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ORIGINAL ARTICLE

Methicillin-resistant *Staphylococcus aureus* with *mecC*: a description of 45 human cases in southern Sweden

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Abstract In 2011, a novel *mecA* gene homologue, *mecC*, was reported in isolates from both humans and dairy cattle. The epidemiology of *mecC* methicillin-resistant *Staphylococcus aureus* (MRSA) in humans is not yet well known. In this retrospective study, we present the epidemiology of human clinical cases with *mecC* MRSA detected in the southern part of Sweden during the period 2005–2014. A total of 45 patients with an isolate positive for *mecC* MRSA were included in the study. Twenty-six isolates were found before 2012 and were retrospectively tested for *mecC*. Nineteen isolates were detected in 2012–2014 through routine testing. Culture results, resistance patterns, Panton–Valentine leukocidin (PVL) genes, and *spa* types were collected from the Clinical Microbiology Laboratory. Epidemiological data were received from the database at the Regional Centre for Communicable Disease Control and the patient's medical files. The majority of the patients with *mecC* MRSA were of Swedish origin, had underlying diseases, and lived in rural areas. The median age was 60 years. Of the *mecC* MRSA, 76 % belonged to *spa* types t373 and t843. The median minimum inhibitory concentration (MIC) value for oxacillin was 16 mg/L (1–64 mg/L) and only one isolate was resistant to other classes of antibiotics. The most common type of infection was skin and soft tissue infections, most often in an existing skin lesion. The patients with *mecC* MRSA were colonized for a short time and gave rise to few secondary cases. *mecC* MRSA in our region appears to have a domestic origin and mainly affects patients with underlying diseases or patients with an existing skin lesion. Our data indicate that it could be a poor colonizer.

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a common pathogen within healthcare facilities and the community, and is a major challenge to the treatment of *S. aureus* infections. Methicillin resistance in *S. aureus* is conferred by the acquisition of a staphylococcal cassette chromosome (SCC) *mec* element, which carries the *mecA* gene encoding a penicillin-binding protein homologue (PBP2a) with reduced affinity for beta-lactam antibiotics. In 2011, a novel *mecA* gene homologue, *mecC*, was reported in isolates from both humans and dairy cattle [1]. Similar to *mecA*, it is located within an SCC*mec* element. This is also novel and given the designation type XI SCC*mec* [2]. *mecC* MRSA have now been reported from 13 European countries and have been isolated from 14 different host species [2]. In humans, *mecC* MRSA have been isolated in a range of infections, predominantly skin and soft tissue infections, but also in severe infections, such as sepsis [2]. In agreement with these epidemiological observations, analysis reveals that *mecC* MRSA isolates encode several known or putative *S. aureus* virulence factors, including several adhesins, superantigens, and toxins [2]. However, where tested, *mecC* MRSA strains have been negative for Panton–Valentine leukocidin (PVL), a prominent virulence factor among

community-associated (CA)-MRSA, and they have been negative for the human immune evasion genes [2]. In Sweden, the first MRSA with *mecC* was isolated in 2003 from a hedgehog but was not described as *mecC* until 2012 [3]. Since then, it has been isolated from dairy cattle [4, 5], cats, and humans (64 cases during the period 2011–2014) [5]. In Denmark, the prevalence of *mecC* MRSA among all MRSA in humans was 1.9 % in 2010, and increased to 2.8 % in 2011 [6]. In the UK and Germany, only a few isolates have been reported [7, 8]. So far, little is known about the epidemiology of *mecC* MRSA in humans.

In Sweden, MRSA is seen as a threat to public health and is, therefore, regulated by the Swedish Communicable Diseases Act. In 2000, MRSA became a mandatory notifiable disease. In 2012, *S. aureus* with *mecC* was included as a notifiable disease and has been, since then, handled in the same way as *S. aureus* with *mecA* regarding follow-up and contact tracing among household and healthcare contacts. However, active screening for *mecC* MRSA is, so far, not yet included in the screening program after healthcare abroad.

In this retrospective study, we present the epidemiology of 45 human clinical cases with *mecC* MRSA detected in Skåne County in the southern part of Sweden during the period 2005–2014.

Patients and methods

Background

Skåne County in southern Sweden has 1.2 million inhabitants. It is considered a low prevalence area for MRSA, with an incidence of 38 MRSA cases per 100,000 inhabitants in 2014 [5]. Since 1999, the Regional Centre for Communicable Disease Control in Skåne County has registered all known cases of MRSA and contact tracing among household members and healthcare contacts has been performed systematically. From 2012 onwards, all cefoxitin-resistant isolates negative for the *mecA* gene have been routinely tested for the presence of the *mecC* gene. The patients with *mecC* MRSA detected between 2012 and 2014 have been registered and followed up in the same way as patients with *mecA* MRSA and contact tracing has been performed.

Patients

A total of 45 patients with an isolate positive for *mecC* MRSA detected at the Clinical Microbiology Laboratory in Skåne between 2005 and 2014 were included in the study. Twenty-six isolates had been saved due to being cefoxitin resistant but *mecA* negative. These isolates were retrospectively tested positive for *mecC*. During the period 2012–2014, 19 *mecC* MRSA isolates were identified through routine testing of clinical *S. aureus* isolates.

The patients detected between 2012 and 2014 were included in the follow-up program with repeated cultures from the nares, throat, perineum, and possible skin lesions, for as long as they were colonized and 6 months thereafter. Contact tracing of household members and healthcare contacts of these patients was performed.

Culture results, resistance patterns, the PVL genes, and *spa* types were collected from the Clinical Microbiology Laboratory. In all patients, epidemiological data were received from the patient's medical files. For patients detected between 2012 and 2014, additional data were collected from the database at the Regional Centre for Communicable Disease Control.

Microbiological methods

Colonies from clinical samples were presumptively identified as *S. aureus* by morphology on blood agar and/or by giving a colored reaction on CHROMagar® Staph aureus (CHROMagar, Paris, France) and confirmed by the agglutination test using the Pastorex® Staph Plus kit (Bio-Rad, Hercules, CA, USA). Positive colonies were subjected to antibiotic susceptibility testing by the disk diffusion method according to instructions by the Swedish Reference Group of Antibiotics (http://www.srga.org) and, later on, the Nordic Committee on Antimicrobial Susceptibility Testing (http://www.nordicast.org). As instructed, disks with cefoxitin (Oxoid, UK) were used in the screening of MRSA. Strains with reduced susceptibility to cefoxitin were tested for the *mecA* gene by polymerase chain reaction (PCR), as described elsewhere [9]. Prior to 2012, *mecA*-negative strains were reported as non-MRSA with reduced susceptibility to isoxazolyl penicillins of unknown course. The strains were frozen at −80 °C for future analysis. In late 2011, the *mecA*-negative isolates were tested for *mecC* using an in house real-time PCR with melting point analysis using forward primer (5′- CAT CAC CAG GTT CAA CCC A -3′) according to García-Álvarez et al. [1] and a new reverse primer (5′- CGC CTT GGC CAT ATC CTG -3′). The PCR reaction contained 0.2 μM of each primer, Brilliant II SYBR®Green QPCR Master Mix (Agilent Technologies, Santa Clara, CA, USA), and 5 μl of template in a final volume of 25 μl. Following an initial incubation at 95 °C for 5 min, there were 40 cycles of 15 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C, followed by a melting program of 95 °C for 10 s, and thereafter gradually increased temperature of 0.5 °C/5 s to 95 °C with step fluorescence acquisition. A PCR product with a melting point of 77.5 ± 0.5 °C was identified as *mecC*. Sequence analysis of PCR products from five randomly selected isolates with different *spa* types showed 100 % homology to *mecC* sequences at the EMBL database (accession number FR821779, http://www.ncbi.nlm.nih.gov/nuccore/FR821779). An *mecC*-positive isolate obtained from Dr. Anders Rhod Larsen, SSI Copenhagen, Denmark, was used as the positive control.

Since 2012, all isolates with reduced susceptibility to cefoxitin that lacked *mecA* have been tested for *mecC*.

Samples from contact tracing were inoculated into mannitol salt broth containing 2.5 % NaCl, 4.0 mg/L cefoxitin, and 8.0 mg/L aztreonam. After overnight incubation at 35–37 °C, an in-house multiplex real-time PCR TaqMan assay targeting *nuc*, Sa442, and *mecA* was performed. Broths positive for *nuc* and/or Sa442 were cultured onto blood agar and CHROMagar® Staph aureus. Cefoxitin disks were placed in the primary streak. Colonies of *S. aureus* with reduced susceptibility to cefoxitin were them tested for *mecC* with PCR as described above.

Minimum inhibitory concentration (MIC) determination was performed using the Etest® (bioMérieux, France), according to the manufacturer's instructions.

Molecular characterization was performed by *spa* typing (sequencing of the polymorphic X region of the *S. aureus* protein A gene), as described elsewhere [9]. The PVL genes *lukS*-PV and *lukF*-PV were detected by PCR, as described elsewhere [9].

The study has been approved by the Regional Ethical Review Board in Lund, approval number 2013/428.

Results

Patients

Forty-five human clinical cases were included in the study. The median age, gender, origin, and the initial culture site is presented in Table 1. The *mecC* isolates were obtained by clinical cultures, 42 from an existing wound (chronic or traumatic), two from sputum, and one from nasopharynx. Most patients had some kind of underlying chronic disease, such as diabetes mellitus, cancer (lung, breast, ovarian, prostate, and brain cancer), autoimmune diseases (rheumatoid arthritis, scleroderma, and glomerulonephritis) and atherosclerotic diseases (hypertension, brain infarction, claudication, arterial insufficiency). Another common denominator was having a chronic wound. Of the ten patients without underlying disease, nine had an infection in an existing wound and one patient was positive in the nasopharynx when cultured during a respiratory tract infection. No primary infection was seen. Many of the patients (47 %) were retired and the others had various occupations. One person worked as a farmer. The majority lived in smaller, rural cities/villages, mostly in individual houses ($n = 38$). Four persons lived in farmhouses and three persons in an apartment. Thirty-nine of the patients were treated with antibiotics after the positive culture. Of these, 24 were treated with cloxacillin and only seven of these patients had their antibiotic treatment changed to non-beta-lactam antibiotics when the culture result was known.

Table 1 Characteristics of the 45 *mecC* methicillin-resistant *Staphylococcus aureus* (MRSA) cases

Median age in years (range)	60 (2–86)
Female gender (*n*)	22 (49 %)
Non-Swedish origin (*n*)	3 (7 %)
Initial culture site (*n*) for detection of *mecC* MRSA	Wound (42), sputum (2), nasopharynx (1)
Underlying diseases (*n*)	35 (78 %) 11 diabetes mellitus, 9 cancer, 4 autoimmune diseases, 11 atherosclerotic disease
spa types (*n*)	CC 130 (38): t373 (23), t843 (11), t10530 (1), t6594 (1), t7538 (1), t11205 (1) CC 2362 (7): t978 (4), t3391 (3)
MIC oxacillin median (mg/L)	16 (1–64)

Contact tracing was performed around the 19 patients that were detected during the period 2012 to 2014. In total, 27 family members of 13 families were cultured and two family members from different families were positive for MRSA *mecC*. Contact tracing of healthcare contacts was also performed and no one was found positive.

Of the patients detected in 2012 to 2014, two died before follow-up and, thus, 17 patients were cultured repeatedly. Of these, 13 were negative in all of the follow-up cultures, with a median time to the first culture of 13 days (7–44 days). One patient was positive after 3 weeks in the nares and the skin lesion, one was positive in the nares for 2 months, one was positive in the nares for 3 months, and one was positive in the nares and the throat for 6 months. The median time for the carriage of *mecC* MRSA (time from the first positive culture to the first negative culture) was 21 days (7–210 days). All of these patients were treated with systemic antibiotics but none were given decolonization treatment.

Microbiology

The *mecC* MRSA isolates belonged to eight different *spa* types, but the majority of the *mecC* MRSA isolates were of either *spa* type t373 or t843. The median MIC value for oxacillin was 16 mg/L (1–64 mg/L) and in 44 of the 45 isolates, no other resistance was found. One of the isolates was resistant to erythromycin and clindamycin. All isolates were PVL negative.

Compared to the total number of clinical cases with MRSA in the period 2012–1014, *mecC* constituted 10/102 (9.8 %) in 2012, 5/94 (5.3 %) in 2013, and 4/116 (3.4 %) in 2014.

Discussion

The discovery of MRSA carrying the *mecC* gene has raised questions about the epidemiology of these strains. In this

retrospective study, we described 45 human clinical isolates collected in Skåne County in the southern part of Sweden between 2005 and 2014.

The median age of the patients with *mecC* MRSA in our study was high (60 years) in comparison to the median age of patients with clinical symptoms of *mecA* MRSA (32 years) in a previous study from the same geographical region in 2005 to 2010 [10]. The majority of patients with *mecC* MRSA in our study were of Swedish origin. In the previous study of patients with *mecA* MRSA [10], only 50 % of the patients with clinical symptoms of *mecA* MRSA were of Swedish origin. The same pattern is described in Denmark [6] and Belgium [11], indicating a different epidemiology compared to *mecA* MRSA. This might indicate that import does not contribute to the emergence of *mecC* MRSA in our county. The majority of the *mecC* patients had an underlying chronic disease, which has not been the case with the *mecA* patients in the same region [10]. A common denominator was having a chronic wound and, most often, the patients without underlying diseases had an existing wound and no primary infection was seen in contrast to what we have seen in patients with *mecA* MRSA. This might reflect that *mecC* MRSA do not possess the PVL genes and other virulence genes as described in previous studies [2].

The *mecC* MRSA isolates in our study can be grouped in two multilocus sequence typing (MLST) clonal complexes (CCs), CC130 and CC2361, which is in concordance to the Danish study. The most common *spa* type, t373, was previously described only from Denmark and Ireland [6, 12]. The second most common *spa* type in our material, t843, is, so far, the most commonly described. Of the *spa* types detected in dairy cattle in Sweden, t524, t911, and t843 [4, 5], only t843 is described in our material. The origin of *mecC* MRSA is not yet clear, but there is evidence that contact with animals poses a risk and that it can be transmitted between species [2]. *mecC* MRSA have been detected from a diverse range of species, including: dairy cattle, seal, chaffinch, dog, sheep, wild hare, cat, wild brown rat, hedgehog, and otter [2]. Most of the patients with *mecC* MRSA lived in rural areas, and at least one person worked with cattle and five persons lived in farms. These factors might indicate exposure to animals and, hence, exposure to *mecC* MRSA. Unfortunately, since the study is retrospective, we lack full information about animal contact.

mecC MRSA have been reported as having lower MICs to oxacillin than their *mecA* counterpart [2], which is also a finding in our study with a median MIC value of 16 mg/L. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), most *mecA* MRSA strains have MIC values above 64 mg/L and the majority 256 mg/L [13]. In accordance with other reports [6], all but one *mecC* MRSA isolate was susceptible to all classes of tested antibiotics. After the positive culture was taken, 39 patients were treated with antibiotics. The majority were treated with cloxacillin and a few of these patients had their treatment switched to non-beta-lactam antibiotics when the culture result was known. This might indicate that treatment with beta-lactam antibiotics can be effective, reflecting the lower MIC values. This is also shown in a study by Mancini et al. [14], who reported that treatment with flucloxacillin was effective in experimental endocarditis caused by *mecC*-positive *S. aureus*.

Our results suggest that *mecC* MRSA isolates might be less prone to be contagious compared to *mecA* MRSA, since only 2 of 27 tested family members were found to be positive. In the previous study from the same region, 39 % of the tested family members were positive for MRSA *mecA* [10]. Among patients with infection caused by *mecA* MRSA from the same geographical region, the median time for carriage in clinical cases was 66 days, while the patients with *mecC* were carriers for a shorter time, 21 days (7–210 days). Since the number of patients with *mecC* MRSA is still small, the above data should be interpreted with caution.

In Denmark, an increase of *mecC* MRSA among all MRSA has been described [6]. This is not the case in our study, with fewer clinical cases with *mecC* MRSA in 2014 than in 2012.

A limitation of our study is that it is retrospective. Compared to other publications, we describe a consecutive material between 2005 and 2014, and it represents all known clinical cases with cefoxitin resistance lacking the *mecA* gene in our region. The finding of 45 cases is, in this context, substantial and adds important material to the knowledge of *mecC* epidemiology.

In conclusion, 45 human clinical cases with *mecC* MRSA were studied retrospectively. *mecC* MRSA mainly affects older patients with underlying diseases or with an existing skin lesion. The *mecC* MRSA in our region appears to have a domestic origin. Data indicate that it could be a poor colonizer with few secondary cases and few people being colonized for a long time.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study has been approved by the Regional Ethical Review Board in Lund, approval number 2013/428.

Informed consent As it was a retrospective study, some of the patients were not alive at study start and the information about the patients were decoded, so informed consent was not required. However, they were informed about the study in a letter and who to contact if they did not want to participate.

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