ORIGINAL ARTICLE

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*.

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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

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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'Etoile, 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

	Isolate 4402/2009	Isolate 5127/2010	Isolate 5590/2012	Isolate 5625/2012	Isolate 5676/2012	Isolate 5752/2013
Disk diffusion test	**Diameter (mm) (category)**	**Diameter (mm) (category)**	**Diameter (mm) (category)**	**Diameter (mm) (category)**	**Diameter (mm) (category)**	**Diameter (mm) (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)	25 (S)	30 (S)
Fusidic acid	30 (S)	31 (S)	26 (S)	30 (S)	30 (S)	30 (S)
Trimethoprim/sulfa	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)**
Ceftaroline	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)	1 (S)	0.25 (S)
Tigecycline	0.25 (S)	0.25 (S)	0.25 (S)	0.25 (S)	0.125 (S)	0.25 (S)
Linezolid	0.5 (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)	1 (S)	0.5 (S)	1 (S)	0.5 (S)	2 (S)
Oxacillin	4	4	2	8	4	2
Cefoxitin	16	32	16	16	32	16
Typing						
Multilocus sequence Type	599	130	SLV of 130	130	599	130
Clonal complex	599	130	130	130	599	130
spa Type	t5930	t3256	t1535	t1535	t5930	t843
Virulence factor gene						
tst	+	–	–	–	+	–
lukED	+	+	+	+	+	+
eta	+	+	+	+	+	+
etd2	–	+	+	+	–	+
edin-B	–	+	+	+	–	+
sel	+	–	–	–	+	–

SLV, single locus variant; MIC, minimum inhibitory concentration.

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