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, $mecA_{LGA251}$ (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 (t843), 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, $mecA_{LGA251}$, *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, $mecA_{LGA251}$, 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 $mecA_{LGA251}$ 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.smittskyddsinstitutet.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.statistics-denmark.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 1. Description of *Staphylococcus aureus* bacterial collections included in the study

Bacterial collection	Search strategy and confirmation	Use in the study
SAB 1960–2011 (n = 45 000) Danish *S. aureus* bacteraemia isolates submitted voluntarily. All isolates typed by various methods and tested for susceptibility to antimicrobials. Clinical and epidemiological information retrieved from hospital discharge summaries	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 received were tested for the presence of *mecC*.	Clinical and bacteriological information
MRSA 1988–2011 (n = 7700) From 1988 to 31 October 2006, all MRSA were systematically collected at SRL. From 1 November 2006, all new MRSA cases were mandatory reportable and isolates were sent to SRL. All isolates were *spa* typed and tested for susceptibility to antimicrobials. Clinical and epidemiological information has been retrieved for all MRSA isolates. A subset consisting of *mecA*-positive *S. aureus* from 2007–2011 categorized as CA-MRSA (n = 2636)	Database search 1988–July 2011: phenotypic MRSA, negative for *mecA*—PCR for detection of *mecC* From 1 August 2011 all isolates received were tested for the presence of *mecC*	Clinical and bacteriological information Clinical and bacteriological information. Used for comparison
Staphylococcus aureus from healthy carriers 2009–2010 Strain collections from: Andersen *et al.* (13) (n = 325) Fode *et al.* (14) (n = 78)	Search for *spa* types previously associated with *mecC*.	
UK isolates Positive *mecC* isolates from the UK (human = 2, animal = 2)		Pulsed-field gel electrophoresis
Danish animal isolates Positive *mecC* isolates from sheep (n = 3) and cow (n = 1)		Pulsed-field gel electrophoresis

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).

SCC*mec* 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, sulphamethoxazole-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, Hillerød, 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

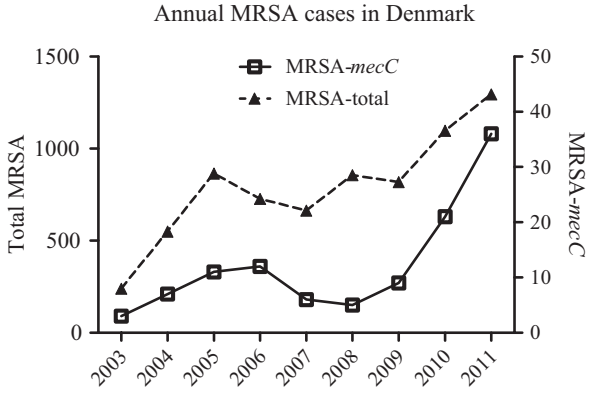

FIG. 1. Number of total MRSA and *mecC* harbouring isolates/year.

TABLE 2. Comparison of MRSA-*mecC* and CA-MRSA patients and isolates (2007–2011)

	***mecC* MRSA total** ***n* = 112**	***mecC* CA-MRSA** ***n* = 64**	**CA-MRSA** ***n* = 2636**
Clinical			
Gender ratio (F/M)	1.04	1.03	1.02
Average age (years)	51 (*n* = 110, 2 NA)	48	36***
Reason for sampling	(*n* = 95)		
Infection	79 (83%)	48 (75%)	55%***
Screen	10 (11%)	9 (14%)	39%
Other/NA	6 (6%)	7 (11%)	6%
Type of infection	(*n* = 79)	(*n* = 47)	
SSTI	63 (80%)	42 (89%)	77%
Blood	8 (10%)	4 (10%)	1%
Urine	1 (1%)	1 (2%)	2%
Postoperative	4 (5%)	–	0
Unknown	3 (4%)	–	20%
Bacteriological			
MLST CC (*spa* types)	CC130 (*n* = 98) t373, t528, **t843**, t1048, t1532, t1535, t1736, t1773, t3218, t3256, t3570, t5970, t6220, t9397 CC2361 (*n* = 14) **t978**, t2345, t3391, t8835, t9395	CC130 (*n* = 60) t528, **t843**, t1048, t1773, t3256, t3570, t6220 CC2361 (*n* = 4) t978, **t2345**	CC8, CC5, CC398, CC80, CC30, CC22, CC1, CC45 and 17 others
PVL (%)	0	0	38
SCC*mec* type	XI	XI	IV, V
Multiresistance (%)[a]	0	0	36

Bold: predominant *spa* type. NA: not available.
[a]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, t3218, t3256, t3570, t5970, t9379 and CC2361: t2345, t3391, t8835, t9395). *blaZ*, *mecI* and *mecR* specific for the SCC*mec* 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,

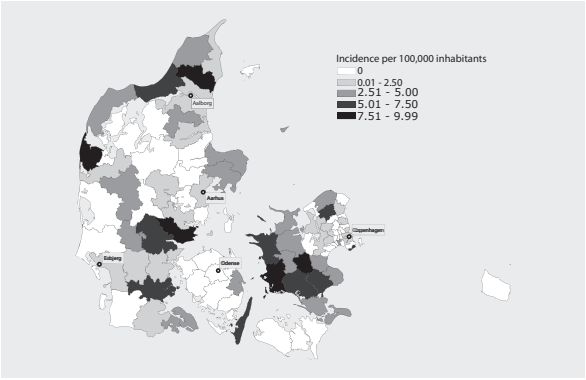

FIG. 2. Accumulated incidence of MRSA-*mecC* cases found in Danish municipalities (2003–2011). The five major cities are shown: Copenhagen (Capital), Aarhus (Central Denmark), Aalborg (North Denmark), Odense and Esbjerg (South Denmark).

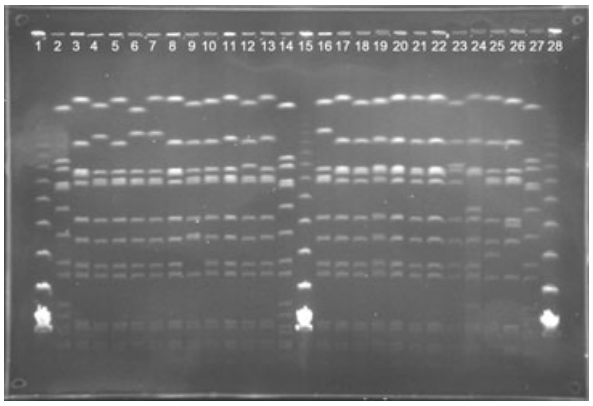

FIG. 3. Pulsed-field gel electrophoresis *Sma*I gel picture of t843 human and animal isolates from Slagelse Clinical Microbiological Department and the United Kingdom. Lanes 1, 15 and 28: Lambda size marker. Lanes 2, 14 and 27: NCTC8325. Lanes 3–13 and 16: human samples from Slagelse. Lanes 17 and 21: animal owners. Lanes 18–20: S (sheep). Lane 22: C (cow). Lanes 23–24: human samples, United Kingdom. Lanes 25–26: animal samples, United Kingdom.

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* type 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 SCC*mec* 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 CC130 isolates and thus substantiate MRSA-*mecC* as a new zoonosis.

The range of infections caused by *mecC*-carrying MRSA is the same as seen for other *S. aureus*, including life-threatening diseases such as bacteraemia.

In conclusion, we present the first nationwide analysis of the prevalence of *S. aureus* isolates carrying *mecC*, which shows that the prevalence has increased in the last few years and is now encompassing 2% of the annual human MRSA cases in Denmark. The most recent and most complete data from 1 August 2011 to 31 December 2011 indicate that the frequency could be as high as 4%. The detected 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.

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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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