Changing characteristics of livestock-associated methicillin-resistant *Staphylococcus aureus* isolated from humans – emergence of a subclone transmitted without livestock exposure, the Netherlands, 2003 to 2014

T Bosch¹, M van Luit¹, GN Pluister¹, D Frentz¹, A Haenen¹, F Landman¹, S Witteveen¹, N van Marm-Wattimena¹, HG van der Heide¹, LM Schouls¹

1. National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control, Bilthoven, the Netherlands

Correspondence: Thijs Bosch (thijs.bosch@rivm.nl)

Citation style for this article:

Bosch T, van Luit M, Pluister GN, Frentz D, Haenen A, Landman F, Witteveen S, van Marm-Wattimena N, van der Heide HG, Schouls LM. Changing characteristics of livestock-associated methicillin-resistant *Staphylococcus aureus* isolated from humans – emergence of a subclone transmitted without livestock exposure, the Netherlands, 2003 to 2014. Euro Surveill. 2016;21(21):pii=30236. DOI: <http://dx.doi.org/10.2807/1560-7917.ES.2016.21.21.30236>

Article submitted on 22 July 2015 / accepted on 11 January 2016 / published on 26 May 2016

Since 2007, livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has become the predominant MRSA clone isolated from humans in the Netherlands. To assess possible temporal changes, we molecularly characterised over 9,000 LA-MRSA isolates submitted from 2003 to 2014 to the Dutch MRSA surveillance. After an initial rapid increase with a peak in 2009 ($n=1,368$), the total number of submitted LA-MRSA isolates has been slowly decreasing to 968 in 2014 and over 80% of LA-MRSA belonged to one of three predominant MLVA/spa-types. Next generation sequencing ($n=118$) showed that MT569/to34 isolates were genetically more diverse than MT398/to11 and MT572/to108. Concurrent with the decrease in LA-MRSA, fewer people reported having contact with livestock and this was most prominent for people carrying MT569/to34 LA-MRSA. The proportion of LA-MRSA isolated from infection-related materials increased from 6% in 2009, to 13% in 2014 and most of these isolates originated from patients older than 50 years of age. Remarkably, 83% of these patients reported not having contact with livestock. The results reveal an ongoing change in the genotypic and epidemiological characteristics of Dutch LA-MRSA isolated from humans with the emergence of a LA-MRSA subclone independent of livestock exposure, suggesting LA-MRSA starts to resemble non-LA-MRSA in terms of transmissibility and pathogenicity.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of hospital-acquired and community-acquired infections [1]. In 2003, a clonal lineage of MRSA cultured from pig farmers and designated as multilocus sequence typing clonal complex 398 (CC398), emerged in the Netherlands and France [2,3].

A large number of countries reported CC398 cultured from animals, revealing a worldwide prevalence [4,5]. CC398 has been found in pigs and other livestock, such as calves, and poultry [6,7], and therefore designated as livestock-associated MRSA (LA-MRSA). LA-MRSA is prevalent in many European countries and LA-MRSA CC398 isolated from humans has become the predominant MRSA clone among isolates submitted for typing in the Dutch MRSA surveillance programme since 2007 [5,8].

Despite its widespread occurrence, the number of reported infections with LA-MRSA among humans remains low. In addition, nosocomial transmission of LA-MRSA in Dutch hospitals was reported to be 72% less likely to occur compared with non-LA-MRSA, although outbreaks of LA-MRSA have been described [9,10]. The reason for this limited transmissibility remains unclear, but several studies suggest that the human innate immunomodulatory genes on bacteriophage $\phi 3$ are important genetic markers for the adaptation of LA-MRSA towards humans and human-to-human transmission of CC398 [11-13].

LA-MRSA represents a homogenous clone with limited differentiation using typing techniques such as multi-locus variable number of tandem repeat analysis (MLVA), and *staphylococcal* protein A (*spa*-) typing [14]. In contrast, whole genome mapping, and next generation sequencing (NGS), revealed more genotypic diversity and suggested a distinction between livestock- and human-associated CC398 clones, although most studies are dominated by isolates obtained from animals and the human-associated isolates were mostly comprised of methicillin-sensitive *S. aureus* [12,14-16].

FIGURE 1

Minimum spanning tree of *Staphylococcus aureus* isolates typed by MLVA, the Netherlands, 2008–14 (n= 22,945)

LA-MRSA: livestock-associated methicillin-resistant *Staphylococcus aureus*; MC: MLVA complex; MLVA: multiple-locus variable number of tandem repeat analysis.

Clustering of MLVA profiles was done using a categorical coefficient and the MLVA types are displayed as circles. The size of each circle indicates the number of isolates with this particular type. Colours denote MLVA types that belong to the same MLVA complex, which are also indicated in characters e.g. MC398. Due to the large number of MC398 isolates, representing LA-MRSA, this complex is displayed separately.

FIGURE 2

Minimum spanning trees based on next generation sequencing of LA-MRSA isolates, the Netherlands, 2003–12 (n=118)

LA-MRSA: livestock-associated methicillin-resistant *Staphylococcus aureus*; MLVA: multiple-locus variable number of tandem repeat analysis; MT: MLVA type; SNP: single nucleotide polymorphism; wgMLST: whole genome multilocus sequence typing.

Panel A displays the minimum spanning tree based on 1,831 genes of the *S. aureus* wgMLST scheme, while panel B shows the tree based on 7,944 SNPs. Colours represent the top 3 representatives of LA-MRSA, MT398/t011 (n=45), MT572/t108 (n=44) and MT569/t034 (n=29). The lengths of the lines between isolates represent either the number of different genes (wgMLST) or the number of SNPs.

In this study, molecular characterisation, including NGS, and epidemiological data of more than 9,000 LA-MRSA isolates submitted to the national MRSA surveillance from 2003 to 2014 were used to assess the characteristics of the most predominant MRSA clade in the Netherlands.

Methods

Bacterial isolates

MRSA isolates, obtained from humans admitted to healthcare centers, were submitted for molecular typing to the National Institute of Public Health and the Environment (RIVM) to the Dutch national MRSA surveillance. All *S. aureus* isolates were subjected to

spa-typing, and MLVA. The MLVA also includes the detection of the genes for *mecA*, *mecC* and the *lukF* gene, indicative for Panton-Valentine leucocidin (PVL) [15,16]. Isolates belonging to MLVA complex 398 (MC398) were classified as LA-MRSA. All isolates not belonging to MC398 were designated as non-LA-MRSA. The discriminatory power of MLVA was assessed using Simpson's index of diversity, while the determination of the confidence intervals (CI) of the Simpson's indices was calculated as described by Grundmann et al. [17,18]. Only the first isolate per person per year was included. Medical microbiologists or infection control practitioners filled out questionnaires regarding epidemiological risk factors for MRSA colonisation or infection, including contact with livestock.

FIGURE 3

Geographic origin of the top three LA-MRSA genotypes by provinces, the Netherlands, 2009 and 2014

B. The geographic demographic origin of LA-MRSA MT569/t034 isolates

2009

2014

LA-MRSA: livestock-associated meticillin-resistant *Staphylococcus aureus*; MLVA: multiple-locus variable number of tandem repeat analysis; MT: MLVA type.

The number of submitted isolates of the top-three LA-MRSA representatives in the four provinces where LA-MRSA was predominant vs the rest of the Netherlands is shown in panel A. The geographic origin of LA-MRSA MT569/t034 isolates in the Netherlands is depicted in panel B.

FIGURE 4

Number of pig farms and number of pigs by provinces, the Netherlands, 2009 and 2014

Data source: statline.cbs.nl/statweb.

Classification of materials

The MRSA isolates were sampled from various materials and sites. Materials were subdivided in four different classes. Swabs from nose and/or throat, and/or perineum were regarded as material indicative for 'carriage'. Blood, cerebrospinal fluid (CSF), pus, sputum, urine, and wounds were considered as 'infection-related' materials. Other human materials were grouped as 'other' and if submitted without information regarding material, they were grouped as 'unknown'.

Next generation sequencing

The first 10 (if available) isolates of the three predominant LA-MRSA MLVA/*spa*-types from 2003 to 2005 and the first five isolates from 2006 to 2012 were used for analysis by NGS. NGS on these 118 isolates was performed as part of the 100k genome project by Davis University using the HiSeq 2000 [19]. Data were used for whole genome multilocus sequence typing (wgMLST), and single nucleotide polymorphism (SNP-) analysis. wgMLST was performed by SeqSphere software version 2.3.0 (Ridom GmbH, Münster, Germany) using the available wgMLST *S. aureus* scheme. For SNP analysis, the core genome, of a complete, circular and annotated reference chromosome of a Dutch LA-MRSA isolate was used. SNPs were identified using the CLCbio Genomics Server/Workbench, version 7.5 (CLCbio, Aarhus, Denmark) and SNP data were imported into Bionumerics version 7.5 for analysis (Applied Maths, Sint-Martens-Latem, Belgium).

φ3-specific PCR

We designed the φ3-specific primers based on the φ3 sequence present in reference strain NCTC8325 (CP000253): fluorescently labelled forward primer Sa3-Int-PET-f (TGATTGTACGGGTTGTC), and the reverse primer Sa3-Int-r (TACTTATGACGTCATAATGTG). The φ3 primers (10 pmol/μl) were added in our MLVA mix2, allowing detection of φ3 as a 160 bp peak. All LA-MRSA and non-LA-MRSA isolates obtained from August 2012 to October 2013 were tested for the presence of φ3.

Results

Genotypic diversity of LA-MRSA vs non-LA-MRSA

During the study period, we identified 17,079 isolates as non-LA-MRSA and 10,318 as LA-MRSA. Inclusion of only the first isolate per person per year resulted in 9,246 LA-MRSA isolates, and 13,699 non-LA-MRSA isolates to be analysed in this study for the period from 2003 to 2014. Questionnaires were available for 5,958 persons from whom LA-MRSA was isolated during the period from 2006 to 2013.

Genotypic diversity among non-LA-MRSA isolates was higher than LA-MRSA (Figure 1). For instance, MC5 (n=3,202), the most frequently found non-LA-MRSA MLVA complex, comprised 244 MLVA-types (MTs). In contrast, MLVA yielded only 144 MTs among the 9,246 LA-MRSA (MC398) isolates, resulting in a diversity index (DI) of 0.64 (95% CI: 0.63–0.65). The predominant MT was MT398 (n=5,111, 55%), followed by MT572 (n=1,872, 20%), and MT569 (n=603, 7%).

FIGURE 5

Changes in the proportion of people carrying LA-MRSA who report having contact with livestock, the Netherlands, 2008–13

LA-MRSA: livestock-associated methicillin-resistant *Staphylococcus aureus*; MLVA: multiple-locus variable number of tandem repeat analysis.

The proportion is expressed as the percentage of people who reported having livestock contact per MLVA/spa-type for the top three LA-MRSA types.

Spa-typing was slightly less discriminatory yielding 120 *spa*-types (DI=0.60, 95% CI: 0.59–0.61). *Spa*-types t011 (n=5,422, 59%), t108 (n=1,860, 20%), and t034 (n=723, 8%) were predominant.

Based on MLVA and *spa*-typing combined, the three predominant LA-MRSA types were MT398/t011 (n=5,043, 55%), MT572/t108 (n=1,742, 19%), and MT569/t034 (n=594, 6%). These top three types accounted for 80% of all LA-MRSA isolates. No other combination of MT and *spa*-type accounted for more than 2% of the LA-MRSA isolates.

Panton-Valentine leukocidin and $\phi 3$ in Dutch LA-MRSA isolates

The presence of the *lukF* gene, indicative for the production of the Panton-Valentine leukocidin (PVL), was determined in all 9,246 LA-MRSA isolates, but found in only 23 (0.2%) with a great variety in MLVA/*spa*-types. Ten of the 23 PVL positives originated from persons younger than 10 years, four of whom were adoption children from China. The proportion of non-LA-MRSA isolates carrying the *lukF* gene was 26% (n=3,585).

Between August 2012 and October 2013 1,538 LA-MRSA and 3,405 non-LA-MRSA isolates were tested for the presence of $\phi 3$. The prevalence of $\phi 3$ among LA-MRSA isolates was 2% (34/1,538). There was a difference in $\phi 3$ prevalence among the top three MLVA/*spa*-types; 7% (11/166) in MT569/t034, 2% (13/838) in MT398/t011, and 0.6% (1/180) in MT572/t108 isolates. In contrast,

prevalence in non-LA-MRSA was much higher with 80% (2,714/3,405) of all tested isolates carrying $\phi 3$.

Whole genome multilocus sequence typing and single nucleotide polymorphism analysis

Both wgMLST and SNP analysis of 118 isolates of the three predominant LA-MRSA types showed that they clustered in three different groups (Figure 2). Of the 1,864 genes of the *S. aureus* wgMLST scheme, 1,831 were present in all 118 isolates and used for comparison and tree construction. LA-MRSA isolates belonging to MT398/t011 (n=45) clustered closely together as did MT572/t108 (n=44) isolates. The average distance between MT398/t011 isolates was allelic variation in 25 genes with a maximum of 60 genes and for MT572/t108, the average distance was 24 genes with a maximum of 52 genes. The closest related isolates of the two groups differed in 145 genes. Compared with MT398/t011 and MT572/t108, the genetic diversity among the MT569/t034 (n=29) isolates was higher. The MT569/t034 isolates differed on average 34 genes with a maximum of 148 genes. The distances between MT569/t034 and MT398/t011, and MT572/t108 were 102 and 136 genes, respectively.

We identified 7,944 SNP positions in the core genome and used these for comparison. The minimum spanning tree was comparable to the wgMLST tree with groups of closely related MT398/t011, and MT572/t108 isolates and a genetic diverse MT569/t034. The average number of SNPs that differed among members of the MT398/t011 group was 43 with a maximum of 117 SNPs, while the MT572/t108 isolates differed on average in 39 SNPs with a maximum of 80 SNPs between the most distant members. The distance between the closest related isolates of MT398/t011, and MT572/t108 was 274 SNPs.

Decrease in the number of submitted LA-MRSA isolates

After its emergence in 2003, the number of LA-MRSA isolates submitted for typing rapidly increased from 20 in 2003 to 1,019 in 2008. At its peak, in 2009, 1,368 of the 3,163 (43%) MRSA isolates (first isolate per person per year) sent to the RIVM were LA-MRSA, but since then the numbers dropped. In 2014, the total number of submitted MRSA isolates was 3,228, of which 968 (30%) were LA-MRSA. The decrease could be largely attributed to the drop in submitted MT398/t011, and MT572/t108 isolates. In contrast, the number of isolates with MT569/t034 has been increasing since 2008. In 2014, 12% (n=117) of the 968 LA-MRSA isolates were of MT569/t034, surpassing MT572/t108 as the second most frequently isolated Dutch LA-MRSA type.

A geographical comparison between 2009 and 2014 showed a steady decrease of MT398/t011 in the four provinces, Noord-Brabant, Gelderland, Limburg, and Overijssel, where LA-MRSA is predominant and a slight increase in the other Dutch provinces (Figure 3A). This was most prominent in the province of Noord-Brabant

FIGURE 6

Sample origin of submitted LA-MRSA and non-LA-MRSA isolates, the Netherlands, 2009 and 2014 (n=5,391)

LA-MRSA: livestock-associated meticillin-resistant *Staphylococcus aureus*.

where a decrease of 40% occurred over time. A similar trend was seen for MT572/t108 LA-MRSA, a type predominantly found in Noord-Brabant resulting in a 69% decrease between 2009 and 2014. In contrast, there was a marked increase until 2013 in the number of submitted MT569/to34 isolates that was not restricted to a particular province. In 2014, the number of isolates slightly decreased in the four provinces, but this did not occur in the rest of the Netherlands. In 2009, 56% of the MT569/to34 isolates originated from Noord-Brabant, but this dropped to 26% in 2014 (Figure 3B).

Concurrent with the drop in the number of submitted LA-MRSA, a decrease in the number of pig farms in the Netherlands occurred (Figure 4). This decline was most prominent in the provinces Noord-Brabant and Gelderland. In addition, the number of people working in the Dutch agricultural sector has declined by 35% between 2000 and 2014 [20]. However, the number of pigs remained stable over time, showing a scale up in the Dutch pig production.

LA-MRSA related to contact with livestock

In 2008, 60–66% of persons carrying any of the three predominant LA-MRSA types reported contact with livestock. When stratified by the top three MLVA/*spa*-types an initial increase of contact with livestock from 2008 to 2010 was reported for people carrying isolates with MT398/to11, and MT572/t108 (Figure 5). After 2010, the proportion of humans reporting contact with livestock decreased again for both types reaching 62% (244/391) and 66% (49/74) for MT398/to11, and MT572/t108, respectively. In contrast, a considerable decrease in reported livestock contact occurred in people with MT569/to34 LA-MRSA where the percentage dropped from 63% (5/8) in 2008 to 52% (44/84) in 2013.

Sample origin of LA-MRSA isolates

Most of the isolates submitted for typing originated from carriage-related materials. In 2009, 6% (76/1,205) of the LA-MRSA isolates were cultured from infection-related materials and despite a drop in the number of LA-MRSA, this proportion increased to 13% (111/841) in 2014 (Figure 6A). In contrast, the number of non-LA-MRSA isolates increased during the same period, yet the proportion of isolates from infection-related materials decreased from 35% (529/1,510) in 2009 to 27% (487/1,835) in 2014.

Of the infection-related LA-MRSA isolates, most samples originated from wounds and sputum (Figure 6B). In 2009, 3% (37/1,205) of the LA-MRSA isolates were cultured from wounds and this increased to 7% (61/841) in 2014. For LA-MRSA isolates originating from sputum, an increase from 1% (16/1,205) in 2009 to 4% (29/841) in 2014 was seen. In contrast, the proportion of wound and sputum samples in the non-LA-MRSA slightly decreased during the same period. The distribution of MLVA/*spa*-types of isolates from infection-related materials did not differ from LA-MRSA isolates obtained from carriage-related materials.

Age-distribution among people carrying LA-MRSA

The median age of people carrying LA-MRSA and those carrying non-LA-MRSA was similar at 48 (range 0 to 105 years), and 49 years (range 2 to 103 years), respectively. However, stratification into age groups revealed a large difference between LA-MRSA carriers and non-LA-MRSA carriers (Figure 7A). In people carrying LA-MRSA, age categories followed a Gaussian distribution with a peak at 41–50 years. The proportion of infection-related isolates increased with increasing age, from 5% in the 0–9 years age group to 44% in the 80–89 years age group. In contrast, there was an almost even age distribution in persons carrying non-LA-MRSA and the proportion of infection-related isolates increased from 18% to 37% in age groups 0–9 years, and 80–89 years, respectively. Remarkably, there was a dip in the age distribution of persons carrying non-LA-MRSA in the age group 11–20 years.

There was a Gaussian age distribution of people carrying LA-MRSA, who reported having contact with livestock, and the vast majority was carriage (Figure 6B). The age groups of people carrying LA-MRSA, who reported not having contact with livestock, were also distributed in a Gaussian fashion, but with lower amplitude. The proportion of infection-related isolates was much higher than in the group of people reporting contact with livestock, increasing from 10% in age group 0–9 years to 59% in the age group 80–89 years. In contrast, in people reporting livestock contact, proportions were only 2%, and 21% in these age groups.

Discussion

In this study, we used a collection of more than 9,000 LA-MRSA isolates originating from humans obtained over the years 2003 to 2014. We showed an increase in the number of MT569/to34 LA-MRSA isolates, despite a decrease in the total number of LA-MRSA isolates in the Netherlands in recent years. NGS demonstrated that MT398/to11 isolates and MT572/t108 isolates partitioned in two genetically homogeneous groups, while MT569/to34 isolates did not partition in a single group and were genetically more diverse. Since 2010, humans carrying LA-MRSA less frequently reported having contact with livestock and this was most prominent for persons carrying MT569/to34 LA-MRSA.

The total number of MRSA isolates submitted for typing to the Dutch MRSA surveillance has been increasing since the start of the surveillance programme in 1989.

Since the first finding in 2003, the number of submitted LA-MRSA isolates increased rapidly until 2009 when the proportion of LA-MRSA was 43%. After that, the number of submitted LA-MRSA isolates dropped from 1,393 in 2009 to 968 in 2014 and as a result, the proportion of LA-MRSA decreased to 30% in 2014. Possible explanations for this decrease could be a reduced number of persons exposed to LA-MRSA, since we observed a concurrent decline in the number of pig farms and

people working the agricultural sector, although the number of pigs did not diminish. Also, there may be a reluctance of medical microbiology laboratories to submit LA-MRSA isolates as current typing poorly discriminates LA-MRSA, transmissibility between humans is considered to be low, and the perception may exist that infections with LA-MRSA occur only sporadically. However, we observed an increase in the number of submitted LA-MRSA isolated from infection-related materials, and this resulted in doubling the proportion of infection-related LA-MRSA from 6% in 2009 to 13% in 2014 and the majority of infection-related LA-MRSA originated from wounds and sputum. This shows that LA-MRSA is not only successful in colonising humans, but is also capable of causing infections. It also suggests that medical microbiology laboratories that have already assessed that an isolate is LA-MRSA, prefer to submit LA-MRSA isolated from infection-related materials rather than carriage isolates. This is not the case for non-LA-MRSA, as the number of submitted carriage-related isolates is increasing, while the number of infection-related isolates remains unchanged.

Analyses of the NGS data of the three predominant LA-MRSA types revealed three different groups. There was no overlap between the types, suggesting that these LA-MRSA types are three unique and independently evolving LA-MRSA clades. The genetically most diverse variant was MT569/to34, the type that rapidly increased in the Netherlands in recent years. This finding suggests that this particular LA-MRSA variant is more adapting towards humans leading to spread to regions of the Netherlands where LA-MRSA is not the predominant MRSA variant and where density of livestock farms is relatively low. Furthermore, the observation that there was a strong decrease in the number of people who reported having contact with livestock while carrying MT569/to34 LA-MRSA suggests that the spread of this LA-MRSA variant also occurs through routes other than livestock-human transmission. Recently, two studies from the Netherlands showed that a large proportion of the MRSA without known origin belonged to the LA-MRSA clade, corroborating our suggestion that LA-MRSA is capable of spreading without livestock exposure [21,22]. The $\phi 3$ phage, proposed by several studies as one of the markers for the adaptation of LA-MRSA towards humans, was nearly absent in our collection, although $\phi 3$ prevalence was highest (7%) among MT569/to34 isolates [11,12,23]. This suggests that $\phi 3$, at least in the Netherlands, plays a limited role in the adaptation of LA-MRSA to the human host.

Previous studies have shown that carriage of LA-MRSA is strongly associated with working in livestock farms. The Gaussian age distribution of people carrying LA-MRSA who reported livestock contact with most of the isolates from people aged between 21–70 years, i.e. the period when most people have an active working career, corroborates this association. The age distribution curve in people who reported not to have contact

FIGURE 7

Age distribution among people carrying LA-MRSA and non-LA-MRSA, the Netherlands, 2003–14

LA-MRSA: livestock-associated meticillin-resistant *Staphylococcus aureus*.

with livestock was flatter and had a dip in the age group 11–20 years similar to the dip in the non-LA-MRSA curve. Furthermore, the proportion of infection-related isolates in those who reported livestock contact was lower than in those reporting not to have contact with livestock. In the latter group, this proportion increased with age. The reasons for this remarkable difference in apparent pathogenicity remain unclear. However, it suggests that LA-MRSA are becoming more adapted to humans and start to resemble non-LA-MRSA in transmissibility and pathogenicity.

Our study has a number of limitations. First, all MRSA isolates originated from humans, limiting a comparison between LA-MRSA obtained from animals and humans. Second, we do not know if the question regarding animal contact in the available questionnaires was answered correctly. It could be that patients failed to

remember livestock contact or misinterpreted the question and answered not having livestock contact. Third, our study only used the $\phi 3$ phage as indicator for animal or human CC398 lineages. Other markers such as tetracycline resistance and canonical SNPs as reported by Stegger et al. could perhaps have provided more differentiation [24]. Finally, we grouped the MRSA isolates in different material classes. However, it is uncertain whether isolates obtained from infection-related materials really caused MRSA infections. For instance, LA-MRSA positive sputum samples could also be the result of a contamination of the sputum sample due to carriage with LA-MRSA in the bacterial flora of the throat.

In conclusion, the emergence of a LA-MRSA subclade transmitted without livestock contact could have important implications for management strategies to control

MRSA in healthcare settings. Possible future adaptations in for instance virulence of LA-MRSA could be unnoticed for prolonged periods if different strategies are used. Therefore, careful monitoring of the different LA-MRSA MC398 types through the national MRSA surveillance and a uniform search and destroy policy regardless which MRSA variant, remains necessary.

Acknowledgements

We would like thank the Dutch medical microbiology laboratories and hospitals for sending in the *Staphylococcus aureus* isolates for molecular typing. Without their contribution this study could not have been performed.

Conflict of interest

None declared.

Authors' contributions

TB was involved in the study design, literature search, data analysis, data interpretation, and writing of the paper. MVL was involved in the data collection, data analysis, and the bioinformatic analysis of the next generation sequencing data. GNP, FL and NMW were involved in the data collection and data analysis. SW and HGJH did the bioinformatics of the next generation sequencing data. DF and AH provided the questionnaires and were involved in the analysis of the epidemiological data. LMS supervised and managed the study and designed the primers for the $\phi 3$ specific PCR. All authors approved the final version of the manuscript.

References

- Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. *Emerg Infect Dis*. 2003;9(8):978-84. DOI: 10.3201/eid0908.030089 PMID: 12967497
- Armand-Lefevre L, Ruimy R, Andremonet A. Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. *Emerg Infect Dis*. 2005;11(5):711-4. DOI: 10.3201/eid1105.040866 PMID: 15890125
- Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant *Staphylococcus aureus* in pig farming. *Emerg Infect Dis*. 2005;11(12):1965-6. DOI: 10.3201/eid1112.050428 PMID: 16485492
- Bhat M, Dumortier C, Taylor BS, Miller M, Vasquez G, Yunen J, et al. *Staphylococcus aureus* ST398, New York City and Dominican Republic. *Emerg Infect Dis*. 2009;15(2):285-7. DOI: 10.3201/eid1502.080609 PMID: 19193274
- van Cleef BA, Monnet DL, Voss A, Krziwanek K, Allerberger F, Struelens M, et al. Livestock-associated methicillin-resistant *Staphylococcus aureus* in humans, Europe. *Emerg Infect Dis*. 2011;17(3):502-5. DOI: 10.3201/eid1703.101036 PMID: 21392444
- Graveland H, Wagenaar JA, Verstappen KM, Oosting-van Schothorst I, Heederik IJ, Bos ME. Dynamics of MRSA carriage in veal calves: a longitudinal field study. *Prev Vet Med*. 2012;107(3-4):180-6. DOI: 10.1016/j.prevetmed.2012.06.006 PMID: 22776914
- Nemati M, Hermans K, Lipinska U, Denis O, Deplano A, Struelens M, et al. Antimicrobial resistance of old and recent *Staphylococcus aureus* isolates from poultry: first detection of livestock-associated methicillin-resistant strain ST398. *Antimicrob Agents Chemother*. 2008;52(10):3817-9. DOI: 10.1128/AAC.00613-08 PMID: 18663024
- Huijsdens XW, Bosch T, van Santen-Verheuevel MG, Spalburg E, Pluister GN, van Luit M, et al. Molecular characterisation of PFGE non-typable methicillin-resistant *Staphylococcus aureus* in The Netherlands, 2007. *Euro surveillance: bulletin European sur les maladies transmissibles = European communicable disease bulletin*. 2009;14(38).

- Huijsdens XW, van Dijke BJ, Spalburg E, van Santen-Verheuevel MG, Heck ME, Pluister GN, et al. Community-acquired MRSA and pig-farming. *Ann Clin Microbiol Antimicrob*. 2006;5(1):26. DOI: 10.1186/1476-0711-5-26 PMID: 17096847
- Wassenberg MW, Bootsma MC, Troelstra A, Kluytmans JA, Bonten MJ. Transmissibility of livestock-associated methicillin-resistant *Staphylococcus aureus* (ST398) in Dutch hospitals. *Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 2011;17(2):316-9.
- McCarthy AJ, van Wamel W, Vandendriessche S, Larsen J, Denis O, Garcia-Graells C, et al. *Staphylococcus aureus* CC398 clade associated with human-to-human transmission. *Appl Environ Microbiol*. 2012;78(24):8845-8. DOI: 10.1128/AEM.02398-12 PMID: 23042163
- Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, et al. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *MBio*. 2012;3(1):e00305-11. DOI: 10.1128/mBio.00305-11 PMID: 22354957
- Foster TJ. Immune evasion by staphylococci. *Nat Rev Microbiol*. 2005;3(12):948-58. DOI: 10.1038/nrmicro1289 PMID: 16322743
- Bosch T, Verkade E, van Luit M, Pot B, Vauterin P, Burgrave R, et al. High Resolution Typing by Whole Genome Mapping Enables Discrimination of LA-MRSA (CC398) Strains and Identification of Transmission Events. *PLoS One*. 2013;8(6):e66493. DOI: 10.1371/journal.pone.0066493 PMID: 23805225
- Harmsen D, Claus H, Witte W, Rothgänger J, Claus H, Turnwald D, et al. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol*. 2003;41(12):5442-8. DOI: 10.1128/JCM.41.12.5442-5448.2003 PMID: 14662923
- Schouls LM, Spalburg EC, van Luit M, Huijsdens XW, Pluister GN, van Santen-Verheuevel MG, et al. Multiple-locus variable number tandem repeat analysis of *Staphylococcus aureus*: comparison with pulsed-field gel electrophoresis and spa-typing. *PLoS One*. 2009;4(4):e5082. DOI: 10.1371/journal.pone.0005082 PMID: 19343175
- Grundmann H, Hori S, Tanner G. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. *J Clin Microbiol*. 2001;39(11):4190-2. DOI: 10.1128/JCM.39.11.4190-4192.2001 PMID: 11682558
- Simpson EH. Measurement of species diversity. *Nature*. 1949;163(4148):688. DOI: 10.1038/163688a0
- UC Davis School of Vet Med. 100k Genome Project [Internet] Davis: available from www.100kgenome.vetmed.ucdavis.edu
- Statistics Netherlands [Internet]. [Accessed 21 Jul 2015]. Dutch. Available from: statline.cbs.nl
- Lekkerkerk WS, van de Sande-Bruinsma N, van der Sande MA, Tjon ATA, Groenheide A, Haenen A, et al. Emergence of MRSA of unknown origin in the Netherlands. *Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 2012;18(7):656-61.
- van Rijen MM, Bosch T, Verkade EJ, Schouls L, Kluytmans JA, CAM Study Group. Livestock-associated MRSA carriage in patients without direct contact with livestock. *PLoS One*. 2014;9(6):e100294. DOI: 10.1371/journal.pone.0100294 PMID: 25000521
- McCarthy AJ, Witney AA, Gould KA, Moodley A, Guardabassi L, Voss A, et al. The distribution of mobile genetic elements (MGEs) in MRSA CC398 is associated with both host and country. *Genome Biol Evol*. 2011;3(0):1164-74. DOI: 10.1093/gbe/evr092 PMID: 21920902
- Stegger M, Liu CM, Larsen J, Soldanova K, Aziz M, Contente-Cuomo T, et al. Rapid differentiation between livestock-associated and livestock-independent *Staphylococcus aureus* CC398 clades. *PLoS One*. 2013;8(11):e79645. DOI: 10.1371/journal.pone.0079645 PMID: 24244535

License and copyright

This is an open-access article distributed under the terms of the Creative Commons Attribution (CC BY 4.0) Licence. You may share and adapt the material, but must give appropriate credit to the source, provide a link to the licence, and indicate if changes were made.

This article is copyright of the authors, 2016.