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# Phenotype and Genotype Characteristics of *Staphylococcus aureus* Resistant to Methicillin/Oxacillin Carrying Gene *mecC* in the Czech Republic from 2002 to 2017

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The aim of this study was to detect and characterize isolates of methicillin-/oxacillin-resistant *Staphylococcus aureus* (MRSA) carrying gene *mecC* (MRSA/*mecC*) and occurring in the Czech Republic within the period from 2002 to 2017. Altogether, 18 from 3,472 isolates of MRSA were *mecC* positive (0.52%). The first detection of MRSA/*mecC* in the Czech Republic is dated to 2004. MRSA/*mecC* isolates were susceptible to almost all tested antibiotics with few exceptions. Resistances to erythromycin ($n=2$), clindamycin ($n=1$), trimethoprim-sulfamethoxazole ($n=1$), and rifampicin ($n=1$) were found in the collection. Multilocus sequence typing and *spa* typing revealed a genetic heterogeneity of MRSA/*mecC* strains: three CCs (130, 425, and 2361), five STs (1245, 130, 2361, 425, and a new ST5480), and eight *spa* types (t843, t978, t1048, t1535, t1736, t6104, t8842, and t17153), which were detected in the study, with the highest prevalence of CC130/t843 lineage ($n=8$, 44%). Except for two strains, none from 18 examined isolates harbored genes encoding any of *S. aureus* toxins: enterotoxins a–u, exfoliative toxins A, B, and D, toxic shock syndrome toxin-1, and the Panton-Valentine leukocidin.

**Keywords:** *Staphylococcus aureus,* MRSA*, mecC*, MLST, *spa* typing

## Introduction

*Staphylococcus aureus* is the most important human *Staphylococcus* species associated with nosocomial and community-acquired infections.[1] Since the first detection of diseases caused by methicillin-/oxacillin-resistant *S. aureus* (MRSA) (1961, UK), this pathogen has expanded into the whole world.[2] MRSA isolates can exhibit resistance to a broad spectrum of antibiotics. Decreased susceptibility to methicillin/oxacillin as well as to other antibiotics is caused by acquired transpeptidase penicillin binding protein 2a (PBP2a) characterized by low affinity to β-lactams, with the exception of ceftaroline and ceftobiprole, so called anti-MRSA cephalosporins. PBP2a is coded by *mecA* occurring in the staphylococcal chromosome cassette *mec* (SCC*mec*). SCC*mec* consists of variable and conserved genetic elements, including *mec* operon with *mecA* and regulatory (*mecI* and *mecR1*) genes, as well as recombinase genes (*ccrA*, *ccrB*, and *ccrC*) responsible for excision/integration of this genetic element within chromosome.[3] Till now, 13 types of SCC*mec* (I–XIII) have been identified.[4]

In 2002 (Scotland) was detected a new variant of *mecA* gene in humans; $mecA_{LGA251}$ (later called *mecC*) producing an altered transpeptidase (PBP2c), also with a low affinity to β-lactam antibiotics.[5] *MecA* and *mecC* homologs share similarity at the DNA level around 70%,[3] and only 63% at amino acid level.[5] Due to a nucleic acid divergence between *mecA* and *mecC* genes, all methods commonly used for *mecA* detection failed in *mecC* uncovering. One of the successful and simple methods used in the detection of MRSA isolates carrying gene *mecC* (MRSA/*mecC*) is PCR in combination with primers specific for gene *mecC*.[5,6]

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Despite the fact that the presence of MRSA isolates carrying gene *mecC* was confirmed almost from all over Europe in humans,[5,7] there is missing information about *mecC* detection in the Czech Republic. Therefore, the objective of this work was to examine the occurrence, an antibiotic susceptibility profile, virulence potential, as well as the genetic diversity of MRSA isolates carrying gene *mecC* in the long-term period, from 2002 to 2017 in the Czech Republic.

## Materials and Methods

### *Bacterial isolates*

Screening of MRSA/*mecA* isolates is provided as routinely in the National Reference Laboratory for Antibiotics (National Institute of Public Health, Prague, Czech Republic) as a part of European Antimicrobial Resistance Surveillance Network (EARS-Net). MRSA strains with an absence of *mecA* gene were further examined for the presence of *mecC* gene. Overall, 3,472 isolates of MRSA were isolated in the National Reference Laboratory for Antibiotics from 2002 to 2017. The majority of the clinical specimen, altogether 78.6% ($n=2,728$), were of invasive origin (blood and cerebrospinal fluid), and 21.4% ($n=744$) of MRSA strains were isolated from noninvasive materials (surgical wound, urine, sputum, and swab: nose, nasopharynx, and throat). All staphylococci were cultivated on nutrient agar (Oxoid) overnight at 36°C aerobically. Identification of *S. aureus* strains was performed by Matrix-assisted laser desorption ionization–time of flight mass spectrometry (Maldi-TOF; Microflex Brucker, Bremen, Germany).

### *Susceptibility testing*

Screening for MRSA was performed by disk diffusion method using cefoxitin disc (30 μg); zone diameter <22 mm was interpreted as oxacillin resistance according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations.[8] Minimal inhibition concentration of oxacillin (OXA), erythromycin (ERY), clindamycin (CLI), trimethoprim-sulfamethoxazole (SXT), rifampicin (RIF), ciprofloxacin (CIP), gentamicin (GEN), vancomycin (VAN), fusidic acid (FUS), chloramphenicol (CMP), linezolid (LNZ), and tigecycline (TGC) was determined by broth microdilution method according to ISO 20776-1. Susceptibility to tetracycline (TET) and tobramycin (TOB) was determined by disc diffusion method.[9] Interpretation of susceptibility testing results was performed as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (later the Clinical & Laboratory Standards Institute: CLSI Guidelines)[10] and EUCAST (version according to a corresponding year).[11] Minimal inhibitory concentration of oxacillin was interpreted according to the NCCLS (later CLSI)[10] and the EUCAST-ECOFF value (version according to a corresponding year).[12]

### *Molecular typing*

PCR. DNA was isolated according to the procedure of isolation kit (GenElute™ Bacterial Genomic DNA Kits, Sigma Aldrich). The presence of *mecA* and *mecC* genes in MRSA isolates was identified by PCR. Gene *mecA* was amplified using primers F 5′ -GTA GAA ATG ACT GAA CGT CCG ATA A- 3′ and R 5′ -CCA ATT CCA CAT TGT TTC GGT CTA A- 3′. Conditions for PCR were 2 minutes at 94°C followed by 30 cycles of 30 seconds at 94°C, 1 minute at 55°C, 1 minute at 72°C, and finished by 10 minutes at 72°C (Bio-rad, DNA Engine Dyad® Dual–Bay Thermal Cycler). Gene *mecC* was detected according to the protocol of National Food Institute.[6] PCR products were resolved in 1% agarose (Agarose SFR, VWR Life Science) in electrophoresis 5 V/cm for 50 minutes. *S. aureus*, NCTC13552 (Salisbury, UK), was used as positive control for *mecC* gene.

Spa and MLST typing. *Spa* typing: MRSA/*mecC* isolates were further analyzed using genotypic methods; repetitive sequences of protein A were amplified using the following primers: 1113f (5′- TAA AGA CGA TCC TTC GGT GAG C -3′) and 1514r (5′-CAG CAG TAG TGC CGT TTG CTT -3′), according to the procedure[13] by analyzer (Applied Biosystems 3130xL). Data were analyzed using Bionumerics 7.6.2 (Applied Maths, Ghent, East Flanders, Belgium). The minimum spanning tree (MST) algorithm (gap creation cost 250%, gap extension cost 50%, duplicate creation cost 25%, and duplicate extension 25%, maximum duplication of three repetitions) used for cluster analysis of spa isolates was carried out also by Bionumerics 7.6.2.

Multilocus sequence typing (MLST) of *mecC* strains: seven housekeeping genes *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqi* were amplified according to the standard procedure described earlier.[14] All strains were sequenced by analyzer (Applied Biosystems 3130xL). Data were analyzed by Bionumerics 7.6.2 and eBurst analysis was performed (database is no longer active).

### *Toxin detection*

Isolates of *S. aureus* carrying *mecC* gene were also subjected to PCR analysis for confirmation or refutation of presence of genes encoding staphylococcus toxins: enterotoxins a-u (*sea–seu*), exfoliative toxins A, B, and D (*eta*, *etb*, and *etd*), staphylococci superantigens: toxic shock syndrome toxin-1 (*tst*), and the Panton–Valentine leukocidin (*lukS/F-PVL*; consisting of the LukS and LukF fragments). The list of primers and PCR conditions used for toxins amplification is in the Supplementary Table S1. S6 (*sea*, *seb*, *seq*, and *sek*), FRI137 (*sec*, *seh*, *sel*, *sem*, *sen*, *seo*, and *seu*), 01HMPL280 (*sed*, *seg*, *sei*, *sej*, *sep*, and *ser*), and FRI326 (*see*) were reference strains used for the detection of enterotoxins (all acquired from the French Food Safety Agency, Paris, France). Strains CCM7056 (*eta* and *etb*), CCM2331 (*etd*) (both acquired from Brno, Czech Republic), CNCTC5931 (Prague, Czech Republic, positive for *tst*,), and ATCC49775 (Virginia, positive for *lukS/F-PVL*,) were used for the detection of exfoliative toxins, *tst*, and the *lukS/F-PVL*.

## Results

### *Antibiotic susceptibility of MRSA/mecC isolates*

Altogether, 3,472 isolates of *S. aureus* resistant to CXT were tested for the presence of *mecA/mecC* gene in the period from 2002 to 2017. Eighteen of them harbored the *mecC* gene (0.52%). Resistance to methicillin/oxacillin in the rest of the MRSA samples (99.48%) was caused by the

Table 1. The List of Methicillin-/Oxacillin-Resistant *Staphylococcus aureus*/*mecC* Isolates Involved in the Study

| | | | | | | *mm* | | | *mg/L* | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Isolate* | *Age* | *Sex* | *Source* | *Year of isolation* | *Location* | *CXT* | *TET* | *TOB* | *OXA* | *ERY* | *CLI* | *SXT* | *RIF* | *CIP* | *GEN* | *VAN* | *FUS* | *CMP* | *LNZ* | *TGC* | *spa* | *ST* | *CC* | *En* | *tst* | *Exf* | *lukS/F-PVL* |
| 2132 | 59 | M | Blood | 2004 | Prague | 12 | 24 | 21 | >8 | 1 | 0.25 | ≤0.125 | ≤0.03 | 0.5 | 0.5 | 1 | 0.25 | 8 | 2 | 0.5 | t1736 | 5480 | 130 | – | – | – | – |
| 4747 | 67 | M | Sputum | 2004 | Ceske Budejovice | 14 | 28 | 22 | >8 | 1 | 0.25 | ≤0.125 | ≤0.03 | 0.25 | 0.5 | 1 | 0.125 | 8 | 2 | 0.5 | t843 | 130 | 130 | – | – | – | – |
| 4259 | 79 | M | Swab | 2010 | Ceske Budejovice | 12 | 27 | 22 | 8 | 0.5 | 0.125 | ≤0.125 | ≤0.03 | 0.5 | 0.25 | 0.5 | 0.125 | 8 | 2 | 0.125 | t843 | 1245 | 130 | – | – | – | – |
| 7468 | 79 | M | Blood | 2010 | Ceske Budejovice | 14 | 25 | 22 | >32 | 0.5 | 0.25 | ≤0.125 | ≤0.03 | 0.5 | 0.25 | 0.5 | 0.06 | 4 | 1 | 0.06 | t843 | 130 | 130 | – | – | – | – |
| 8223 | 79 | M | Blood | 2011 | Ceske Budejovice | 13 | 28 | 23 | 8 | 0.25 | 0.125 | ≤0.125 | ≤0.03 | 0.5 | 0.5 | 1 | 0.06 | 4 | 2 | 0.125 | t843 | 130 | 130 | – | – | – | – |
| 18063 | 73 | M | Chirurgical wound | 2013 | Benesov | 17 | 26 | 21 | 2 | >8 | 0.125 | ≤0.125 | ≤0.03 | 0.5 | 0.5 | 2 | 0.125 | 3 | 2 | 0.06 | t978 | 2361 | 2361 | # | + | – | – |
| 25255 | 65 | M | Swab | 2014 | Pribram | 18 | 25 | 22 | 1 | 0.5 | 0.125 | ≤0.125 | ≤0.03 | 0.5 | ≤0.125 | 1 | 0.125 | 8 | 2 | 0.06 | t843 | 1245 | 130 | – | – | – | – |
| 25994 | 6 | M | Swab | 2014 | Kyjov | 11 | 27 | 22 | 16 | 0.5 | 0.125 | ≤0.125 | ≤0.03 | 0.5 | 0.25 | 1 | 0.125 | 8 | 2 | 0.06 | t6104 | 425 | 425 | – | – | – | – |
| 26510 | 68 | F | Blood | 2014 | Plzen | 16 | 27 | 21 | 8 | 0.25 | 0.06 | ≤0.125 | ≤0.03 | 0.5 | ≤0.125 | 1 | 0.125 | 8 | 2 | 0.5 | t8842 | 1245 | 130 | – | – | – | – |
| 29732 | 62 | M | Chirurgical wound | 2015 | Prerov | 14 | 23 | 20 | 4 | 0.25 | 0.25 | ≤0.125 | ≤0.03 | 0.5 | 0.25 | 1 | ≤0.03 | 8 | 2 | 0.5 | t1048 | 130 | 130 | – | – | – | – |
| 30299 | 65 | M | Chirurgical wound | 2015 | Prerov | 17 | 27 | 21 | 4 | 0.25 | 0.125 | >4 | >2 | 0.5 | 0.25 | 1 | 0.125 | 4 | 2 | 0.06 | t1048 | 130 | 130 | – | – | – | – |
| 30720 | 37 | F | Swab | 2015 | Pribram | 16 | 25 | 21 | 8 | 0.25 | 0.125 | ≤0.125 | ≤0.03 | 0.5 | 0.5 | 1 | 0.06 | 8 | 2 | 0.06 | t843 | 1245 | 130 | – | – | – | – |
| 30721 | 5 | F | Swab | 2015 | Pribram | 16 | 28 | 23 | 8 | 0.5 | 0.25 | ≤0.125 | ≤0.03 | 0.5 | 0.25 | 1 | 0.125 | 8 | 2 | 0.125 | t843 | 1245 | 130 | – | – | – | – |
| 30722 | 5 | F | Swab | 2015 | Pribram | 15 | 27 | 21 | 4 | 0.5 | 0.25 | ≤0.125 | ≤0.03 | 0.5 | 0.5 | 1 | 0.125 | 8 | 2 | 0.125 | t843 | 1245 | 130 | – | – | – | – |
| 30730 | 84 | M | Chirurgical wound | 2015 | Uherske Hradiste | 16 | 27 | 22 | 4 | >8 | >4 | ≤0.125 | ≤0.03 | 0.5 | 0.5 | 2 | 0.06 | 8 | 2 | 0.125 | t1048 | 130 | 130 | – | – | – | – |
| 30792 | 57 | M | Swab | 2015 | Pribram | 15 | 26 | 22 | 8 | 0.5 | 0.25 | ≤0.125 | ≤0.03 | 0.5 | 0.5 | 1 | 0.06 | 8 | 2 | 0.125 | t1535 | 1245 | 130 | – | – | – | – |
| 35220 | 71 | F | Swab | 2016 | Prerov | 13 | 28 | 23 | 8 | 0.5 | 0.125 | ≤0.125 | ≤0.03 | 1 | 0.5 | 1 | 0.06 | 4 | 2 | 0.125 | t17153 | 130 | 130 | – | + | – | – |
| 37553 | 53 | M | Chirurgical wound | 2017 | Brno | 11 | 26 | 21 | 8 | 1 | 0.25 | 0.5 | ≤0.03 | 0.5 | ≤0.125 | 1 | 0.125 | 8 | 2 | 0.125 | t1048 | 130 | 130 | – | – | – | – |

CIP, ciprofloxacin; CLI, clindamycin; CMP, chloramphenicol; CXT, cefoxitin; ERY, erythromycin; FUS, fusidic acid; GEN, gentamicin; LNZ, linezolid; OXA, oxacillin; RIF, rifampicin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; TGC, tigecycline; TOB, tobramycin; VAN, vancomycin.

#, genes for enterotoxins (*sec*, *seg*, *sei*, *sel*, and *sem)* detected in MRSA/*mecC* isolates; +, positive; –, negative; CC, clonal complex; En, enterotoxins; Exf, exfoliative toxins; F, female; *lukS/F-PVL*, Panton–Valentine leukocidin; M, man; *spa*, Staphylococcus protein A; ST, sequence type; *tst*, toxic shock syndrome toxin-1.

presence of *mecA* gene (data not shown). The magnitude of spreading differed from year to year, but the first detection of *mecC* in the Czech Republic was dated to the year 2004 (Table 1, isolate number 2132). MRSA/*mecC* isolates were detected in Ceske Budejovice, Pribram, Prostejov, Kyjov, Uherske Hradiste, Benesov, Prague, and Plzen. Positive strains were isolated especially from noninvasive material: swab (nose, nasopharynx, and throat) ($n=8$), chirurgical wound ($n=5$), and sputum ($n=1$). Only four strains were acquired from blood.

Antimicrobial susceptibility testing revealed that the majority of isolates was susceptible to most of tested antibiotics. Altogether, two isolates of MRSA/*mecC* (18063 and 25255) were resistant to CXT, but sensitive to OXA. One isolate showed resistance to ERY. The isolate 30730 was resistant to both ERY and CLI. The strain 30299 was resistant to SXT and RIF (Table 1).

### *Molecular typing*

Among 18 isolates, eight different *spa* types were found in the collection. *Spa* type t843 ($n=8$), followed by t1048 ($n=4$) were the most frequent *spa* types detected in the study. The remaining *spa* types of MRSA/*mecC* isolates were as follows: t978, t1535, t1736, t6104, t8842, and t17153 (Table 1). The core of the group/the closest genetic relationship (one difference in repeat sequence) was observed between four *spa* types: t843, t1048, t1535, and t8842. The rest of isolates (t978, t1736, t6104, and t17153) differed from the core of the group by more than just one repeat sequence in the *spa* gene. The cluster analysis (MST) based on similarity of *spa* profiles is shown in Fig. 1.

Altogether, there were five sequence types (STs) detected in MRSA/*mecC* isolates: ST130 ($n=8$), ST425 ($n=1$), ST1245 ($n=7$), and ST2361 ($n=1$). A new ST5480 (a new allele *tpi* 611) was found in the first MRSA-/*mecC*-positive isolate (number 2132) detected in the Czech Republic. MLST analysis revealed the prevalence of clonal complex (CC) 130 (89%, $n=16$), including STs 1245, 130, and 5480. Other STs detected in the study, ST2361 and ST425, belonged to different CCs: CC2361 (ST2361) and CC425 (ST425) (Table 1).

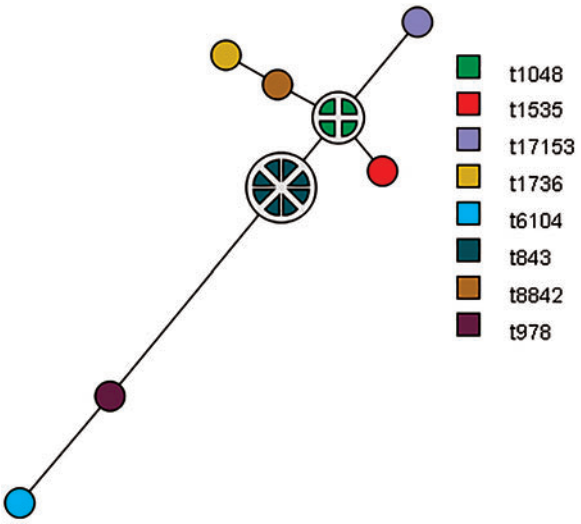


**FIG. 1.** MST analysis of MRSA/*mecC* isolates ($n=18$) illustrating the relationship within cluster. The nodes/*one-colored circles* consist of isolates with the same genotype, the size of node/*circle* correlates with the number of genetically identical isolates, and the distance between nodes represents the genetic relationship between nodes. MST, minimum spanning tree; MRSA, methicillin-/oxacillin-resistant *Staphylococcus aureus*. Color images are available online.

### *Toxins*

Strains were negative for virulence toxin genes encoding exfoliative toxins, and *lukS/F-PVL* (Table 1), with the exception of genes for enterotoxins *sec, seg, sei, sel, sem* (found in 18063), and *tst* that were detected in two isolates (18063 and 35220) (Table 1).

## Discussion

Identification of MRSA isolates is key for controlling dissemination of this pathogen in human population and it is important for suitable management of colonized and infected patients. Except for *mecA*, there is also a new homologue of this gene, *mecC*, which has been already sporadically reported in MRSA strains all over Europe.[15,16] Except for *S. aureus*, *mecC* has been already detected in other *Staphylococcus* species, namely *Staphylococcus stepanovicii*,[17] *Staphylococcus xylosus*,[18] and *Staphylococcus sciuri*.[19]

The most reliable tools used in the MRSA/*mecC* detection are PCR with specific primers for *mecC* gene and disc diffusion test with CXT. Two (18063 and 25255) from 18 strains were susceptible to OXA and resistant to CXT, along with a positive detection of *mecC* gene by PCR. The majority of *mecC*-positive MRSA was susceptible to the most of examined antibiotics, which was in line with results of other authors.[7,20,21]

Molecular typing is an important tool for control and prevention of infections. One of such methods used especially for analysis of genetic variability of *S. aureus* is *spa* typing. *Spa* analysis revealed the presence of eight *spa* types associated with *mecC* strains in the trial. The most prevalent *spa* type t843 (44%),[22] altogether with t978, t1535, t8842,[23] t1736,[24] and t1048,[7,25] has been already confirmed in *mecC*-positive strains in epidemiological studies all over Europe. Except for a susceptible *S. aureus* from France (Ridom SpaServer, Ridom GmbH, Würzburg, Germany), *spa* type t6104 was detected in this study too. MLST is also an epidemiology tool useful for understanding the molecular evolution of bacteria. Isolates of MRSA were assigned to three CCs: 130, 425, and 2361. CC130 has been previously reported in MRSA isolates of animal and human origin and it is a CC commonly associated with the occurrence of MRSA isolates carrying gene *mecC* in Europe.[6,25,26] CC2361 and 425 were previously observed in MRSA/*mecC* isolates in Denmark and Spain.[27,28]

A virulence potential of *mecC*-positive MRSA strains has been also studied. A whole genome sequencing analysis has been pointed at an absence of genes coding exfoliative toxins as well as staphylococcal *tst*, and enterotoxins (*sea–see* and *seg–sej*).[7,16] The absence of *lukS/F-PVL* in MRSA/*mecC* isolates was confirmed.[25] On the other hand, it seems that ST profile could be associated with toxin production; an

absence of toxin genes in the most samples of CC130 has been already described before.[7,16] The only two toxins-positive isolates (enterotoxins *sec*, *seg*, *sei*, *sel*, *sem*, and *tst*) belonged to the CC130/t17153 and CC2361/t978, indicating that this toxin producing CCs/*spa* types might represent a risk to humans.

In line with our results, the spreading of MRSA/*mecC* isolates becomes more frequent in people older than 50 years.[7,16,27] This part of human population is considered high risk due to the weakening of immune system and frequent consumption of antibiotics.[27] Also, a hospitalization or contact with animals (zoonotic transmission) as well as travel activities belong to the risk factors for MRSA spreading.[16]

The limitation of this study lies in the kind of analyzed clinical specimen. The most of MRSA isolates was acquired from invasive material, preferentially sent to the National Reference Laboratory for Antibiotics. However, the majority of *mecC*-positive MRSA isolates, as showed this study, has been found in noninvasive clinical specimen. Most of isolates were acquired from swab (nose/throat).

The aim of this study was to detect and describe MRSA/*mecC* isolates occurring in the Czech Republic within a 15-year long period. And to our knowledge, this is the first report concerning the detection of these strains in the Czech Republic. The first detection of MRSA/*mecC* isolate was dated to 2004 (59 years, man, Prague), a new ST5480, a spa type t1763. The most of MRSA-positive *mecC* strains belonged to the susceptible lineage CC130/t843. A rare occurrence of MRSA/*mecC* isolates confirmed in this study (0.52%) was comparable with findings in Europe (2%, in Denmark).[25]

Due to a clinical relevance of MRSA strains and their spreading possibilities, there should be attention on a right detection as well as monitoring of abovementioned strains associated, especially with hospital-acquired infections.

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### Disclosure Statement

No competing financial interests exist.

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### Supplementary Material

Supplementary Table S1

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