broth enrichment for the accurate detection of MRSA in clinical samples [12–13].

It is difficult to compare our findings to other studies as there are few clinical studies that have used the Oxoid **Brilliance™** MRSA agar. However, other chromogenic media have been studied. For example, a study by Nonhoff et al. evaluated the performance of MRSA ID, MRSA-Screen and MRSA **Select™** in combination with broth enrichment for the detection of MRSA in 1,002 mucocutaneous swabs from 639 hospitalized patients. Swabs were plated on the three chromogenic media. Subsequently, broth enrichment was subcultured after overnight incubation onto the different agar plates. MRSA strains were isolated from 68...
Detection and susceptibility testing of LA-MRSA specimens from 44 patients. The sensitivity of all chromogenic media was <50% after 18 h of incubation, but increased with prolonged incubation at 42 h to 75, 80.9 and 72.1% for MRSA ID, MRSA-Screen and MRSA \textit{Select}, respectively. The difference in sensitivity between MRSA-Screen and MRSA \textit{Select} after 42 h was significant. After enrichment, the sensitivity for all media was 85.3% at 24 h of incubation. The specificity was excellent for MRSA ID (99.9%) and MRSA \textit{Select} (100%) after 18 h, and lower for MRSA-Screen (97.2%), albeit not statistically significant.

Another recently published study that reported similar findings was performed by Malhotra-Kumar et al. They evaluated and compared the potential for MRSA detection of five chromogenic media – \textit{Brilliance} \texttrademark\ MRSA agar (Oxoid), chromID\textsuperscript{®} (bioM"{e}rieux), MRSA \textit{Select} \textsuperscript{™} (Bio-Rad), CHROMagar\textsuperscript{™} (CHROMagar-Microbiology), and BBL\textsuperscript{™}-CHROMagar\textsuperscript{™} (BD Diagnostics). Media were tested on log serial dilutions of pure isolates of MRSA (\(n = 60\)), non-MRSA (\(n = 27\)), and their defined mixtures simulating clinical samples (\(n = 213\)) from 165 hospitalized patients. Cumulative average sensitivity on isolates, mixtures and clinical samples was highest for \textit{Brilliance} \texttrademark\ (97%), and similar for the other four media (U92%). Cumulative average specificity was highest for BBL-CHROMagar (99%), followed by MRSA \textit{Select} \textsuperscript{™} (98%), CHROMagar (97%), chromID\textsuperscript{®} (89%), and \textit{Brilliance} \texttrademark\ (86%). False-positive results were mainly due to methicillin-resistant \textit{S. epidermidis}. The two above-mentioned studies confirm the additional yield of broth enrichment and are comparable to our findings.

In contrast, Peterson et al. reported different sensitivities from our findings. They compared the performance of Oxoid \textit{Brilliance} \texttrademark\ MRSA agar to traditional media in detecting MRSA. In this study, 767 nasal samples from a multi-center study and 667 nasal samples from a tertiary hospital were screened for the presence of MRSA. After 24 h of incubation, the sensitivity and specificity were as follows: multi-center study: Oxoid \textit{Brilliance} \texttrademark\ MRSA agar, 95.4%, 99.7%; Tryptic Soy Agar with 5% Sheep Blood (SBA), 93.6%, 100%; tertiary hospital: Oxoid \textit{Brilliance} \texttrademark\ MRSA agar, 95.2%, 99.5%; Mannitol Salt Agar (MSA), 88.7%, 94.0%, respectively. Extending the incubation to 48 h did not significantly improve the recovery of MRSA and resulted in a decreased specificity. The higher sensitivity found in this study can easily be explained by the lack of broth enrichment in this comparison. However, this cannot explain the much higher specificity. The discrepancy between the sensitivities obtained in the above mentioned study in comparison to the direct plating in our study is most likely caused by the addition of broth enrichment in our culture procedure. The differences in performance values may be predominantly due to the use of sub-optimal media as “gold standard”. Another explanation for the discrepancy between the specificity found in our evaluation and in some other studies may be that we screened a specific population, i.e. veterinarians and their household members, who carry a specific clone of MRSA, named livestock-associated MRSA. Therefore, our results do not necessarily apply to other types of MRSA.
In the present work, the evaluation of the performance of chromogenic media for MRSA detection in screening swab samples in a high-prevalence population demonstrated a low sensitivity (64–65%) of the two media after 20 to 24 h of incubation. However, the sensitivity of both media was increased significantly by using an enrichment step.

A selection of the false-positive colonies that grew on the Oxoid Brilliance™ MRSA agar were identified. Sixty percent were of the genus Bacillus, 24% were coagulase-negative Staphylococci (CNS) and 16% were methicillin-susceptible Staphylococcus aureus (MSSA). After performing the additional identification tests recommended by the manufacturers, specificity was improved significantly.

The results of our study have one important limitation. The investigators did not use a non-selective blood agar plate as the “gold standard”. This could have increased the amount of recovered MRSA isolates from the samples. However, this would have resulted in even lower sensitivities compared to the current evaluation.

Despite commercial efforts to develop new media with high sensitivity, the present study, as well as others [12-13], showed a substantial increase in sensitivity following enrichment compared with direct cultures. Therefore, broth enrichment remains necessary for reliable MRSA detection. Indeed, in our study, if screening samples had only been directly plated and inspected after 20 to 24 h, as suggested by the manufacturers, 29.9% of the MRSA positive samples detected would have been missed. On the other hand, only one MRSA positive sample (0.9%) would have been missed if samples had exclusively been grown after enrichment.

In summary, the Oxoid Brilliance™ MRSA agar performed similar to the MRSA ID medium with respect to the sensitivity, but with a significantly lower specificity with and without broth enrichment. An advantage of the Oxoid Brilliance™ MRSA agar is that this medium is less light sensitive than MRSA ID, making it easier to work within the laboratory. The additional yield of the broth enrichment was comparable to what has been reported before and has to be considered as the standard for screening of MRSA.

In conclusion, Oxoid Brilliance™ MRSA agar in combination with broth enrichment is a sensitive method for MRSA detection in people exposed to livestock. However, the specificity is relatively low and suspected colonies need to be confirmed by further testing.
Acknowledgments

The chromogenic medium Oxoid Brilliance™ MRSA agar was kindly provided by Thermo Fisher Scientific (Basingstoke, UK).

Conflicts of interest

Jan Kluytmans has received research grant support from Thermo Fisher Scientific and bioMérieux. The other authors have none to declare.

Reference List


Detection and susceptibility testing of LA-MRSA
Clinical evaluation of Bio-Rad MRSA Select™ medium for detection of livestock-associated methicillin-resistant Staphylococcus aureus

Erwin Verkade
Carlo Verhulst
Brigitte van Cleef
Jan Kluytmans

Abstract

Bio-Rad MRSA.Select™ medium was evaluated for its ability to recover methicillin-resistant Staphylococcus aureus (MRSA) from nasal samples of pig farmers and their household members. In total, 257 samples were inoculated on Bio-Rad MRSA.Select™ medium with and without broth enrichment and on bioMérieux MRSA ID with broth enrichment. A sample was considered to be positive if at least one of the media grew MRSA. The sensitivity of Bio-Rad MRSA.Select™ medium without broth enrichment was 63.9%. With broth enrichment the sensitivity increased to 98.4%. The specificity was 95.4% both with and without broth enrichment. In conclusion, Bio-Rad MRSA.Select™ medium as well as MRSA ID medium are reliable methods to detect MRSA carriage when used in combination with broth enrichment. The directly inoculated MRSA.Select™ medium was statistically significantly less sensitive than the two media after broth enrichment.

Introduction

First described in 1961, methicillin-resistant Staphylococcus aureus (MRSA) has emerged worldwide as a nosocomial pathogen of major importance, and the incidence of infections caused by MRSA continues to increase [1, 2]. Since the nineteen-nineties MRSA has also emerged in the community [3]. In addition, a new clone has been identified, which is related to an extensive reservoir in animals. Persons who are in direct contact with pigs frequently carry this livestock-associated MRSA (LA-MRSA) [4, 5].

Laboratory-based screening for MRSA colonization of patients and health care workers remains a cornerstone of infection control measures to limit the spread of this organism [6]. Methods to detect MRSA in clinical samples ideally should have a high sensitivity and specificity combined with a short time to reporting of the results. To identify S. aureus from contaminated samples more easily and reliably, selective media have been developed [7]. The extension of MRSA beyond its previously known boundaries to livestock-related persons poses a challenge for microbiological laboratories to improve their screening strategies. The purpose of this study is to evaluate the in vivo sensitivity and specificity of a new selective medium for the detection of MRSA from nasal samples taken from people living or working on pig farms.
MRSA.Select™ is a chromogenic medium for identification of MRSA in human specimens and was supplied as prepoured culture plates from Bio-Rad, Hercules, California, USA. The composition of the chromogenic and selective mix is proprietary. On MRSA.Select™, MRSA strains form distinctive pink colonies. The selective mixture inhibits MSSA strains, most bacteria not belonging to the genus *Staphylococcus*, and yeasts. Results can be read after 18-28 hours, according to the manufacturer.

--- Materials and methods ---

Nasal samples were taken from pig-farmers, their co-workers and their household members as part of an ongoing study and send by mail to the Laboratory for Microbiology and Infection Control in the Amphia Hospital. Samples were inoculated onto MRSA.Select™ medium and diluted with a sterile loop using a three-streak dilution method. In addition, a broth enrichment containing Mueller-Hinton broth with 6.5% NaCl, was inoculated using the same swab, and this was incubated overnight at 35°C. Subsequently, 10 μl of the broth enrichment was inoculated onto both MRSA.Select™ and MRSA ID (bioMérieux, La Balme Les Grottes, France). Both sets of plates were read after 18-28 hours of incubation at 35-37°C. Growth of colonies on the MRSA.Select™ agar plates showing pink coloration or growth of colonies with green coloration on the MRSA ID agar plates were considered to be indicative for MRSA. No growth, or colonies with colors other than pink or green were considered negative. Presumptive *S. aureus* colonies were further identified by a latex agglutination test (Slidex™ Staph-Kit; bioMérieux, La Balme Les Grottes, France), and by the detection of DNase (Oxoid DNase agar; Thermo Fisher Scientific, Basingstoke, UK). When isolates were identified as *S. aureus* the methicillin susceptibility was tested using the cefoxitin disk diffusion test according to CLSI (formerly NCCLS) standards [8]. All cefoxitin resistant isolates were confirmed to be MRSA using a duplex PCR for the mecA gene and coagulase gene as described previously [9, 10] and this was considered the “gold standard”. When MRSA was recovered from one or more of the media, the sample was considered to be positive.
A total of 257 freshly collected nasal samples originating from different individuals were analyzed in this study. When combined results for all media were analyzed, 61 of the 257 (23.7%) samples were identified as positive for MRSA. The results obtained with MRSASelect™ medium and MRSA ID medium are shown in Table 1. When used in combination with broth enrichment the sensitivity of MRSASelect™ was comparable to MRSA ID. The directly inoculated MRSASelect™ was statistically significantly less sensitive than the two media after broth enrichment. After broth enrichment, MRSASelect™ grew 9 and MRSA ID grew 10 false positive strains. These false positive strains were not further identified. This results in specificities of 95.4% and 94.9% respectively ($p = 1.0$). The specificity of the directly inoculated MRSASelect™ medium was also 95.4%. In combination with broth enrichment, the positive predictive value (PPV) of MRSASelect™ and of MRSA ID were 87.0% and 85.9% respectively ($p = 1.0$). The negative predictive values (NPV) were 99.5% and versus 100.0% respectively ($p = 1.0$). The PPV and NPV of directly inoculated MRSASelect™ were 81.3% and 89.5% respectively.

Table 1 Analytical performance of MRSASelect™ and MRSA ID medium in a prospective clinical study of nasal swabs ($n = 257$, including $n = 61$ MRSA-positive samples)

<table>
<thead>
<tr>
<th>Medium</th>
<th>Method</th>
<th>True positive</th>
<th>False-positive</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>PPV %</th>
<th>NPV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSASelect™</td>
<td>Direct</td>
<td>39</td>
<td>9</td>
<td>63.9</td>
<td>95.4</td>
<td>81.3</td>
<td>89.5</td>
</tr>
<tr>
<td></td>
<td>Enrichment</td>
<td>60</td>
<td>9</td>
<td>98.4</td>
<td>95.4</td>
<td>87.0</td>
<td>99.5</td>
</tr>
<tr>
<td>MRSA ID</td>
<td>Enrichment</td>
<td>61</td>
<td>10</td>
<td>100</td>
<td>94.9</td>
<td>85.9</td>
<td>100</td>
</tr>
</tbody>
</table>

PPV, positive predictive value; NPV, negative predictive value
We evaluated the in vivo performance of MRSA.Select™ medium and MRSAID medium with and without broth enrichment in a population with a high prevalence of MRSA carriage. Both media had high sensitivities and adequate specificities when used in combination with broth enrichment. However, without broth enrichment the sensitivities were significantly lower. This confirms the importance of broth enrichment for the accurate detection of MRSA in clinical samples [11].

The sensitivity and specificity found in this clinical evaluation are significantly lower than recent findings in other studies [12, 13]. Stoakes et al. [12] compared MRSA.Select™ medium with CHROMagar, mannitol-salt agar with oxacillin, and mannitol-salt agar with cefoxitin. In this study, nasal and perineal swabs were routinely collected from all patients admitted to the hospital who had been admitted to any health care facility in the previous 6 months. They processed a total of 2,125 (1,243 nasal and 882 perineal) consecutive swabs and found that 3 from the 111 confirmed MRSA strains were not detected by MRSA.Select™ medium (sensitivity of 97.3%). Ben Nsira et al. [13] screened 666 samples using MRSA.Select™ medium and three other selective media. Ninety-nine samples were positive for MRSA on at least one selective medium. Only one MRSA isolate was missed on MRSA.Select™ medium (sensitivity of 99.8%). The discrepancy between the sensitivities obtained in these two studies in comparison to the direct plating in our study is most likely caused by the addition of broth enrichment in our culture procedure. This improves the sensitivity of the reference method substantially and thereby reduces the sensitivity of the test that is evaluated (in this case the direct plating method). Another explanation for the discrepancy between the specificity found in our evaluation and in some other studies may be that we screened a specific population, i.e. pig farmers and their household members, who carry a specific clone of MRSA, named livestock-associated MRSA and these strains all belong to multilocus sequence type 398 (ST398) [4, 5, 16, 17]. Therefore, our results do not necessarily apply to other types of MRSA.

A study that reported similar results as our findings was performed by van Loo et al. [11]. In total, they tested 3,000 samples from 409 patients for the presence of MRSA. Every sample was directly inoculated onto an MRSA.Select™ medium. Fifty-five out of 70 true positive samples (sensitivity 78.6%) were detected by the MRSA.Select™ medium after 48 h of incubation. Subsequently, the investigators subcultured 225 samples from a broth enrichment containing Mueller-Hinton broth with 6 μg/ml oxacillin and 6 μg/ml aztreonam on the MRSA.Select™ medium. This increased the yield of MRSA containing samples with 12%. Our increase was much larger which may be explained by the choice of the type of broth enrichment. However, the present study used a less selective broth enrichment than van Loo et al. A recent study found that the broth enrichment as used by van Loo et al. resulted in suboptimal performance [14].
In another recent study, Nonhoff et al. [15] evaluated the performance of three chromogenic media, MRSA ID, MRSA Screen and MRSA Select™ in combination with broth enrichment for the detection of MRSA in 1,002 mucocutaneous swabs from 639 hospitalized patients. A sample was considered to be positive when MRSA was isolated from any of the three media tested. MRSA strains were isolated from 68 (6.8%) specimens from 44 patients. The sensitivity of all chromogenic media was <50% after 18 h of incubation, but increased with prolonged incubation at 42 h to 75, 80.9 and 72.1% for MRSA ID, MRSA Screen and MRSA Select™, respectively. The difference in sensitivity between MRSA Screen and MRSA Select™ after 42 h was significant. After enrichment, the sensitivity for all media was 85.3% at 24 h of incubation. The specificity was excellent for MRSA ID (99.9%) and MRSA Select™ (100%) after 18 h, and lower for MRSA Screen (97.2%), albeit not statistically significant. This study confirms the additional yield of broth enrichment and is comparable to our findings. Comparison of the results of all above mentioned studies with our current evaluation is not easy because of differences in the study design: swab type, body site sampled, direct inoculation versus prior homogenisation in saline or broth, incubation time, use or not of broth enrichment and, most important, the nature of the test chosen as the gold standard.

The results of our study have one important limitation. We sampled a specific population, i.e. pig farmers, who carry a specific clone of MRSA, namely LA-MRSA. Therefore, the performance maybe different for other types of MRSA. There are several studies that reported that persons working with pigs frequently carry LA-MRSA [4, 16, 17]. Van Loo et al. [4] performed a case-control study and showed that carriers of LA-MRSA were more often pig or cattle farmers. Thirty-four of 35 case-patients carried ST398; one had ST9. In addition, Wulf et al. [16] reported that veterinarians at an international conference on pig health frequently carried MRSA. Thirty-one of the 34 isolates were non-typeable by pulsed-field gel electrophoresis following Smal digestion of chromosomal DNA. All of the non-typeable isolates belonged to spa-types (t011, t034, t108, t571, t567 and t899) that correspond to ST398. Finally, van den Broek et al. [17] demonstrated that intensive and repeated exposure to pigs is an important factor in MRSA colonization and that this is almost always ST398. Although we did not performed molecular typing, it is beyond reasonable doubt that the vast majority of the MRSA strains recovered in our study belonged to ST398. The conclusion is that MRSA Select™ medium and MRSA ID medium in combination with broth enrichment are useful tools for the detection of LA-MRSA in nasal samples. In this study, the MRSA Select™ medium and MRSA ID medium were equivalent and can be used indifferently. The additional yield of the broth enrichment was comparable to what has been reported before and is considered to be the standard for screening of MRSA. After direct inoculation or subculture of broth enrichment, typical MRSA colonies need to be confirmed by further testing.
Acknowledgments

The chromogenic medium MRSA Select™ was kindly provided by Bio-Rad (Hercules, California, USA).

Reference List


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

Brigitte van Cleef*†
Erwin Verkade*†
Mireille Wulf
Alton Buuring
Andreas Voss†
Xander Huisdens
Wilind van Poit
Mick Mulders
Jan Kluytmans

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* These authors contributed equally to this work
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.
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 *Smal*, 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.
Materials 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 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.

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. 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.

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 extend 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). 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.
Prevalence of LA-MRSA in communities with high pig-densities

Figure 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.
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 1).

**Table 1** Spa-types and antibiotic resistance patterns of the recovered MRSA strains

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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 person who work with animals or live on farms.

--- **Acknowledgments**

The authors would like to thank all the employees of the following laboratories for their unselfish participation: the Laboratory for Microbiology and Infection control of the Amphia Hospital in Breda, the Laboratory for Medical Microbiology and Immunology of the St. Elisabeth Hospital in Tilburg, the Department of Medical Microbiology and Infection Diseases of the Canisius-Wilhelmina Hospital in Nijmegen and the Laboratory for Medical Microbiology of the PAMM foundation in Veldhoven.
Prevalence of LA-MRSA in communities with high pig-densities

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Prevalence of LA-MRSA in the community
Prevalence of LA-MRSA in the community
Recent emergence of *Staphylococcus aureus* sequence type 398 in human blood cultures
Abstract

Background

Recently a new clone of MRSA sequence type 398 (ST398) has emerged, which is related to an extensive reservoir in animals, especially pigs and veal calves. It has been reported previously that methicillin-susceptible variants of ST398 circulate among humans at low frequency, and these have been isolated in a few cases of bloodstream infections (BSI). The purpose of this study was to determine the prevalence of *S. aureus* ST398 in blood cultures taken from patients in a geographic area with a high density of pigs.

Methodology/Principal Findings

In total, 612 consecutive episodes of *S. aureus* BSI diagnosed before and during the emergence of ST398 were included. Three strains that were isolated between 2010-2011 from bacteremic patients were positive in a ST398 specific PCR. There was a marked increase in prevalence of *S. aureus* ST398 BSI compared to strains that were isolated between 1996-1998 and 2002-2005 (3/157, 1.9% vs 0/455; 0.0%; *p* = 0.017).

Conclusions/Significance

In conclusion, in an area with a relative high density of pigs, *S. aureus* ST398 was found as a cause of BSI in humans. This indicates that certain *S. aureus* ST398 subclones, especially spa-type t571, are able to cause invasive infections in humans and that the prevalence is rising. Careful monitoring of the evolution and epidemiology of *S. aureus* ST398 in animals and humans is therefore important.
Introduction

Traditionally, methicillin-resistant *Staphylococcus aureus* (MRSA) has been considered a hospital associated pathogen. Since the end of the last century, MRSA has expanded its territory to the community causing severe infections in previously healthy persons all over the world [1–2]. In 2003 a new clone of MRSA was first identified that was related to an extensive reservoir in pigs and cattle [3]. People who are in direct contact with pigs and veal calves have high carriage rates of MRSA (23% and 29%, respectively) [3–4]. The livestock-associated MRSA ST398 strains are characterized by being non-typeable by Pulsed-Field Gel Electrophoresis (PFGE) and are, therefore, sometimes referred to as non-typeable MRSA (NT-MRSA) [5]. Using Multi Locus Sequence Typing (MLST) these strains all belong to the sequence type 398 (ST398). Remarkably, *S. aureus* ST398 harbors several Staphylococcal Cassette Chromosome mec (SCCmec) types, suggesting that the resistance cassette was acquired on multiple unrelated occasions [5]. A typing method for *S. aureus*, based upon length polymorphism of the 16S-23S spacer region has been described previously. Although shown to be effective and reproducible [6], this method has not been widely implemented. It is based upon the fact that every *S. aureus* strain has 5 or 6 interspace regions in its chromosome. The length of the individual regions varies within the chromosome, so when amplified and sorted by length using agarose gel electrophoresis, each strain produces an individual pattern of bands. Typing based on these spacer patterns has been shown to correlate well with traditional typing methods [7], with discriminatory power comparable to that of MLST [8–9]. To confirm the results of the 16S-23S spacer fingerprint analysis, we additionally performed two specific polymerase chain reactions (PCR) for the detection of *S. aureus* ST398 isolates as described previously [10].

Livestock is the first clearly defined non-human reservoir of MRSA in the community. In the Netherlands MRSA ST398 was first detected in 2003, and by the end of 2007 nearly 30% of all newly identified MRSA in humans in the Netherlands were of this type, suggesting a recent and very rapid spread [11]. MRSA ST398 is now considered a zoonotic pathogen, affecting mainly people who work with pigs and veal calves. If this strain can spread successfully from human to human and also cause diseases in healthy individuals it will likely constitute a significant public health problem in the near future. Recently, van Belkum and colleagues reported that methicillin-susceptible *S. aureus* (MSSA) isolates homologous to the MRSA ST398 were found among isolates derived from bacteremic patients (3 [2.1%] of 146) [12]. However, this study was based on a strain collection obtained from inhabitants of a densely populated, urban area. Moreover, the strains were not from consecutive episodes of bloodstream infections (BSI), which may have caused selection bias. The purpose of the current study is to determine the prevalence of *S. aureus* ST398 in consecutive BSI episodes from patients in an area with a high density of pigs.
Materials and Methods

Strain collections

Three independently collections with a total of 612 S. aureus isolates from patients with BSI were tested. The patients were hospitalized in one of two hospitals (St. Elisabeth Hospital, Tilburg or Amphia Hospital, Breda, the Netherlands). The cities of Tilburg and Breda are located in the southeast of the Netherlands with a high pig-density, i.e. approximately 1,000 pigs per square kilometer [13]. The first collection consisted of 250 S. aureus strains that were isolated from consecutive episodes of BSI that occurred between January 1996 and February 1998, before MRSA ST398 had been reported. The second collection consisted of 205 S. aureus strains that were isolated from consecutive episodes of BSI that occurred between August 2002 and August 2005, when MRSA ST398 emerged in the Netherlands. The third collection comprised 157 S. aureus isolates that were isolated from consecutive episodes of BSI that occurred between January 2010 and April 2011, when MRSA ST398 was the most frequently observed MRSA variant in the hospitals involved. Only one isolate was included per patient per bactereemic episode. All isolates were identified by a latex agglutination test (Staphaurex Plus; Murex Diagnostics Ltd., Dartford, England), and by the detection of DNase (DNase agar; Oxoid Ltd., Basingstoke, England). The blood cultures isolates were classified as methicillin-susceptible (MIC of oxacillin ≤ 2 µg/ml) at the time of collection by broth micro-dilution susceptibility testing performed according to CLSI standards [14]. Resistance profiles to 21 antimicrobial agents of all confirmed S. aureus strains were determined with the VITEK system (bioMérieux SA, Craponne, France) according to the manufacturer’s instructions.

Samples and microbiological procedures

The isolates were stored at -80°C in Microbank™ (Pro-Lab Diagnostics) preservation system until they were tested. All strains were cultured overnight at 35°C on Columbia agar plates with 5% sheep blood to obtain fresh growth. A suspension with an optical density of 1.0 McFarland was made in 2 ml of 0.75% NaCl. From this suspension 500 µl was centrifuged at 14,000 rpm for 3 minutes in an eppendorf centrifuge. The supernatant was removed and the pellet was resuspended in 200 µl distilled water (Baxter Healthcare SA, Zurich, Switzerland) by vortexing. This hypotonic fluid caused sufficient lysis of S. aureus. Subsequently, the suspension was centrifuged at 14,000 rpm for 3 minutes. The supernatant was used for PCR amplification without further processing.
16S-23S spacer fingerprint analysis

For amplification of the 16S-23S rDNA spacer regions, two primers were constructed in conserved regions of the 16S and 23S rRNA genes, as described previously [7]. Each PCR mixture with a final volume of 15 μl contained, 1 μl of DNA, 1x SuperTaq Buffer (HT Biotechnology Ltd, Cambridge, England), 0.25 U of SuperTaq polymerase (HT Biotechnology Ltd), 1.5 mM MgCl2, 67 μM deoxynucleotide triphosphates (HT Biotechnology Ltd), 0.5 μl of 1.25% bovine serum albumin and 1 μM of each primer [15]. The amplification was carried out on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, USA). Cycling conditions for PCR were: 72°C for 2 min, 35 cycles of 94°C for 30 sec, 56°C for 45 sec, 72°C for 1 min and a final extension at 72°C for 10 min.

Subsequently, 7 μl of PCR product was mixed with 5 μl of loading buffer and separated on a 2.0% agarose gel. Electrophoresis was performed at 300V during 90 minutes. Fragments lengths were sized using a 100 bp ladder DNA marker (Invitrogen, Carlsrad, USA). Next, band patterns were visualized using a UV Transilluminator (BioDoc-It System UV, Upland, USA). Total time needed from cultured strains to spacer fingerprints was about 4 hours. This typing method for *S. aureus* has recently been validated using an extensive collection of strains and could detect all non-typeable MRSA in that collection [15]. The MRSA ST398 control strain used in our assay was isolated from a pig farmer and was non-typeable with PFGE using the restriction enzyme *SmaI*. This strain had a spa-type t108 and contained the SCCmec type 5.

Discrimination of MRSA ST398 from other *S. aureus* strains using spacer fingerprints is easy and straightforward, due to the easy detection of the 539 bp fragment that is unique to MRSA ST398 strains. This method was only applied on the strains isolated before 2007.

ST398 specific PCR

The ST398 specific primers C01F and C01R were used as described previously in order to detect *S. aureus* ST398 strains by PCR [10]. In addition, a ST398 specific probe C01P-FAM (FAM-GTCAGTATGAATTGCGGTATG-BHQ1) was constructed to visualize DNA amplification by real-time PCR. For amplification of *S. aureus* sequence, the primers A04F and A04R were used as described previously to check all tested strains for amplifiable *S. aureus* DNA [10]. Furthermore, probe A04P-YY (Yakima Yellow-GAGATTGAGTTGAGTTGATACCTGA-BHQ1) was constructed to visualize DNA amplification in real-time. Each PCR mixture with a final volume of 25 μl contained, 2 μl of DNA and 12.5 μl of TaqMan® Fast Universal PCR Master Mix (Applied Biosystems, Foster City, USA). For the ST398 specific PCR, concentrations of 600 nM of primer C01F, 600 nM of primer C01R and 150 nM of probe C01P-FAM were used. For the *S. aureus* specific PCR, concentrations of 900 nM of primer A04F, 400 nM of primer A04R and 100 nM of probe A04P-YY were used. The amplification was carried out on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, USA). PCRs were performed using the following cycling conditions...
protocol: 95°C for 10 min, followed by 35 cycles of 95°C for 15 sec, 58°C (C01 primers) or 54°C (A04 primers) for 30 sec, 60°C for 1 min. All 612 *S. aureus* strains were analyzed with the ST398 specific PCR.

**Ethics statement**

Medical ethics review was not required for this study according to the Dutch Medical Research Involving Human Subjects Act (WMO) due to the fact that patients were not physically involved in this study. In addition, privacy of patients was provided by coding all tested isolates according to the requirements of the National Privacy Regulations in the Netherlands and thus waived the need for consent. All blood cultures were taken routinely from patients with body temperatures higher than 39°C and symptoms of BSI for screening of micro-organisms.

**Results**

In total, 612 *S. aureus* BSI were diagnosed in 367 males and 245 females. The vast majority were methicillin-susceptible (MSSA), 610 cases (99.7%) versus 2 cases of MRSA (0.3%). The MRSA cases were both from the most recent collection. The strains from the first and second collection were typed using the 16S-23S spacer fingerprint analysis, which showed that none of the 455 *S. aureus* strains had a fingerprint that corresponded to the MRSA ST398. For more accurate analysis, the spacer fingerprints were digitalized and compared using the BioNumerics software package (Applied Maths, Sint-Martens-Latem, Belgium). Typically, MRSA ST398 strains were characterized by the presence of fragments of 369, 386, 399, 520, 539 and 601 bp in length. The 539 bp fragment was encountered only in livestock-associated strains [15].

To confirm the results of the 16S-23S spacer fingerprint analysis, a ST398 specific PCR protocol was performed on all 612 *S. aureus* strains. All strains were positive in the *S. aureus* specific PCR, demonstrating that all 612 DNA samples used for PCR contained amplifiable *S. aureus* DNA. In addition, ST398 specific PCR analysis demonstrated that 3 (2 MSSA, 1 MRSA) out of these 612 *S. aureus* isolates (0.5%) were positive using the protocol. Prevalence was the highest among MRSA (1/2, 50% vs 2/610, 0.3% for MSSA; *p* = 0.010). The three *S. aureus* ST398 isolates all belonged to the third collection which was harvested between January 2010 and April 2011.
This study showed a marked increase in prevalence of *S. aureus* ST398 BSI originating from recently isolated strains compared to the other collections (3/157, 1.9% vs 0/455, 0.0%; *p* = 0.017). Patients with *S. aureus* ST398 BSI did not differ from patients with *S. aureus* non-ST398 BSI by gender nor age. Examination of patient history did not reveal any exposure to animal husbandry among the three ST398 cases. Microbiological study of the three ST398 isolates revealed the following antibiotic susceptibility patterns. The MRSA isolate was found resistant to tetracycline, trimethoprim/sulfadiazine and gentamicin; the two MSSA isolates were only resistant to erythromycin. Spa-typing of the three ST398 isolates identified two related spa-types. Both MSSA isolates were assigned to spa-type t571, a very infrequently found spa-type within clonal complex 398. The MRSA isolate was a typical pig-borne spa-type t011. None of the three *S. aureus* ST398 isolates were positive for the Panton Valentine Leukocidin (PVL) gene.

To ensure that both protocols used in this study can detect *S. aureus* ST398, the five ST398 strains found in the study of van Belkum et al. [12] were analyzed using the 16S-23S spacer fingerprint analysis protocol and the ST398 specific PCR. Both strains isolated from the nose swabs and the three strains isolated from blood cultures showed a 16S-23S spacer fingerprint that corresponded to our MRSA ST398 control strain (data not shown). Moreover, these five strains as well as our positive control strain were also positive in both the *S. aureus* specific and the ST398 specific PCR.

**Discussion**

A large collection of consecutive isolates of *S. aureus* BSI episodes in an area with a high pig-density revealed the recent emergence of *S. aureus* ST398 in blood cultures. We demonstrated that three of the 612 *S. aureus* strains isolated from bacteremic patients living in an area with a high pig-density belong to ST398. These three strains were all isolated between 2010–2011. Conversely, none of the 455 strains that were isolated between 1996-1998 and 2002–2005 belong to ST398. Therefore, the present study confirms the recent emergence among Dutch patients of BSI due to ST398, a *Staphylococcus aureus* strain formerly found primarily in association with pigs [12].

In the present study, we found only one MRSA isolate with spa-type t011. Examination of this patient history revealed no known contact with livestock. The patient was a 72-year-old woman with an infected shoulder prosthesis. On the other hand, the two MSSA ST398 isolates found in our study had both spa-type t571, which is not a very regular spa-type among ST398 strains considering the recent report of Huijsdens et al. [16] in which only 1.9% of the MRSA ST398 that were sent to the National Institute of Public Health and the Environment in the Netherlands in 2007 had spa-type t571. These two cases were hospital-
acquired and included 1 case of catheter-associated bloodstream infection observed in a 71-year-old woman with complicated perforation of the intestinal, and 1 case following cholecystitis in a 84-year-old woman. Overall, the mode of acquisition of the *S. aureus* ST398 isolates by our 3 patients remains unclear.

In accordance to our findings, van Belkum and colleagues [12] found that ST398 is rare among Dutch MSSA strains colonizing healthy persons (2 [0.2%] of 829 strains). However, a number of MSSA isolates homologous to the MRSA ST398 were found among bacteremic patients (3 [2.1%] of 146; *p* = 0.026). This MSSA ST398 prevalence in human blood cultures is comparative with the prevalence we found in our study (*p* = 1.00). Interestingly, all three *S. aureus* ST398 strains found by van Belkum and colleagues were isolated from bacteremic patients in Rotterdam had also spa-type t571. The authors were unable to find an epidemiological link between these 3 patients. The strains were isolated from different patients in different medical departments over an extended period. Although no link was found it could be that an unusual common source may be the explanation for the origin of these 3 strains.

Another recently published study demonstrated that the incidence of *S. aureus* ST398 BSI is rapidly increasing. Valentin-Domelier et al. reported 18 *S. aureus* ST398 BSI during a four-year period in three noncontiguous French regions [17]. *S. aureus* ST398 incidence showed a seven-fold increase during the study period (0.002 per 1,000 patients days in 2007 vs 0.014 in 2010). Seventeen out of the 18 BSI isolates were methicillin-susceptible and additional spa-typing identified seven related spa-types. The two spa-types t571 and t1451, that are infrequently associated with pig-borne isolates, were found with 10 and 4 BSI isolates, respectively. No isolate was assigned to classical pig-borne spa-types, such as t011, t034 or t108. None of the 18 ST398 isolates were positive for the PVL gene.

At present, it is unclear whether MRSA ST398 is easily transmissible from human to human. However, recent published studies demonstrate that MRSA ST398 is less transmissible than other MRSA strains [18–19]. Also the virulence of *S. aureus* ST398 is still a matter of debate. There are indications that MRSA ST398 causes infections less frequently than other MRSA strains [19–20]. On the other hand, there are increasing case reports of severe invasive infections due to MRSA ST398, such as endocarditis and necrotizing pneumonia [21–22]. In addition, these recent invasive infections are mostly associated with non-pig-borne isolates [11, 17, 23]. To determine whether the potential impact of MRSA ST398 can be substantiated by the existence of genetically homologous MSSA strains among bacteremic patients, we determined the prevalence of *S. aureus* ST398 in positive blood cultures taken from patients in an area with a high prevalence of pigs using 16S-23S spacer fingerprint analysis and also a ST398 specific PCR. The historical occurrence of *S. aureus* ST398 in blood culture isolates provides an estimate for the potential impact of MRSA ST398 on public health.
The results found in recent published studies that the prevalence of *S. aureus* ST398 in BSI is increasing over time, was confirmed in our study. These data suggest that *S. aureus* ST398 have recently been adapted toward human hosts. One possible explanation is that *S. aureus* ST398 have a deficiency in the *Sau*I type I restriction system, resulting in hypersusceptibility to acquisition of foreign genes [24]. The recent observation of the genome content of *S. aureus* ST398 BSI isolates suggest that the emergence could have arisen as a consequence of recent horizontal transfers in diverse pig-borne isolates [17]. Horizontal transfer of phage allows bacteria to adapt to specific niches, and may lead to the emergence of new clones. Another plausible explanation is that *S. aureus* ST398 BSI isolates harbor the Sa3int phage, which includes *hlb*-converting genes that are associated with colonization and virulence in humans [17, 25]. This supports the hypothesis that a human-adapted ST398 has recently evolved from pig-borne strains, and is able to accept genes conferring the ability to colonize humans, possibly by virtue of the Sa3int phage with which it is associated. This would explain the sudden ability of the emerging ST398 to infect humans in the absence of exposure to livestock. Nevertheless, further investigations are needed to better understand the emergence and spread of different ST398 subclones into the general population.

The results of our study have two important limitations. Firstly, approximately one third of the *S. aureus* BSI of the earlier period 1996–1998 were community-associated. The portion of community-associated BSI increased to 50% in the period 2002–2005 and 2010–2011, respectively. One might expect that MRSA ST398 would be found more frequently in community-associated cases as compared to healthcare-associated cases based on the epidemiology known to date. Therefore, the likelihood to demonstrate the presence of *S. aureus* ST398 in blood culture is reduced by also including healthcare-associated cases. Secondly, the first collection of strains was isolated between 1996–1998. This may have been too early as the first case of MRSA ST398 was reported only in 2003. It is possible that *S. aureus* ST398 was not present in livestock in the Netherlands at that time. Considering the findings of Armand-Lefèvre et al. this possibility is highly unlikely [26]. They found MSSA ST398 strains that were isolated from swine infections and also healthy pig farmers between 1996–2002. MSSA ST398 was retrieved from 6 out of the 44 (13.6%) studied isolates from healthy pig farmers. In addition, four of the 14 (28.6%) isolates which were derived from a swine infection were MSSA ST398. The second collections of strains were isolated between 2002–2005 and 2010–2011 when MRSA ST398 was present in our country.

In conclusion, the rapid emergence of MRSA ST398 in the Netherlands is worrying [3]. The first isolate was found in 2003, and by the end of 2007 nearly 30% of all newly identified MRSA in humans reported to the National Institute of Public Health and the Environment in the Netherlands were MRSA ST398 [27]. Approximately one third of people who are in direct contact with pigs and veal calves carry MRSA [28] and there is no indication that the extensive reservoir in animals can be brought under control in the near future. *S. aureus* ST398 is a clonal complex that has been associated with pigs and pig farmers before [26]. Our results demonstrated that *S. aureus* ST398 is an increasing cause of invasive
Prevalence of LA-MRSA in the community. These results are in line with other findings that certain \textit{S. aureus} ST398 isolates, especially \textit{spa}-type t571, can cause invasive infections in humans [12, 17, 22]. This human-adapted ST398 subclone is now increasingly identified in hospitals [23]. It appears to be highly receptive for horizontal gene transfer. Therefore, further adaptation to humans may occur and if \textit{S. aureus} ST398 can successfully spread from human to human it may pose a significant public health problem in the future. In addition, further acquisition of genetic elements harboring virulence and antibiotic resistance could arise. Therefore, careful monitoring of the evolution and epidemiology of MRSA ST398 is important.

\textbf{Acknowledgments}

The authors thank Veronica Weterings and Jolanda van Baal-Visser for performing the 16S-23S spacer region PCRs and gel electrophoresis, as well as Marie-Louise van Leest for developing and optimizing the real-time \textit{S. aureus} specific and ST398 specific PCR.
Recent emergence of MSSA ST398 in human blood cultures

Reference List


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Recent emergence of MSSA ST398 in human blood cultures
Dynamics and transmission of LA-MRSA
Recent emergence of MSSA ST398 in human blood cultures

Dynamics and transmission of LA-MRSA
Dynamics and transmission of LA-MRSA
Dynamics and determinants of *Staphylococcus aureus* carriage in livestock veterinarians: a prospective cohort study

Erwin Verkade
Birgit van Benthem
Marjolein Kluytmans - van den Bergh
Brigitte van Cleef
Miranda van Rijen
Thijs Bosch
Jan Kluytmans

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Abstract

Background

Since 2003, a new clade of methicillin-resistant \textit{Staphylococcus aureus} (MRSA) belonging to clonal complex (CC) 398 and associated with animal husbandry has emerged in the Netherlands. The purpose of this study was to determine the dynamics of carriage in persons with direct contact to livestock.

Methods

A 2-year prospective cohort study was performed in which the anterior nares and oropharynx of 137 livestock veterinarians were sampled for the presence of \textit{S. aureus} every 4 months during the first year and again 1 year later. All \textit{S. aureus} isolates were genotyped by staphylococcal protein A (spa) typing and with Multiple-locus variable-number tandem repeat analysis (MLVA).

Results

The mean prevalence of MRSA CC398 carriage was 44\% (range 42–46\%), and for \textit{S. aureus} the prevalence was 72\% (range 69–75\%). Thirty-two veterinarians (23\%) were always carrying MRSA CC398 and 18 of those (56\%, 13\% of all veterinarians) had identical MLVA types at all sampling moments.

Conclusions

A high proportion of veterinarians had persistent MRSA CC398 carriage during the 2-year study period, indicating that this variant may colonize humans for prolonged periods. Furthermore, prevalence of \textit{S. aureus} carriage was extremely high, indicating that MRSA CC398 is not replacing the susceptible strains, but comes on top of it.
Infections with methicillin-resistant *Staphylococcus aureus* (MRSA) are associated with increased morbidity, mortality, and healthcare costs [1, 2]. Traditionally, MRSA has been considered a hospital-associated pathogen [3, 4]. In the last 10–15 years, MRSA has expanded its territory to the community causing severe infections in previously healthy persons worldwide [5]. Surveillance data of MRSA in the Netherlands show that MRSA prevalence upon hospital admission is still extremely low (0.1%) [6].

A new clade of MRSA associated with animal husbandry has emerged in the Netherlands since 2003 [7, 8]. This so-called livestock-associated MRSA belongs to multilocus sequence type clonal complex 398 (MRSA CC398) [9]. After its emergence, an active screening program was developed, and subsequently a strong increase in MRSA prevalence was observed in humans [10, 11]. By the end of 2008, 42% of all newly identified MRSA strains in humans belonged to this clade (www.rivm.nl/mrsa). The main risk groups for MRSA CC398 carriage are humans with professional exposure to pigs and veal calves [12, 13]. MRSA CC398 is rarely found outside of these risk groups [14].

*Staphylococcus aureus* nasal carriage has been extensively studied in patients and healthy individuals [15–17]. Cohort studies distinguish 3 carriage patterns among healthy individuals. Persistent carriage occurs in about 20% (range 12%-30%) of the population; about 30% (range, 16%-70%) are intermittent carriers, and about 50% (range, 16%-69%) are noncarriers [15, 18, 19]. Persistent carriers usually carry the same strain for extended periods of time, whereas intermittent carriers tend to host different strains over time [17, 19]. The underlying mechanisms are largely unknown.

Veterinarians who regularly work with pigs and veal calves are daily exposed to extreme high loads of methicillin-susceptible *S. aureus* (MSSA) and MRSA CC398. Therefore, the dynamics of *S. aureus* carriage in these persons might be different due to competition for the binding site in the anterior nares between MSSA and MRSA strains [20]. The purpose of this prospective cohort study was to elucidate the dynamics of *S. aureus* carriage and its determinants in Dutch livestock veterinarians.
Materials and methods

Study design and setting

A 2-year prospective cohort study was conducted in Dutch veterinarians who mainly work with pigs and veal calves. Veterinarians were recruited from April until December 2008 and were followed for 2 years after enrolment. Data were collected at baseline (0 months), and at 4, 8, 12, and 24 months after inclusion. This study was approved by the medical ethics committee of the St Elisabeth Hospital in Tilburg, the Netherlands (protocol number 0749).

Study population

In April 2008, all veterinarians associated with the Pig Health Department (approximately 95% of Dutch swine veterinarians) of the Royal Dutch Veterinary Society were invited by mail to participate in the study and asked to complete a questionnaire to determine the eligibility for the study. Veterinarians were eligible for participation if they (1) were aged between 18 and 65 years, (2) had 1 or more household members who were willing to participate in another study in which the transmission of MRSA CC398 between veterinarians and household members was studied, (3) had professional contact with pigs or veal calves at least once every 2 weeks in the previous year, (4) did not live on a farm with pigs or veal calves, (5) had no household members with professional contact with pigs or veal calves, (6) had not been treated for colonization with MRSA in the previous 3 months, and (7) had provided written informed consent.

During a home visit, cultures were taken from the anterior nares and the oropharynx (Supplementary Figure 1). Additional data were collected using a questionnaire that comprised information on age, sex, smoking, composition of the household, exposure to livestock, antibiotic treatment 4 months prior to sampling, and infections. Veterinarians were asked to take nasal and oropharyngeal cultures in the morning before visiting the stables and additionally to complete a short questionnaire on the presence of active infections and antibiotic usage at 4, 8, 12, and 24 months. Appropriate transport material with Amies medium (Transwab, Medical Wire & Equipment), instructions for sampling, and questionnaires were provided during the baseline home visit.
Microbiological procedures

Nasal and oropharyngeal samples were directly plated on chromID® *S. aureus* and chromID® MRSA agar plates (bioMérieux, La Balme, France), and subsequently placed in a Mueller–Hinton (MH) broth supplemented with 6.5% sodium chloride. The overnight MH broth was subcultured onto both chromID® *S. aureus* and chromID® MRSA agar plates. All agar plates were read after 18-24 hours of incubation at 35-37°C according to manufacturer’s instructions [21]. All cefoxitin resistant isolates were tested using a polymerase chain reaction for the presence of the *mecA* and *nuc* genes [22, 23]. All *S. aureus* isolates were genotyped by staphylococcal protein A (*spa*) typing [24] and multilocus variable number of tandem repeat analysis (MLVA) [25] and were stored at -80°C in the Microbank (Pro-Lab Diagnostics) preservation system.

Definitions of the carrier state

Determination of the MRSA and MSSA carrier state was based on the 5 sampling moments during the 2-year study period (0, 4, 8, 12, and 24 months after inclusion). For each sampling moment, veterinarians were considered MRSA or MSSA-positive when either the nasal or oropharyngeal swab harbored MRSA or MSSA. Veterinarians who were MRSA or MSSA positive at all 5 sampling moments were considered persistent carriers. In the case that one sampling moment was missing and the veterinarian was MRSA or MSSA positive at all other sampling moments, the veterinarian was regarded as a persistent carrier as well. Veterinarians for whom only 1-4 samples yielded MRSA or MSSA were identified as intermittent carriers, and veterinarians who did not have any positive sample were considered noncarriers.

Statistical analyses

All analyses were performed using SPSS 19.0 for Windows (SPSS Inc, Chicago, Illinois). The mean prevalence was calculated by averaging the prevalences of all sampling moments. Differences in continuous variables between groups were tested with Student *t* test or Mann-Whitney *U* test when applicable, and differences in categorical variables between groups were tested with the Pearson **χ**² test. The statistical tests were 2-tailed and *P* < 0.05 was considered statistically significant. All univariate analysis were performed in a generalized estimating equations (GEE) model using a Poisson distribution with robust covariance estimators, to control for violation of the distribution assumption that the variance equals the mean, and an independent correlation matrix for the multiple samples per veterinarian [26]. The carrier state of a given sampling moment was used as a possible predictor for the carrier state of the next sampling moment, corrected for the fact that multiple samples came from
one person (clustered data). A sensitivity analysis was performed to establish the effect of missing samples on the carrier state.

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### Results

**Enrollment**

In April 2008, 361 livestock veterinarians were asked to participate in the study. Two hundred and twenty-five (62%) veterinarians responded and, 137 (61%) veterinarians were eligible and included in the study (Figure 1).

**Figure 1** Flowchart of the recruitment of livestock veterinarians

Legend:

- **Potentially eligible**
  - $n = 361$

- **Non-response**
  - $n = 136 \,(37.7\%)$

- **Examined for eligibility**
  - $n = 225 \,(62.3\%)$

- **Not eligible**
  - $n = 88$

**Reasons**

- No professional contact with pigs or veal calves: 35
- No household members: 24
- Household contacts have pigs or veal calves: 15
- No informed consent: 9
- Living on a pig farm: 5
Staphylococcus aureus carriage

During the 2-year study period, 137 veterinarians were screened for MRSA and MSSA in nares and oropharynx on 5 consecutive sampling moments. Among 1348 samples, 724 (54%) harbored *S. aureus* strains. Table 1 describes the carrier state and mean prevalence of MRSA, MSSA, and *S. aureus*. When broth enrichment would not have been used, 187 (26%) *S. aureus*-positive samples would have been missed. MRSA CC398 was recovered in 294 of 674 sampling moments (44%). There was a limited additional yield of oropharyngeal samples for the detection of MRSA CC398, as 17 (5.8%) samples originating from 9 different veterinarians were only positive in the oropharynx. Consequently, 6 veterinarians would have been defined as intermittent carriers instead of persistent carriers and 2 veterinarians would have been classified as noncarriers instead of intermittent carrier.

Table 1  Mean prevalence and carrier state of methicillin-resistant *Staphylococcus aureus* (MRSA) CC398, MRSA non-CC398, methicillin-susceptible *S. aureus* and *S. aureus* among 137 livestock veterinarians

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Mean prevalence % (Range)</th>
<th>Persistent No. (%)</th>
<th>Intermittent No. (%)</th>
<th>Noncarrier No. (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA CC398</td>
<td>43.6 (41.6–46.3)</td>
<td>32 (23.4)</td>
<td>56 (40.9)</td>
<td>49 (35.8)</td>
<td>137 (100%)</td>
</tr>
<tr>
<td>MRSA non-CC398</td>
<td>1.0 (0.7–1.5)</td>
<td>1 (0.7)</td>
<td>1 (0.7)</td>
<td>135 (98.6)</td>
<td>137 (100%)</td>
</tr>
<tr>
<td>MSSA</td>
<td>27.2 (22.1–30.5)</td>
<td>18 (13.1)</td>
<td>57 (41.6)</td>
<td>62 (45.3)</td>
<td>137 (100%)</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>71.7 (69.1–74.8)</td>
<td>65 (47.4)</td>
<td>57 (41.6)</td>
<td>15 (10.9)</td>
<td>137 (100%)</td>
</tr>
</tbody>
</table>

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*

a Based on 5 consecutive sampling moments during the 2-year study period

b A persistent carrier was a person with all nasal or oropharyngeal cultures positive for MRSA (5 sampling moments); noncarriers had no positive cultures; intermittent carriers were the remaining persons

c Because MRSA and MSSA could coexist in one sample, the numbers do not add up

Throughout the study, 11 of 685 (1.6%) sampling moments were not received. Sensitivity analysis on the effect of missing samples had no relevant consequences on the conclusions (data not shown). A total of 133 (97%) veterinarians completed and returned the general questionnaire. The general characteristics and the frequencies of contact with livestock during the study period are shown in Table 2.
Table 2  Baseline and study characteristics of livestock veterinarians by methicillin-resistant *Staphylococcus aureus* CC398 carrier state \((n = 133)^a\)

<table>
<thead>
<tr>
<th>General characteristics</th>
<th>persistent MRSA CC398 carrier ((n = 32))</th>
<th>intermittent MRSA CC398 carrier ((n = 55))</th>
<th>MRSA CC398 non-carrier ((n = 46))</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR)</td>
<td>47.0 (41.0–52.5)</td>
<td>50.0 (42.0–53.0)</td>
<td>46.0 (39.5–53.3)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Male sex</td>
<td>31 (96.9)</td>
<td>51 (92.7)</td>
<td>41 (89.1)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Smoking</td>
<td>4 (12.5)</td>
<td>14 (25.5)</td>
<td>5 (10.9)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

**Contact with livestock**\(^b\)

<table>
<thead>
<tr>
<th>Duration with livestock</th>
<th>persistent MRSA CC398 carrier ((n = 32))</th>
<th>intermittent MRSA CC398 carrier ((n = 55))</th>
<th>MRSA CC398 non-carrier ((n = 46))</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 3 months contact with pigs</td>
<td>30 (93.8)</td>
<td>48 (87.3)</td>
<td>29 (63.0)</td>
<td>&lt;0.05(^d)</td>
</tr>
<tr>
<td>&gt; 3 months with veal calves</td>
<td>3 (9.4)</td>
<td>3 (5.5)</td>
<td>5 (10.9)</td>
<td>n.s.</td>
</tr>
<tr>
<td>&gt; 3 months contact with horses</td>
<td>4 (12.5)</td>
<td>15 (27.3)</td>
<td>9 (19.6)</td>
<td>n.s.</td>
</tr>
<tr>
<td>&gt; 3 months contact with other poultry</td>
<td>1 (3.1)</td>
<td>1 (1.8)</td>
<td>1 (2.2)</td>
<td>n.s.</td>
</tr>
<tr>
<td>&gt; 3 months contact with other animals</td>
<td>15 (46.9)</td>
<td>31 (56.4)</td>
<td>29 (63.0)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

**Household characteristics**

<table>
<thead>
<tr>
<th>Household characteristics</th>
<th>persistent MRSA CC398 carrier ((n = 32))</th>
<th>intermittent MRSA CC398 carrier ((n = 55))</th>
<th>MRSA CC398 non-carrier ((n = 46))</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of household members, median (IQR)</td>
<td>3.0 (2.3–4.0)</td>
<td>3.0 (1.0–4.0)</td>
<td>3.0 (1.0–4.0)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Number of companion animals(^c), median (IQR)</td>
<td>1.0 (1.0–2.0)</td>
<td>1.0 (0.0–2.0)</td>
<td>1.0 (0.8–2.3)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Data are presented as No. (%) unless otherwise specified. Statistically significant relationships are bolded.

\(A \ p\)-value <0.05 was considered statistically significant.

Abbreviations: IQR, interquartile range (p25–p75); MRSA, methicillin-resistant *Staphylococcus aureus*; n.s., not significant.

\(^a\) General characteristics of 4 veterinarians were missing.

\(^b\) Total cumulative duration of contact with livestock during the first year of the study period.

\(^c\) Total amount of cats and dogs in the household.

\(^d\) Both persistent and intermittent MRSA CC398 carriers compared with MRSA CC398 non-carriers.

Persistent and intermittent MRSA CC398 carriers reported significantly more intensive contact with pigs than veterinarians who did not carry MRSA CC398 \((p = 0.016\) and \(p = 0.001\), respectively). The vast majority of veterinarians who carried MRSA CC398 or MSSA during the first year were still positive 1 year later for MRSA CC398 \((31/39 [79\%]; 1\ sample missing) or MSSA \((17/19 [89\%]; 1\ sample missing)\), respectively.
Results of spa typing and MLVA

A total of 450 MRSA and 274 MSSA strains from nares and oropharynx were genotyped by spa typing and MLVA. Seventeen different spa types and 23 different MLVA types (MTs) were found among the 450 MRSA isolates; the majority of spa types and MTs identified belonged to CC398 (97.6%). Two dominant spa types, t011 and t108, accounted for 83% of all MRSA CC398 isolates. Spa types t034 and t571 (originated from 4 veterinarians) were found in 15 (3.4%) and 6 (1.4%) of the 439 MRSA CC398 strains, respectively. MLVA profiles clustering revealed the presence of 3 different MLVA complexes (MCs; Supplementary Figure 2). In persistent carriers with MRSA CC398, different spa types and MTs could be detected over time. Eleven strains (2.4%) were identified as non-CC398 types. One veterinarian was identified as a persistent carrier with MRSA non-CC398 (spa type t127; MC1). Overall, the mean prevalence of MRSA non-CC398 carriage was 1.0% (range, 0.7%–1.5%).

A total of 71 different spa types and 109 different MTs were found among 274 MSSA isolates. The majority of the strains (81%) did not belong to the CC398 lineage. Notably, spa types t034 and t571 were found in 29 (56%) and 3 (5.8%) of the 52 MSSA CC398 strains, respectively. Spa type t034 was significantly more present in MSSA CC398 isolates compared with MRSA CC398 strains (relative risk [RR], 16.1; 95% confidence interval [CI], 9.1–28.4; \( p < 0.001 \)). MLVA profiles clustering revealed 16 different MLVA complexes (Supplementary Figure 2). The CC398 lineage in MSSA is the largest single clade representing 52 isolates (19%). When genotyping results of MRSA isolates were taken into account, 18 of 32 veterinarians (56%) were found to carry MRSA CC398 with the same MT throughout the 2-year study period. Additionally, 11 of 18 veterinarians (61%) with persistent MSSA carriage had the same MT.

Determinants for MRSA CC398 carriage

The result of the baseline sample was highly predictive for subsequent findings. Thirty-one of 59 (52.5%) veterinarians with a baseline sample that contained MRSA CC398 were carrying MRSA CC398 in all 4 subsequent samples. These rates were significantly lower in veterinarians that harbored MSSA at baseline (3/40 [7.5%]; \( p < 0.001 \)) and in veterinarians who did not carry \textit{S. aureus} (3/37, [8.1%]; \( p < 0.001 \)). In contrast, 46 of 77 (59.7%) veterinarians who did not carry MRSA CC398 at the baseline sample harbored no MRSA CC398 in the 4 subsequent samplings. Furthermore, veterinarians that carried MRSA CC398 in the baseline oropharyngeal sample had significantly more MRSA-positive test results in the next 4 sampling moments than those who did not carry MRSA CC398 in the baseline oropharyngeal sample, irrespective of nasal results (17/27 vs 20/109; RR, 3.4; 95% CI, 2.0–5.4).
Carriage of MSSA (but not MRSA) was not found to protect veterinarians from the acquisition of MRSA CC398 (RR, 0.98; 95% CI, 0.56–1.71) as compared to veterinarians who carried no \textit{S. aureus} (Table 3).

**Table 3** Methicillin-resistant \textit{Staphylococcus aureus} CC398 and methicillin-susceptible \textit{S. aureus} carriage in relation to the result of the next sampling moment among 135 livestock veterinarians \((n=535\) sampling moments)

<table>
<thead>
<tr>
<th>MSSA carriage on current sampling moment(^a)</th>
<th>No. of observations</th>
<th>MRSA CC398 positive No. (%)</th>
<th>MRSA CC398 negative No. (%)</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nasal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA positive</td>
<td>116</td>
<td>16 (14)</td>
<td>100 (86)</td>
<td>0.95</td>
<td>0.53–1.70</td>
</tr>
<tr>
<td>MSSA negative</td>
<td>193</td>
<td>28 (15)</td>
<td>165 (85)</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td><strong>Oropharyngeal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA positive</td>
<td>78</td>
<td>8 (10)</td>
<td>70 (90)</td>
<td>0.68</td>
<td>0.33–1.39</td>
</tr>
<tr>
<td>MSSA negative</td>
<td>325</td>
<td>49 (15)</td>
<td>276 (85)</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td><strong>Nasal and/or oropharyngeal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA positive</td>
<td>151</td>
<td>30 (20)</td>
<td>121 (80)</td>
<td>0.98</td>
<td>0.56–1.71</td>
</tr>
<tr>
<td>MSSA negative</td>
<td>155</td>
<td>24 (16)</td>
<td>131 (84)</td>
<td>Ref</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Only persons at risk for MRSA acquisition were considered, that is, MRSA negative on current sampling moment

**Discussion**

We found a high prevalence of MRSA CC398 colonization (44%) in 137 veterinarians who work mainly with pigs and veal calves. In total, 88 veterinarians (65%) carried MRSA CC398 at least transiently. Others have also found high carriage rates of MRSA CC398 by humans with professional contact to pigs [7, 14, 27, 28].
The mean prevalence of MSSA among veterinarians per measurement was 27%, approximately equal to what is normally found. The combined MSSA and MRSA prevalence was 72%, which is extremely high compared to the general population [6, 15, 16, 18, 19, 29]. We conclude that MRSA CC398 carriage does not replace the susceptible strains and adds up to the total carriage rate of 

S. aureus. High rates of 

S. aureus carriage in healthy pig farmers have been described before by Aubry-Damon et al in 2004 [30]. The prevalence of 

S. aureus among pig farmers (44.6% [50/112]) was significantly higher compared to nonfarmers (24.1% [27/112]). Five of the 50 

S. aureus isolates (10%) were methicillin-resistant. The exact figures are difficult to compare with our findings as they did not use broth enrichment in their culture method, and thereby probably underestimated the 

S. aureus prevalence [21]. Nevertheless, people who work with pigs and veal calves have much higher carriage rates of 

S. aureus than those who do not. As shown in our study, this is partly determined by the level and duration of exposure. Persistent carriers reported more frequent contact with pigs. This was also found in a recent study among veal calf farmers [31]. In addition to the high levels of exposure during work, carriage of MRSA CC398 may also be determined by characteristics of the individual. The importance of host characteristics for the nasal carriage state is widely accepted and has been reviewed recently [32]. Thirty-two veterinarians (23%) had MRSA-positive test results throughout the entire study period and 18 of those (56%, 13% of all veterinarians) had 5 identical MTs and can therefore be considered as actual persistent MRSA CC398 carriers. This prolonged carriage pattern was also found by others. A recent report demonstrated that the majority of pig farmers (59%) did not lose their MRSA CC398 carriage after the holidays [33]. Furthermore, when volunteers were actively colonized with MSSA CC398, they often carried it for prolonged periods [34]. Conversely, several studies stated that MRSA CC398 is not a good colonizer in humans. A recent study among field workers with short-term occupational exposure to pigs and veal calves suggested a high rate of transient contamination, without many persistent colonization [35]. Another study showed that MRSA prevalence among veal calf farmers was strongly reduced (58%) after absence of animal contact [31]. The reasons for these discordant findings are unclear. Different levels of exposure in stables and duration of exposition may have contributed.

Carriage of MSSA could theoretically offer a protective effect for the acquisition of MRSA CC398 as bacterial interference among 

S. aureus strains has been described before [15, 31], possibly through competition of the binding site [20]. However, veterinarians who carried MSSA at a specific moment did not have a lower risk to be MRSA CC398 positive in the next sampling moment compared to veterinarians who did not carry 

S. aureus at all. A possible explanation for the observed differences might be that veterinarians are exposed to extremely high amounts of MRSA CC398 [31, 36], which overrules the normal protective effect.
The MRSA non-CC398 prevalence among veterinarians was significantly higher compared to the general population (2/137 [1.5%] vs 7/6496 [0.1%]; RR, 13.5; 95% CI, 2.0–69.9; \( p = .014 \)) [6]. However, a recent study also showed that a surprisingly high fraction of MRSA strains (7.3%) from veal calf farmers did not belong to CC398 [31]. The 2 veterinarians that carried MRSA non-CC398 isolates did not have any known MRSA risk factors, such as visits to healthcare facilities. Nonetheless, one veterinarian reported frequent contact with companion animals during the study period. Different prevalence studies have found very diverse prevalences in small/companion animals [37, 38]. A recent study demonstrated that transmission of MRSA between companion animals and humans can occur [39].

Among the isolated MSSA strains from veterinarians, the CC398 lineage was the largest single clade representing 52 isolates (19%), which is significantly more prevalent than in MSSA originating from the general population [40]. A possible explanation is that MSSA CC398 is prevalent among pigs in Europe as well [41], and transmission to veterinarians can occur frequently due to direct contact with pigs. Moreover, spa type t571 was found in both MRSA and MSSA strains. Several studies demonstrated that this spa type has recently emerged in human blood cultures in diverse countries, indicating more virulent strain properties [40, 42, 43]. The present study also identified 3 MRSA spa type t571 isolates, showing that this specific subclone could indeed acquire the \( mecA \) gene. This could become a potential treat for the public health in the near future.

To the best of our knowledge, this investigation is the first large, long-term, prospective cohort study among livestock veterinarians. Furthermore, we have analyzed all samples for the presence of both MRSA and MSSA, and all isolates were genotyped by spa-typing and MLVA. Thereby, we were able to evaluate the dynamics of \( S. aureus \) carriage among livestock veterinarians in detail.

There are several limitations to our study. First, we did not take any samples from the stables that had been visited by the participating veterinarians. In general, livestock veterinarians visit multiple pig and veal calf farms daily. Because it is very likely that multiple MRSA subclones are present in the stables, we could not determine the exact source and transmission routes. Second, we might have missed some MSSA strains in samples with predominant MRSA growth, due to the use of \( S. aureus \)-selective plates. This may have resulted in an underestimation of MSSA prevalence, although MRSA and overall \( S. aureus \) prevalences were not affected. Finally, study subjects were relatively healthy individuals that were exposed to an extremely high load of MRSA. Therefore, our data on development of disease in carriers are not representative for other populations.
In summary, we found a mean MRSA CC398 prevalence of 44% among livestock veterinarians. Furthermore, 23% of veterinarians were persistent MRSA CC398 carriers, and 56% of those veterinarians had always identical MTs and can therefore be considered as actual persistent MRSA CC398 carriers. This indicates that regular livestock contact can indeed lead to persistent colonization. Further research is required to elucidate the exact host factors and strain characteristics responsible for persistent MRSA CC398 carriage among individuals.

Supplementary data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgements

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Disclaimer

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Potential conflicts of interest

J.K. has received honoraria from Destiny Pharma, 3M, Cepheid, Phico Therapeutics, and Pfizer for consultancy tasks. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Reference List


Dynamics and transmission of LA-MRSA


Transmission of MRSA CC398 from livestock veterinarian to their household members

Erwin Verkade
Marjolein Kluytmans - van den Bergh
Birgit van Bentheim
Brigitte van Cleef
Miranda van Rijen
Thijs Bosch
Leo Bosch
Jan Kluytmans

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Abstract

There are indications that livestock-associated MRSA CC398 has a reduced human-to-human transmissibility, limiting its impact on public health and justifying modified control measures. This study determined the transmissibility of MRSA CC398 from livestock veterinarians to their household members in the community as compared to MRSA non-CC398 strains.

A one-year prospective cohort study was performed to determine the presence of MRSA MC398 in four-monthly nasal and oropharyngeal samples of livestock veterinarians \((n = 137)\) and their household members \((n = 389)\). In addition, a cross-sectional survey was performed to detect the presence of MRSA non-MC398 in hospital derived control patients \((n = 20)\) and their household members \((n = 41)\). *Staphylococcus aureus* isolates were genotyped by staphylococcal protein A \((spa)\) typing and multiple-locus variable-number tandem repeat analysis (MLVA).

Mean MRSA MC398 prevalence over the study period was 44% (range 41.6–46.0%) in veterinarians and 4.0% (range 2.8–4.7%) in their household members. The MRSA MC398 prevalence in household members of veterinarians was significantly lower than the MRSA non-MC398 prevalence in household members of control patients \((\text{PRR} 6.0; 95\% \text{ CI} 2.4–15.5)\), indicating the reduced transmissibility of MRSA MC398.

The impact of MRSA CC398 appears to be low at the moment. However, careful monitoring of the human-to-human transmissibility of MRSA CC398 remains important.
Infections with methicillin-resistant *Staphylococcus aureus* (MRSA) are associated with increased morbidity, mortality, and (healthcare) costs [1-2]. Traditionally, MRSA has been considered as a hospital-associated pathogen [3-4]. Since approximately 15–20 years, MRSA has expanded its territory to the community causing severe infections in previously healthy persons all over the world [5]. In 2003, a new clade of MRSA emerged in the Netherlands which was related to an extensive reservoir in pigs and cattle [6-7]. This so-called livestock-associated MRSA (LA-MRSA) mostly belongs to clonal complex 398 (MRSA CC398). National Dutch guidelines were adapted in June 2006, recommending persons in contact with live pigs or cattle to be screened upon hospital admission [8]. Since then the number of MRSA CC398 found in the Netherlands has dramatically increased. In 2010, 38% of all newly identified MRSA strains in humans in the Netherlands were of this type, up from 16% by the end of 2006 [9].

Several studies have reported the transmission of healthcare-associated MRSA (HA-MRSA) strains between patients and their household members, with transmission rates varying from below 10% up to 36% [10-14]. Recent surveys showed that MRSA CC398 was 4 to 6-fold less transmissible than other MRSA strains in a hospital-setting [15-17]. At present, the human-to-human transmissibility of MRSA CC398 in a community setting is still unclear. Considering the extensive reservoir in animals and people who work with livestock, the occurrence of MRSA CC398 in people who are not directly involved in farming is strikingly low. So far, there are no indications that MRSA CC398 has spread extensively into the general population. A cross-sectional survey in a livestock-dense region found that only 0.2% of adult individuals without livestock contact were positive for MRSA CC398 [18]. On the other hand, there are observations that proximity of farms is a potential risk factor, even in absence of direct contact between humans and animals [19-21]. In addition, a recent study suggest that incomplete cooking of meat, but also by consumption of simultaneously prepared food products, such as salads, using contaminated kitchen equipment can cause transmission [22].

Studying the human-to-human transmissibility of MRSA CC398 is hampered by the fact that the reservoir of MRSA CC398 is limited to the livestock agriculture setting, and that the majority of individuals working in this sector live on the farms together with their families, who mostly have direct animal contact themselves. Therefore, livestock veterinarians are an excellent group for studying human-to-human transmissibility of MRSA CC398 since their household members do not have direct contact with pigs or veal calves themselves.
The aim of this study was to determine the transmissibility of MRSA CC398 from livestock veterinarians to their household members compared to other MRSA strains in a community setting.

--- Materials and methods ---

Study design and setting

A one-year prospective cohort study was conducted in Dutch livestock veterinarians and their household members. Individuals were sampled for the presence of MRSA and methicillin-susceptible *S. aureus* (MSSA) in the anterior nares and oropharynx every four months (total study period July 2008 through December 2009). In addition, to compare transmissibility of MRSA CC398 strains with MRSA non-CC398 isolates, a cross-sectional survey was performed in MRSA-positive hospital-based patients and their household members.

Study population

Veterinarians associated with the Dutch Pig Health Department (VGV) were asked to participate in the study in April 2008. Control patients, defined as newly identified carriers of MRSA non-CC398, and their household members were recruited from a network of 16 hospitals and their affiliated microbiological laboratories between July 2009 and May 2011. Veterinarians and control patients were asked to take a nasal and oropharynx swab and to complete a questionnaire, which was used to determine the eligibility for the study. Subjects were eligible for participation if they (1) were aged between 18 and 65 years, (2) had one or more household members who were willing to participate, (3) did not live on a farm with pigs or veal calves, (4) did not have household members with professional pigs or veal calf contact, (5) were not treated for colonisation with MRSA in the previous three months, and (6) had provided written informed consent.

At baseline, veterinarians and control patients were visited at home and cultures were taken from the anterior nares and the oropharynx. Additional data were collected using a questionnaire that comprised information on age, gender, smoking, composition of the household, exposure to livestock, antibiotic treatment 4 months prior to sampling, and infections. Subsequently, veterinarians and their household members were asked to take nasal and oropharyngeal samples and complete a short questionnaire on the presence of active infections and antibiotic usage at 4, 8, and 12 months and return these by mail to the investigator. Appropriate transport material with Amies medium (Transwab®, Medical Wire
& Equipment), instructions for sampling and questionnaires were provided during the baseline home visit.

**Microbiological procedures**

Nasal and oropharyngeal samples were directly plated on chromID® *S. aureus* and chromID® MRSA agar plates (bioMérieux, La Balme, France), and subsequently placed in a Mueller–Hinton (MH) broth supplemented with 6.5% NaCl. The overnight MH broth was subcultured onto both chromID® *S. aureus* and chromID® MRSA agar plates. All agar plates were read after 18–24 h incubation at 35–37°C according to manufacturer’s instructions [23]. All cefoxitin resistant isolates were tested using a PCR for the presence of the *meca* and *nuc* gene [24-25]. All *S. aureus* strains were genotyped by staphylococcal protein A (*spa*) typing [26] and multiple-locus variable-number tandem repeat analysis (MLVA) as described previously [27]. MLVA types (MTs) were clustered using a categorical clustering coefficient and a minimum spanning tree was constructed to display the relationships between the various MTs.

**Definitions**

Subjects were considered positive when either a nasal or an oropharynx swab harboured MRSA or MSSA. Subjects that were MRSA MC398 positive at all four sampling moments were defined as persistent MRSA MC398 carriers. Subjects that yielded MRSA MC398 in one to three samples out of all samples were defined as intermittent MRSA MC398 carriers and subjects that did not have any positive sample during the one-year study were defined as MRSA MC398 non-carriers.

A transmission event was confirmed when MRSA isolates of the same MLVA type were detected in veterinarians and in their household members on a specific sampling moment. A dyad was defined as a set of two household members, which could be MRSA positive or negative.

**Statistical analyses**

All analyses were performed using SPSS 19.0 for Windows (SPSS Inc. Chicago, IL, USA). Differences in continuous variables between groups were tested with Student’s t-test or Mann-Whitney U test when applicable and differences in categorical variables between groups were tested with the Pearson Chi-square test. Univariate backwards analysis for MRSA carriage were performed in a generalized estimated equations (GEE) model using a
Poisson distribution with robust covariance estimators to calculate prevalence risk ratios (PRR) [28] with 95% confidence interval (95% CI).

Results

Enrolment

Veterinarians

Two hundred and twenty-five of 361 (62%) veterinarians responded and were examined for their eligibility. One-hundred thirty-seven (61%) veterinarians were eligible and included in the one-year follow-up study (Figure 1). These veterinarians had a total of 389 household members (mean number of household members per veterinarian 2.8 persons).

Figure 1  Flow chart of the recruitment of livestock veterinarians and control patients
Control patients

A total of 279 newly identified MRSA patients were reported to the central laboratory. After assessment of the inclusion – and exclusion criteria, 87 (31%) eligible patients were available (Figure 1). After consultation by the investigator, 31 (36%) control patients were included and visited at home. Ten control patients that were visited at home were found to be MRSA-negative, and one control patient carried MRSA MC398. These eleven control patients were excluded from the analysis. A total of 20 (65%) control patients harboured MRSA non-MC398 and these subjects were included for the analysis. These patients had a total of 41 household members (mean number of household members per patient 2.1 persons).

Results of MLVA and spa-typing

In total, 4246 samples were analysed in this study with the following distribution: 1086 samples from a total of 137 veterinarians and 3036 cultures originating from 389 household members. In addition, there were 42 samples from 21 control patients and 82 cultures originating from their household members. Throughout the study, only 5 out of 548 (response rate 99.1%) sampling events from veterinarians and 38 out of 1556 (response rate 97.6%) of their household members were not received. Sensitivity analysis on the effect of missing samples had no relevant consequences on the conclusions (data not shown).

A total of 1790 isolates were genotyped by spa-typing and MLVA: 365 MRSA and 211 MSSA strains from veterinarians, 84 MRSA and 1097 MSSA strains from household members of a veterinarian, 21 MRSA strains from control patients and 12 MRSA strains from household members of a control patient. In total, 341 different MTs belonging to 24 different MLVA complexes (MC) were found among the S. aureus isolates (Figure 2). Thirteen isolates (39%) from control patients and their household members had MCs that belonged to hospital-associated MRSA (HA-MRSA) strains. Two isolates (6%) were placed into MRSA MC398 and 18 isolates (45%) clustered into community-acquired MRSA (CA-MRSA). Eleven of these strains (33%) were Panton-Valentine leukocidin (PVL) positive. In contrast, none of the S. aureus MC398 strains originated from veterinarians and their household members were PVL-positive. Two-hundred twenty-two (12.4%) isolates did not belong to a known MLVA complex.
Figure 2  Genotypic relatedness of 482 MRSA (A) and 1308 MSSA (B) isolates derived from livestock veterinarians (green), household members of veterinarians (red), control patients (blue) and household members of control patients (yellow), represented as a minimum spanning tree based on MLVA types (MT). Clustering of MLVA profiles was obtained using a categorical coefficient to create a minimum spanning tree in which the MLVA types are displayed as circles. The size of each circle indicates the number of isolates with this particular type. MLVA complexes were previously assigned if 2 neighbouring types did not differ in more than 1 variable number tandem repeat (VNTR) locus and if at least 5 neighbouring types fulfilled this criterion. MLVA complexes are indicated in characters e.g. MC398 denotes MLVA complex 398.
The distribution of the different spa-types found in *S. aureus* MC398 isolates among veterinarians and their household members are summarized in Table 1. Two dominant spa-types, t011 and t108, accounted for 84% of all MRSA MC398 isolates and 4 spa-types were found only once. There were 54 MSSA isolates that belong to MC398; 42 isolates were derived from veterinarians (78%) and 12 isolates were recovered from household members (22%). Most of the MSSA strains had spa-type t034 (57%) or t081 (20%), which is in large contrast to the MRSA strains, where 9/430 (2.1%) MRSA MC398 isolates had spa-type t034 and none had spa-type t081.

**Table 1** Distribution of spa-types of MRSA MC398 isolates (n=430) and MSSA MC398 (n = 54) derived from livestock veterinarians and household members during the one-year study period

<table>
<thead>
<tr>
<th>Spa-type</th>
<th>No. of MRSA MC398 isolates (%)</th>
<th>No. of MSSA MC398 isolates (%)</th>
<th>p-value[a]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Veterinarians</td>
<td>Household members</td>
</tr>
<tr>
<td>t011</td>
<td>241 (56.0%)</td>
<td>210</td>
<td>31</td>
</tr>
<tr>
<td>t108</td>
<td>121 (28.1%)</td>
<td>99</td>
<td>22</td>
</tr>
<tr>
<td>t567</td>
<td>17 (4.0%)</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>t1184</td>
<td>14 (3.3%)</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>t081</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>t034</td>
<td>9 (2.1%)</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>t1456</td>
<td>9 (2.1%)</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>t571</td>
<td>5 (1.2%)</td>
<td>5</td>
<td>none</td>
</tr>
<tr>
<td>t1451</td>
<td>3 (0.7%)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>t8333</td>
<td>3 (0.7%)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>t899</td>
<td>2 (0.5%)</td>
<td>2</td>
<td>none</td>
</tr>
<tr>
<td>t3479</td>
<td>2 (0.5%)</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>t4652</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>t1606, t2287, t4628, t6606</td>
<td>1 each (0.2%)</td>
<td>1 each</td>
<td>none</td>
</tr>
<tr>
<td>t5902</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Total</td>
<td>430 (100%)</td>
<td>355</td>
<td>75</td>
</tr>
</tbody>
</table>

[a] Proportions of MRSA MC398 isolates vs proportions of MSSA MC398 isolates.

*A p-value ≤0.05 was considered statistically significant.*
Carriage of MRSA MC398 among veterinarians and their household members

Table 2 depicts the distribution of all MRSA and MSSA isolates that belong to MLVA complex 398 or non-MC398 isolates found in nasal and oropharynx samples derived from veterinarians and their household members. From the 576 *S. aureus* isolates derived from veterinarians 365 isolates were MRSA (63%), while the 1181 *S. aureus* strains isolated from household members only 84 strains (7.1%) were methicillin-resistant. The vast majority of MRSA strains recovered from veterinarians (97%) and household members (89%) belonged to MC398. The mean prevalence of MRSA MC398 carriage among veterinarians was 44% (range 41.6–46.0%) and that of their household members was 4.0% (range 2.8–4.7%).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Assignment to MLVA complex 398 (MC398), non-MC398 or no complex of 449 MRSA and 1308 MSSA isolates derived from livestock veterinarians and household members during the one-year study period</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Veterinarians (n = 137)</strong></td>
<td><strong>Mean prevalence % (range)</strong></td>
</tr>
<tr>
<td>MRSA isolates</td>
<td>44.6 (43.1–47.1)</td>
</tr>
<tr>
<td>MSSA isolates</td>
<td>26.3 (22.1–29.2)</td>
</tr>
<tr>
<td>All <em>S. aureus</em> isolates</td>
<td>70.9 (69.1–73.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Household members (n = 389)</strong></th>
<th><strong>Mean prevalence % (range)</strong></th>
<th><strong>MC398 n(%)</strong></th>
<th><strong>non-MC398 n(%)</strong></th>
<th><strong>no complex n(%)</strong></th>
<th><strong>Total</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA isolates</td>
<td>4.4 (3.1-5.0)</td>
<td>75 (89.3)</td>
<td>9 (10.7)</td>
<td>0 (0)</td>
<td>84</td>
</tr>
<tr>
<td>MSSA isolates</td>
<td>48.6 (45.9–50.3)</td>
<td>12 (1.1)</td>
<td>912 (83.1)</td>
<td>173 (15.8)</td>
<td>1097</td>
</tr>
<tr>
<td>All <em>S. aureus</em> isolates</td>
<td>53.0 (50.9–54.8)</td>
<td>87 (7.4)</td>
<td>921 (78.0)</td>
<td>173 (14.6)</td>
<td>1181</td>
</tr>
</tbody>
</table>

Table 3 shows the carrier state of veterinarians and their household members during the one-year study period. Two veterinarians had samples positive with MRSA non-MC398 strains and were excluded for further analysis. Forty veterinarians had four MRSA-positive test results (30%) and were defined as persistent MRSA MC398 carriers. Furthermore, 41 (30%) veterinarians were intermittent MRSA MC398 carriers and 54 (40%) veterinarians never carried MRSA. Altogether 36 from 386 non-exposed household members (9.3%), originating from 28 families (20.4%) carried MRSA MC398 intermittently. The prevalence of MRSA MC398 carriage in household members was statistically significantly higher for veterinarians with persistent MRSA MC398 carriage as compared to veterinarians with MRSA MC398 non-carriage (PRR 9.3; 95% CI 2.8–38.5) as well as veterinarians with intermittent MRSA.
MC398 carriage (PRR 2.1; 95% CI 1.0–4.6). MLVA genotyping data were not taken into account here. Results of confirmed transmission events are depicted in Table 3.

Table 3  Carriage of MRSA MC398 and transmission events among livestock veterinarians and their household members during the one-year study period

<table>
<thead>
<tr>
<th></th>
<th>Total n(%)</th>
<th>Persistent MRSA MC398 carriage n(%)</th>
<th>Intermittent MRSA MC398 carriage n(%)</th>
<th>Non-carriers n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veterinarians</td>
<td>135 (100)</td>
<td>40 (29.6)</td>
<td>41 (30.4)</td>
<td>54 (40.0)</td>
</tr>
<tr>
<td>Household members</td>
<td>386</td>
<td>123</td>
<td>114</td>
<td>149</td>
</tr>
<tr>
<td>MRSA carriers among household members</td>
<td>36 (9.3)</td>
<td>23 (18.7)</td>
<td>10 (8.8)</td>
<td>3 (2.0)</td>
</tr>
<tr>
<td>Families with a transmission events</td>
<td>28 (20.7)</td>
<td>16 (40.0)</td>
<td>9 (22.0)</td>
<td>3 (5.6)</td>
</tr>
<tr>
<td>Confirmed transmission events to household membersa</td>
<td>31 (8.0)</td>
<td>22 (17.9)</td>
<td>9 (7.9)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

a A confirmed transmission event was defined as that veterinarian and household members were both MRSA-positive during one sampling moment with the same MLVA type

Transmissibility of MRSA MC398 compared to other MRSA strains

The prevalence of MRSA carriage among household members of livestock veterinarians and control patients was measured. The baseline characteristics of veterinarians and control patients are depicted in Table 4. There were significant differences between the two groups: gender, educational level, ethnicity, age, number of household members, and number of companion animals. The 59 veterinarians that were found MRSA MC398 positive at the initial sampling moment had a total of 180 household members. During the home visit 8 household members (4.4%) harboured MRSA MC398 with the same MLVA type as the index veterinarian, i.e. 4 partners and 4 children. This result was compared with the prevalence of MRSA non-MC398 in household members of 20 control patients, which had a total of 41 household members. Eleven household members (26.8%) carried MRSA non-MC398 with the same MTs as the control patients during the home visit. The MRSA MC398 prevalence in household members of veterinarians was significantly lower than the MRSA non-MC398 prevalence in household members of control patients (8/180 vs. 11/41, p < 0.001). Hence, the transmission rate of MRSA non-MC398 strains is significantly higher than that of MRSA MC398 strains (PRR 6.0; 95% CI 2.4–15.5).
Table 4  Characteristics of livestock veterinarians with MRSA MC398 carriage and control patients with MRSA non-MC398 carriage at the initial sampling moment

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Veterinarians (n = 59)</th>
<th>Control patients (n = 20)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age – median (IQR)</td>
<td>47.0 (41.0–52.0)</td>
<td>39.0 (24.3–57.8)</td>
<td>0.295</td>
</tr>
<tr>
<td>Male sex - no. (%)</td>
<td>55/59 (93.2)</td>
<td>7/20 (35.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking - no. (%)</td>
<td>10/58 (17.2)</td>
<td>4/15 (26.7)</td>
<td>0.467</td>
</tr>
<tr>
<td>Educational levela – median (IQR)</td>
<td>6.9 (7.0–7.0)</td>
<td>3.6 (2.0–5.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Born in the Netherlands - no. (%)</td>
<td>55/59 (93.2)</td>
<td>15/20 (75.0)</td>
<td>0.023</td>
</tr>
<tr>
<td>Age of household members – median (IQR)</td>
<td>16.0 (8.3–39.0)</td>
<td>34.0 (15.0–52.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of household members – median (IQR)</td>
<td>3.0 (2.0–4.0)</td>
<td>1.5 (1.0–3.0)</td>
<td>0.006</td>
</tr>
<tr>
<td>Number of companion animalsb – median (IQR)</td>
<td>1.0 (0.8–2.0)</td>
<td>0.0 (0.0–1.0)</td>
<td>0.023</td>
</tr>
</tbody>
</table>

IQR: interquartile range (p25–p75); no.: number
A p-value < 0.05 was considered statistically significant

a Highest educational level is a bachelor or master title and was valued with a maximum of 7
b Total amount of cats and dogs in the household

The MRSA MC398 prevalence per family was 13.6% for veterinarians and 45% for control patients (8/59 vs. 9/20, p = 0.009). At family level, the transmission rate of MRSA non-MC398 strains to other household members is significantly higher in comparison with MRSA MC398 (PRR 3.3; 95% CI 1.3–8.0). In addition, household member pairs (dyads) colonised with the same MLVA complex were identified to estimate the spread of different MRSA clades. In 59 families with MRSA MC398 colonisation, 8 MRSA MC398-positive member dyads out of 422 possible dyads (1.9%) were detected. By comparison, there were significant more concordant dyads among MRSA non-MC398 carriers (13/78 [16.7%], PRR 8.8; 95% CI 3.5–22.6).

Discussion

Our prospective cohort study demonstrates that the mean prevalence of MRSA MC398 colonisation among household members of livestock veterinarians is relatively high (4.0%). None of these families were living on a farm or raising livestock. In total, 36 household members (9.3%), originating from 28 families (20.4%), harboured MRSA MC398 at least once during the one-year study period. These data confirm the results from a previous study performed in Germany in which an MRSA CC398 prevalence of 9.0% among household members of veterinarians was reported [29]. In our study, the prevalence of MRSA MC398
carriage among household members was shown to be highly dependent on the carrier state of the veterinarian. In addition, the prevalence of MRSA among household members was significantly higher for control patients carrying MRSA non-MC398 strains than for veterinarians carrying MRSA MC398 (PRR 6.0; 95% CI 2.4–15.5). These data suggest that MRSA MC398 spread less easily from humans with professional livestock contact to their household members than other MRSA non-MC398 isolates in a community setting. A possible explanation for this reduced transmissibility is that MRSA CC398 originates in humans as MSSA [30], and then spread to livestock, where it subsequently acquired the SCC\_mec cassette and methicillin-resistance. The jump of CC398 was also accompanied by the loss of phage-carried human virulence genes, making this clade less adapted to humans. In a hospital setting, Wassenberg and colleagues found that MRSA CC398 was 5.0 times less transmissible than other healthcare-associated MRSA (HA-MRSA) strains [15]. In general, transmission of MRSA within families seems to be common [10–14]. Several other studies showed high prevalences of MRSA among household members of individuals with HA-MRSA strains [11, 14, 31-34].

Guidelines underlying the Search & Destroy policy have been adapted in the Netherlands since 2006 based on conclusions from a case-control study [8]. Humans that work with live pigs and veal calves were defined as new risk populations for MRSA carriage and are now actively screened when admitted to a hospital. These guidelines were revised in December 2012, and all household members of confirmed MRSA patients have to be screened for MRSA on hospital admission. At present, household members of livestock veterinarians are not screened upon admission to a hospital. However, this study showed that they have a relatively high MRSA carriage in comparison to the Dutch general population [35]. Consequently, we advocate that household members of MRSA-positive veterinarians should also be screened for the presence of MRSA carriage upon hospital admission.

Despite the reduced human-to-human transmissibility of MRSA CC398, there are a few recent studies indicating that MRSA CC398 might have spread into the general population. A recent study found that MRSA with no link to established risk factors for acquisition, so-called MRSA of unknown origin (MUO), has now emerged [36]. Two distinct genotypic MUO groups were distinguished: MUO CC398 (26%) and MUO non-CC398 (74%), which suggests spread of MUO CC398, not by direct contact with livestock (pigs, veal calves), but through human-to-human transmission or by incomplete cooking of meat, but also by consumption of simultaneously prepared food products, such as salads, using contaminated kitchen equipment [22]. Furthermore, there are observations that proximity of farms is a potential risk factor, even in absence of direct contact between humans and animals [19-21].
To our knowledge, this study is the largest detailed survey among household members of livestock veterinarians. Unlike previous studies on transmissibility of MRSA CC398 [15, 29, 37], we performed this survey among veterinarians and their household members in a community setting for a prolonged period. Moreover, household members of veterinarians did not have livestock contact themselves during the study period. Therefore, we could estimate the human-to-human transmissibility of MRSA CC398 in detail. Thus the finding of 4.0% of household members carrying MRSA MC398 very likely represents the frequency of intrafamilial transmission. There are, of course, other possibilities of acquisition such as from pets and/or from horses. In addition, a cross-sectional survey to determine the transmissibility of MRSA CC398 in comparison with MRSA non-CC398 in the community was conducted. Here, the same inclusion - and exclusion criteria were used for control patients and veterinarians. Finally, we performed genotyping of all recovered \( S. \) \emph{aureus} isolates, confirming the similarity between MRSA strains isolated from the veterinarians or control patients and their household members.

There are some limitations to our study. First, there was a large difference between response rate of veterinarians compared to control patients which may have caused selection bias. In addition, there were significant differences between gender, educational level and ethnicity, age and number of household members, and number of companion animals between control patients and veterinarians. However, to which extent these differences have influenced transmission rates remains unclear. Second, this study does not provide data on the exact transmission route to the household members (e.g. via direct physical contact or via contaminated household, i.e. doorknob, remote control, chairs, etc.). Third, MRSA CC398 isolates are hard to discriminate when using current molecular typing techniques, such as \emph{spa} -typing and MLVA. This hampers studies that investigate possible transmission events and outbreaks caused by this MRSA clade. Finally, a minor limitation is that the results are somewhat outdated because data collection started in August 2008. However, we believe that human-to-human transmissibility of MRSA CC398 has not changed much in 5 years. Since this was a prospective cohort study with one-year follow-up it was inevitable to report the results after several years.

In summary, MRSA CC398 colonisation was common among household members of livestock veterinarians and this was shown to be highly dependent on the carrier state of the veterinarian. Moreover, the transmissibility of MRSA CC398 in the community setting was found to be substantially lower than that of MRSA non-CC398 strains. Therefore, we believe that screening of household members of MRSA-positive veterinarians upon hospital admission is justified and that the current Dutch MRSA guidelines can be maintained. The present situation is a widespread resistant bacterium with an enormous reservoir in livestock. The impact of MRSA CC398 appears to be low at the moment. However, when MRSA CC398 acquires genetic elements harbouring virulence factors it may pose a significant public health problem in the future. Careful monitoring of the human-to-human transmissibility of MRSA CC398 is therefore important.
Acknowledgements

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Ethical approval and funding

This study was approved by the medical ethics committee of the St. Elisabeth Hospital in Tilburg, the Netherlands (protocol number 0749).

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Potential conflicts of interest

All authors declare not to have any conflict of interest concerning this article. Regarding other activities outside this paper, J.K. declares having received honoraria from Destiny Pharma, 3M, Cepheid, Phico Therapeutics, and Pfizer for consultancy tasks. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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with methicillin-resistant Staphylococcus aureus (MRSA) CC398 with and without exposure to 
Outbreak of methicillin-resistant
*Staphylococcus aureus* ST398 in a Dutch nursing home

Erwin Verkade
Thijs Bosch
Yvonne Hendriks
Jan Kluytmans

*Infect Control Hosp Epidemiol* 2012; 33:624–626
Abstract

We describe an outbreak of MRSA ST398 in a nursing home in the Netherlands. Seven residents and 4 healthcare workers (HCW) with MRSA were identified but 2 of the HCW carried other strains. This study demonstrates that MRSA ST398 can spread in nursing homes.

Introduction

Traditionally, methicillin-resistant *Staphylococcus aureus* (MRSA) has been considered a hospital-associated pathogen. Recently, MRSA has expanded its territory to the community causing severe infections in previously healthy persons all over the world [1]. In 2003, a new clone of MRSA was identified that was related to an extensive reservoir in pigs and veal calves [2, 3]. People who are in direct contact with pigs and veal calves have a high carriage rate of this MRSA (23% and 29%, respectively) [2, 4]. Using multi locus sequence typing (MLST) the vast majority of these strains belong to sequence type 398 (ST398). Transmission within families, as well as single cases of colonized healthcare workers, have been described [2, 5]. However, up to now there have been few reports of transmission of MRSA ST398 in health care settings. In the hospital setting MRSA ST398 is reported to be less transmissible than other MRSA types [6]. We describe an outbreak of MRSA ST398 in a nursing home.

Materials and methods

Setting

This is a prospective epidemiologic analysis of an outbreak of MRSA ST398 that occurred in a nursing home in the Netherlands from October 2010 to February 2011. The nursing home is located in the southeast of the Netherlands in a region with a high density of pig (~3,000 pigs per square kilometer). The nursing home consists of 3 separate wards, with a total of 51 residents living in individual units. Incident cases were defined as residents and healthcare workers with MRSA obtained from clinical cultures (ie, wound) or surveillance cultures (ie, anterior nares, throat and perineum).
Outbreak Investigation

In October 2010, MRSA was cultured from a wound on the leg of a resident. Subsequently, more extensive screening cultures of this resident were obtained in November 2010, which showed that he was also colonized in throat, nose, and perineum. At the same time, another resident of the same ward had a wound culture with MRSA-positive test results. Subsequent screening in December 2010 of contacts among residents and healthcare workers of this ward revealed additional residents and healthcare workers with MRSA. Because of the high prevalence of MRSA on this ward, a screening of the other 2 wards was performed in January 2011.

Infection Control Measures

According to the current national guidelines for the control of MRSA in nursing homes, transmission-based precautions were taken when there was physical contact with residents who carried MRSA. This means that gowns and gloves were worn when contact with the residents or their equipment was anticipated [7]. Also, instructions on hand hygiene were given. The healthcare workers who carried MRSA were temporarily suspended from work, and decolonization of all colonized subjects was initiated with mupirocin nasal ointment, chlorhexidine wash, and systemic treatment with clarithromycin and rifampicin.

Microbiologic Methods

Nose, throat, and perineum swab samples were taken from residents and healthcare workers. Samples were directly inoculated onto chromID® MRSA (bioMérieux SA, Craponne, France). In addition, broth enrichment containing Mueller-Hinton broth supplemented with 6.5% NaCl was inoculated using the same swabs. Directly as well as overnight enriched inoculated plates were read after 18-24 h incubation at 35-37°C.

From the 11 individuals that were found to harbour MRSA, 16 MRSA isolates were genotyped by staphylococcal protein A (spa) typing. In addition, all isolates were genotyped by pulsed-field gel electrophoresis (PFGE) using the restriction enzyme Cfr9I according to previously described methods [8].
Epidemiology of MRSA

The additional screening of the first ward in December 2010 revealed 3 residents and 1 healthcare worker with MRSA. Subsequent screening of the other 2 wards in January 2011 revealed another 2 residents and 3 healthcare workers that were colonized with MRSA. During the 2 months preceding the sampling, the 4 colonized healthcare workers had worked on all 3 wards and had been in contact with all residents. Altogether the rate of MRSA carriage within residents was 7 of 51 (13.7%). In healthcare workers this was 4 of 76 (5.3%).

In total, 6 of the 7 affected residents were successfully decolonized with a single course. However, one resident failed initial treatment and was treated again with the same regimen, which failed also. This resident had been living on a pig farm until recently and reported regular visits to his son at the pig farm. In contrast, none of the other residents had contact with livestock.

Two of the 4 colonized healthcare workers reported contact with livestock. Healthcare worker 1 lived on the grounds of a pig farm, but she only sporadically had contact with pigs herself. After receiving treatment she was recolonized within 1 month. Healthcare worker 2 lived on a veal calves farm, and she reported frequent contact with livestock. Eradication of colonization was not attempted in this healthcare worker due to the anticipated risk of recolonization. Healthcare worker 3 who did not have livestock contact was successfully treated with mupirocin nasal ointment and chlorhexidine wash. At present, she has MRSA-negative test results for 3 months. Healthcare worker 4 who did not have livestock contact became MRSA negative without receiving any treatment. In March 2011, all healthcare workers and residents who had MRSA-positive test results were consecutively screened for the present of MRSA. Only the index case and the healthcare workers who had contact with livestock were still colonized with MRSA. All other healthcare workers and residents had MRSA-negative test results 3 times.

All isolated strains were resistant to tetracycline. The resistance profiles of all confirmed MRSA strains are depicted in Figure 1.
Molecular Typing

Relatedness of the MRSA strains was confirmed by PFGE with Cfr9I restriction digestion in 12 of the 16 isolates [8]. Only the MRSA isolates originating from the 2 healthcare workers who reported livestock contact carried MRSA that had a different PFGE cluster type (Figure 1). Strains can also be subdivided into 3 different resistance profiles. Each PFGE cluster corresponds to a unique resistance profile.

Moreover, spa-typing showed that 14 of the 16 strains were spa-type t011. Only the isolates originating from healthcare worker 1 were spa-type t108. Both spa-types are very frequently found within MRSA ST398.
**Figure 1** Dendrogram of the PFGE data from sixteen MRSA ST398 isolates. Next to the dendrogram the PFGE of Cfr macrorestriction fragments, host, sample date, spa-type, PFGE cluster type and antibiotic resistance patterns are given.

<table>
<thead>
<tr>
<th>Resident/HCW No.</th>
<th>Date</th>
<th>Spa-Type</th>
<th>PFGE cluster</th>
<th>te</th>
<th>tr</th>
<th>er</th>
<th>cl</th>
<th>to</th>
<th>ci</th>
<th>va</th>
<th>ri</th>
<th>fu</th>
<th>li</th>
<th>mu</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCW 1</td>
<td>Dec 2010</td>
<td>t108 A2</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>HCW 1</td>
<td>Feb 2011</td>
<td>t108 A2</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Resident 1</td>
<td>Oct 2010</td>
<td>t011 A</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Resident 4</td>
<td>Dec 2010</td>
<td>t011 A</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Resident 5</td>
<td>Dec 2010</td>
<td>t011 A</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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</tr>
<tr>
<td>Resident 3</td>
<td>Dec 2010</td>
<td>t011 A</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>HCW 3</td>
<td>Jan 2011</td>
<td>t011 A</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
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</tr>
<tr>
<td>Resident 7</td>
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<td>t011 A</td>
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<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
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<td>S</td>
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<tr>
<td>Resident 6</td>
<td>Jan 2011</td>
<td>t011 A</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
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<td>S</td>
<td>S</td>
<td>S</td>
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</tr>
<tr>
<td>HCW 4</td>
<td>Jan 2011</td>
<td>t011 A</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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</tr>
<tr>
<td>Resident 1</td>
<td>Nov 2010</td>
<td>t011 A</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
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</tr>
<tr>
<td>Resident 2</td>
<td>Nov 2010</td>
<td>t011 A</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
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<td>S</td>
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</tr>
<tr>
<td>Resident 3</td>
<td>Jan 2011</td>
<td>t011 A</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
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<tr>
<td>Resident 3</td>
<td>Feb 2011</td>
<td>t011 A</td>
<td>R</td>
<td>R</td>
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<td>Apr 2009</td>
<td>t011 B</td>
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HCW = healthcare worker, S = sensitive, R = resistant, I = intermediate sensitivity, te = tetracyclin, tr = trimethoprim/sulfamethoxazole, er = erythromycin, cl = clindamycin, to = tobramycin, ci = ciprofloxacin, va = vancomycin, ri = rifampicin, fu = fusidic acid, li = linezolid, mu = mupirocin
To date, only one outbreak of MRSA ST398 in a Dutch hospital has been reported [9]. We report the first outbreak to our knowledge of MRSA ST398 in a nursing home that comprised 7 residents and 2 healthcare workers. The MRSA strain responsible for this outbreak was spa-type t011, which belongs to MLST type ST398. The most likely source for this outbreak was the 98-year-old male resident number 3. The index case had been living on a pig farm until recently, before he moved to the nursing home. He reported regular visits to his son at the pig farm. We assume that healthcare workers transmitted the outbreak strain to other residents because the index case did not have direct contact with the other MRSA-positive residents. Moreover, there was repeated intense physical contact between colonized healthcare workers and the index case due to his obesity and immobility. Furthermore, none of the other colonized residents had contact with pigs or veal calves.

Although we did not assess the compliance to hand hygiene of healthcare workers, this is generally low in nursing homes and may have contributed to the spread of MRSA. When the outbreak was detected, the importance of hand hygiene was communicated to all healthcare workers. Hand sanitizer dispensers were placed at the entrance of all patients’ rooms. By doing this the compliance to proper hand hygiene was probably increased. Two additional healthcare workers had MRSA-positive test results during the outbreak period, but they carried other strains. These healthcare workers reported contact with livestock and had worked for a long time in the nursing home. One of the healthcare workers that had contact with livestock had a similar spa-type of the outbreak-related strain, but the PFGE pattern was clearly different and also the resistance profile showed major differences. We concluded that they were not involved in this outbreak based on these differences. The MRSA ST398 strains isolated from these healthcare workers were not found in any other residents, who all had been screened. This suggests that healthcare workers who are colonized with MRSA ST398 and comply with proper hygiene precautions are not a significant risk for transmission. It is unclear if host adaptation of this animal-derived strain plays a role in its transmissibility.

In conclusion, several studies have demonstrated that transmissibility of MRSA ST398 is probably lower than hospital-associated MRSA strains [5, 6]. However, this outbreak of MRSA ST398 in a community setting shows that substantial human-to-human transmission can occur. Further adaptation to humans may occur, and if MRSA ST398 can successfully spread from human to human, it may pose a significant public health problem in the future. Therefore, careful monitoring of the evolution and epidemiology of MRSA ST398 is important.
Acknowledgements

Potential conflicts of interest

All authors report no conflicts of interest relevant to this article. All authors submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and the conflicts that the editors consider relevant to this article are disclosed here.

Reference List

7. WIP guidelines. MRSA in nursing homes and homecare, 2007
High resolution typing by whole genome mapping enables discrimination of LA-MRSA (CC398) strains and identification of transmission events
After its emergence in 2003, a livestock-associated (LA-)MRSA clade (CC398) has caused an impressive increase in the number of isolates submitted for the Dutch national MRSA surveillance and now comprises 40% of all isolates. The currently used molecular typing techniques have limited discriminatory power for this MRSA clade, which hampers studies on the origin and transmission routes. Recently, a new molecular analysis technique named whole genome mapping was introduced. This method creates high-resolution, ordered whole genome restriction maps that may have potential for strain typing. In this study, we assessed and validated the capability of whole genome mapping to differentiate LA-MRSA isolates. Multiple validation experiments showed that whole genome mapping produced highly reproducible results. Assessment of the technique on two well-documented MRSA outbreaks showed that whole genome mapping was able to confirm one outbreak, but revealed major differences between the maps of a second, indicating that not all isolates belonged to this outbreak. Whole genome mapping of LA-MRSA isolates that were epidemiologically unlinked provided a much higher discriminatory power than spa-typing or MLVA. In contrast, maps created from LA-MRSA isolates obtained during a proven LA-MRSA outbreak were nearly indistinguishable showing that transmission of LA-MRSA can be detected by whole genome mapping. Finally, whole genome maps of LA-MRSA isolates originating from two unrelated veterinarians and their household members showed that veterinarians may carry and transmit different LA-MRSA strains at the same time. No such conclusions could be drawn based on spa-typing and MLVA. Although PFGE seems to be suitable for molecular typing of LA-MRSA, WGM provides a much higher discriminatory power. Furthermore, whole genome mapping can provide a comparison with other maps within 2 days after the bacterial culture is received, making it suitable to investigate transmission events and outbreaks caused by LA-MRSA.
Staphylococcus aureus and in particular methicillin resistant S. aureus (MRSA) is a bacterial pathogen that is associated with serious hospital- and community-acquired infections [1,2]. In the Netherlands, the incidence of MRSA infections is still low due to the restricted use of antibiotics and the successful implementation of the ‘search and destroy’ policy. However, the number of MRSA isolates sent to the national institute for public health and the environment (RIVM) in the context of the national MRSA surveillance, has been gradually increasing in the last years [3]. This increase is mainly caused by the emergence of a new single MRSA clade multi-locus sequence type ST398 originating from livestock, mainly pigs. ST398 was first described by Voss et al. in 2005 and since then ST398 has been found in numerous countries worldwide [4-8]. ST398 has been isolated from different types of domesticated animals and therefore is ST398 also known as livestock-associated MRSA (LA-MRSA) [9,10]. After the first reports in 2005, LA-MRSA (ST398) has spread very rapidly in the Netherlands and has become the predominant MRSA clade since 2007. In 2010, 38% of all isolates sent to the RIVM were LA-MRSA [3].

Typing of LA-MRSA however, has turned out to be a challenge. One of its characteristics is that LA-MRSA are non-typeable with regular pulsed-field gel electrophoresis (PFGE) due to methylation of the \( S\beta a \) recognition site [11]. In recent years, a number of reports have shown that PFGE with restriction enzyme \( Cfr91 \), a neoehizomer of \( S\beta a \), can be used to overcome the problems with DNA-methylation [12,13]. Although PFGE with \( Cfr91 \) yields a relatively high discriminatory power for LA-MRSA isolates, PFGE remains a time-consuming, laborious and non-portable method. Other typing techniques, such as staphylococcal protein A (\( spa \))-typing and multiple-locus variable number of tandem repeat analysis (MLVA), can be used to characterize LA-MRSA, but yield very limited discrimination within this clade. From 2008-2012, 17,869 MRSA isolates were characterized by \( spa \)-typing and MLVA within our national MRSA surveillance. The predominant MLVA complex was MC398, representing LA-MRSA and comprising of 7,066 isolates. Although 96 different \( spa \)-types and 78 different MLVA-types (MT) were found within this clade, the 2 dominant types, \( spa \)-type t011, MT398 (\( n = 4093 \)) and \( spa \)-type t108, MT572 (\( n = 1282 \)), accounted for 76% of all LA-MRSA isolates. In contrast, MCS (MRSA, \( n = 2520 \)), the most isolated clade after MC398, yielded 113 different \( spa \)-types and 205 different MLVA-types with \( spa \)-type t003, MT130 (\( n = 182 \)) and \( spa \)-type t002, MT5 (\( n = 126 \)) as the predominant types, but accounting only for 12% of all MC5 isolates. Strains within MC398 show limited variability and the absence of a highly discriminating typing method to characterize MC398 (LA-MRSA) isolates has hampered studies on the origin and transmission routes of this MRSA clade.
In 2007, a molecular analysis technique was introduced initially called optical mapping and now designated as whole genome mapping (WGM), although whole chromosome mapping would be more appropriate. A whole genome map is a high-resolution, ordered, whole genome restriction map and for *S. aureus* isolates these maps consists of 200–300 restriction fragments. In contrast, in PFGE of *S. aureus* only 10–15 non-ordered restriction fragments are used for the analysis [14,15]. Although Shukla et al. have previously successfully used WGM to characterize MRSA belonging to the USA300 clade [16], the number of reports in which WGM was used for molecular typing of bacterial pathogens is very limited [16-18].

In this study, we assessed and validated the capability of whole genome mapping to differentiate LA-MRSA isolates. For this purpose, we used epidemiologically related and non-related MRSA and LA-MRSA isolates.

**Materials and methods**

**Strain selection**

For this study a total of 18 MRSA and 45 LA-MRSA isolates were selected to create 84 different whole genome maps (WGMs). Two MRSA strains (NCTC8325, N315) and one LA-MRSA strain (S0385), which are often used as reference strains and for which published genomes are available [25-27], were used for reproducibility experiments and comparison of whole genome maps created in our laboratory with *in silico* maps. In addition, two LA-MRSA (MC398) isolated from Dutch veterinarians (VET78 (t = 0m) and VET35 (t = 0m)) were used for reproducibility experiments. The capability to identify transmission events was studied using isolates obtained during three well-documented outbreaks in the Netherlands of CA-MRSA (USA300), HA-MRSA (MC45) and LA-MRSA (MC398) and 22 LA-MRSA isolates from Dutch veterinarians and their household members [6,19,20]. Finally, 16 LA-MRSA isolates originating from a longitudinal survey of veterinarians frequently visiting livestock farms were used to investigate the discriminatory power of the whole genome mapping method for LA-MRSA (Table 1). All isolates used in this study originated from pre-existing collections and the isolates used to create WGMs were also characterized by MLVA, *spa*-typing and PFGE as described previously [21-24].
Table 1  Bacterial strains used in this study

<table>
<thead>
<tr>
<th>Experiment</th>
<th>MRSA strains</th>
<th>LA-MRSA strains</th>
<th>Reference</th>
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<tr>
<td>Re-assembly of raw data</td>
<td>NCTC8385</td>
<td>S0385</td>
<td>[25], [27]</td>
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<td>Optimal comparison settings</td>
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<td>VET78 (t = 0m)</td>
<td>[25], VET study</td>
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<td>VET78 (t = 0m), VET35 (t = 0m)</td>
<td>[25], [26], VET study</td>
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<tr>
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<td>S0385</td>
<td>[25], [26], [27]</td>
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<tr>
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<td>CA-MRSA (n = 8), HA-MRSA (n = 8)</td>
<td></td>
<td>[19], [20]</td>
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<tr>
<td>Discriminatory power for LA-MRSA</td>
<td>VET isolates (n = 16)</td>
<td>VET-study</td>
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<td>LA-MRSA transmission events</td>
<td>Transmission isolates (n = 4)</td>
<td>[6]</td>
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<tr>
<td>Suspected LA-MRSA transmission</td>
<td>VET isolates (n = 22)</td>
<td>VET-study</td>
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* VET study; isolates collected for a longitudinal MRSA carriage study among veterinarians and written consent was provided by all participants (E. Verkade personal communication).

Isolation of HMW DNA

Whole genome mapping requires the input of high molecular weight DNA (HMW DNA) with an average molecule size of approximately 250,000 bp. The Argus™ HMW DNA isolation kit (OpGen, Gaithersburg, USA) provides reagents and a protocol specifically designed for the isolation of HMW DNA. Briefly, a single colony was picked from a plate and suspended in cell wash buffer. Bacteria were treated to form spheroplasts and subsequently lysed to release the HMW DNA. For the isolation of HMW DNA of *S. aureus* this protocol required small but essential adaptations. First, we doubled the amount of lysostaphine (15 units/sample) used during the spheroplasting step and tripled the incubation time (3 hrs) recommended by the manufacturer. Furthermore, to obtain sufficient yield of HMW DNA for WGM we empirically determined that the isolated DNA required to relax and go into solution for at least 24 hrs at room temperature before proceeding to the dilution step. In our protocol, a 1:40 dilution was usually optimal for *S. aureus*. The quality (e.g. the average molecule size (AMS)) and the concentration of the DNA samples were checked using so-called Quality Control cards (Argus™ QCard kit, OpGen, Gaithersburg, USA). We found that for *S. aureus* a minimum of 5–10 DNA molecules of approximately 250,000 bp should be present per image in order to obtain good WGMs.
Creating whole genome maps

Whole genome maps were created using the manufacturer’s instructions. Shortly, HMW DNA was applied to Mapcards containing micro channels in which DNA molecules were stretched, bound to a glass surface, and subsequently digested with AflII and stained with a fluorescent agent in a micro fluids system. The restriction fragments were sized in the whole genome mapper and assembled into a whole genome map in which the restriction sites are mapped in the order in which they occur in the chromosome using MapManager software version 1.1 (OpGen). For assembly, only DNA molecules larger than 150,000 bp and with a minimum of 12 restriction sites were included. In a complete map, each assigned restriction site should have been found in at least 30 single molecules (coverage) and typically an average depth of 50 to 80 molecules is found. To assess whether assembly using the settings recommended by OpGen resulted in reproducible maps, the same raw data obtained from DNA of reference strains NCTC8325 and S0385 were assembled into WGMs 5 times each. Comparison of the generated maps revealed identical WGMs demonstrating the reproducibility of the assembly under the recommended conditions. After assembly, the generated restriction maps were imported into MapSolver software version 3.0 (OpGen, Gaithersburg, USA) to create the final circular whole genome map and without further manipulation the map was subsequently saved in .xml file format.

Analyzing whole genome maps

The .xml files containing the ordered maps were imported into a database created with an alpha version of BioNumerics version 7.0 (Applied Maths, Sint-Martens-Latem, Belgium). For clustering and alignment of the maps, we chose the first restriction fragment after the origin of replication in the chromosome as the starting point of the map, using a map rotation plugin in BioNumerics. For rotation of LA-MRSA maps, the in silico whole genome map of LA-MRSA strain S0385 (AM990992) was used as a template and for regular MRSA strains an in silico map of NCTC8325 (CP000253) was utilized. The BioNumerics software allowed alignment and clustering of WGMs using filtering of small fragments and size tolerance settings. The background on the algorithms used for the comparisons of whole genome maps in BioNumerics will be described in detail elsewhere. In the alignment, two fragments were considered to be identical if their sizes differed no more than the value set for the absolute tolerance. If this criterion was not met, two fragments were still considered identical if they met the relative tolerance criterion. This relative tolerance is defined as the difference between two fragments dived by their average; (size fragment 1 - size fragment 2) / ((size fragment 1 + size fragment 2)/2). The similarity between the whole genome maps was calculated by dividing the number of matched fragments by the total number of fragments. The method we chose for cluster analysis was UPGMA. In the BioNumerics software WGMs are represented as linear maps displaying the fragments in randomly chosen colours, with matching fragments sharing the same colour. Fragments of maps that were excluded from the
comparison as a result from the fragment filtering were not deleted from the maps, but displayed as blocks with reduced height in the maps.

Results

Assessing optimal settings for comparisons

The whole genome mapping encompasses procedures in which the restriction fragments are sized and subsequently assembled into ordered restriction maps. However, there is experimental variation in the sizing and also smaller restriction fragments may variably be lost during the procedure. The reason for this loss is that smaller DNA fragments have a relatively low net charge and as a result a weaker bond to the glass surface of the Mapcard. To compensate for both the variation in sizing and the presence or absence of smaller fragments during clustering and alignment, tolerance and filtering were employed in the BioNumerics software. In order to assess the optimal filtering and tolerance settings, DNA isolated from a MRSA and a LA-MRSA isolate was repeatedly used to create WGMs on 4 consecutive days and analyzed in BioNumerics using an range of filtering and tolerance settings. A combination of a filtering setting that excluded fragments <3,000 bp from the comparison and a relative tolerance of 15% combined with an absolute tolerance of 1,000 bp resulted in maps with a similarity of >99% for replicates of the DNA samples, whilst unrelated samples yielded distinct maps. Settings that were more stringent resulted in artificial differences between maps of the replicates while less stringent settings resulted in loss of the ability to discriminate unrelated samples.

Stability of whole genome maps and comparison with in silico maps

To assess the temporal stability of MRSA genomes as reflected in whole genome maps under laboratory conditions, single colonies from 2 LA-MRSA and 2 MRSA isolates were subcultured for 30 consecutive days. The similarity between the WGMs obtained from the DNA isolated on day 1 and day 30 ranged between 99.4% and 100%, showing that under laboratory conditions the MRSA genome was stable enough to yield virtually identical maps.

To determine to what extend WGMs accurately reflect the composition of a whole genome sequence, maps obtained from NCTC8325 (MRSA) [25], N315 (MRSA) [26] and S0385 (LA-MRSA) [27] were compared to their in silico counterparts generated in the Mapsolver software. The similarity between the WGMs created in the laboratory and their in silico counterparts varied between 95.5% and 98.7% for these isolates. Close inspection of the
differences between the real WGMs and the in silico maps revealed that in general, sizing of the restriction fragments by the whole genome mapper in general results in slightly smaller fragments than those predicted based on the whole genome sequences. Furthermore, there were several locations where the composition of the predicted maps clearly differed from the maps generated in the laboratory (Figure 1).

**Figure 1** Examples of differences between the WGMs created in the lab and their in-silico counterparts. The figure shows details of the comparisons of the maps of LA-MRSA strain S0385 and MRSA strains N315 and NCTC8325. The WGMs are represented as linear maps displaying the fragments in randomly chosen colours, with matching fragments sharing the same colour.

**Capability of WGM to identify transmission events of MRSA**

To determine whether whole genome mapping is capable of identifying transmission events, two earlier reported MRSA outbreaks were investigated. The first outbreak comprised 8 community-acquired MRSA (CA-MRSA) isolates obtained during an outbreak in a Dutch beauty salon in 2006 [19]. According to the authors of this study, PFGE showed that, all isolates belonged to the so-called USA300 cluster and had indistinguishable PFGE banding patterns. However, renewed inspection of the PFGE profiles during our study revealed that 5 isolates had identical PFGE profiles, but 3 isolates had an additional band of approximately 80 kb. Molecular typing characterized all isolates as Panton-Valentine Leukocidin (PVL)-positive, _spa_-type t024 and MT308 (MC8). The whole genome maps of the isolates were also highly similar with a similarity of 97.9% between the most distinct maps. Remarkably, the 3 isolates that had an additional band in PFGE also carried an additional, approximately 40 kb DNA segment which was absent in the 5 other isolates (Figure 2).

Based on the results with the replicates, that yielded >99% similar profiles, and the result of the above described outbreak we chose to set the cut-off value at 98% for indistinguishable profiles. This will allow for the variation in WGMs due to the presence or absence of mobile elements.
Figure 2  Detail of the whole genome maps of an outbreak of CA-MRSA (USA300) showing an additional DNA segment in 3 isolates. All isolates had \textit{spa}-type t024 and MLVA-type MT308. The gel image on the right hand side shows the PFGE profiles with an additional 80 kb band in the lower 3 isolates.

The second set of isolates were presumed hospital-acquired MRSA (HA-MRSA) and originated from an outbreak in two large medical care facilities in the Netherlands that started in 2001 and persisted for a period of 20 months [20]. Since many MRSA were isolated during this outbreak, we randomly selected 8 isolates with identical genotypes (\textit{spa}-type t445 and MT512 (MC45) and indistinguishable PFGE) from one facility for whole genome mapping. Although the WGMs of 4 of the isolates were closely related with similarities of >98.5%, there were major differences with the maps of the other isolates resulting in only 90.4% similarity between the most distinct maps (Figure 3).

Figure 3  Detail of whole genome maps showing differences between HA-MRSA outbreak isolates obtained from a large medical care center in the Netherlands. Based on molecular typing (\textit{spa}-typing, MLVA and PFGE) all isolates were indistinguishable.

Discriminatory power of whole genome mapping for LA-MRSA

LA-MRSA isolates obtained from 16 epidemiologically unrelated veterinarians frequently visiting livestock farms were subjected to molecular typing, including whole genome mapping. \textit{Spa}-typing and MLVA could hardly discriminate these isolates yielding only 5 different \textit{spa}- and 4 MLVA-types caused by variations in the number of \textit{spa}-repeats only. In contrast, whole genome mapping was able to discriminate these isolates and the average similarity between maps ranging from 77.0% to 98.3%. However, 2 of the 16 WGMs were nearly identical, with a similarity of 99.6%. Considerable variation was seen in the SCC\textit{mec} region of these isolates with 11 different compositions of the SCC\textit{mec} region among the 16 isolates (Figure 4).
Figure 4  Detail of the WGMs of 16 LA-MRSA isolates originating from unrelated veterinarians showing
the discriminatory power of whole genome mapping. The limited variation obtained by MLVA- and spa-
typing is displayed on the right hand side of the WGMs. The blowup of the WGMs displays considerable
variation in the SCC.mec region.

Capability to identify transmission events of LA-MRSA

We investigated the capability of whole genome mapping to identify LA-MRSA transmission
events using LA-MRSA isolates obtained during a LA-MRSA outbreak that occurred in 2004
[6]. The 4 isolates used in the study presented here were obtained from a pig farmer’s family
and originated from a mother suffering from LA-MRSA mastitis, from the infant that she
nursed, from the farmer who is the father of the child and from one of the pigs that were
sampled during the study. All isolates were spa-type t108, MT572 and PFGE profiles were
identical. The WGMs of the 4 isolates were virtually indistinguishable yielding a similarity of
99.1%, showing that the transmission event within this family could be confirmed by WGM
(Figure 5).
**Figure 5** Complete WGMs of LA-MRSA isolates obtained from a confirmed transmission event. The 4 isolates represent 3 household members and 1 isolate originated from a pig on the farm. All isolates were identical in PFGE, spa-typing and MLVA.

**WGM to study suspected LA-MRSA transmission events**

A set of samples originating from two families of epidemiologically unrelated veterinarians frequently visiting livestock farms that were screened for MRSA carriage on 5 time points during a fourteen-month survey period were used to assess the capability of WGM to track transmission of LA-MRSA from veterinarians to their household members. After the first screening ($t = 0$), sampling took place during home visits after 2 months ($t = 2m$) and by the veterinarians themselves after 6, 10 and 14 months ($t = 6m, t = 10m, t = 14m$). During this study period, 24 LA-MRSA isolates were cultured from the two families. Thirteen LA-MRSA isolates were obtained from veterinarian VET45 and his household members, while 11 isolates were cultured from veterinarian VET66 and his household members. Spa-typing and MLVA characterized 21 of 24 isolates as spa-type t011 and MT398 (MC398). The spa and MLVA profiles from the 3 other isolates, all originating from the family of VET66, differed only slightly from the 21 other isolates. PFGE yielded indistinguishable banding patterns for 11 of the 13 isolates obtained from the family of VET45 and the profiles of the other 2 isolates, differed from that of the 11 isolates in a single band and in 2 bands, respectively. Nine of the 11 isolates from VET66 and his household members had identical PFGE patterns, while the remaining 2 isolates were clearly different. The PFGE profiles of the two families represented two distinct groups, which was corroborated by WGM. Within the isolates from VET45 and his household members, two different WGM-clusters were identified. The first cluster (cluster A) was comprised of 8 isolates with a similarity of 97.9% between the most distinct WGMs and 6 of the maps were more than 99.5% similar. The second cluster (cluster B) was comprised of WGMs from 4 isolates and was distinct from the first cluster with a similarity between the first and second cluster of 93.3%. PFGE profiles of 11 of the 12 isolates belonging to these clusters were identical, showing the high discriminatory power of WGM. The remaining WGM obtained from the first isolate ($t = 0m$) cultured from VET45, was quite distinct and did not belong to cluster A or cluster B. This isolate also had a distinct PFGE.
profile, indicating that the veterinarian carried a different strain at this time. Within the 11 LA-MRSA isolates obtained from the second family (VET66), the maps of 9 isolates were nearly identical with a similarity of 96.2% between the most distinct maps within this cluster (cluster C). These 9 isolates were also indistinguishable with PFGE. The remaining 2 isolates differed considerably in all typing analyses (Figure 6).

Figure 6  Detail of the WGMs of two veterinarians and their household members showing transmission events. A and B denote the clusters with highly similar WGMs of isolates obtained from VET45 and his household members (light red block). C denotes the cluster with highly similar WGMs of isolates obtained from VET66 and his household members (blue block). Sampling time-points, sampling sites, spa-type, MLVA-type and PFGE-type are indicated on the right hand side of the maps. The PFGE-type numbers are arbitrary numbers.

The sample from veterinarian VET66 taken at t = 0m contained LA-MRSA with a WGM that was identical to those found for the LA-MRSA from the same veterinarian up to 14 months later. At sampling points from 6 to 14 months this LA-MRSA was also isolated from the household members at sampling moments 6 to 14 months. Remarkably, the veterinarian apparently carried 2 distinct LA-MRSA strains in nose and throat at the 6 month sampling point and it was the strain found in the nose that had been transmitted to its household members. In the family of VET45, WGM suggested that transmission with 2 different strains had taken place at different time points. The first strain (cluster A) was isolated from VET45 and his household members at the 6 months sampling point and thereafter. The second strain (cluster B) was isolated from VET45 at the 2 months (nose) and 10 months sampling point (nose and throat). This strain was also found in contact45.1 (HHM45.1) at 10 months (throat), but remarkably, a different strain was isolated from the same contact at the same sample moment, albeit in a different anatomic site (nose) (Figure 6).
LA-MRSA isolates are hard to discriminate when using current molecular typing techniques, such as spa-typing, MLST and MLVA. Although the PFGE using Cfr9I provides a much better differentiation of MC398 isolates this method is laborious and yields data that are not easily electronically exchanged. This hampers the study of possible transmission events and outbreaks caused by this MRSA clade. The whole genome mapping presented here provides a typing method with high discriminatory power that appears to be suitable to identify LA-MRSA transmission events.

The discriminatory power of WGM was illustrated by the ability to type and differentiate LA-MRSA isolates obtained from epidemiologically unrelated veterinarians frequently visiting livestock farms for which spa- and MLVA-typing failed to provide clear distinction. Among these unrelated isolates many different compositions of the SCCmec region existed indicating that variation in this locus significantly contributes to observed genomic diversity among LA-MRSA. Furthermore, WGM was able to confirm well documented CA-MRSA, HA-MRSA and LA-MRSA transmission events. In the first outbreak, involving transmission of a USA300 strain in a beauty salon, WGM identified a DNA segment of approximately 40 kb present in only 3 of the 8 isolates. Although this was not reported as such in the original paper, the additional fragment was also detected by PFGE. This additional fragment most likely represents a bacteriophage which usually has a genome size of approximately 40 kb and has the ability to jump in and out of bacterial genomes [28]. This shows that variation may occur within the same strain by gain or loss of mobile elements. Such events will lead to very localized changes whereas differences between distinct strains are the result of various genetic events and therefore in general occur scattered throughout the chromosome. This is an important criterion to decide whether two isolates may represent the same strain and thus might indicate the occurrence of a transmission event.

WGM of isolates presumably belonging to a HA-MRSA outbreak in the Netherlands revealed that several isolates did not belong to the outbreak. Although, this multi-center outbreak expanded over a long period of time and involved many different patients, the isolates we selected for this study originated from one center and were received within 2 weeks of each other. We believe that we were unable to assign all isolates as part of the outbreak because multiple strains yielding the same molecular characteristics (spa-typing, MLVA and PFGE) were circulating at the time of the outbreak. Indeed in 2002, 19% (n = 265) of all MRSA isolates sent to the RIVM had the PFGE-type that was identified as the outbreak-type. Of these isolates one-third originated from health care centers other than the two that were identified as the outbreak centers. This shows that the higher discriminatory power of WGM makes it possible to better assess whether isolates belong to an outbreak or not.
We employed WGM to assess whether the technique is suitable to identify transmission events of LA-MRSA in a community setting (i.e. transmission from veterinarians to their household members). Indeed, we obtained virtually identical WGMs of the isolates obtained from the veterinarians and their household members. However, not all isolates were identical and two different clusters were identified among the isolates of one veterinarian and his household members. These results suggest that veterinarians may pick up different LA-MRSA strains during their visits to animal farms and become colonized for a longer time period. Apparently, veterinarians may carry different LA-MRSA strains in their nose and throat at the same time and both may be transmitted to their direct contacts. We conclude that WGM now enables us to identify transmission events of LA-MRSA which would be impossible using spa-typing or MLVA and with much more uncertainty when using PFGE. We are currently conducting WGM of isolates obtained from a larger number of veterinarians and their household members to study LA-MRSA transmission among this group in further detail.

Based on the comparisons made of replicates of both MRSA and LA-MRSA isolates in this report and allowing for the presence of occasional mobile elements we now consider MRSA isolates with WGMs that have similarities of 98% or higher as indistinguishable. Isolates with WGMs with similarities between 95–98% may represent the same strain or should be regarded as highly related strains and those with maps that have similarities below 95% are deemed different strains. These cut-off values are supported by a recent report of Shukla et al. in which WGM of *S. aureus* was described and where a map distance of 5% was chosen as a cut-off point to define a WGM cluster [16]. This cut-off value was used for WGM of clonally related USA300 MRSA isolates using *Xba*I as restriction enzyme, but seems to be valid for LA-MRSA and other restriction enzymes as well.

We do acknowledge that based on our cut-off criteria the *in silico* maps and the maps created in our lab would not be designated as identical as would be expected. Although showing good concordance, we found inconsistencies in the size, number and order of fragments between the WGMs created in the lab and the *in silico* maps. The fragments present in the *in silico* maps were generally larger, but that should have been compensated for by the tolerance settings. The subcultures of the reference strains from which DNA was isolated to generate the whole genome sequences which were used to create the *in silico* maps and the subcultures used in our lab to create the real WGMs were not identical. Since the isolates used for comparison of *in silico* and real maps may have been subcultured for many years in various laboratories, changes in the genome may have occurred over time and this might explain for some of the observed differences. Another possible explanation could be that either the whole genome sequences of the isolates used for generating *in silico* maps contain sequence errors or that the assembly of the WGMs is inaccurate. However, the reproducibility of the WGM procedure assessed by repeated mapping of the same DNA sample on 4 consecutive days turned out to be excellent. To exclude that subculture of the reference isolates caused the observed differences we are currently assessing the complete
Molecular typing is used to characterize pathogens like MRSA in order to provide evidence that will support epidemiological studies on transmission and outbreaks. Furthermore, it is used to study changes in bacterial population structures e.g. to assess the effects of human intervention such as widespread antibiotic treatment and vaccination. WGM seems to be suitable to type LA-MRSA and identify transmission events where existing typing methods usually fail. An alternative that was not included in the analysis, but is rapidly gaining interest as typing tool, is whole genome sequencing (WGS). WGS has many advantages over WGM e.g. it will be very difficult to infer phylogenetic relationships and assess population structures using WGM, whereas WGS is very well suited for these purposes. Furthermore, sequencing can potentially reveal all details on gene composition, the presence of virulence factors, etc. Although we believe that WGS is the ultimate typing method, there may be a number of drawbacks for outbreak investigations leaving a niche for methods like WGM. WGS has been suggested as the best tool for typing during outbreak investigations and a number of papers have been published supporting this claim [29-32]. However, these studies are all retrospective investigations and they do not yet show the utility of real time WGS. Due to the relatively simple data analysis, WGM can provide a comparison with other maps within 2 days after the bacterial culture has been received, making it suitable to investigate real time transmission events and outbreaks involving pathogens such as LA-MRSA. Furthermore, the generated WGM data can be stored as a table containing the ordered restriction fragments e.g. in .xml-format and therefore are easy accessible, allowing users to quickly exchange data and compare isolates.

......Acknowledgements

The authors would like to thank W. K. van der Zwaluw (RIVM) and S. Kuiling (RIVM) for their contribution in collecting the whole genome mapping data. We would also like to thank G. N. Pluister (RIVM) for her technical assistance in the laboratory and K. Knecht (Applied Maths) for his valuable contributions to the development of the BioNumerics software. Finally, we thank T. Wagner (OpGen) for his much-appreciated advice during the optimization of the whole genome mapping method.


Morbidity associated by S. aureus carriage

Virulence of LA-MRSA
Morbidity associated with *Staphylococcus aureus* carriage in livestock veterinarians: a prospective cohort study

Erwin Verkade
Marjolein Kluytmans-van der Bergh
Birgit van Benthem
Brigitte van Cleef
Miranda van Rijen
Jan Kluytmans

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**Abstract**

**Background**

The purpose of this prospective cohort study was to determine the morbidity associated with *Staphylococcus aureus* carriage in livestock veterinarians.

**Methods**

A two-year prospective cohort study was conducted in 137 livestock veterinarians who were sampled for the presence of *S. aureus* every four months during the first year and again one year later. Furthermore, they completed a questionnaire on the presence of active infections and antibiotic usage at 4, 8, 12, and 24 months.

**Results**

Sixty-five (47%) veterinarians persistently carried *S. aureus* (MRSA CC398 or MSSA) during the study period, 57 (42%) carried *S. aureus* intermittently and 15 (11%) were defined as *S. aureus* non-carriers. Persistent *S. aureus* carriers reported more skin and soft tissue infections (SSTIs) than intermittent *S. aureus* carriers (RR 4.66; 95% CI 1.39–19.7) and *S. aureus* non-carriers (RR 3.30; 95% CI 0.81–∞; p = 0.058). Also, *S. aureus* carriage at a given sampling moment was significantly associated with the development of SSTIs within the following 4 months compared with *S. aureus* non-carriage (RR 1.42; 95% CI 1.30–1.56; p <0.001). No difference in SSTI rates was observed between MRSA CC398 and MSSA carriers.

**Conclusions**

Livestock veterinarians have extremely high *S. aureus* carriage rates. *S. aureus* carriers reported significantly more SSTIs than non-carriers. Likewise, the SSTI rates for MRSA CC398 and MSSA carriers were comparable. The overall incidence of SSTI in veterinarians is enlarged, mainly due to the increased *S. aureus* carrier rates rather than methicillin-resistance.
Introduction

The epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) has changed significantly over the past 15–20 years. Being initially a nosocomial pathogen, during the last decades new clones have emerged in the community, leading to infections in relatively young and healthy individuals lacking contact with healthcare [1]. More recently, a specific clone of MRSA with clonal complex (CC) 398 emerged, which has spread extensively in animal food production, and is therefore called livestock-associated MRSA (LA-MRSA) [2]. Epidemiological investigations have shown that MRSA CC398 does not only colonize livestock, but is able to cross the species barrier resulting in transmission to humans with direct livestock contact. This may occasionally result in the development of an infection [3, 4]. A spectrum of infections with MRSA CC398 have been documented, ranging from relatively minor or localized infections, including abscesses [5–7], various skin and soft tissue infections (SSTIs) [8–10], urinary tract infections [6], wound infections [6, 11], mastitis [12], and conjunctivitis [13], as well as more serious or invasive infections, including bacteremia [6, 14, 15], endocarditis [16], pneumonia [9, 11] (including necrotizing pneumonia [17]), osteomyelitis [9, 13], pyomyositis [7], and surgical site infections [10]. Despite the diverse array of infection types reported, it has been suggested that MRSA CC398 is less virulent than other MRSA strains in hospitalized patients. However, the morbidity associated with livestock-associated MRSA CC398 carriage in relatively healthy individuals is largely unknown. Considering the huge spread of MRSA CC398 among persons with livestock contact, there is a need to monitor the occurrence of infections caused by MRSA CC398 in the community. The purpose of the present study was to determine the morbidity associated with MRSA CC398 carriage in Dutch livestock veterinarians in a prospective cohort study.

Materials and methods

Study design and setting

A two-year prospective cohort study was conducted in Dutch livestock veterinarians who work mainly with pigs and veal calves. Veterinarians were recruited from April until December 2008, and followed for two years after enrolment. This study was approved by the medical ethics committee of the St Elisabeth Hospital in Tilburg, the Netherlands (protocol number 0749).
Study population

In April 2008, all veterinarians associated with the Pig Health Department (approximately 95% of Dutch swine veterinarians) of the Royal Dutch Veterinary Society were invited by mail to participate in the study and asked to complete a questionnaire to determine the eligibility for the study. Veterinarians were eligible for participation if they (1) were aged between 18 and 65 years, (2) had professional contact with pigs or veal calves at least once every 2 weeks in the previous year, (3) did not live on a farm with pigs or veal calves, (4) had not been treated for colonization with MRSA in the previous 3 months, and (5) had provided written informed consent.

Microbiological procedures

Veterinarians were asked to take nasal and oropharyngeal cultures in the morning before visiting the stables at baseline (0 months), and at 4, 8, 12, and 24 months. Appropriate transport material with Amies bediuβ (Transwab, Medical Wire & Equipment), instructions for sampling, and questionnaires were provided during the baseline home visit. Nasal and oropharyngeal samples were directly plated on chromID® *S. aureus* and chromID® MRSA agar plates (bioMérieux, La Balme, France), and subsequently placed in a Mueller–Hinton (MH) broth supplemented with 6.5% sodium chloride. The overnight MH broth was subcultured onto both chromID® *S. aureus* and chromID® MRSA agar plates. All agar plates were read after 18–24 hours of incubation at 35–37°C according to manufacturer’s instructions [18]. All cefoxitin resistant isolates were tested using a polymerase chain reaction for the presence of the *mecA* and *nuc* genes [19, 20]. All *S. aureus* isolates were genotyped by staphylococcal protein A (*spa*) typing [21] and multilocus variable number of tandem repeat analysis (MLVA) [22] and were stored at –80°C in the Microbank (Pro-Lab Diagnostics) preservation system.

Questionnaires

Data on background variables were collected during the home visit using a general questionnaire, which comprised questions on age, gender and contact with animals (which kind and how often). Furthermore, veterinarians were asked to complete a self-reported morbidity questionnaire on the presence of active infections and antibiotic usage at 4, 8, 12, and 24 months. The following variables were included in this questionnaire: allergy, eczema, impetigo, furunculosis, abscesses, common cold, pharyngitis, otitis, sinusitis, pneumonia, bacteraemia, bone or joint infections and antibiotic use. Comorbidity at the time of completion of the questionnaire was evaluated using a validated list of chronic conditions from the annual Dutch National Health Survey [23]. Major diseases such as lung diseases
(COPD), diabetes mellitus, myocardial infarction, stroke, and malignancy were included in this list.

Definitions

Determination of the MRSA and MSSA carrier state was based on the five sampling moments during the two-year study period (0, 4, 8, 12, and 24 months after inclusion). For each sampling moment, veterinarians were considered MRSA or MSSA-positive when either the nasal or oropharyngeal swab harbored MRSA or MSSA. Persistent carriers were defined as persons with five sampling moments positive for MRSA or MSSA, non-carriers had no positive cultures for that specific isolate, and intermittent carriers were the remaining persons. SSTI was defined as having impetigo, furunculosis or abscesses. Upper respiratory tract infection (URTI) was defined as having a common cold, pharyngitis, otitis or sinusitis. Finally, invasive infections were defined as having a bacteraemia, bone or joint infection.

Statistical analyses

All analyses were performed using SPSS 19.0 for Windows (SPSS Inc Chicago, Illinois). Differences between carrier state groups were tested by means of the Student t test in case of continuous variables. Where the distribution of scores deviated from normality, nonparametric methods were used (Mann-Whitney U test). Categorical variables were compared using the Fisher Exact test. Results were reported as relative risks (RR) with 95 percent confidence interval (95% CI). The statistical tests were two-tailed and \( P \leq .05 \) was considered statistically significant.

The carrier state of a given sampling moment (0, 4 and 8 months) was used as a possible predictor for the development of infections during the next 4 months of follow-up (4, 8 and 12 months). This univariate analysis was performed in a generalized estimating equations (GEE) model using a Poisson distribution with robust covariance estimators, to control for violation of the distribution assumption that the variance equals the mean, and an independent correlation matrix for the multiple samples per veterinarian (clustered data) [24]. In addition, when individuals reported having had a SSTI the next sampling moment was excluded from the analysis.
Results

Patient characteristics

One-hundred and thirty-seven veterinarians were included and followed for two years. Throughout the study, 11 of 685 (1.6%) potential samples were not received. Sensitivity analysis on the effect of missing samples did not change the conclusions (data not shown). A total of 131 (95.6%) veterinarians completed all morbidity questionnaires.

Sixty-five (47%) veterinarians carried *S. aureus* at all five sampling moments and were defined as persistent *S. aureus* carriers. Another 57 veterinarians (42%) carried *S. aureus* at 1 to 4 sampling moments and were defined as intermittent *S. aureus* carriers. Only 15 veterinarians (11%) never tested positive for *S. aureus* during the two-year study period, and were defined as *S. aureus* non-carriers. Population characteristics, including the frequency of contact with livestock during the two-year study period are shown in Table 1. Persistent *S. aureus* carriers were more frequently male than intermittent *S. aureus* carriers (63/65 vs. 48/57; *p* = 0.023). Age, smoking habits and contact with livestock were comparable for the three carrier state groups.

Population characteristics and frequencies of intense contact with pigs, veal calves, horses and poultry did not differ significantly between MRSA and MSSA carriers (data not shown).

Morbidity

*S. aureus* carrier state groups did not differ with respect to healthcare use, i.e. visiting the general practitioner, visits to the outpatient clinic, or hospital, including undergoing surgical procedures (Table 2). The presence of chronic disorders such as lung diseases (COPD), diabetes mellitus, myocardial infarct, stroke, and malignancies was comparable for all *S. aureus* carrier state groups (data not shown). The proportion of veterinarians reporting allergies, eczema, URTIs, or pneumonia during the two-year study period was not statistically different between the *S. aureus* carrier state groups. However, persistent *S. aureus* carriers reported more skin and soft tissue infections (SSTIs) than intermittent *S. aureus* carriers (RR 4.67; 95% CI 1.39–19.7; *p* = 0.005) and *S. aureus* non-carriers (RR 3.30; 95% CI 0.81–∞; *p* = 0.058), although the latter was not statistically significant (Table 2). In addition, persistent *S. aureus* carriers reported significantly more antibiotic use compared with intermittent *S. aureus* carriers (RR 1.75; 95% CI 1.00–3.17; *p* = 0.036).
Carriage of *S. aureus* at a given sampling moment was found to be associated with a higher probability of developing a SSTI in the following 4 months as compared with *S. aureus* non-carriage (RR 1.42; 95% CI 1.30–1.56; \( p < 0.001 \)). In order to assess the additive effect of methicillin-resistance on the morbidity, infections associated with MRSA CC398 carriage at a given sampling were compared with MSSA carriage. The SSTI rate was comparable for MRSA CC398 and MSSA carriers (RR 1.05; 95% CI 0.70–1.60).
### Table 1  Main characteristics of the veterinarians based on *S. aureus* carrier state during the two-year study period

<table>
<thead>
<tr>
<th>Population characteristics</th>
<th>Persistent <em>S. aureus</em> carriage</th>
<th>Intermittent <em>S. aureus</em> carriage</th>
<th><em>S. aureus</em> non-carriage</th>
<th>p-value* persistent vs. intermittent</th>
<th>p-value* persistent vs. non-carriage</th>
<th>p-value* intermittent vs. non-carriage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age – year (SD)</td>
<td>46.4 (8.0)</td>
<td>47.2 (8.6)</td>
<td>46.7 (8.3)</td>
<td>0.600</td>
<td>0.881</td>
<td>0.858</td>
</tr>
<tr>
<td>Male sex – no. (%)</td>
<td>63/65 (96.9)</td>
<td>48/57 (84.2)</td>
<td>15/15 (100)</td>
<td>0.023</td>
<td>1.00</td>
<td>0.189</td>
</tr>
<tr>
<td>Smoking - no. (%)</td>
<td>8/63 (12.7)</td>
<td>14/56 (25.0)</td>
<td>1/14 (7.1)</td>
<td>0.101</td>
<td>1.00</td>
<td>0.273</td>
</tr>
<tr>
<td>Contact with livestock</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than 6 months contact with pigs – no. (%)</td>
<td>51/63 (81.0)</td>
<td>46/56 (82.1)</td>
<td>10/14 (71.4)</td>
<td>1.00</td>
<td>0.472</td>
<td>0.457</td>
</tr>
<tr>
<td>More than 6 months with veal calves – no. (%)</td>
<td>7/63 (11.1)</td>
<td>4/56 (7.1)</td>
<td>0/14 (0.0)</td>
<td>0.537</td>
<td>0.338</td>
<td>0.577</td>
</tr>
<tr>
<td>More than 6 months with horses – no. (%)</td>
<td>10/63 (15.9)</td>
<td>14/56 (25.0)</td>
<td>4/14 (28.6)</td>
<td>0.256</td>
<td>0.270</td>
<td>0.745</td>
</tr>
<tr>
<td>More than 6 months contact with poultry – no. (%)</td>
<td>1/63 (1.6)</td>
<td>2/56 (3.6)</td>
<td>0/14 (0.0)</td>
<td>0.601</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*p-values were calculated using 2-tailed Fisher’s Exact tests. A p-value <0.05 was considered statistically significant*
Table 2  Morbidity associated with the *S. aureus* carrier state during the two-year study period

<table>
<thead>
<tr>
<th></th>
<th>Persistent <em>S. aureus</em> carriage</th>
<th>Intermittent <em>S. aureus</em> carriage</th>
<th><em>S. aureus</em> non-carriage</th>
<th>Relative Risk (95% CI)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Relative Risk (95% CI)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>p-value&lt;sup&gt;c&lt;/sup&gt; persistent vs. intermittent</th>
<th>p-value&lt;sup&gt;c&lt;/sup&gt; persistent vs. non-carriage</th>
<th>p-value&lt;sup&gt;c&lt;/sup&gt; intermittent vs. non-carriage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit to general practitioner - no. (%)</td>
<td>45/65 (69.2)</td>
<td>45/56 (80.4)</td>
<td>11/14 (78.6)</td>
<td>0.86 (0.71-1.09)</td>
<td>0.88 (0.70-1.44)</td>
<td>0.211</td>
<td>0.747</td>
<td>1.00</td>
</tr>
<tr>
<td>Visit to the outpatient clinic - no. (%)</td>
<td>33/65 (50.8)</td>
<td>27/56 (48.2)</td>
<td>8/13 (61.5)</td>
<td>1.05 (0.71-1.57)</td>
<td>0.83 (0.55-1.66)</td>
<td>0.856</td>
<td>0.553</td>
<td>0.540</td>
</tr>
<tr>
<td>Visit to the hospital - no. (%)</td>
<td>9/63 (14.3)</td>
<td>7/55 (12.7)</td>
<td>3/13 (23.1)</td>
<td>1.12 (0.41-3.19)</td>
<td>0.62 (0.19-2.76)</td>
<td>1.00</td>
<td>0.421</td>
<td>0.389</td>
</tr>
<tr>
<td>Surgical procedures - no. (%)</td>
<td>12/61 (19.7)</td>
<td>10/54 (18.5)</td>
<td>5/13 (38.5)</td>
<td>1.06 (0.46-2.48)</td>
<td>0.51 (0.22-1.52)</td>
<td>1.00</td>
<td>0.160</td>
<td>0.146</td>
</tr>
<tr>
<td>Allergies - no. (%)</td>
<td>16/63 (25.4)</td>
<td>19/57 (33.3)</td>
<td>3/13 (23.1)</td>
<td>0.76 (0.41-1.41)</td>
<td>1.10 (0.39-4.54)</td>
<td>0.422</td>
<td>1.00</td>
<td>0.742</td>
</tr>
<tr>
<td>Eczema - no. (%)</td>
<td>7/63 (11.1)</td>
<td>13/56 (23.2)</td>
<td>1/13 (7.7)</td>
<td>0.48 (0.18-1.19)</td>
<td>1.44 (0.21-31.1)</td>
<td>0.090</td>
<td>1.00</td>
<td>0.278</td>
</tr>
<tr>
<td>Skin and soft tissue infections - no. (%)</td>
<td>16/63 (25.4)</td>
<td>3/55 (5.5)</td>
<td>0/13 (0.0)</td>
<td>4.66 (1.39-19.7)</td>
<td>3.30 (0.81-∞)</td>
<td>0.005</td>
<td>0.058</td>
<td>1.00</td>
</tr>
<tr>
<td>Upper respiratory tract infections - no. (%)</td>
<td>54/65 (83.1)</td>
<td>52/57 (91.2)</td>
<td>15/15 (100.0)</td>
<td>0.91 (0.81-1.06)</td>
<td>0.83 (0.83-1.11)</td>
<td>0.282</td>
<td>0.113</td>
<td>0.577</td>
</tr>
<tr>
<td>Pneumonia - no. (%)</td>
<td>3/63 (4.8)</td>
<td>0/55 (0.0)</td>
<td>1/13 (7.7)</td>
<td>2.62 (0.40-∞)</td>
<td>0.62 (0.06-15.4)</td>
<td>0.247</td>
<td>0.536</td>
<td>0.191</td>
</tr>
<tr>
<td>Invasive infections - no. (%)</td>
<td>1/61 (1.6)</td>
<td>1/54 (1.9)</td>
<td>0/13 (0.0)</td>
<td>0.89 (0.02-32.2)</td>
<td>2.13 (0.01-∞)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Antibiotic use - no. (%)</td>
<td>28/63 (44.4)</td>
<td>14/55 (25.5)</td>
<td>7/13 (53.8)</td>
<td>1.75 (1.00-3.17)</td>
<td>0.83 (0.50-1.81)</td>
<td>0.036</td>
<td>0.558</td>
<td>0.091</td>
</tr>
</tbody>
</table>

<sup>a</sup> persistent *S. aureus* carriage versus intermittent *S. aureus* carriage

<sup>b</sup> persistent *S. aureus* carriage versus *S. aureus* non-carriage

<sup>c</sup> p-values were calculated using 2-tailed Fisher’s Exact tests. A p-value <0.05 was considered statistically significant
Our two-year prospective cohort study demonstrates that livestock veterinarians have extremely high *S. aureus* carriage rates and almost half carried *S. aureus* throughout the study period. In addition, persistent *S. aureus* carriers reported significantly more often the occurrence of a SSTI than intermittent *S. aureus* carriers and *S. aureus* non-carriers during the two-year study period, although the latter did not reach statistical significance. However, the above mentioned observation does not rule out that the SSTIs were responsible for the maintenance of the *S. aureus* carriage. Therefore, the development of infections associated with *S. aureus* carriage during the next 4 months of follow-up was analyzed. Carriage of *S. aureus* at a given sampling moment was found to be associated with a higher probability of developing a SSTI in the following 4 months as compared with *S. aureus* non-carriers. Comparison of the occurrence of medical events following carriage of MRSA CC398 and MSSA revealed no difference in the risk of developing SSTIs, indicating that the morbidity was caused by *S. aureus* carriage and not by the fact that these strains were methicillin-resistant.

A recent study [25] described an extremely high prevalence of *S. aureus* carriage (72%) among livestock veterinarians, compared to the general population [26]. The carriage rate of MSSA was comparable to the general population and MRSA came on top of that. This high *S. aureus* carriage rate places livestock veterinarians at a higher risk for the development of a SSTI compared to the general population and *S. aureus* carriage (MRSA CC398 and MSSA) could be seen as an occupational hazard among livestock veterinarians. So far, there are only few studies that described the occurrence of disease associated with livestock-associated MRSA CC398 versus other MRSA clones [27, 28] and none have been performed in a prospective cohort study. Köck and colleagues investigated the occurrence of MRSA CC398 from clinical and screening specimens derived from human patients treated in German hospitals, and by general practitioners. This study reported that apart from the detection of MRSA CC398 from screening samples, MRSA CC398 accounted for a significant proportion of MRSA isolates from clinical specimens including blood cultures (8%) and deep respiratory tract secretions (14%), clearly documenting that MRSA CC398 is able to cause severe human infections in hospitals [28].

Another recent study demonstrated that MRSA CC398 was not less pathogenic for humans than *S. aureus* in general [29]. Cuny et al. found that MRSA CC398 accounts for approximately 15% of all MRSA isolates from deep-seated SSTIs in the community and for about 0.8-2% of all MRSA isolated from clinical specimens obtained in hospital settings. When introduced into the hospital it can cause postoperative wound infections and even septicemia. Our study in a relatively healthy population showed two invasive bone or joint infections among livestock veterinarians during two-year follow-up, but no bacteraemia. However, the micro-organism responsible for these bone or joint infections was not known.
Morbidity associated by *S. aureus* carriage

Our prospective cohort study enables a quantitative assessment of the risk for development of infections. We clearly demonstrated that SSTIs are associated with *S. aureus* carriage but invasive infections are rare in livestock veterinarians.

Despite the diverse spectrum of infection types reported, it has been suggested that MRSA CC398 is less virulent than other human MRSA strains. MRSA CC398 strains generally lack virulence factors that are commonly detected in human *S. aureus* strains (PVL, TSST-1, enterotoxins) [30, 31]. However, some sporadic cases of PVL-producing MRSA CC398 have been described in Europe [32, 33]. So far, only few studies reported the presence of enterotoxin encoding genes in MRSA CC398 [30, 34]. As toxin genes may be present on mobile elements such as phages or plasmids, it is possible that isolates of MRSA CC398 may acquire such genes in the future. This could have consequences for the impact on morbidity of the CC398 lineage for humans. Recently, several phylogenetic studies support the existence of three subpopulations in the CC398 lineage: an ancestral clade that originated in humans as MSSA [3], a lineage composed predominantly of animal-related MRSA isolates [4], and one that emerged recently by clonal expansion in humans [35]. A strong increase over time in the prevalence of the ancestral MSSA CC398 strain in bloodstream infection was observed in several studies [14, 15, 33, 36]. Moreover, these MSSA CC398 bloodstream infections were diagnosed in patients lacking livestock exposure and are in general associated with MSSA CC398 *spa*-type t571 [14, 37]. The reservoir for these strains is not clear at the moment.

To the best of our knowledge, this investigation is the first long-term, prospective cohort study that described the morbidity associated with persistent MRSA CC398 as well as persistent *S. aureus* carriage among livestock veterinarians. A limitation is that we did not take samples from acute infections to confirm that the infections were actually caused by MRSA CC398. So the link between SSTI and carriage is an epidemiological association, which was not objectified by culture proven infections. Furthermore, a good explanation for the higher antibiotic use among persistent *S. aureus* carriers compared to intermittent *S. aureus* carriers is not easy to find. Although we conducted a prospective cohort study, a recall bias could not completely be excluded. Therefore, it remains unclear whether the increased antibiotic use is due to infections or that the antibiotic use has caused the prolonged *S. aureus* carriage.

In summary, we found that *S. aureus* (MRSA CC398 and MSSA) carriers had significantly more SSTIs than *S. aureus* non-carriers during a two-year study period among relatively healthy livestock veterinarians. The occurrence of medical events was comparable for MRSA CC398 and MSSA carriers. The impact of MRSA CC398 appears to be low at the moment. However, the extremely high carriage rates may pose a significant public health threat in the future if this clade acquires genetic elements that make them more virulent. Therefore, careful monitoring of the evolution and epidemiology of MRSA CC398 is important. Further research is required to characterize the full scope of the genetic changes associated with
increasing virulence. Cohorts, like the one described in this study, are useful to monitor the effect of genetic changes on the occurrence of disease in an early phase.

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Potential conflicts of interest

All authors declare not to have any conflict of interest concerning this article. Regarding other activities outside this paper, J.K. declares having received honoraria from Destiny Pharma, 3M, Cepheid, Phico Therapeutics, and Pfizer for consultancy tasks. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.
CHAPTER 13

General discussion and summary
For several decades, methicillin resistance in *S. aureus* has been considered a strictly human problem. Initially, it was confined to the healthcare sector but later it became a matter of concern in the general population too. This changed in 2005 with the isolation of MRSA CC398 from pigs and pig farmers in the Netherlands [1]. This unexpected discovery prompted research worldwide, showing the presence of MRSA CC398 in a variety of farm animals [2, 3], especially in production sectors characterized by intensive animal farming practices with high antibiotic use, being the pig, veal, dairy and broiler industry [4]. Persons in direct contact with affected animals frequently carry MRSA CC398 [5–8]. Considering the huge spread of MRSA CC398 among food production animals globally, it is unlikely that this will be eradicated easily. 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 MRSA CC398 it is essential to know the dynamics, transmissibility and virulence of this subtype of MRSA. The research described in this thesis aimed to gain more insight into dynamics of MRSA CC398 carriage and morbidity associated with MRSA CC398 carriage in livestock veterinarians. In addition, we aimed to determine the transmission of MRSA CC398 from livestock veterinarians to their household members and its determinants.

### Detection and susceptibility testing of MRSA CC398

A large diversity of antimicrobial resistance pheno- and genotypes has been observed in MRSA CC398 isolates worldwide [9, 10]. This diversity likely results from the enhanced capability of the CC398 lineage to acquire external DNA combined with the fact that MRSA CC398 isolates worldwide may have been subjected to different antimicrobial selection pressures. Consequently, a wide variety of resistance genes has been detected in MRSA CC398, including resistance genes that are typical for human and animal staphylococci. However, the vast majority of MRSA CC398 strains are relatively susceptible for most non-beta-lactam antibiotics, with the exception of tetracycline for MRSA CC398, for which they are almost always resistant. This is most likely due to the extensive use of this antimicrobial agent in animal husbandry. Likewise, zinc and other metals are frequently used in animal feed formulations and may co-select for MRSA CC398 strains that carry the czrC zinc resistance gene, as suggested previously [11]. This hypothesis is supported by the fact that the vast majority of MRSA CC398 strains carry SCCmec type Vc, which contains the czrC gene. Because tigecycline is a derivative of tetracycline, it is important to determine the activity of this new drug for MRSA CC398.
Tigecycline was shown to exhibit broad *in vitro* activity against a collection of MRSA strains collected in the Netherlands, including livestock-associated strains (chapter 2). Using the recommended methodology, we found three strains to be resistant. However, the results for tigecycline may be influenced by the concentration of manganese in the medium [12]. Additional testing showed that these strains were susceptible when Iso-Sensititest medium was used. This discrepancy warrants further investigations into the preferred test conditions because the interpretation of the *in vitro* susceptibility of tigecycline is affected significantly.

The extension of MRSA beyond its known boundaries poses an additional challenge for microbiological laboratories to improve their screening strategies. Methods to detect MRSA in clinical samples ideally should have a high sensitivity and specificity combined with a short time to reporting of the results. To identify *S. aureus* from contaminated samples more easily and reliably, selective media have been developed [13]. Chapter 3 describes the evaluation of the *in vitro* sensitivity and specificity of a new selective medium, called Oxoid *Brilliance*™ MRSA for the identification of MRSA, using a well-defined collection consisting of 266 MRSA strains, 257 methicillin-susceptible *S. aureus* (MSSA) strains, and 265 coagulase-negative staphylococci (CNS). Oxoid *Brilliance*™ MRSA was shown to be a highly reliable screening tool for the detection of MRSA.

Further clinical studies have been performed to evaluate different selective media for the detection of MRSA directly from clinical samples. Oxoid *Brilliance*™ MRSA Agar and bioMérieux chromID® MRSA medium were evaluated for their abilities to identify methicillin-resistant *Staphylococcus aureus* in clinical samples (chapter 4). Nasal and oropharyngeal samples (*n* = 629) were taken from veterinarians and their household members. The sensitivities of Oxoid *Brilliance*™ MRSA Agar and chromID® MRSA medium after 20 hour of incubation were 63.6 and 64.5%, and the specificities were 94.1 and 99.4%, respectively. After an enrichment step the sensitivities increased to 96.3 and 97.2%, but the specificity decreased to 88.7 and 98.5%, respectively. In another clinical evaluation described in this thesis, Bio-Rad MRSA *Select*™ medium was evaluated for its ability to recover MRSA from nasal samples of pig farmers and their household members (chapter 5). In total, 257 samples were inoculated on Bio-Rad MRSA *Select*™ medium with and without broth enrichment and on chromID® MRSA with broth enrichment. The sensitivity of Bio-Rad MRSA *Select*™ medium without broth enrichment was 63.9%. With broth enrichment, the sensitivity increased to 98.4%. The specificity was 95.4% both with and without broth enrichment. In conclusion, Oxoid *Brilliance*™ MRSA Agar, Bio-Rad MRSA *Select*™ medium and chromID® MRSA medium are all sensitive method for the screening of MRSA in combination with broth enrichment, but positive results require confirmation. Despite commercial efforts to develop new media with high sensitivity, the studies described in this thesis, as well as others [14, 15], showed a substantial increase in sensitivity following enrichment compared with direct cultures. Therefore, broth enrichment remains necessary for reliable MRSA detection.
A case-control study performed by van Loo et al. [16] showed that contact with pigs and veal calves as the major risk factor for MRSA CC398 carriage. This study lead to an amendment in the Dutch search and destroy policy in July 2006 which stated that patients who come into contact with live pigs or veal calves have to be isolated and screened upon hospital admission. Some years later, several Dutch studies report prevalences of positive farms varying from 23% to 81%, whereas the prevalence in individual pigs varies from 11 to 39% [17, 18]. In veal production, high prevalence of MRSA CC398 were found as well: 88% of the farms and 28% of the calves tested positive [7]. The extremely high MRSA CC398 prevalence among livestock results in very high carriage in humans in contact with these animals. Broens et al. have shown that pig herd prevalences rose from 30% in the beginning of 2007 to 75% in the end of 2008 [19]. Similarly, the prevalence of MRSA CC398 in pig farmers found in more recently published studies are higher compared to older studies on pig farmers: from 29% in 2007 [20] to 63% in 2010 (van Cleef et al. personal communication). In addition, the carriage rate of MRSA CC398 in livestock veterinarians as reported in chapter 8 is higher than any other studies on veterinarians that has been described to date (MRSA prevalences in veterinarians vary from 12.5% to 45% in various international studies [8, 21].

In order to assess how widespread the dissemination of this unusual type of MRSA in the general population was, we screened individuals that would have had the highest risk of carriage when MRSA CC398 would have been spread into the community. This 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. The results of this study are shown in chapter 6. Using a random mailing in 3 selected municipalities in the Netherlands, adult persons were asked to fill in a questionnaire and to take a nasal swab. In total, complete information was obtained on 583 persons. Of the 534 persons without livestock-contact only one person (0.2%; 95% CI <0.01–1.2) tested positive for MRSA. 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. All recovered MRSA strains had spa-types that belong to the known livestock-associated clone CC398 [22].

--- Origin and epidemiology of the CC398 lineage ---

Apart from the occurrence of livestock-associated MRSA CC398 among humans, there is a second epidemiological event (i.e. the emergence of MSSA CC398 infections) for which an epidemiological link to livestock is lacking. Microarrays and whole-genome sequencing approaches applied to a large number of CC398 isolates distinguished two clades within the
CC398 lineage [23–25]: the classical livestock-associated clade, isolates of which have long been responsible for frequent colonization, and rare infections among farmers and veterinarians [26]; and a human clade. This human clade is comprised of two subpopulations: the ancestral human subpopulation, and the emerging human-adapted non-LA CC398 subpopulation that has recently and increasingly been causing invasive infections worldwide in humans living in animal-free environments [24, 27, 28], and that readily colonize and spread between humans [23]. Price et al. suggested that livestock-associated MRSA CC398 was derived from a human MSSA CC398 lineage that only recently acquired mecA on repeated occasions when it disseminated into animal populations. The human-to-livestock jump was accompanied by the loss of immune evasion cluster (IEC) genes and the acquisition of tetracycline resistance due to tet(M).

The two clades of the CC398 lineage are characterized by different prophages. Livestock-associated CC398 isolates commonly carry φ2 and φ6 [25], or a φAvB prophage [24]. By contrast, isolates belonging to the human clade contain β-converting φ3 prophage variants that encode and express two human-specific virulence genes (chp and scn) [22, 23, 25]. There is now evidence that the emerging subpopulation differs from the ancestral human subpopulation by additional prophage features (φ1, φ2, φ5 or φ7) relevant to its epidemiology [23, 25]. More recently, the φMR11-like helper prophage has been described, that may facilitate the expression of the φ3 prophage virulence genes chp and scn [29].

This human-adapted CC398 subclone is now increasingly identified in hospitals. Also, a strong increase over time in the prevalence of S. aureus CC398 in bloodstream infection was observed in a recent study [27]. Seventeen of the 18 bloodstream infection isolates were methicillin-susceptible and none had the common pig-borne spa-types t011, t034 or t108. The mode of acquisition of the S. aureus CC398 isolates by the patients remains unclear. Moreover, all S. aureus CC398 bloodstream infections were diagnosed in patients lacking livestock exposure. These invasive infections are in general associated with MSSA CC398 spa-type t571 [16, 27, 30]. These results were confirmed by our study (chapter 7). In total, 612 consecutive episodes of S. aureus bloodstream infections (BSI) diagnosed before and during the emergence of CC398 were included. Three strains (2 MSSA and 1 MRSA) that were isolated from bacteremia patients between 2010–2011 were positive in a CC398 specific PCR. There was a marked increase in prevalence of S. aureus CC398 BSI isolated between 2010–2011 compared to the combined collections that were isolated between 1996–1998 and 2002–2005 (3/157, 1.9% vs. 0/455, 0.0%; \( p = 0.017 \)). We conclude that S. aureus CC398 might be an increasing cause of invasive staphylococcal disease. Our results are in line with other findings that certain S. aureus CC398 isolates, especially spa-type t571, can cause invasive infections in humans.
Dynamics of MRSA CC398 carriage

There is still debate about the dynamics of MRSA CC398 carriage in persons with regular contact to livestock. Question is whether an MRSA CC398 positive person is truly colonized or merely inhaled MRSA contaminated dust during work, without being truly colonized. This is an important distinction, because contamination with MRSA CC398 is less likely thought to lead to infection, and can probably easily be eliminated by a period of non-exposure. Cuny et al. [21] found an extreme high MRSA CC398 nasal colonization of 45% among livestock veterinarians. Carriage may be prolonged, as shown in a recent report from Germany, where the majority of pig farmers (59%) did not lose their MRSA CC398 carriage after the holidays [31]. Furthermore, when volunteers were actively colonized with methicillin-susceptible *S. aureus* (MSSA) CC398, they often carried it for prolonged periods [32]. We conducted a two-year prospective cohort study with 137 veterinarians that mainly work with pigs and veal calves (chapter 8). This study demonstrated a mean MRSA CC398 prevalence of 44% (range 42-46%). In total, 88 veterinarians (65%) carried MRSA CC398 at least once. Thirty-two veterinarians (23%) had MRSA-positive test results throughout the entire study period and 18 of those (56%, 13% of all veterinarians) had five identical MLVA types and can therefore be considered as true persistent MRSA CC398 carriers. On the other hand, there are several studies that stated that MRSA CC398 is not a good colonizer in humans. A study among field workers with short-term occupational exposure to pigs and veal calves suggested a high rate of transient contamination, without substantial persistent colonization [33]. Another study showed that MRSA prevalence among veal calf farmers was strongly reduced (58%) after absence of animal contact [10]. The reasons for these discordant findings are unclear and require further investigation.

We conclude that regular livestock contact can indeed lead to true persistent colonization with MRSA CC398. Veterinarians that mainly work with pigs and veal calves frequently carried the same strain for prolonged periods.

Human-to-human transmission is the main determinant for the spread through a human population. The magnitude of the public health threat depends mainly on this characteristic, in combination with strain virulence. Several recent studies have shown that MRSA CC398 was 4 to 6-fold less transmissible compared with HA-MRSA strains in a hospital-setting [34–36], limiting its impact on public health and justifying modified control measures. At present, the human-to-human transmissibility of MRSA CC398 in a community setting is still unclear. In addition, studying the human-to-human transmissibility of MRSA CC398 is hampered by
the fact that the reservoir of MRSA CC398 is restricted to humans having direct contact with livestock, and that the majority of individuals working in this sector live on the farms together with their families. The household members mostly have direct animal contact themselves. Therefore, livestock veterinarians are an excellent group for studying human-to-human transmissibility of MRSA CC398 since their household members do not have direct contact with pigs or veal calves themselves. We conducted a prospective cohort study in Dutch livestock veterinarians and their household members (chapter 9). Our study demonstrates a relatively high mean prevalence of MRSA CC398 colonization among household members of 4.0%. In total, 36 household members (9.3%), originating from 28 families (20.4%), harbored MRSA CC398 at least once during the one-year study period. These data confirm the results from a previous study performed in Germany in which an MRSA CC398 prevalence of 9.0% among household members of veterinarians was reported [21]. In our study, the prevalence of MRSA CC398 carriage among household members was shown to be highly dependent on the carrier state of the veterinarian.

In addition, to compare transmissibility of MRSA CC398 strains with other MRSA isolates, a cross-sectional survey was performed in MRSA-positive hospital-based patients and their household members. The prevalence of MRSA among household members was significantly higher for control patients carrying MRSA non-MC398 strains than for veterinarians carrying MRSA CC398 (PRR 6.0; 95% CI 2.4–15.5). These data suggest that MRSA CC398 spreads less easily from humans with professional livestock contact to their household members than other MRSA isolates in a community setting.

Considering the extensive reservoir in animals and people who work with livestock, the occurrence of MRSA CC398 in people who are not directly involved in farming is strikingly low. One of the first studies that examined the role of living in a livestock-dense region as a risk factor did not find it to be a risk factor (chapter 6). This cross-sectional survey found that only 0.2% of adult individuals without livestock contact were positive for MRSA CC398. Meanwhile, there are a few recent studies indicating that MRSA CC398 already have spread into the general population. The ways in which MRSA CC398 can be transmitted to humans without livestock contact are direct contact MRSA-positive individuals, environmental contamination [37–39], and eating or handling contaminated meat [40]. A recent study by Lekkerkerk et al. [41] found that MRSA with no link to established Dutch risk factors for acquisition, so-called MRSA of unknown origin (MUO), has now emerged. National MRSA surveillance data (2008–2009) were analyzed for epidemiological determinants and genotypic characteristics. A quarter (24%) of the 5545 MRSA isolates registered were MUO, i.e. not from defined risk groups. Two distinct genotypic MUO groups were distinguished: MUO CC398 (352; 26%) and MUO non-CC398 (998; 74%), which suggests spread of MUO CC398, not by direct contact with livestock (pigs, veal calves), but through other risk factors.
Transmission of MRSA CC398 within the healthcare setting has been observed in several studies. The first documented hospital-associated outbreak of MRSA CC398 occurred in the Netherlands [42]. The authors found five patients with MRSA CC398 colonization and/or infection. All strains belonged to CC398 and were spa-type t567. Likewise, an outbreak of MRSA CC398 in a nursing home in the Netherlands is described in chapter 10. Seven residents and two healthcare workers with MRSA CC398 were identified. The MRSA strain responsible for this outbreak was spa-type t011, which belong to CC398. The most likely source had been living on a pig farm until recently, before he moved to the nursing home. He reported regular visits to his son at the pig farm.

However, MRSA CC398 isolates are hard to discriminate when using current molecular typing techniques, such as spa-typing, MLST and MLVA [43]. Although the PFGE using Cfr9I provides a much better differentiation of CC398 isolates this method is laborious and yields data that are not easily electronically exchanged [44]. This hampers studies that investigate possible transmission events and outbreaks caused by this MRSA clade. The whole-genome mapping (WGM) described in chapter 11 provides a typing method with high discriminatory power that appears to be suitable to identify MRSA CC398 transmission events. The discriminatory power of WGM was illustrated by the ability to type and differentiate MRSA CC398 isolates obtained from epidemiologically unrelated veterinarians frequently visiting livestock farms for which spa- and MLVA-typing failed to provide clear distinction. Furthermore, WGM was performed to assess whether this technique was suitable to identify transmission events of MRSA CC398 in a community setting (i.e. transmission from veterinarians to their household members). Indeed, we obtained virtually identical WGMs of the isolates obtained from livestock veterinarians and their household members. We conclude that WGM now enables us to identify transmission events of MRSA CC398 which would be impossible using spa-typing or MLVA and with much more uncertainty when using PFGE. We are currently conducting WGM of isolates obtained from a larger number of veterinarians and their household members to study MRSA CC398 transmission among this group in further detail.

Virulence of MRSA CC398

The capacity of livestock-associated MRSA CC398 to cause infections in humans has been demonstrated, ranging from relatively minor or localized infections [34, 45-47], as well as more serious or invasive infections [30, chapter 6]. Despite the diverse array of infection types reported, it has been suggested that MRSA CC398 is less virulent than other human MRSA strains. Nevertheless, the exact morbidity associated with MRSA CC398 carriage in relatively healthy individuals is largely unknown. Considering the huge spread of MRSA
CC398 among persons with livestock contact, there is a need to monitor the occurrence of infections caused by MRSA CC398 in the community.

Our prospective cohort study demonstrates that persistent MRSA CC398 as well as persistent *S. aureus* carriers had significantly more skin and soft tissue infections (SSTIs) than *S. aureus* non-carriers during a two-year study period (chapter 12). Moreover, carriage of MRSA CC398 and MSSA at a given sampling moment were found to be associated with a higher chance of developing a SSTI in the next 4 months as compared with *S. aureus* non-carriers. Our data confirm the results of several studies that MRSA CC398 carriage mainly causes moderate to severe SSTIs [30, 45, 46, 48]. Another recent study demonstrated that MRSA CC398 was not less pathogenic for humans than *S. aureus* in general [49].

We found that the risk of having SSTIs during the study period among MRSA CC398 and MSSA carriers was comparable, indicating that the SSTIs were caused by *S. aureus* carriage alone and not by the fact that these strains were methicillin-resistant. Noteworthy, chapter 8 describes an extremely high mean prevalence of *S. aureus* carriage (72%) among livestock veterinarians, which is high compared to the general population [50]. Therefore, livestock veterinarians are more likely to develop a SSTI compared to the general population, and *S. aureus* carriage (MRSA CC398 and MSSA) can now be seen as an occupational hazard.

The previous paragraphs have shown that a public health threat may arise from livestock-associated MRSA CC398, which needs to be controlled. At present, in healthcare settings MRSA CC398 appears to be under control. The impact on public health of MRSA CC398 appears to be low at the moment, illustrated by the limited spread into the community and the minimal amount of serious invasive infections caused by MRSA CC398, as shown in chapters 6 and 7. While prevalences in livestock farming probably have increased close to a saturation point, MRSA CC398 seldom seems to cause serious problems in hospitals. Nonetheless, experts worry that the rapid evolution of MRSA CC398 may result in gaining new characteristics in the near future [23, 24], since MRSA CC398 has proven to be able to exchange many mobile genetic elements between strains, suggesting that this clade can rapidly adapt to changes [25, 51]. Also, a study based on whole-genome sequencing provided evidence that the clade of MRSA CC398 originated in humans, and lost some immune evasion genes when it entered the livestock population, while acquiring resistance genes. This creates worries about a possible reverse event (acquisition of virulence factors while maintaining resistance traits) from the immense reservoir that has been created in livestock. Indeed, there is now evidence from a recent study that livestock-associated MRSA CC398 is now readapting to humans [29]. In this study, hybridization of genomic DNA with
microarrays revealed no livestock-associated prophages remnants in the genomes of the ancestral human subpopulation. In contrast, they also demonstrate that the human-adapted isolates and the livestock-associated isolates share some prophage elements, suggesting an animal origin of the newly described \( \Phi \)MR11-like helper prophage. However, further investigations are required to confirm these findings.

\textit{Future research}

The two-year prospective cohort study described in chapters 8, 9 and 12 contains valuable epidemiology information. MRSA as well as MSSA isolates have been collected from veterinarians and their household members that had no livestock contact, and further molecular characterization with sophisticated typing methods can give more insight into the exact mechanisms of persistent carriage of MRSA CC398, higher transmissibility and virulence factors. We are currently monitoring a large number of MRSA and MSSA CC398 isolates using whole-genome sequencing to assess the risk for the emergence of a sustainable community reservoir for livestock-associated \textit{S. aureus} CC398.

In addition, we have identified several veterinarians that never were colonized with \textit{S. aureus} during the two-year study period despite an extremely high exposure to MRSA CC398 and MSSA in pig and veal calf stables. These individuals seem to be "immune" to colonization. Determination of the genetic characteristics and the microbiome of the nares of these non-carriers may result in targets for new drugs or vaccines to prevent colonization and subsequent infection.

To evaluate whether the MRSA CC398 prevalence in people without livestock contact is rising in an area with a high density of pig farms a cross-sectional survey can be performed once again after five years.

\textit{Conclusions}

The research in this thesis gained more insight into the detection, dynamics, transmissibility and virulence of MRSA CC398 in veterinarians with livestock contact, and contributed to a better understanding of the possible public health threat. The following main conclusions can be drawn from this thesis: (1) Oxoid \textit{Brilliance™} MRSA Agar, Bio-Rad MRSA \textit{Select™} medium and chromID® MRSA medium are all sensitive method for the screening of MRSA CC398 in combination with broth enrichment, but positive results require confirmation. Our studies showed a substantial increase in sensitivity following enrichment compared with direct
cultures. Therefore, broth enrichment are indispensable for reliable MRSA detection. (2) Regular livestock contact can indeed lead to true persistent colonization with MRSA CC398. Veterinarians that mainly work with pigs and veal calves frequently carried the same strain for prolonged periods. (3) A relatively high mean prevalence of MRSA CC398 colonization of 4.0% was found among household members. In addition, prevalence of MRSA CC398 carriage among household members was shown to be highly dependent on the carrier state of the veterinarian. Also, MRSA CC398 spread less easily from humans with professional livestock contact to their household members than other MRSA isolates in a community setting (RR 6.0; 95% CI 2.4–15.5). (4) The cross-sectional survey we performed in areas with an extremely high pig-density found that only 0.2% of adult individuals without livestock contact were positive for MRSA CC398, which is comparable to the carriage level of the general population. (5) Whole-genome mapping enables discrimination of MRSA CC398 and identification of transmission events, which would be impossible using spa-typing or MLVA and with much more uncertainty when using PFGE. (6) Veterinarians with persistent MRSA CC398 and persistent S. aureus carriers had significantly more skin and soft tissue infections than S. aureus non-carriers during a two-year study period.

Recommendations

The Dutch search and destroy policy was revised in December 2012, and all household members of confirmed MRSA patients have to be screened for MRSA on hospital admission. Nonetheless, household members of livestock veterinarians are not yet screened upon admission to a hospital. However, we showed that they have a relatively high MRSA carriage in comparison to the Dutch general population [50], independent of the MRSA state of the veterinarian. Consequently, we advocate that household members of all livestock veterinarians should also be screened for the presence of MRSA carriage upon hospital admission.

From the public health perspective, continuous surveillance of the virulence potential, antimicrobial resistance profiles and human colonization capacity of MRSA CC398 is strongly recommended. Simultaneous adaptation of CC398 to humans and animals would represent a considerable threat to public health, because of the huge CC398 reservoir in livestock, combined with the fact that this lineage seems to acquire foreign DNA quite easily.

When MRSA CC398 eventually will readapt to humans and can successfully spread from human to human it will constitute a significant public health problem in the near future. This will necessitate major adaptations of the existing guidelines for control in the community and in healthcare institutions. It is questionable if the current control strategy in the Netherlands can be maintained in this scenario. At least the associated costs will increase significantly.
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196  General discussion and summary


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Samenvatting en discussie
Samenvatting en discussie
Sinds enkele decennia wordt methicilline-resistentie bij _S. aureus_ gezien als een menselijk probleem. Vroeger was het beperkt tot de zorgsector, maar later werd het ook een probleem in de algemene bevolking. Dit veranderde in 2005 door veegerelateerde MRSA CC398 bij varkens en varkenshouders in Nederland. Deze onverwachte ontdekking gaf aanleiding tot een wereldwijd onderzoek en toonde MRSA CC398 in verschillende dieren aan, voornamelijk in de intensieve veehouderij met een hoog antibioticagebruik, zoals in de varkens-, vleeskalveren-, melkvee- en vleeskuikenindustrie. Mensen die direct contact hebben met besmette dieren, dragen vaak MRSA CC398. Gezien de wereldwijde verspreiding van MRSA CC398 onder voedseleproductie dieren, is het onwaarschijnlijk dat dit eenvoudig kan worden vermindert. De opkomst van MRSA CC398 vormt een bedreiging voor de succesvolle bestrijdingsstrategie in Nederland. Om de mogelijke gevolgen van MRSA CC398 te bepalen is het noodzakelijk de dynamiek, overdraagbaarheid en virulentie van deze MRSA kloon te onderzoeken. Het onderzoek in dit proefschrift heeft als doel inzicht te krijgen in de dynamiek en ziektelast van MRSA CC398 dragerschap bij dierenartsen. Daarnaast hebben we geprobeerd om de overdracht van MRSA CC398 van dierenartsen naar hun gezinsleden en de determinanten te bepalen.

Detectie en gevoeligheidsbepalingen van MRSA CC398

Bij MRSA CC398 isolaten is wereldwijd een grote diversiteit van feno- en genotypische antimicrobiële resistentie waargenomen. Deze verscheidenheid komt waarschijnlijk door de verhoogde capaciteit van de CC398 kloon om extern DNA op te nemen, gecombineerd met het feit dat MRSA CC398 isolaten wereldwijd zijn onderworpen aan verschillende antimicrobiële selectiedruk. Er zijn een groot aantal resistentiegenen in MRSA CC398 waargenomen, zoals genen die typerend zijn voor zowel menselijke en dierlijke stafylokokken. De overgrote meerderheid van de MRSA CC398 stammen zijn relatief gevoelig voor de meeste niet-beta-lactam antibiotica. Behalve tetracycline, waarvoor deze stammen bijna altijd resistent zijn. Dit komt waarschijnlijk door het uitgebreide gebruik van dit antibiotica in de veehouderij. Ook zijn zink en andere metalen vaak in diervoer gebruikt en hierdoor worden MRSA CC398 stammen die het czrC zink resistentiegen dragen uitgeselecteerd. Deze hypothese wordt ondersteund doordat de meeste MRSA CC398 stammen SCC_mec type Vc dragen die het czrC gen bevat. Omdat tigecycline een derivaat is van tetracycline, is het belangrijk om de activiteit van dit nieuwe geneesmiddel voor MRSA CC398 te bepalen.
In het onderzoek werd aangetoond dat tigecycline een brede *in vitro* activiteit heeft tegen een verzameling MRSA isolaten uit Nederland, waaronder veegeassocieerde stammen (hoofdstuk 2). Met de aanbevolen methode werden drie resistenten stammen gevonden. Echter, de resultaten voor tigecycline worden beïnvloed door de concentratie van mangaan in het medium. Aanvullend onderzoek toonde aan dat deze stammen gevoelig waren wanneer Iso-Sensitest medium werd gebruikt. Dit verschil verdient nader onderzoek naar de beste testomstandigheden omdat de interpretatie van de *in vitro* gevoeligheid voor tigecycline wezenlijk anders is.

De uitbreiding van MRSA buiten de bekende grenzen vormt een extra uitdaging voor microbiologische laboratoria om hun screeningstrategieën te verbeteren. De methoden om MRSA aan te tonen in klinische monsters hebben idealiter een hoge sensitiviteit en specificiteit in combinatie met een korte rapportagetijd. Er zijn selectieve medias ontwikkeld om *S. aureus* eenvoudiger en betrouwbaar uit verontreinigde monsters te identificeren. **Hoofdstuk 3** beschrijft de evaluatie van de *in vitro* sensitiviteit en specificiteit van het nieuwe selectieve medium Oxoid *Brilliance™* MRSA. Hiervoor werd een goed gedefinieerde verzameling gebruikt bestaande uit 266 MRSA stammen, 257 methicilline-gevoelige *S. aureus* (MSSA) stammen en 265 coagulase-negatieve stafylokokken (CNS). Oxoid *Brilliance™* MRSA bleek een zeer betrouwbare screeningsmethode te zijn voor de detectie van MRSA.

Er zijn meerdere klinische studies uitgevoerd om verschillende selectieve medias te evalueren voor de detectie van MRSA uit klinische monsters. Oxoid *Brilliance™* MRSA Agar en bioMérieux chromID® MRSA medium werden geëvalueerd op hun vermogen om methicilline-resistente *Staphylococcus aureus* te identificeren in klinische monsters (hoofdstuk 4). Er werden neus- en keelmonsters (*n* = 629) van dierenartsen en hun gezinsleden genomen. De sensitiviteit van Oxoid *Brilliance™* MRSA Agar en chromID® MRSA medium waren na 20 uur incubatie 63,6 en 64,5% en de specificiteit waren 94,1 en 99,4%. Na een ophopingsstap werd de sensitiviteit verhoogd tot 96,3 en 97,2%, maar de specificiteit daalde naar 88,7 en 98,5%. In een andere klinische evaluatie werd Bio-Rad MRSA.Select™ medium beoordeeld op haar vermogen om MRSA aan te tonen van neusmonster van varkenshouders en hun gezinsleden (hoofdstuk 5). In totaal werden 257 monsters geënt op Bio-Rad MRSA.Select™ medium met één zonder bouillonophoping en op chromID® MRSA met bouillonophoping. De sensitiviteit van Bio-Rad MRSA.Select™ medium zonder bouillonophoping was 63,9%. Met bouillonophoping werd de sensitiviteit verhoogd tot 98,4%. De specificiteit was 95,4% zowel met als zonder bouillonophoping. Oxoid *Brilliance™* MRSA Agar, Bio-Rad MRSA.Select™ medium en chromID® MRSA medium zijn allen gevoelige methoden voor het screenen van MRSA in combinatie met bouillonophoping. Positieve resultaten dienen nog wel bevestigd te worden. Ondanks de commerciële inspanningen om nieuwe media te ontwikkelen met een hoge sensitiviteit, toonden de studies in dit proefschrift, evenals anderen, een aanzienlijke toename van de sensitiviteit na ophoping in vergelijking met directe kwenen. Daarom blijft bouillonophoping noodzakelijk voor een betrouwbare detectie van MRSA.
Prevalentie van MRSA CC398

Uit een case-control studie van Loo et al. is gebleken dat direct contact met varkens en vleeskalveren de belangrijkste risicofactor is voor MRSA CC398 dragerschap. Deze studie leidde in juli 2006 tot een wijziging van het Nederlandse ‘search and destroy’ beleid. Hierin werd gesteld dat patiënten die in contact komen met levende varkens of vleeskalveren bij een ziekenhuisopname gescreeën en geïsoleerd dienen te worden. Enkele jaren later rapporteerden diverse Nederlandse studies MRSA CC398 prevalenties van positieve bedrijven variërend van 23% tot 81%, terwijl de prevalentie bij individuele varkens varieert van 11 tot 39%. Ook werd in de vleesklaverensector een hoge MRSA CC398 prevalentie gevonden: 88% van de bedrijven en 28% van de kalveren waren namelijk positief getest. De extreem hoge MRSA CC398 prevalentie onder vee resulteert in een zeer hoog dragerschap bij mensen die in contact komen met deze dieren. Broens et al. hebben aangetoond dat de prevalentie in de varkensstapel van 30% in begin 2007 naar 75% aan het eind van 2008 is gestegen. Ook de prevalentie van MRSA CC398 bij varkenshouders in recentere gepubliceerde studies zijn hoger dan oudere studies: van 29% in 2007 tot 63% in 2010. Bovendien, het dragerschap percentage van MRSA CC398 bij dierenartsen in hoofdstuk 8 is hoger dan alle eerdere studies (MRSA prevalentie in dierenartsen varieert van 12,5 % tot 45% in verschillende internationale studies).

Om te beoordelen hoe de verspreiding van deze nieuwe variant van MRSA in de algemene bevolking is, zijn er mensen gescreeën die het hoogste risico van dragerschap hebben. Deze studie onderzocht of MRSA CC398 verspreid is van de boerderijen naar de rest van de gemeenschap in gebieden met een extreem hoge dichtheid van varkensbedrijven. De resultaten van deze studie worden beschreven in hoofdstuk 6. Met behulp van een mailing in 3 geselecteerde gemeenten in Nederland, werden volwassenen gevraagd een vragenlijst in te vullen en een neuskweek af te neven. In totaal werd er van 583 personen volledige informatie verkregen. Slechts één van de 534 personen zonder veecontact werd (0,2%; 95% CI <0,01–1,2) positief getest op MRSA. In tegenstelling, 13 (26,5%; 95% CI 16,1–40,4) van de 49 personen met veecontact (ofwel werken op of leven op een veehouderij) werd positief getest op MRSA. Alle gevonden MRSA stammen hadden spa-typen die tot de bekende veegeassocieerde CC398 kloon behoren.

Oorsprong en epidemiologie van de CC398 kloon

Op een groot aantal CC398 isolaten werden microarrays en whole genome sequencing toegepast en hierbij worden twee varianten binnen de CC398 kloon onderscheiden. Het betreft de klassieke veegeassocieerde variant, deze isolaten zijn verantwoordelijk voor

De twee CC398 varianten worden gekenmerkt door verschillende profagen. Veegeassocieerde CC398 isolaten dragen vaak φ2 en φ6, of een φAv profaag. Isolaten die daarentegen behoren tot de menselijke variant bevatten een β-omzetting φ3 profaag die coderen voor twee menselijke specifieke virulentiegenen (\textit{chp} en \textit{scn}). Er is nu bewijs dat de aan de mens aangepaste subpopulatie verschilt van de voorouderlijke menselijke subpopulatie door de extra profaag functies (φ1, φ2, φ5 of φ7).

Deze menselijke CC398 variant wordt nu steeds vaker gevonden in ziekenhuizen. Ook is er een sterke stijging in de prevalentie van \textit{S. aureus} CC398 in bloedbaaninfectie waargenomen in een recente studie. Zeventien van de 18 bloedbaaninfecctie isolaten waren methicilline-gevoelig en geen enkele behoorde tot de veel voorkomende veegeassocieerde \textit{spa}-types t011, t034 of t108. De wijze waarop de \textit{S. aureus} isolaten CC398 door de patiënten zijn verkregen blijft onduidelijk. Bovendien werden alle \textit{S. aureus} CC398 bloedbaaninfecties gediagnosticeerd bij patiënten die niet blootgesteld waren aan vee. Deze ernstige infecties zijn geassocieerd met MSSA CC398 \textit{spa}-type t571. Deze resultaten werden ook bevestigd door onze studie (hoofdstuk 7). Er werden in totaal 612 opeenvolgende episoden van \textit{S. aureus} bloedbaaninfecties geïncludeerd. Drie stammen (2 MSSA en MRSA 1) die werden geïsoleerd uit bacteriëmiëse patiënten tussen 2010–2011 waren positief met een CC398-specifieke PCR. Er was een duidelijke toename in prevalentie van \textit{S. aureus} CC398 bloedbaaninfecties tussen 2010–2011 ten opzichte van de collecties die werden geïsoleerd tussen 1996–1998 en 2002–2005 (3/157, 1.9% vs. 0/455, 0.0%; \( p = 0,017 \)). Wij concluderen dat \textit{S. aureus} CC398 een toenemende oorzaak van invasieve stafylokokken ziekte zou kunnen zijn. Onze resultaten zijn gelijk aan andere bevindingen dat bepaalde \textit{S. aureus} isolaten CC398, vooral \textit{spa}-type t571, invasieve infecties kunnen veroorzaken bij mensen.

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Er is nog steeds discussie over de dynamiek van MRSA CC398 dragerschap bij mensen met regelmatig veecontact. Vraag is of een MRSA CC398 positief persoon echt gekoloniseerd is of alleen maar MRSA besmette stof heeft ingeademd tijdens het werk, zonder daarbij echt gekoloniseerd te zijn. Dit is een belangrijk onderscheid, omdat kolonisatie met MRSA CC398 waarschijnlijk minder vaak tot infecties leidt en dat personen deze mogelijk sneller weer kwijt raakt na een periode van niet-blootstelling. Cuny et al. vonden een extreem hoge MRSA CC398 neuskolonisatie van 45% bij dierenartsen. Dragerschap kan ook langdurig zijn, zoals in een recent rapport uit Duitsland, waar de meerderheid van de varkenshouders (59%) hun MRSA CC398 niet kwijt raakte na de vakantie. In tegenstelling tot dit, als vrijwilligers actief werden gekoloniseerd met meticilline-gevoelige S. aureus (MSSA) CC398 droegen ze deze vaak voor langere perioden. We hebben een prospectieve cohort studie gedurende twee jaar uitgevoerd met 137 dierenartsen die vooral werken met varkens en vleeskalveren (hoofdstuk 8). Deze studie toonde een gemiddelde MRSA CC398 prevalentie van 44% aan (bereik 42-46%). In totaal werden er bij 88 dierenartsen (65%) tenminste eenmaal MRSA CC398 gevonden. Tweeëndertig dierenartsen (23%) hadden MRSA-positieve testresultaten gedurende de gehele onderzoeksperiode en 18 van hen (56%, 13% van alle dierenartsen) hadden vijf identieke MLVA types en kunnen dus worden beschouwd als persisterende MRSA CC398 dragers. Aan de andere kant zijn er diverse studies die aantonen dat MRSA CC398 geen goede kolonisator van mensen is. Een studie onder veldwerkers met kortdurende blootstelling aan varkens en vleeskalveren vond een hoge mate van voorbijgaande verontreiniging, zonder substantiële persisterende kolonisatie. Een andere studie toonde aan dat de prevalentie van MRSA bij vleeskalverenhouder sterk afnam (58%) na afwezigheid van dierlijk contact. De redenen voor deze tegenstrijdige bevindingen zijn onduidelijk en vereisen nader onderzoek.

Wij concluderen echter dat regelmatig contact met vee inderdaad kan leiden tot persisterende kolonisatie met MRSA CC398. Dierenartsen die vooral werken met varkens en vleeskalveren dragen vaak dezelfde stam voor langere periodes.

Mens-op-mens overdracht van MRSA CC398

Mens-op-mens overdracht is de belangrijkste determinant voor de verspreiding naar de algemene populatie. De omvang van de bedreiging van de volksgezondheid is voornamelijk afhankelijk van deze eigenschap, in combinatie met stamvirulentie. Verschillende recente studies hebben aangetoond dat MRSA CC398 in een ziekenhuis omgeving 4-6 keer minder vaak overdraagt in vergelijking met ziekenhuisgerelateerde MRSA stammen. Dit beperkt de...
gevolgen voor de volksgezondheid en het aanpassen van de controlemaatregelen zou gerechtvaardigd zijn. Op dit moment is de mens-op-mens overdraagbaarheid van MRSA CC398 in een huishoudelijke omgeving echter nog onduidelijk. Daarbij is het bestuderen van de mens-op-mens overdraagbaarheid van MRSA CC398 lastig, omdat het reservoir van MRSA CC398 beperkt is tot mensen die direct contact hebben met dieren. De meerderheid van deze personen leven op boerderijen samen met hun gezinnen. Deze gezinsleden hebben meestal zelf direct diercontact. Daarom zijn dierenartsen een uitstekende groep voor het bestuderen van de mens-op-mens overdraagbaarheid van MRSA CC398 omdat hun gezinsleden zelf geen direct contact hebben met varkens of vleeskalveren. We hebben een prospectieve cohort studie bij Nederlandse dierenartsen en hun gezinsleden uitgevoerd (hoofdstuk 9). Ons onderzoek toonde een relatief hoge gemiddelde MRSA CC398 kolonisatie van 4,0% bij de gezinsleden. In totaal werd er bij 36 gezinsleden (9,3%), afkomstig uit 28 gezinnen (20,4%), tenminste één keer MRSA CC398 aangetoond tijdens de studieperiode van één jaar. Deze gegevens bevestigen de resultaten van een in Duitsland eerdere uitgevoerde studie waar een MRSA CC398 prevalentie van 9,0% onder de gezinsleden van dierenartsen werd gevonden. In ons onderzoek bleek de MRSA CC398 dragerschap bij gezinsleden sterk afhankelijk van de dragerschap status van de dierenarts te zijn.

Om daarnaast de overdraagbaarheid van MRSA CC398 stammen te vergelijken met andere MRSA isolaten, werd een cross-sectionele studie uitgevoerd in MRSA-positieve patiënten en hun gezinsleden. De MRSA prevalentie onder de gezinsleden was significant hoger bij controle patiënten met MRSA niet-CC398 stammen dan bij dierenartsen met MRSA CC398 (prevalentie risico ratio 6,0; 95% CI 2,4–15,5). Deze gegevens suggereren dat MRSA CC398 zich minder eenvoudig verspreidt tussen mensen dan andere MRSA isolaten in een huishoudelijke omgeving.

Ondanks het omvangrijke reservoir bij dieren én mensen die werken met vee, is de MRSA CC398 prevalentie onder mensen die niet direct betrokken zijn in de veeteelt opvallend laag. Eén van de eerste studies die het leven in een veerij gebied onderzocht vond dit geen risicofactor (hoofdstuk 6). In deze cross-sectionele studie bleek dat slechts 0,2% van de volwassenen zonder veecontact positief waren voor MRSA CC398. Inmiddels zijn er een aantal recente studies waaruit blijkt dat MRSA CC398 zich al wel heeft verspreid in de algemene bevolking. De manieren waarop MRSA CC398 kan worden overgedragen aan mensen zonder veecontact zijn direct contact met MRSA-positieve personen, omgevingsontreiniging en eten of hanteren van besmet vlees. In een recente studie van Lekkerkerk et al. bleek dat MRSA van onbekende oorsprong (MUO) aan het toenemen is. De nationale gegevens van de MRSA surveillance (2008–2009) werden geanalyseerd op epidemiologische determinanten en genotypische kenmerken. Een kwart (24%) van de 5545 MRSA isolaten waren MUO, d.w.z. niet behorend tot één van de bekende risicogroepen. Twee verschillende genotypische MUO groepen werden onderscheiden: MUO CC398 (352, 26%) en MUO niet-CC398 (998, 74%). Dit suggerereert een verspreiding van MUO CC398, echter niet door direct contact met dieren (varkens, vleeskalveren), maar door andere risicofactoren.
Ook is verspreiding van MRSA CC398 binnen een gezondheidszorginstelling in verschillende studies waargenomen. De eerste beschreven ziekenhuisgeassocieerde uitbraak van MRSA CC398 heeft zich voorgedaan in Nederland. De auteurs vonden vijf patiënten met MRSA CC398 kolonisatie en/of infectie. Alle stammen behoorden tot CC398 en hadden spa-type t567. Verder wordt er in hoofdstuk 10 een uitbraak van MRSA CC398 in een verpleeghuis in Nederland beschreven. Er werden zeven bewoners en twee werknemers in de gezondheidszorg met MRSA CC398 geïdentificeerd. De MRSA stam die verantwoordelijk was voor deze uitbraak had spa-type t011, welke behoorde tot de CC398 kloon. De meest waarschijnlijke menselijk bron woonde tot voor kort op een varkensbedrijf, voordat hij naar het verpleeghuis verhuisde. Hij meldde nog regelmatig op bezoek te gaan naar het varkensbedrijf van zijn zoon.

Echter zijn MRSA CC398 isolaten moeilijk te onderscheiden door de huidige moleculaire typeringstechnieken, zoals spa-typen, MLST en MLVA. Hoewel de PFGE met restrictie enzym Cfr een veel betere differentiatie van CC398 geeft, is deze methode omslachtig en levert gegevens op die niet eenvoudig elektronisch uitgewisseld kunnen worden. Dit belemmert studies naar mogelijke verspreidingen en uitbraken door deze MRSA kloon. In hoofdstuk 11 wordt de typeringsmethode whole genome mapping (WGM) beschreven. WGM heeft een hoog onderscheidend vermogen waardoor deze methode lijkt om MRSA CC398 verspreiding te identificeren. Het onderscheidend vermogen werd bevestigd doordat WGM de MRSA CC398 isolaten van epidemiologisch ongerelateerde dierenartsen kon differentiëren waarvan spa- en MLVA-typering geen duidelijk onderscheid kon maken. Verder werd WGM uitgevoerd om te beoordelen of deze techniek geschikt was om overdracht van MRSA CC398 in een huishoudelijke omgeving te identificeren. Er werden vrijwel identieke WGMs van de isolaten afkomstig van dierenartsen en hun gezinsleden gevonden. Wij concluderen dat WGM een geschikte methode is om overdracht van MRSA CC398 te identificeren, die niet mogelijk zou zijn geweest met behulp van spa-typering of MLVA. Bij gebruik van PFGE zou er veel meer onzekerheid zijn geweest. We zijn momenteel bezig WGM uit te voeren van een groter aantal isolaten van dierenartsen en hun gezinsleden om MRSA CC398 overdracht nader te bestuderen.

Virulentie van MRSA CC398

De capaciteit van veegerelateerde MRSA CC398 om infecties bij de mens te veroorzaken is reeds beschreven. Deze variëren van relatief kleine of gelokaliseerde infecties tot meer ernstige of invasieve infecties (hoofdstuk 6). Ondanks de diversiteit aan soorten infecties wordt er gesuggereerd dat MRSA CC398 minder virulent is dan andere menselijke MRSA stammen. Toch is de exacte ziektelast die geassocieerd is met MRSA CC398 dragerschap bij relatief gezonde personen grotendeels onbekend. Gezien de grote verspreiding van MRSA...
CC398 bij mensen met veecontact is er behoefte aan het bestuderen van infecties die veroorzaakt worden door MRSA CC398.

Onze prospectieve cohort studie toont aan dat persisterende MRSA CC398 evenals persisterende *S. aureus* dragers significant meer huid- en weke delen infecties hadden dan *S. aureus* niet-dragers tijdens een studie van twee jaar (hoofdstuk 12). Onze gegevens bevestigen de resultaten van verschillende studies dat MRSA CC398 dragerschap vooral matige tot ernstige huid- en weke delen infecties veroorzaakt. Een andere recente studie heeft aangetoond dat MRSA CC398 niet minder pathogeen was voor de mens dan *S. aureus* in het algemeen.

Wij vonden een vergelijkbaar risico op huid- en weke delen infecties tijdens de onderzoekspériode onder MRSA CC398 en MSSA dragers. Dit betekent dat de huid- en weke delen infecties alleen door *S. aureus* dragerschap zijn veroorzaakt en niet doordat deze stammen methicilline-resistent zijn. Hoofdstuk 8 beschrijft een opmerkelijk hoge gemiddelde *S. aureus* dragerschap (72%) bij dierenartsen, dit is zeer hoog in vergelijking met de algemene bevolking. Dierenartsen hebben meer kans om een huid- en weke delen infectie te ontwikkelen in vergelijking met de algemene bevolking en hierdoor zou *S. aureus* dragerschap (MRSA CC398 en MSSA) kunnen gezien worden als een beroepsrisko.

Bedreiging van de volksgezondheid door MRSA CC398

De voorgaande alinea’s hebben aangetoond dat er een gevaar voor de volksgezondheid kan ontstaan door veegerelateerde MRSA CC398. Op dit moment lijkt MRSA CC398 in de gezondheidszorginstellingen onder controle te zijn. De gevolgen voor de volksgezondheid van MRSA CC398 lijken op het moment laag te zijn, dit blijkt uit de beperkte verspreiding in de gemeenschap en de minimale hoeveelheid van ernstige invasieve infecties door MRSA CC398, zoals beschreven in hoofdstukken 6 en 7. Terwijl de prevalentie in de veehouderij waarschijnlijk dicht bij het verzadigingspunt is, leidt MRSA CC398 zelden tot ernstige problemen in ziekenhuizen. Toch maken experts zich zorgen dat de mogelijke snelle evolutie van MRSA CC398 kan resulteren in nieuwe eigenschappen in de nabije toekomst, omdat MRSA CC398 mobiele genetische elementen tussen stammen kan uitwisselen. Dit suggereert dat deze kloon zich snel zou kunnen aanpassen aan veranderingen. Een studie met whole genome sequencing heeft aangetoond dat veegerelateerde MRSA CC398 ontstaan in de mens, en bij het overgaan naar de veestapel een aantal genen verloor, terwijl het resistentiegenen erbij kreeg. Dit baart zorgen over een mogelijke omgekeerde gebeurtenis (overname van virulentiefactoren met behoud van resistentie eigenschappen) vanuit het immense reservoir in de veestapel. Er is nu bewijs uit een recente studie dat veegerelateerde MRSA CC398 zich kan aanpassen aan de mens. Hybridisatie van DNA met microarrays
toonde in deze studie geen resten van veegeassocieerde profagen in het genoom van de voorouderlijke menselijke subpopulatie. Daarentegen delen de aan de mens aangepaste isolaten en de veegeassocieerde isolaten bepaalde profaag elementen wat een dierlijke oorsprong suggereert. Deze bevindingen vereisen echter nader onderzoek om dit te bevestigen.

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**Toekomstig onderzoek**

De twee jaar durende prospectieve cohort studie die in *hoofdstukken 8, 9 en 12* wordt beschreven bevat waardevolle epidemiologische informatie. MRSA en MSSA isolaten werden verzameld uit dierenartsen en hun gezinsleden die geen contact bet vee hadden. Verdere moleculaire karakterisatie met geavanceerde typeringsmethoden kunnen meer inzicht geven in de exacte mechanismen van persisterend MRSA CC398 dragerschap, hogere overdraagbaarheid en virulentie factoren. Op dit moment proberen we met behulp van whole genome sequencing van een groot aantal MRSA en MSSA CC398 isolaten het risico in te schatten van het ontstaan van een groot reservoir van veegeassocieerde *S. aureus* CC398 in de algemene bevolking.

Daarnaast hebben we een aantal dierenartsen gevonden die nooit gekoloniseerd zijn geweest met *S. aureus* gedurende de studie van twee jaar, ondanks een zeer hoge blootstelling aan MRSA CC398 en MSSA in de varkens- en vleeskalveren stallen. Deze individuen lijken "immun" voor kolonisatie te zijn. De bepaling van de genetische kenmerken en het microbiome van de neus van deze niet-dragers zouden kunnen leiden tot doelstellingen voor nieuwe geneesmiddelen of vaccins om kolonisatie en infecties te voorkomen.

Om te evalueren of de MRSA CC398 prevalentie bij mensen zonder veecontact in een gebied met een hoge dichtheid van varkensbedrijven toeneemt, dient het cross-sectionele onderzoek na vijf jaar nogmaals te worden uitgevoerd.

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**Conclusies**

Het onderzoek in dit proefschrift heeft geleid tot meer inzicht in de detectie, dynamiek, overdraagbaarheid en de virulentie van MRSA CC398 in dierenartsen met veecontact. Het heeft ook bijgedragen aan een beter begrip van de mogelijke bedreiging voor de volksgezondheid. De volgende belangrijke conclusies kunnen worden getrokken uit dit proefschrift: (1) Oxoid Brilliance™ MRSA Agar, Bio-Rad MRSA Select™ medium en chromID®
MRSA medium zijn allen gevoelige methoden voor het screenen van MRSA CC398 in combinatie met bouillonophoping, echter positieve resultaten hebben bevestiging nodig. Onze studies toonden een aanzienlijke verhoging van de sensitiviteit na ophoping in vergelijking met directe kweken. Daarom blijft een bouillonophoping onmisbaar voor een betrouwbare MRSA detectie. (2) Regelmatig veecocontact kan inderdaad leiden tot persisterende kolonisatie met MRSA CC398. Dierenartsen die vooral werken met varkens en vleeskalveren dragen vaak dezelfde stam voor langere periodes. (3) Een relatief hoge gemiddelde prevalentie van MRSA CC398 kolonisatie van 4,0% werd gevonden onder gezinsleden. Bovendien bleek de prevalentie van MRSA CC398 dragerschap bij gezinsleden sterk afhankelijk te zijn van de dragerschap status van de dierenarts. MRSA CC398 verspreidt zich minder eenvoudig tussen mensen met beroepsmatig veecocontact en hun gezinsleden dan andere MRSA isolaten in een huishoudelijke omgeving (prevalentie risico ratio 6,0; 95% CI 2,4–15,5). (4) De cross-sectionele studie in gebieden met een extreem hoge varkendsdichtheid vond dat slechts 0,2% van de volwassenen zonder diercontact positief waren voor MRSA CC398. Dit is vergelijkbaar met de dragerschap prevalentie van de algemene bevolking. (5) Whole genome sequencing kan MRSA CC398 onderscheiden en identificatie van overdracht. (6) Dierenartsen met persisterende MRSA CC398 en persisterende *S. aureus* dragerschap hadden significant meer huid- en weke delen infecties dan *S. aureus* niet-dragers tijdens een studie van twee jaar.

--- Aanbevelingen ---

Het Nederlandse ‘search en destroy’ beleid werd in december 2012 herzien, en alle gezinsleden van bevestigde MRSA patiënten moeten nu worden gescroond op MRSA bij een ziekenhuisopname. Toch worden gezinsleden van dierenartsen nog niet onderzocht bij een opname in een ziekenhuis. Maar wij hebben aangetoond dat ze relatief vaak MRSA dragen in vergelijking met de Nederlandse bevolking, onafhankelijk van de MRSA dragerschap status van de dierenarts. Daarom pleiten wij ervoor dat gezinsleden van dierenartsen ook moeten worden gescroond op de aanwezigheid van MRSA dragerschap bij een ziekenhuisopname.

Vanuit het volksgezondheid oogpunt wordt een permanente monitoring van de virulentie, antimicrobiële resistentie profielen en menselijke kolonisatie capaciteit van MRSA CC398 sterk aanbevolen. Aanpassing aan de mens door veegerelateerde CC398 zou een grote bedreiging voor de volksgezondheid kunnen zijn vanwege het enorme reservoir bij vee, en omdat dat deze kloon vrij eenvoudig vreemd DNA lijkt te verwerven. Dit zou grote aanpassingen van de bestaande richtlijnen in de gemeenschap en zorginstellingen vereisen. Het is de vraag of de huidige strategie in Nederland in dit scenario kan worden gehandhaafd. In ieder geval zullen de kosten dan aanzienlijk gaan stijgen.

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Ongeveer vijf jaar geleden ben ik begonnen met het promotieonderzoek dat geleid heeft tot dit proefschrift. Hoewel dit op papier een lange periode lijkt, is deze tijd voor mijn gevoel voorbij gevlogen. Het was een periode van (soms) hard werken, maar ook met veel gezelligheid. Dit proefschrift had niet afgerond kunnen worden zonder de inzet en betrokkenheid van een groot aantal mensen, die ik daar allen hartelijk voor wil bedanken. Naast de dierenartsen en hun gezinsleden, van wie we de gegevens mochten gebruiken, wil ik een aantal mensen graag in het bijzonder noemen.

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Natuurlijk was dit proefschrift nooit gevuld zonder de enorme bereidheid, inzet en medewerking van de deelnemende dierenartsen en hun gezinsleden. Ontzettend bedankt! Daarnaast is de samenwerking met diverse bestuursleden van de Vakgroep Gezondheidszorg Varken altijd prettig verlopen.

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Erwin
Curriculum Vitae
List of publications


