Epidemiology of methicillin-resistant *Staphylococcus aureus* carrying the novel *mecC* gene in Denmark corroborates a zoonotic reservoir with transmission to humans

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**Abstract**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of healthcare-associated (HA), community-associated (CA) and livestock-associated (LA) infections. Recently, the discovery of human and bovine MRSA isolates carrying a new *meca* gene homologue, *mecALGA251* (now designated *mecC*), has caused concern because they are not detected by conventional, confirmatory tests for MRSA. Very little is known about their frequency, epidemiology and possible transmission between livestock and humans. In this study, the epidemiology of the *mecC* isolates in Denmark was investigated by screening the national collections of MRSA cases (from 1988 onwards) and *S. aureus* bacteraemia cases (from 1958 onwards). Isolates carrying *mecC* were only recovered infrequently before 2003 (*n* = 2) but now seem to be increasing, with 110 cases in 2003–2011. Clinical data on *mecC*-carrying MRSA demonstrated that *mecC*-MRSA were primarily community-acquired (CA-MRSA) and affected persons typically living in rural areas, being older than other CA-MRSA patients. Among 22 cases in Region Zealand, four reported contact with cattle and sheep. Two of these persons lived on farms with livestock positive for *mecC*-carrying MRSA, sharing spa type (*t*843), MLVA (MT429) and PFGE pattern with the human isolates. These observations indicate that *mecC*-carrying MRSA can be exchanged between humans and ruminants.

**Keywords:** Cattle, CC130, CC1943, epidemiology, livestock, *mecALGA251*, *mecC*, methicillin-resistant *Staphylococcus aureus*, sheep

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**Introduction**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital acquired (HA-MRSA) infections as well as an increasing cause of infections in non-hospitalized persons (community acquired, CA-MRSA) (1–3). Since 2006 detection of MRSA in livestock, especially pigs, but also in calves, chickens, horses, turkey and dairy cattle, has shown that livestock constitute a reservoir of MRSA belonging to clonal complex (CC) 398 (LA-MRSA), which can be transmitted to humans (4–6). Interchange of isolates between the reservoirs may be accomplished by adaptation to the host as well as to differences in antibiotic pressure. Price et al. (6) suggested that CC398 originated as methicillin susceptible in humans, jumped to pigs, wherein methicillin resistance was acquired, and is now seen re-infecting humans.

In 2011, a new *meca* gene homologue, *mecaALGA251*, was found in isolates from both humans and dairy cattle (7). It has been suggested by the International Working Group on the Classification of Staphylococcal Cassette Chromosome (SCC) Elements (8) that the *mecaALGA251* gene should be renamed *mecC*, and this term will be used herein. The *mecC*-containing MRSA isolates were found to belong to lineages typically reported in cattle (i.e. CC130, CC1943 and sequence type (ST) 425), which indicated the existence of a zoonotic MRSA reservoir (7). Bovine isolates belonging to
CC130 and CC1943 have been found in the United Kingdom, Ireland, France and Sweden (7–10) (http://www.smitsskyddsinstitutet.se/nyhetsarkiv/2012/ny-variant-av-antibiotikaresistent-bakterie and F. Laurent personal communication). However, in a Danish study of S. aureus isolated from cattle, pigs and chicken, no such isolates were found (11).

In humans, 11 mecC-positive CC130 isolates were detected among a total of 12 691 (prevalence of 0.1%) in Germany (12) and sporadic human cases have also been detected in Norway, Sweden, Belgium and the Netherlands (L. Marstein, S. Hagman, A. Petersson, O. Denise, personal communications).

In Denmark, we suggested a frequency of mecC-positive strains between 0.2 and 1% of all MRSA isolates, based on initial findings of human isolates with spa type t843 (7). This may, however, be an underestimate because these isolates have not previously been recognized as MRSA and no systematic surveillance studies have been carried out as yet.

This study provides insight into the emergence, frequency and epidemiology of mecC-containing strains in an MRSA low-prevalence country. Investigations of individual cases further substantiated the hypothesis of a zoonotic reservoir and transmission to humans.

Materials and Methods

Setting

Denmark has 5.58 million inhabitants, in five different regions: North Denmark (0.58 million), Central Denmark (1.27 million), Southern Denmark (1.20 million), Zealand (0.82 million) and Capital (1.72 million) (http://www.statisticsdenmark.dk/, visited March 11, 2012). Since 1958, S. aureus has been surveyed at the Staphylococcus reference laboratory (SRL) at Statens Serum Institut (SSI). A low prevalence (<2%) of MRSA has been detected among Staphylococcus aureus bacteraemia cases (SAB) since 1975. Nationwide surveillance of SAB has been carried out since 1958 and for MRSA since 1988. One isolate from each case has prospectively been submitted to the SRL, where it has been typed, susceptibility tested and stored. spa typing has been performed since 2007 (n = 17 969) and on additionally selected isolates prior to 2007 (n = 2116).

Search strategy

Staphylococcus aureus isolates were selected for this study based on being phenotypic MRSA but negative for mecA using conventional in-house PCR (13) from the national collection of SAB isolates 1960–2011 (n = 45 000), the national collection of MRSA irrespective of origin isolated between 1988 and 2011 (n = 7700), and a healthy nasal carriers in 2009–2010 (n = 403) (13,14). In addition, SAB and presumed MRSA isolates routinely received in our laboratory after 1 August 2011 (n = 643) were included in the study. See Table 1 for description of collections.

mecC detection and characterization of isolates

The isolates were confirmed as mecC positive by a recently developed multiplex PCR assay (16).

Isolates were spa-typed and subjected to MLST typing if spa types were not previously directly linked to CC130.

<table>
<thead>
<tr>
<th>Bacterial collection</th>
<th>Search strategy and confirmation</th>
<th>Use in the study</th>
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<tbody>
<tr>
<td>SAB 1960–2011 (n = 45 000)</td>
<td>Database search 1960–July 2011: (1) phenotypic MRSA, negative for mecA—PCR for detection of mecC, (2) search for spa types previously associated with mecC carriage. From 1 August 2011 all isolates were received for the presence of mecC.</td>
<td>Clinical and bacteriological information.</td>
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<td>MRSA 1988–2011 (n = 7700)</td>
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<td>Clinical and bacteriological information.</td>
</tr>
<tr>
<td>Staphylococcus aureus from healthy carriers 2009–2010</td>
<td>Search for spa types previously associated with mecC.</td>
<td>Pulsed-field gel electrophoresis.</td>
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<tr>
<td>Strain collections from:</td>
<td></td>
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<tr>
<td>Andersen et al. (13) (n = 325)</td>
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<tr>
<td>Fode et al. (14) (n = 78)</td>
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<tr>
<td>UK isolates</td>
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<tr>
<td>Positive mecC isolates from the UK (human = 2, animal = 2)</td>
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<tr>
<td>Danish animal isolates</td>
<td></td>
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<tr>
<td>Positive mecC isolates from sheep (n = 3) and cow (n = 1)</td>
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CC2361 or ST425 (17,18). Selected isolates were subjected to pulsed-field gel electrophoresis (PFGE) and multi-locus VNTR analysis (MLVA) as previously described (19, 20).

SCCmec typing was performed as previously described (7,21). Antimicrobial susceptibility was determined using disc diffusion according to EUCAST methodology for 12 antimicrobials (penicillin, cefoxitin, norfloxacin, erythromycin, clindamycin, kanamycin, tetracycline, linezolid, fusidic acid, rifampicin, sulphonmethoxazole-trimethoprim and mupirocin) (http://www.eucast.org).

Clinical information

MRSA in humans became notifiable in Denmark on 1 November 2006. Because mecC-positive isolates previously were defined as borderline oxacillin-resistant or modified S. aureus, no standardized notification was made by the submitting hospitals. On such isolates, regular notifications were received for all mecC-carrying isolates from 1 January 2010 onwards. From the notifications, reason for sampling (infection or carriage) and place of acquisition (HA, CA or travel, or healthcare-associated with a community onset (HACO)) were registered. Acquisition was determined as previously described based on the time of sampling after admission to hospital (< or >48 h), travel activities and occupation (22). In addition, contact with other MRSA-positive persons was registered. Residential county and Danish or foreign origin (defined as being born outside or having a parent who was born outside of Denmark) was investigated through the Danish civil registration system. Clinical information for isolates from before 2010 was retrieved from hospital discharge summaries or general practitioners’ notes, when available.

In Region Zealand (820 000 inhabitants) and North Denmark (580 000 inhabitants), 22 and 12 mecC-positive persons were contacted by the MRSA infection control nurse, at Slagelse and Aalborg Hospitals, respectively, and interviewed about occupation and contact with animals. If contact with animals was confirmed, animal samples were taken, whenever possible, from the animals after the owner’s informed consent. Samples were taken from the muzzles/nostrils of sheep, goats, cows, dogs, horses and rabbits. The time between human and animal sampling was 3–12 months.

Presence of S. aureus was confirmed by growth in selective broth (Tryptic Soy Broth + 7.5% NaCl), morphology on 5% horse blood agar (SSI Diagnostika, Hillered, Denmark), positive agglutination by Slidex Staph Plus (Biomérieux, Marcy l’Etoile, France) and detection of the spa gene fragment.

The human mecC MRSA cohort was compared with other MRSA cases from our database, using data from 1 January 2007 onwards (i.e. since MRSA became notifiable).

Statistics

Statistical significance was assessed using the Fisher’s exact test and Student’s t-test, with a p value of <0.05 indicating significance.

Results

The retrospective search was made among all isolates in Table 1. A total number of 87 were mecC-positive MRSA isolates. Two mecC-positive isolates were from 1975 and 1992, respectively. Prospective testing for presence of mecC in SAB and presumed MRSA isolates during 5 months (1 August to 31 December 2011) identified 25 unique mecC-positive isolates among 643 MRSA (3.9%). The combined annual incidence between 2003 and 2011 was: n = 3, 7, 11, 12, 6, 5, 9, 21 and 36, respectively (Fig. 1). Clinical and microbiological description of the mecC-positive isolates included in the study is shown in supplementary Table S1. Compared with the total number of MRSA in the period 2003–2011, mecC constituted 112/6960 (1.5%), with an increasing tendency, the frequency reaching 21/1097 (1.9%) and 36/1294 (2.8%) in 2010 and 2011, respectively (Fig. 1). By searching for isolates with spa types related to carriage of mecC, we identified 12 MSSA isolates (t528, n = 11 and t1048, n = 1) among more than 45 000 entries in our databases (<0.03%). No mecC-positive isolates were found among healthy carriers (n = 403, sampled in 2009 and 2010, Table 1).

Clinical information

In addition to the notifications received after 1 January 2010, information regarding 40 patients before this date was obtained on request; however, clinical information varied in quality. Annotation of the reason for sampling was possible for 95 cases: infection in 79 cases, screening in 10 cases and another reason in six cases (Table 2). Infection was predominantly skin and soft tissue infections (SSTI) (n = 63); the

<table>
<thead>
<tr>
<th></th>
<th>mecC MRSA total n = 112</th>
<th>mecC CA-MRSA n = 64</th>
<th>CA-MRSA n = 2636</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Gender ratio (F/M)</td>
<td>1.04</td>
<td>1.03</td>
<td>1.02</td>
</tr>
<tr>
<td>Average age (years)</td>
<td>51 (n = 110, 2 NA)</td>
<td>48</td>
<td>36**</td>
</tr>
<tr>
<td>Reason for sampling</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Infection</td>
<td>79 (83%)</td>
<td>48 (75%)</td>
<td>55%***</td>
</tr>
<tr>
<td>Screen</td>
<td>10 (11%)</td>
<td>9 (14%)</td>
<td>39%</td>
</tr>
<tr>
<td>Other/NA</td>
<td>6 (6%)</td>
<td>7 (11%)</td>
<td>6%</td>
</tr>
<tr>
<td>Type of infection</td>
<td></td>
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<tr>
<td>SSTI</td>
<td>63 (80%)</td>
<td>42 (89%)</td>
<td>77%</td>
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<tr>
<td>Blood</td>
<td>8 (10%)</td>
<td>4 (10%)</td>
<td>1%</td>
</tr>
<tr>
<td>Urine</td>
<td>1 (1%)</td>
<td>1 (2%)</td>
<td>2%</td>
</tr>
<tr>
<td>Postoperative</td>
<td>4 (5%)</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (4%)</td>
<td>–</td>
<td>20%</td>
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<tr>
<td>Bacteriological</td>
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<td></td>
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<tr>
<td>MLST CC (spa types)</td>
<td>CC130 (n = 98)</td>
<td>CC130 (n = 60)</td>
<td>CC8, CC5, CC398,</td>
</tr>
<tr>
<td></td>
<td>t373, t528, t843, t1048,</td>
<td></td>
<td>CC80, CC30, CC22,</td>
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<tr>
<td></td>
<td>t1532, t1535, t1736,</td>
<td></td>
<td>CC1, CC45 and 17</td>
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<td></td>
<td>t1773, t218, t2256,</td>
<td></td>
<td>others</td>
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<tr>
<td></td>
<td>t3570, t3970, t2220,</td>
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<td></td>
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<tr>
<td></td>
<td>t9397</td>
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<td></td>
<td>CC2361 (n = 14)</td>
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<td></td>
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<tr>
<td></td>
<td>t978, t2345, t3391, t8835,</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>t3995</td>
<td></td>
<td></td>
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<tr>
<td>PVL (%)</td>
<td>0</td>
<td>0</td>
<td>38</td>
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<tr>
<td>SCC mec type</td>
<td>XI</td>
<td>XI</td>
<td>IV, V</td>
</tr>
<tr>
<td>Multiresistance (%)**</td>
<td>0</td>
<td>0</td>
<td>36</td>
</tr>
</tbody>
</table>

Bold: predominant spa type. NA: not available.

*Resistance to three or more groups of antimicrobials in addition to beta-lactams.
**p < 0.001

remainder were SAB (n = 8), postoperative wound infections (n = 4), one urinary tract infection, and three that remained unknown.

Epidemiological classification into CA, HA, HACO or imported cases was possible for 78 cases (n = 64, 3, 10 and 1 cases, respectively). Six patients who had MRSA carrying mecC had been born, or had parents born, outside Denmark (Iran, Norway, the Netherlands, Sweden, Thailand and the USA).

As the majority of the mecC isolates were CA-MRSA, we compared cases with mecA-positive CA-MRSA cases, 2007–2011 (n = 2636) (Table 2). No differences were observed between the two cohorts regarding gender (p = 1.00, Fisher’s exact test); however, CA-MRSA mecC patients were significantly older than other CA-MRSA cases (p <0.001, Student’s t-test). Moreover, CA-MRSA-mecC patients had a significantly higher frequency of infections (p <0.001, Fisher’s exact test). However, no significant difference in the proportion of infections was seen from 2011 onwards when compared with mecA-positive CA-MRSA cases, 2007–2011.

Typing and antimicrobial susceptibility testing of isolates harbouring mecC

The mecC-carrying MRSA isolates could be grouped into MLST clonal complexes CC130 (n = 98) and CC2361 (n = 14), based on spa typing and MLST of representative isolates (n = 10). Two new MLST types were found, ST2173 (allelic profile 18-313-260-18-235-223-5) and ST2174 (18-313-279-18-7-223-5), both STs belonging to clonal complex CC2361, to which other mecC-positive spa types already belonged. spa typing revealed 19 different spa types (Table 2), dominated by t843 belonging to CC130 (n = 70), t528/CC130 (n = 9) and t978/CC2361 (n = 7). Several spa types not previously reported to be mecC positive were found to harbour mecC (CC130: t373, t528, t1048, t3256, t3570, t6220 CC2361 (n = 4) t978, t2345, t3995). blaz, mecl and mecR specific for the SCCmec XI were detected in all isolates, but amplification of ccrA1 and ccrB3 failed for some isolates belonging to different spa types. Except for one isolate resistant to norfloxacin, mecC MRSA isolates only expressed resistance to beta-lactams. No isolates carried the PVL gene (lukF-PV).

Rural distribution and relation to bovine or ovine reservoirs mecC-positive isolates were found in all regions, with the majority in more rural areas (n = 86) and only six isolates from the Capital region (Fig. 2).

The highest number of cases was found in Region Zealand and North Denmark (n = 29 and 15, respectively). In Region Zealand, 14 were healthy carriers and the remaining had infections: SSTI (n = 4), wound infections (n = 7), blood (n = 2), urinary tract infections (n = 1), and unknown (n = 1).

Twenty-two patients (22/29) from Region Zealand were interviewed at follow-up about occupation and possible contact with livestock animals.

In four cases contact with animals was reported.

Case 1: female, 53 years, with bacteraemia; teacher; living on a small farm with two cows and a horse, only one cow carried mecC MRSA in the nostrils. Case 2: female, 69 years,
with SSTI; nurse in old peoples’ home; living on a farm with a herd of sheep and a dog, three of 10 sheep carried mecC MRSA (t843) but not the dog.

Case 3 and 4: both male, 64 years, presenting with SSTI and positive uriculture, respectively; working as butchers (cattle); worked and lived apart and no animals were available for screening.

The mecC-carrying MRSA isolates from cows and sheep in proximity to case 1 and case 2, respectively, had the same spa type (t843) and MLVA profile (MT429) as the owners. MT429 was the predominant MLVA profile found among other humans in Region Zealand, as well as in other regions. PFGE of the isolates confirmed the similarity between animal and human isolates; however, other t843 isolates from Region Zealand with no known contact with these cases revealed similar patterns. The same was true for isolates from the United Kingdom included as likely outliers and the PFGE results were therefore inconclusive (Fig. 3). Four persons infected with t843 isolates from North Denmark reported contact with live cows. Subsequent screening of ten cows in the vicinity of three of the patients revealed no MRSA but a single MSSA isolate (t591-CC1).

Discussion

The discovery of MRSA carrying the mecC gene has caused speculations about the origin, epidemiology and impact of these isolates. In this study these questions were addressed by searching the Danish S. aureus collections for isolates harbouring the mecC gene and collecting demographic and clinical information from the affected patients. This search revealed 112 such cases. The majority of isolates were found in recent years and there has been an increase in numbers and frequency to a total of 36, comprising 2.8% of all new MRSA cases in 2011.

The national MRSA guidelines advocate eradication of MRSA in carriers and close contacts, which means that a large proportion of Danish MRSA cases (approximately 40%) are found by active screening. This procedure has not been practised systematically for MRSA being negative for mecA before August 2011, meaning that the true prevalence of mecC-carrying MRSA may be underestimated. This is compatible with results of prospective testing since August 2011, which showed a proportion of mecC-carrying MRSA of 3.9% among new MRSA isolates. However, only 20% of these cases were found by active screening, meaning that (i) screening procedures still perform differently for mecC MRSA cases compared with other MRSA cases, (ii) mecC-carrying MRSA isolates cause infections with a relatively higher frequency or (iii) other preferred colonization niches exist that were not screened. This difference may also explain why a higher frequency of infection was found in the retrospective period compared with the situation after August 2011. Contacts with mecC-positive persons would then routinely have been screened and more non-infected carriers would be detected. Countries with a low prevalence of MRSA have the opportunity to survey every MRSA case in detail, which may explain the higher prevalence found in this study compared with the findings in Germany (11). However, a high number of isolates of both MSSA and MRSA with the
various spa types detected in this study was found in the Ridom spa server (http://spaserver.ridom.de/) and likewise ovine, bovine and caprine isolates with sequence type (ST) 130, 151, 425, 1245 and 1526 from Norway, the Netherlands, Italy, Spain and the UK, are registered in the MLST database. (http://www.MLST.net). These registrations indicate that mecC-carrying MRSA may be more widely distributed, but not recognized yet. Our search among bacteraemia patients and healthy carriers only revealed a few MSSA spa types (t528 and t1048, but none of the most frequent spa types (t843) associated with mecC. Further studies of S. aureus carriage in ruminants could be of interest to confirm if a non-human reservoir of mecC MRSA exists in, for example, cows or sheep.

The initial characterization of mecC-carrying MRSA strains showed that they in general were susceptible to most other classes of antibiotics. The lack of mecA detection could, however, have been associated with initial treatment failures using, for example, dicloxacillin. In countries with an infection control system where MRSA cases are registered based on mecA confirmation, a number of cases have definitely not been recognized, resulting in a risk of continued spread. The SCCmec XI element lacks non-beta-lactam resistance mechanisms and the high susceptibility of these isolates is in accordance with other bovine S. aureus, indicating that the antimicrobial pressure is low (8). Penicillin is the preferred antibiotic used in cattle in Denmark (22). The isolates therefore differ from LA-MRSA (CC398) isolates (related to pigs), which are highly resistant to tetracycline and heavy metals used in pig production.

Comparison of clinical data with those of CA-MRSA showed differences regarding patient characteristics. For example MRSA-mecC patients were older than other CA-MRSA patients, indicating that mecC MRSA has a different origin and epidemiology to typical CA-MRSA. We have previously reported a high degree of suggested import of CA-MRSA in Denmark, as 35% of CA-MRSA patients were born or had parents born outside Denmark (22). Among the mecC-carrying MRSA, only six patients (5%) were found to have a non-Danish origin, suggesting that import does not contribute substantially to the emergence of mecC-carrying MRSA in Denmark. The finding of mecC-carrying MRSA in human, bovine and ovine samples indicates the existence of several distinct reservoirs. The higher frequency of human cases in rural areas compared with the capital region (Fig. 2) may also indicate that contact with livestock could be a risk factor for acquiring mecC-carrying MRSA whereas transmission via food is not. Possible zoonotic transmissions were inferred from detection of seven human cases with bovine or ovine contact and the subsequent finding of mecC-carrying MRSA in animals with contact with two of the patients. The carriage rate among tested animals confirmed that only a few animals in a cohort were colonized in the snouts (24). Therefore sampling a larger number of animals in especially North Denmark could possibly have resulted in findings of mecC-carrying MRSA. We need comprehensive, veterinary studies to specify the optimal sites for culture of S. aureus in different animals. Early UK retrieval of MRSA-mecC in pooled milk samples suggests that milk or udders should be tested.

Furthermore, three patients carried MRSA-mecC for 2–4 years and household transmission events were observed in five cases (data not shown), indicating that mecC-carrying MRSA are well adapted as human colonizers. However, in a S. aureus collection from healthy carriers no isolates had spa types matching the mecC-carrying isolates, pointing to a low frequency.

Our results confirm that ruminants (dairy cattle and sheep) are healthy carriers of mecC MRSA, including life-threatening diseases such as bacteraemia.

In conclusion, we present the first nationwide analysis of mecC-carrying MRSA in Denmark. The most recent and complete data from 1 August 2011 to 31 December 2011 indicate that the frequency could be as high as 4%. The mecC isolates belonged to only two genetic lineages, CC130 and CC2361. We found cows and sheep to be carrying mecC MRSA isolates and in two cases evidence suggested transmission and development of bacteraemia and skin infection in humans.

Acknowledgements


Transparency Declaration

The authors declare that they have no conflicting interests in relation to this work.
Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Clinical and microbiological description of the mecC-positive isolates included in the study.

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