First report of mecC MRSA in human samples from Austria: molecular characteristics and clinical data

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Abstract

Reports of mecC methicillin-resistant Staphylococcus aureus (MRSA) strains have been published from several European countries. We describe the first six mecC MRSA isolates of human origin from Austria and report the application of a rapid PCR test. Candidate isolates \((n = 295)\) received between 2009 and 2013 were investigated phenotypically by cefoxitin screening and streaking on ChromID MRSA plates. The presence of mecC was confirmed in six isolates from blood cultures, wound swabs and screening samples of four female and two male patients (age range 7–89 years) by an in-house PCR method and the new Genspeed MRSA test (Greiner Bio-One, Kremsmünster, Austria). The mecC MRSA were further characterized by whole genome sequencing, multilocus sequence and spa typing.

Antimicrobial susceptibility testing was performed by Eucast disk-diffusion method and Vitek 2. The six mecC MRSA isolates were from two clonal lineages (CC130, including a new single-locus variant, and CC599) and four different spa types (t843, t1535, t3256, t5930). Analysis for virulence factor genes yielded lukED, eta, etd2 and edin-B (CC130 isolates) and tst, lukED, eta and sel (ST599 isolates). The Genspeed MRSA test identified mecC in all isolates whereas Vitek 2 failed to detect methicillin resistance in one isolate. The strains were susceptible to a wide range of non-β-lactam antibiotics. All patients were successfully treated or decolonized.

mecC MRSA are present in Austria as colonizers but may also cause infections. Thus, laboratories must choose appropriate test methods such as cefoxitin screening and confirmation using molecular assays specifically targeting mecC.

Keywords: Austria, cefoxitin, Genspeed, mecC, MLST, MRSA, PCR, Staphylococcus aureus, whole genome sequencing

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Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) isolates carrying the mecA homologue mecC have been reported from all over Europe [1–8]. They may be detected phenotypically by routine cefoxitin screening and by PCR using specific primers; however, standard molecular diagnostic systems based on amplification of mecA fail to recognize these strains due to nucleic acid divergences between mecA and mecC. Published clinical data concerning mecC MRSA in humans include reports about colonization as well as skin and soft tissue infections [4], but also include fatal bacteremia [7] and osteomyelitis [9]. Thus, reliable detection of these strains in diagnostic microbiology routine is important [10].

The National Reference Centre for Antimicrobial Resistance and Nosocomial Infections at the Elisabethinen Hospital Linz receives bacterial isolates of human origin for identification, confirmation and typing from Austrian laboratories. Its strain collection contains over 5000 isolates of Staphylococcus spp. many of which have been extensively studied and typed using molecular methods [11–14]. We searched this strain collection for S. aureus carrying mecC using the conventional phenotypic
approach followed by molecular confirmation with an in-house PCR method as well as one of the first commercially available systems also able to detect mecC, the Genspeed MRSA test (Greiner Bio-One, Kremsmünster, Austria). In addition, clinical and molecular typing data on four mecC-positive isolates detected as part of routine screening are presented, describing for the first time the presence of mecC MRSA in human samples from Austria.

Materials and Methods

Bacterial isolates, phenotypic and molecular antibiotic susceptibility testing and typing

Candidate S. aureus isolates (n = 295) that had tested negative for mecA and positive for femA using previously published primer sets between the years 2003 and 2012 were chosen from the strain collection [15,16]. Additionally, four strains received from Austrian laboratories in 2012–2013 for further testing regarding mecC were included in this study. Strains were subcultured overnight on trypticase soy agar containing 5% sheep’s blood (Oxoid, Wesel, Germany) at 36 ± 1°C in an aerobic atmosphere. Species identification of all isolates was done by matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry using the IVD MALDI Biotyper (Bruker Daltonik, Bremen, Germany) and the Vitek 2 system (bioMérieux, Marcy l’Étoile, France).

Susceptibility testing was performed according to the Eucast disk-diffusion method. All strains were screened phenotypically for methicillin susceptibility using cefoxitin 30 μg disks in quintuplicate. They were also inoculated onto ChromID MRSA agar plates (bioMérieux) that were read after 24 hours of incubation at 37°C. The broader antimicrobial susceptibility of mecC MRSA for a panel of substances (Table 1) was assessed by disk diffusion testing, and for selected substances, minimum inhibitory concentrations (MICs) were determined by gradient diffusion testing (Etest; bioMérieux) (Table 1). The susceptibility profiles of the mecC MRSA were also assessed using Vitek 2 Gram-positive antimicrobial susceptibility testing cards (bioMérieux).

All isolates showing a mean cefoxitin zone diameter <22 mm and/or growth on selective media underwent confirmatory PCR testing using a protocol published by Stegger et al. [17] to detect mecA, mecC and lukF-PV after extraction of bacterial DNA with InstaGene Matrix (BioRad, Hercules, CA, USA), with

| TABLE 1. Phenotypic and molecular typing data of six mecC methicillin-resistant Staphylococcus aureus isolates |
|---------------------------------------------------------------|
| Disk diffusion test                  | Diameter (mm) | Diameter (mm) | Diameter (mm) | Diameter (mm) | Diameter (mm) | Diameter (mm) |
|                                  | (category)    | (category)    | (category)    | (category)    | (category)    | (category)    |
| Cefoxitin                        | 18 (mean, R)  | 16 (mean, R)  | 21 (mean, R)  | 18 (mean, R)  | 17 (mean, R)  | 20 (mean, R)  |
| Gentamicin                       | 20 (S)        | 22 (S)        | 20 (S)        | 22 (S)        | 24 (S)        | 22 (S)        |
| Erythromycin                     | 26 (S)        | 26 (S)        | 24 (S)        | 28 (S)        | 30 (S)        | 30 (S)        |
| Clindamycin                      | 25 (S)        | 26 (S)        | 22 (S)        | 26 (S)        | 30 (S)        | 30 (S)        |
| Tetracycline                     | 25 (S)        | 26 (S)        | 22 (S)        | 27 (S)        | 30 (S)        | 30 (S)        |
| Fusidic acid                     | 30 (S)        | 31 (S)        | 26 (S)        | 30 (S)        | 30 (S)        | 30 (S)        |
| Trimethoprim/sulfadiazine        | 32 (S)        | 31 (S)        | 26 (S)        | 34 (S)        | 30 (S)        | 30 (S)        |
| Rifampicin                       | 31 (S)        | 30 (S)        | 27 (S)        | 32 (S)        | 30 (S)        | 30 (S)        |
| Gradient MIC test                | MIC (mg/L)    | MIC (mg/L)    | MIC (mg/L)    | MIC (mg/L)    | MIC (mg/L)    | MIC (mg/L)    |
| Cefoxolin                        | 1 (S)         | 1 (S)         | 1 (S)         | 1 (S)         | 0.5 (S)       | 0.5 (S)       |
| Vancomycin                       | 1 (S)         | 1 (S)         | 2 (S)         | 1 (S)         | 1 (S)         | 2 (S)         |
| Teicoplanin                      | 1 (S)         | 1 (S)         | 2 (S)         | 1 (S)         | 0.25 (S)      | 0.25 (S)      |
| Tigecycline                      | 0.25 (S)      | 0.25 (S)      | 0.25 (S)      | 0.25 (S)      | 0.125 (S)     | 0.125 (S)     |
| Linezolid                        | 0.3 (S)       | 0.5 (S)       | 1 (S)         | 0.5 (S)       | 0.5 (S)       | 2 (S)         |
| Daptomycin                       | 0.25 (S)      | 0.125 (S)     | 0.125 (S)     | 0.125 (S)     | 0.25 (S)      | 0.25 (S)      |
| Fosfomycin                       | 1 (S)         | 0.5 (S)       | 1 (S)         | 0.5 (S)       | 2 (S)         | 2 (S)         |
| Oxacillin                        | 4             | 4             | 2             | 8             | 4             | 2             |
| Cefoxitin                        | 16            | 32            | 16            | 16            | 32            | 16            |

Typing

| Multilocus sequence | Isolate 4402/2009 | Isolate 5127/2010 | Isolate 5590/2012 | Isolate 5625/2012 | Isolate 5676/2012 | Isolate 5752/2013 |
|---------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| SLV of 130          | 599               | 130               | 130               | 130               | 599               | 130               |
| Clonal complex      | 599               | 130               | 130               | 130               | 599               | 130               |
| spa Type            | cS930             | cS1256            | cS1253            | cS1253            | cS930             | cS443             |
| Virulence factor gene |                  |                   |                   |                   |                   |                   |
| tst                  | +                 | –                 | –                 | –                 | –                 | –                 |
| lukED                | +                 | –                 | –                 | –                 | +                 | +                 |
| eta                  | +                 | +                 | +                 | +                 | +                 | +                 |
| eta2                 | +                 | +                 | +                 | +                 | +                 | +                 |
| edinA                | –                 | –                 | –                 | +                 | –                 | +                 |
| sel                  | +                 | –                 | –                 | –                 | +                 | –                 |

SLV, single locus variant; MIC, minimum inhibitory concentration.
modified primer concentrations. Additionally, a commercially available rapid PCR system, the Genspeed MRSA (Greiner Bio-One, Kremsmünster, Austria) was evaluated. This system uses rapid bacterial lysis followed by conventional PCR and detection of mecA and mecC, including specific probes for *S. aureus*, via hybridization on a chip.

Isolates carrying mecC were further analyzed by pulsed-field gel electrophoresis of Smol restriction fragments. They were also typed by amplification of the polymorphic X region of the protein A gene (spa) followed by sequencing and assignment of the spa type [18]. Whole genome sequencing of mecC-positive isolates was performed as previously described [19] to confirm their mecC gene status, to determine their multilocus sequence type (ST) and to identify virulence factor and antibiotic resistance genes. Nucleotide sequences have been deposited in the European short-read archive as ERR387183, ERR387184, ERR387185, ERR387090, ERR490343 and ERR490433 for isolates 4402, 5127, 5590, 5625, 5675 and 5752, respectively.

**Clinical information**

Clinical information on patients with mecC MRSA was obtained from medical records retrospectively. Consent of the local ethics committee was provided (C-60-13).

**Results**

All strains were confirmed as *Staphylococcus aureus* with MALDI-TOF scores of $>2.0$. Phenotypic screening of the 295 isolates from the strain collection revealed two cefoxitin screening–positive isolates (0.7%) with mean diameters of 14 mm (range 13–14 mm) and 15 mm (all 15 mm), respectively. Fourteen isolates (4.7%), including the two that were cefoxitin screening positive, showed growth on ChromID MRSA agar. Cefoxitin diameters of the other 12 isolates were in the susceptible category (range 27–32 mm); thus, sensitivity of the ChromID MRSA agar for phenotypic meticillin resistance detection was 100% and specificity was 95.9%.

Further analysis by molecular testing was performed on these fourteen isolates. The two cefoxitin-resistant isolates were positive for mecC and negative for mecA by conventional PCR as well as with the new Genspeed MRSA test. In accordance with their susceptibility to cefoxitin, the other 12 isolates carried neither mecA nor mecC. Additionally, the four strains that were received in our capacity as reference laboratory were analyzed. All four isolates had a positive cefoxitin screening test with diameters of 17, 18, 20 and 21 mm, respectively. Presence of mecC and absence of mecA was confirmed with both PCR assays, and *lukF-PV* was not detected in any of the strains.

Analysis of spa sequencing data showed four different spa types among the six isolates: t5930 ($n = 2$), t1535 ($n = 2$), t843 ($n = 1$) and t3256 ($n = 1$). Whole genome sequencing of all isolates confirmed that each isolate encoded mecC within an SCCmec type XI. Sequence types derived from the genome sequences included three ST130, a single-locus variant of ST130 and two ST599. All six isolates were positive for the genes encoding leukocidin ED (*lukED*) (GenBank accession no. Y13225) and exfoliative toxin A (eta) (GenBank accession no. CAQ49592). The four isolates belonging to CC130 additionally carried the epidermal cell differentiation inhibitor B gene (edn-B) (GenBank accession no. AHCS4577) and the exfoliative toxin D gene (*etd2*) (GenBank accession no. HFS63069). The two ST599 isolates were also positive for the toxic shock syndrome toxin-1 (tst) (GenBank accession no. Q9F0L4) and the enterotoxin-L (*set*) gene (GenBank accession no. CAI80052) (Table 1). Other than mecC and *blaZ*, no other acquired resistance genes were identified.

Analysis of Smol pulsed-field gel electrophoresis patterns showed the strains to be unrelated, with the exception of isolates 5590/2012 and 5625/2012, which were isolated in the same area of Austria within 8 weeks of each other. The Dice coefficient for this pair was 85.9%, indicating possible relatedness.

Subsequently, the six mecC MRSA were subjected to antimicrobial susceptibility testing and yielded susceptible results for all non-β-lactam antibiotics (Table 1). Two isolates (5127/2010 and 5752/2013) had oxacillin MICs below the breakpoint usually used to infer meticillin resistance. Vitek 2 identified all six isolates correctly as *S. aureus* and gave a positive result for the cefoxitin screen in five isolates, whereas all isolates were given a susceptible result for oxacillin. One isolate (5752/2013) was identified as meticillin-susceptible *Staphylococcus aureus* (MSSA) with a negative cefoxitin screen also upon repetition of the test. The remaining susceptibility data of the AST GP card matched the results of manual testing.

Clinical data are summarized in Table 2, and the geographic location of the laboratories that provided the mecC MRSA strains as well as the year of detection are indicated in Fig. 1.

**Discussion**

To our knowledge, this is the first report concerning the detection of mecC MRSA in humans in Austria. We can confirm the presence of such strains back to the year 2009 resulting from the investigation of our strain collection. The only other data on mecC MRSA in Austria covers livestock- and wildlife-origin strains [20,21].

All mecC-positive isolates in this study were phenotypically recognizable as MRSA using cefoxitin in the Eucast disk.
diffusion test, which has been shown to reliably detect these strains [22]. However, the built-in cefoxitin screen of the Vitek 2 GP AST card detected only five of the six isolates, which is in contrast to a recent study using the same system successfully on 62 mecC MRSA isolates [23]. However, the atypical Vitek 2 oxacillin-susceptible/cefoxitin-resistant profile of mecC MRSA as described by Cartwright et al. [23] was observed in the remaining five isolates. Isolate 5752/2013, which was misidentified as MSSA by Vitek 2, had the largest cefoxitin diameter (21 mm) of all isolates and thus was also challenging for disk-diffusion test reading. All isolates were universally susceptible to non-β-lactam antibiotics, which compares to data on mecC MRSA from other European countries [6,7,24].

Previous reports have shown reduced efficiency of growth on commercially available selective chromogenic media, also including ChromID agar [6], for ST130 mecC MRSA. In contrast, in our small sample also containing ST130 strains, all isolates grew well on ChromID agar. However, the cefoxitin MICs of our isolates were considerably higher than those reported by Cuny et al. [6] (all ≥16 mg/L). The published cefoxitin content of this medium is 4 mg/L [25,26], so efficient growth was not unexpected. Also, the observed false-positive rate of 4.1% is in line with published data [27].

Up to now, PCR methods for MRSA detection have successfully covered all known SCCmec types as a result of the high homology of the mecA gene. However, the newly discovered mecC element shares only 69% identity of its DNA sequence with mecA, leading to negative results using mecA-based PCR protocols and a need for additional primers to detect mecC. At the moment, not all commercially available assays cover this requirement. The Genspeed MRSA test that we used in this study can be completed in 75 minutes from

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**TABLE 2. Clinical information of six mecC methicillin-resistant Staphylococcus aureus isolates**

| Characteristic | Isolate 4402/2009 | Isolate 5127/2010 | Isolate 5590/2012 | Isolate 5625/2012 | Isolate 5676/2012 | Isolate 5752/2013 |
|---------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Patient age/sex | 89/female | 54/female | 7/female | 72/female | 83/male | 70/male |
| Site of isolation | Blood culture | Myelodysplastic syndrome, sepsis | Methicillin screen | Wound swab (outer ear) | Wound swab (leg ulcer) | Ulcus cruris |
| Underlying disease | Diabetes, Eczema | Eczema | Otis externa | Ulcus cruris | Stroke | Blood culture |
| Therapy | Unknown | Octenidine/mupirocin | Topical ofloxacin | Topical silver-sulfas-diazine/povidone-iodine | Mupirocin | Mupirocin |
| Outcome | Recovered | Eradication | Unknown | Improvement | Unknown | Eradication |
| Risk factors | Unknown | Pet rabbit (not screened) | Unknown | Ulcer | Unknown | Unknown |
| Geographic location | Upper Austria | Vienna | Vorarlberg | Vorarlberg | Salzburg | Upper Austria |

No contact could be established between patients.

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**FIG. 1.** Location and year of isolation of six mecC methicillin-resistant Staphylococcus aureus isolates. Most patients lived in rural areas of Austria. (Map is a public domain file downloaded from [http://www.mygeo.info/karten/austria_pol99.jpg](http://www.mygeo.info/karten/austria_pol99.jpg)).
Thus, reliable detection and monitoring of MRSA at the moment, which requires information methods is needed.

The capability of mecC MRSA to cause clinical disease in a wide range of patients has been established by several reports. In a Spanish case series, most patients were elderly men who were colonized, but a patient with lethal sepsis was also described [7]. Petersen et al. [4] reported on 112 mecC MRSA isolates from Denmark, with equal sex distribution and a mean patient age of 51 years. Interestingly, the majority of the isolates were from infections, mostly skin and soft tissue infection, and not from colonization screening. In a recent report on the prevalence of human mecC MRSA in England, more than half of the isolates were identified from screening samples [24], whereas in a case series from Germany, most strains were isolated from wounds but also included was an isolate from nosocomial pneumonia [6]. A French case report described a patient with mediastinitis and sternal osteitis due to mecC MRSA [9]. In two of our six clinically diverse study patients, mecC MRSA were identified via routine screening, but in the remaining four patients, it was clearly associated with their clinical presentation.

Overall, mecC MRSA do not seem to be highly prevalent in Austria at the moment, which reflects the situation in most other European countries [10]. On the other hand, the frequency of mecC MRSA has increased significantly to a proportion of 2% of the total annual human MRSA cases in Denmark [4]. As mecC is carried on a mobile genetic element (SCCmec), it has the potential to spread into different lineages [29–31]. Thus, reliable detection and monitoring of mecC MRSA with appropriate phenotypic and molecular screening and confirmation methods is needed.

**Conflict of interest**

None declared.

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