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Molecular characterisation of methicillin-resistant *Staphylococcus pseudintermedius* from dogs and the description of their SCC*mec* elements

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ABSTRACT

In recent years an increasing number of methicillin-resistant *S. pseudintermedius* (MRSP) has been observed in both, healthy and clinically infected dogs. The aim of the study was the characterisation of MRSP isolates from clinical routine diagnostics of a German laboratory in order to assess the abundancy of resistance genes and SCC*mec* elements. 97 isolates from 96 dogs were analysed using microarrays detecting resistance genes and SCC*mec*-associated markers.

All isolates harboured *mecA* and *blaZ*. Other abundant resistance markers (in > 80% of isolates) included *aacA-aphD, aphA3* and *sat* as well as *erm*(B). Tetracycline resistance genes (*tet*(K), *tet*(M)) and *cat* also were common (in > 20%). The vast majority (n = 59) of isolates carried SCC*mec* III elements. SCC*mec* IV and V elements were identified in 21 and 15 isolates, respectively. Irregular or pseudo-SCC*mec* elements were found in 2 isolates.

The high degree of uniformity of hybridisation patterns of tested strains suggest that the majority of MRSP infections was caused by one single strain and comparison to previously published reports and sequences suggest that this was the ST71-SCC*mec* III strain that also predominates elsewhere in Western Europe.

1. Introduction

Staphylococcus pseudintermedius is a coagulase-positive species of *Staphylococcus*. It forms, together with *Staphylococcus intermedius* and *Staphylococcus delphini* the "*S. intermedius* group (SIG)" of closely related species that are difficult to differentiate phenotypically (Sasaki et al., 2007). It colonises - or infects - dogs and cats. In a similar way as *Staphylococcus aureus* in humans, it is carried by a high percentage of healthy, asymptomatic individuals, but it is also one of the most common pathogens causing clinical infection in its host species (Bannoehr and Guardabassi, 2012; Fazakerley et al., 2010). *S. pseudintermedius* can cause a wide variety of different infections, including pyoderma, wound infections, otitis, urinary tract infections and osteomyelitis (Cabassu and Moissonnier, 2007; Fitzgerald, 2009; Huerta et al., 2011; Weese and van Duijkeren, 2010). Most, or possibly all, isolates carry genes encoding a bi-component leukocidin akin to the Panton-Valentine leukocidin of *S. aureus* (Abouelkhair et al., 2018; Kaneko and Kamio, 2004; Szmigielski et al., 1999).

S. pseudintermedius can be transmitted to humans, especially to those who had close contact to dogs. Zoonotic infections in humans include infected dog bites, but also bloodstream infections, abscesses, pneumonia and septic arthritis (Borjesson et al., 2015; Frank et al., 2009; Pantosti, 2012; Riegel et al., 2011; Robb et al., 2017; Somayaji et al., 2016). It is easily confused with *S. aureus* when isolated from human samples (Borjesson et al., 2015; Frank et al., 2009; Pantosti, 2012; Riegel et al., 2011; Robb et al., 2017; Somayaji et al., 2016).

In recent years an increasing number of methicillin-resistant *S. pseudintermedius* (MRSP) has been observed in both, healthy and clinically infected dogs (Bannoehr and Guardabassi, 2012; Bergstrom et al., 2012; Loeffler et al., 2007; van Duijkeren et al., 2011). For instance, the rate of MRSP in The Netherlands increased from 0.9% (2004) to 7% MRSP (2013) (Duim et al., 2016), while in the USA an increase from 5% (2001) to 30% (2007) was noted (Kadlec and Schwarz, 2012).

Dogs can be colonised by MRSP for months whether or not they

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show clinical symptoms (Bergstrom et al., 2012; Kadlec et al., 2011; van Duijkeren et al., 2011; Laarhoven et al., 2011; Windahl et al., 2012). Previous studies indicated that skin, fur, nose/nostrils, muzzle and anus can be colonized by *S. pseudintermedius*/MRSP (Bannoehr and Guardabassi, 2012). Likely risk factors for MRSP colonization, infection and transmissions include previous animal hospital admissions, surgical interventions, administration of antibiotics and corticosteroids (Weese et al., 2012; Windahl et al., 2012).

Methicillin resistance indicates general beta-lactam resistance, and this property causes significant therapeutic and infection control challenges. As in *S. aureus,* methicillin resistance in *S. pseudintermedius* is encoded also by the gene *mecA*. Its gene product is a modified penicillin binding protein that has a low affinity for most beta-lactams.

The *mecA* gene is situated on potentially mobile genetic elements, the SCC*mec* elements (staphylococcal cassette chromosome *mec*). Besides *mecA*, SCC*mec* elements also harbour regulatory genes, recombinase genes and a wide range of variable accessory genes. So far, thirteen main types of SCC*mec* elements have been described (Ito et al., 2001, 2004; IWG-SCC, 2009; Shore and Coleman, 2013; Wu et al., 2015) as well as a high number of variants (Monecke et al., 2016; Shore et al., 2005) The definition of SCC*mec* types relies on the identity of the *mec* complex, i.e., genes directly accompanying *mecA*, including its regulatory genes, and on the identity of the recombinase genes (*ccr*) (http://www.sccmec.org/Pages/SCC_TypesEN.html) (IWG-SCC, 2009). SCC*mec* elements are mobile and thus identical elements can be found in different strains of *S. aureus* and in other staphylococcal species. Several classes of these elements have already been found also in methicillin-resistant *S. pseudintermedius* (MRSP).

MRSP often carry additional resistance markers resulting in resistance towards additional classes of antibiotic compounds (Beever et al., 2015; Borjesson et al., 2012; Gharsa et al., 2013; Gold et al., 2014; Siak et al., 2014). Significantly reduced therapeutic options warrant efforts in infection control including screening and molecular typing (Bannoehr and Guardabassi, 2012; Beck et al., 2012; Laarhoven et al., 2011; van Duijkeren et al., 2011; Windahl et al., 2012).

The aim of our study was the characterisation of MRSP isolates from clinical routine in order to assess the abundancy of resistance genes and SCC*mec* elements in a Central European setting.

A second aim was to review the diversity of currently known SCC*mec* elements in MRSP.

2. Material and methods

2.1. Isolates and phenotypic identification

MRSP isolates originated from canine samples that were analysed at the Laboklin GmbH & Co KG laboratory, Bad Kissingen, Germany, as part of its routine diagnostic tasks. All MRSP isolates were included that were found in a sampling period from October 2014 to April 2015 and originated mostly from Germany, Austria and Switzerland (for details, see Supplemental file 1) All isolates were subjected to standard culture procedures, and the species was identified using MALDI-TOF (Shimadzu Deutschland GmbH, Duisburg) and API ID32 Staph (bioMérieux, Nürtingen, Germany) in parallel. Resistance tests were performed by broth dilution in commercially available, prefabricated microtiter plates (Merlin Diagnostika, Bornheim-Hersel, Germany). Phenotypically methicillin-resistant isolates were subjected to a *mecA*-specific PCR as described previously (McDonald et al., 2005).

2.2. Microarray-based molecular characterisation

All isolates were characterised using microarrays (Abbott (Alere Technologies GmbH), Jena, Germany) designed for *S. aureus* typing (Monecke et al., 2011). This allowed a rapid detection of resistance genes and SCC-related markers. An assignment to strains and clonal complexes, as done for *S. aureus*, was not possible for *S. pseudintermedius* because the typing markers on the array were designed only for *S. aureus*.

Representative isolates (n = 23) were additionally tested with another microarray-based test that allowed SCC*mec* subtyping (Monecke et al., 2016).

This array also included experimental probes for the detection of an *S. intermedius/pseudintermedius* specific bi-component leukocidin, *lukF* $_{(int)}$ and *lukS* $_{(int)}$ (for probe and primer sequences, see Supplemental file 2).

Details on hybridisation procedures, on probe and primer sequences as well as on data analysis have been described previously (Monecke et al., 2011, 2016; Monecke et al., 2008).

3. Results

The proportion of MRSP among clinical *S. pseudintermedius* isolates in routine diagnostic was 9.3% in the study time (unpublished data from Laboklin routine diagnostics). Out of the routine procedures, 96 phenotypically resistant *S. pseudintermedius* isolates were obtained for this study. Isolates originated from 96 dogs, out of which 12 were not treated with antibiotics four weeks prior to admission. Details on sample types are shown in Table 1. Upon genotyping, one isolate was revealed to be a mixed culture of two distinct MRSP strains (one SCC*mec* III and one SCC*mec* V/VT strain) that were analysed separately henceforth. Full hybridisation profiles are provided in Supplemental file 1.

3.1. Resistance markers and SCCmec elements

The absolute numbers and the prevalence rates of resistance genes among the 97 isolates are summarized in Table 2.

An overview on SCC*mec* types is provided in Table 3.

The majority of isolates, n = 59, harboured SCC*mec* III elements or composite elements of SCC*mec* III parentage, as defined by a presence of *mecA*, *ugpQ, mecR1, mecI, xylR/mecR2*, *ccrA-3* and *ccrB-3*. Twenty-one isolates carried SCC*mec* IV elements of different subtypes. Fifteen isolates had SCC*mec* V/VT elements or composites thereof.

One isolate was identified with an irregular element with a *mec* complex that included *mecA, ugpQ, mecR* and *mecI* but that lacked *psmMEC* and *xylR*. Another isolate was found to carry a pseudo-SCC*mec*

Table 1
Samples (n = 96) from which MRSP have been isolated, and previous exposure to antibiotics (during four weeks prior to admission).

Localisation/Sample type	Samples per localisation	Animals with previous antibiotic treatment	Animals without previous antibiotic treatment
Abscess	**2**	2	0
Bone	**1**	1	0
Ear	**17**	15	2
Eye	**3**	3	0
Fistula	**5**	5	0
Joint	**3**	3	0
Milk	**1**	1	0
Nose/Nasal swab	**5**	4	1
Pharynx	**4**	4	0
Sarcoma	**1**	1	0
Skin swab	**26**	19	7
Synovial bursa	**1**	1	0
Synthetic implant	**1**	1	0
Thoracic swab (unspecified)	**1**	1	0
Tumor/swelling (unspecified)	**1**	1	0
Urine	**8**	7	1
Vaginal swab	**2**	2	0
Wound swab	**14**	13	1

Table 2
Resistance genes and their numbers and prevalence rates.

Gene	Explanation	Number	Percent
mecA	Modified penicillin binding protein (PBP2a)	**97**	**100.00**
mecC	Alternate gene encoding a modified penicillin binding protein. Present in, and characteristic for, SCC*mec* XI	0	0.00
blaZ	Beta-lactamase	**97**	**100.00**
***erm*(A)**	rRNA adenine N-6-methyltransferase, erythromycin/clindamycin resistance	0	0.00
***erm*(B)**	rRNA adenine N-6-methyltransferase, erythromycin/clindamycin resistance	**91**	**93.81**
***erm*(C)**	rRNA adenine N-6-methyltransferase, erythromycin/clindamycin resistance	0	0.00
***linA/lnu*(A)**	Lincosamid-nucleotidyltransferase	0	0.00
msrA	Energy-dependent efflux of erythromycin	0	0.00
mefA	Macrolide efflux protein A	0	0.00
***mph*(C)**	Probable lysylphosphatidylglycerol synthetase, macrolide resistance	0	0.00
***vat*(A)**	Virginiamycin A acetyltransferase	0	0.00
***vat*(B)**	Acetyltransferase inactivating streptogramin A	0	0.00
***vga*(A)**	ATP binding protein, streptogramin-A-resistance	0	0.00
vgb	Virginiamycin B hydrolase	0	0.00
aacA-aphD	Bifunctional enzyme Aac/Aph, resistance towards gentamicin, tobramycin, kanamycin and amikacin	**81**	**83.51**
aadD	Aminoglycoside adenyltransferase, resistance towards tobramycin kanamycin and neomycin	0	0.00
aphA3	3'5'-aminoglycoside phospho-transferase, resistance to neomycin, kanamycin and amikacin	**89**	**91.75**
sat	Streptothricine-acetyltransferase	**89**	**91.75**
dfrA	Dihydrofolate reductase type 1	0	0.00
fusC	SCC-born fusidic acid resistance gene	**1**	**1.03**
far1	Plasmid-born fusidic acid resistance gene (=*fusB*)	0	0.00
mupA	Mupirocin resistance protein	0	0.00
***tet*(K)**	Tetracycline resistance	**25**	**25.77**
***tet*(M)**	Tetracycline resistance	**36**	**37.11**
cat	Chloramphenicol acetyltransferase	**24**	**24.74**
cfr	23S rRNA methyltransferase	0	0.00
fexA	Chloramphenicol/florfenicol exporter	0	0.00
qacA	Quaternary ammonium compound resistance protein A	0	0.00
qacC	Quaternary ammonium compound resistance protein C	**6**	**6.19**
vanA	Vancomycin resistance gene	0	0.00

Table 3
SCC*mec* types and their occurrence among tested isolates.

SCC*mec* main type	Number
SCC*mec* III	58/97
SCC [*mec* III + *fus* + *ccrC*]	1/97
SCC*mec* IV	21/97
SCC*mec* V/VT	8/97
SCC [*mec* V/VT + *ccrA(B)*1]	7/97
"Irregular"*	1/97
Pseudo-SCC*mec* class C	1/97

* For details, see text and Table 4.

element class C, *i.e.*, a derivative of a SCC*mec* V/VT element that lost its recombinase gene *ccrC*.

Twenty-three representative isolates were additionally tested with another microarray allowing determination of SCC*mec* subtypes and a direct comparison to published MRSP sequences.

180 *S. pseudintermedius* sequences harbouring *mecA,* as retrieved in February 2019 from GenBank and BioSample databases, carry SCC*mec* elements or pseudoelements that could, using the present set of probes, be assigned to 17 different subtypes (Table 4). The 23 isolates subtyped herein belonged to ten different subtypes (Table 4). Out of these, two have previously been sequenced in *S. aureus* (SCC*mec* IVa $_{(MW2)}$ and SCC*mec* IVc $_{(TCH60)}$), although we did not identify them among published MRSP sequences. For another three (one SCC*mec* III/copper resistance and one SCC*mec* III/fusidic acid resistance composite element and one "irregular" element), no corresponding sequences were identified.

3.2. Virulence markers

Enterotoxin genes were not identified, at least none that could be detected by probes designed for *S. aureus* enterotoxin sequences (*sea, sea* (320E), *sea* (N315)*/sep, seb, sec, sed, see, seh, sej, sek, sel, seq, ser* as well as *tst*1, ORF CM14 and *egc* genes *seg, sei, selm, seln, selu*). The *S. intermedius/pseudintermedius* specific bi-component leukocidin, *lukF* $_{(int)}$ and *lukS* $_{(int)}$, was detected in 23 out of those 23 isolates that were tested with the additional assay.

4. Discussion

A proportion of MRSP among clinical *S. pseudintermedius* isolates in routine diagnostic of about 10% is a rather low rate. In a Finnish study, 14% MRSP in clinical isolates were found (Grönthal et al., 2017) while in Korea, 18% MRSP (but 33% *mec*A-positives) have been found among dogs with pyoderma or otitis (Yoo et al., 2010; Yoon et al., 2010). In general, comparisons between different studies cannot easily be made as the prevalence of MRSP varies depending not only on the geographic region but also on sample localisation, and population studied. Higher MRSP rates are found in pyoderma than among other clinical manifestations.

A variety of different SCC*mec* types and subtypes was observed. The most common one was SCC*mec* III as in the genome sequence of strain KM1381, AM904732.1. This type has been observed in MRSP before. "SCC*mec* II-III" as previously described (Descloux et al., 2008) and what is named SCC*mec* III $_{(KM1381)}$ here can be regarded synonymous. The reason for this discrepant nomenclature is apparently that Descloux et al. considered the *mec* complex (*i.e.*, *mecA* and the surrounding genes) of *S. aureus* strain 85/2082 (GenBank AB037671.1) prototypic for SCC*mec* III and noted that the one from MRSP KM1381 was more related to the *mec* complex from SCC*mec* II (*e.g.*, from *S. aureus* Mu50 or N315 and *Staphylococcus epidermidis* RP62A). Hence the designation "SCCmec II-III" indicating that it harboured a *mec* complex as in SCC*mec* II while the other parts of the element such as the *ccr* genes being SCC*mec* III. However, 85/2082 is an atypical strain, with a deletion within *mecR1*, representing a local and possibly near-extinct clade within CC239-MRSA-III (Monecke et al., 2018). Nearly all other *S. aureus* SCC*mec* III elements sequenced afterwards harbour the same *mec* complex as KM1381, RP62A, Mu50 and N315. For that reason we suggest naming the MRSP element just SCC*mec* III $_{(KM1381)}$. Contrarily

Table 4
SCC*mec* types and subtypes in publicly available sequences, their predicted hybridisation patterns and their occurrence among tested isolates.

SCC*mec* subtype	Reference strains and sequences for SCC*mec* subtype (Strain / GenBank or BioSample accession number)*	*mec* complex	Other payload	Recombinase genes	SCC termini	Other published MRSP sequences assigned to the respective SCC*mec* subtype (BioSample or GenBank accession numbers)	Subtyped isolates from this study
SCC*mec* III (KM1381) **	KM1381/AM904732.1; 081661/CP016073.1	*mecA*, *ugpQ*, *mecR*, *mecI*, *psm*MEC, *xylR*	*mvaS*, *cstB*-SCC1, Q933A2	*ccrA/B-3*	*dcs*	SAMEA1463306; -1463463; -1484740; -1710360; -1904197; −1904198; -1929653; -2167882; -2167884; -2167886; −2167887; -2167889; -2167899; -2167900; -2167901; −2167902; -2167903; -2167904; -2167905; -2167906; −2167907; -2167908; -2168040; -3252850; -3252851; −3252852; -3252853; -3252854; -3252855; -3252856; −3252857; -3252858; -3252859; -3252860; -3252861; −3252862; -3252863; -3252864; -3252865; -3252866; −3252867; -3252868; -3252869; -3252870; -3252871; −3252872; -3252873; -3252874; SAMN02471704; -03366842; -07415006; -07415007; −07415009; -07415010; -07415012; -07415014; -07415015; -07415016; -07415019; -07415020; -07415021; -07415022; −07415023; -07415024; -07415025; -07415030; -07415035; −07415036; -07415037; -07415040; -07415044; -07415054; −08028732; -08028736; -08028737; -08028738; -08028739; −08741526; -08741588; -10142584	**8/23**
SCC*mec* III (KW81ST71)	KW81ST71/SAMN08741581	*mecA*, *ugpQ*, *mecR*, *mecI*, *psm*MEC, *xylR*	*mvaS*, *cstB*-SCC1	*ccrA/B-3*	*dcs*	N/A	**0**
SCC [*mec* III + Cd] (KM241) ***	KM241/AM904731	*mecA*, *ugpQ*, *mecR*, *mecI*, *psm*MEC, *xylR*	*mvaS*, *cadD*$_{(R35)}$	*"ccrA/B-5" (ccrA3/B1)*	—	N/A	**0**
SCC [*mec* III + Cu]	N/A	*mecA*, *ugpQ*, *mecR*, *mecI*, *psm*MEC, *xylR*	*mvaS*, *cstB*-SCC1, Q933A2, *copA2*-SCC	*ccrA/B-3*	*dcs*	N/A	**1/23**
SCC [*mec* III + *fus* + *ccrC*]	N/A	*mecA*, *ugpQ*, *mecR*, *mecI*, *psm*MEC, *xylR*	*mvaS*, *cstB*-SCC1, Q933A2, *fusC*	*ccrA/B-3, ccrC*	*dcs*, SCCterm10	N/A	**1/23**
truncated /fragmentary SCC*mec* III sequences	N/A	—	—	—	—	SAMN08741522; -08741527; -08741552; -08741569; -08741574	**N/A**
Pseudo-SCC*mec* [class A + Cd] (KW21st497)	KW21ST497/SAMN08741547	*mecA*, *ugpQ*, *mecR*, *mecI*, *psm*MEC, *xylR*	*mvaS*, *cadD*$_{(R35)}$	—	—	SAMN08741553	**0**
SCC [*mec* complex class A + Cd + *czrC* + *ccrA1B*?] (AI16)	AI16/LN864705	*mecA*, *ugpQ*, *mecR*, *mecI*, *psm*MEC, *xylR*	*mvaS*, *czrC*, *cadD*$_{(R35)}$	*ccrA1/B?*	SCCterm1	N/A	**0**
SCC*mec* IVa (MW2)	*S. aureus* MW2/BA000033.2	*mecA*, *ugpQ*, Delta *mecR1*	*mvaS*, *cstB*-SCC2	*ccrA/B-2*	*dcs*	N/A	**1/23**
SCC*mec* IVc (TCH60)	*S. aureus* TCH60/CP002110.1	*mecA*, *ugpQ*, Delta *mecR1*	*mvaS*, B2Y834	*ccrA/B-2*	*dcs*	N/A	**1/23**
SCC*mec* IVg (SA40)	*S. aureus* SA40/CP003604.1	*mecA*, *ugpQ*, Delta *mecR1*	*mvaS*, Q3YK51	*ccrA/B-2*	*dcs*	SAMEA1463488; SAMN07415011; -07415013; -07415026; −07415028; -07415031; -07415032; -07415033; -07415034; −07415039; -07415041; -07415042; -07415043; -07415045; −07415046; -07415049; -07415050; -07415051; -07415052; −07415053; -08741523; -08741555; -08741556; -09708406	**5/23**
SCC*mec* IVg (2120306040011)	Strain 2120306040011/ SAMN07415038	*mecA*, *ugpQ*, Delta *mecR1*	Q3YK51	*ccrA/B-2*	*dcs*	N/A	**0**
Pseudo-SCC*mec* [class B]	KW20ST71/SAMN08741577	*mecA*, *ugpQ*, Delta *mecR1*	*mvaS*	—	*dcs*	N/A	**0**

(*continued on next page*)

Table 4 (*continued*)

SCC*mec* subtype	Reference strains and sequences for SCC*mec* subtype (Strain / GenBank or BioSample accession number)*	*mec* complex	Other payload	Recombinase genes	SCC termini	Other published MRSP sequences assigned to the respective SCC*mec* subtype (BioSample or GenBank accession numbers)	Subtyped isolates from this study
SCC [*mec*V + As/Cu] (RIVM3897) and SCC*mec* (NA45) ****	*S. aureus* RIVM3897/ CP013621.1 NA45/ CP016072.1	*mecA*, *ugpQ*	*mvaS*, *ydhK copA2*-SCC, *arsB*-SCC, *arsC*-SCC	*ccrAA*, *ccrC*	SCCterm10	SAMEA1463490; SAMN08028731; -08028740; -08741524; −08741557; -08741558; -08741559; -08741560; -08741562; −08741564; -08741566; -08741590; -09708397; -09708398; −09708400; -09708401; -09708409; -09708411; -09708412	**0**
SCC [*mec* V + *czrC* + *ccrA1*] (K57ST496)	K57ST496/SAMN08741542	*mecA*, *ugpQ*	*mvaS*, Q4LAG7, *czrC*	*ccrA/(B)-1* *ccrAA*, *ccrC*	—	N/A	**0**
SCCmec VT (GR1)	*S. aureus* GR1/AJLX	*mecA*, *ugpQ*	*mvaS*	*ccrAA*, *ccrC*	SCCterm2	FJ544922	**0**
SCC [*mec* VT + *cas*] (K7)	K7/BARM	*ugpQ*, *mecA*	*mvaS*, D1GU38, Q4LAG7, *cas1* (MSHR1132)	*ccrAA*, *ccrC*	SCCterm2	CP015626.1; SAMN06052361; -07415048; -08475774; -09708399	**0**
SCC [*mec* VT + *cas* + Cu] (VET0460R)	*S. aureus* VET0460R/JIKU	*mecA*, *ugpQ*	*mvaS*, D1GU38, Q4LAG7, *cas1* (MSHR1132), *copA2*-SCC	*ccrAA*, *ccrC*	SCCterm2	N/A	**1/23**
SCC [*mec* VT + *cas*, *ccrAA* + /*ccrC*-] (23929)	23929/SAMEA1529840	*ugpQ*, *mecA*	*mvaS*, D1GU38, Q4LAG7, *cas1* (MSHR1132)	*ccrAA*	SCCterm2	SAMEA1710351	**0**
SCC [*mec* VT + *cas* + *czrC* + *ccrA1*] (SL/154)	SL/154/SAMN06054141	*mecA*, *ugpQ*	*mvaS*, D1GU38, *czrC*, Q4LAG7, *cas1* (MSHR1132)	*ccrA/(B)-1* *ccrAA*, *ccrC*	SCCterm2	SAMN08475772; -08741539; -08741546; -09708394; -09708395; -09708396; -09708402; -09708403; -09708405	**2/23**
Pseudo-SCC*mec* [class C + As] (GD2010-090)	*S. aureus* GD2010-090/JIYL	*mecA*, *ugpQ*	*mvaS*, *arsB*-SCC, *arsC*-SCC	—	SCCterm 10	SAMN09708404	**0**
Pseudo-SCC*mec* [class C + As/Cd] (2100503050011)	2100503050011/ SAMN07415029	*mecA*, *ugpQ*	*arsB*-SCC, *cadX* (JCSC6943)	—	—	N/A	**0**
Pseudo-SCC*mec* [class C + As/Cd/Cu] (57395)	Strain 57395/HE984157	*mecA*, *ugpQ*	*ydhK*, *copA2*-SCC, *arsB*-SCC, *cadX* (JCSC6943)	—	*****	SAMN06054118; -06054137; -06054138; -06054139; −06054140; -06054142; -07415008; -07415017; -07415018; −07415027; -07415047; -07415055; -08028756; -08028820; −08475775; -08741536; -08741537; -08741538	**1/23**
Pseudo-SCC*mec* [class C + As/Cd/Cu] (K23ST45)	K23ST45/SAMN08741534	*mecA*, *ugpQ*	*copA2*-SCC, *arsB*-SCC, *cadX* (JCSC6943)	—	—	N/A	**0**
"Irregular"; SCC [(*mecA/R/I* +, *xylR*-) + *ccrC*]	N/A	*mecA*, *ugpQ*, *mecR*, *mecI*	*mvaS*, Q8CU82	*ccrC*	—	N/A	**1/23**

* Strains belong to *S. pseudintermedius*, unless indicated otherwise.
** Identical to SCC*mec* II-III (Descloux et al., 2008).
*** Identical to "SCC*mec* VII" (Descloux et al., 2008).
**** SCC [*mec*V + As/Cu] (RIVM3897) and SCC*mec* (NA45) differ in at the 3′ end of the SCC*mec* cassette, see (Worthing et al., 2018), but the region in question is not covered by the probes used herein.
***** Harbours a unique terminal sequence being present in *S. pseudintermedius* 57395, HE984157.2, and *S. haemolyticus* Sh29_312_L2, CP011116.1.

to most *S. aureus* SCC*mec* III elements, it does not harbour additional *ccr* recombinase genes, heavy metal resistance genes and other additional markers so that it can be regarded as a prototypical "plain" SCC*mec* III element (Monecke et al., 2018). Two of the isolates tested herein harboured composite SCC*mec* elements that derived from SCC*mec* III, but we did not find matching sequences in publicly accessible databases.

Several studies from Europe and North America (Perreten et al., 2010; Ruscher et al., 2010, 2009) as well as Australia (Worthing et al., 2018) identified SCC*mec* III as the most prevalent SCC*mec* element in MRSP as we did in our study. Among published sequences of *S. pseudintermedius*, SCC*mec* III, also clearly predominates. 89 out of 180 *S. pseudintermedius* sequences carry SCC*mec* III elements (out of which 82 are unambiguously identical to KM1381, see Table 4) and one harbours a SCC*mec* III derived pseudo-SCC*mec* element (Worthing et al., 2018). This high prevalence is in striking contrast to the situation in *S. aureus*, where SCC*mec* III is clearly not as common, being largely restricted to two clones, one of which is pandemic but declining (CC239) while the other one is rare and localised (CC5).

The abundant presence of KM1381-like SCC*mec* III elements and of genes *aacA-aphD*, *aphA3* and *sat* as well as of *erm*(B), as present in CP016073.1 ((Riley et al., 2016); see Supplemental file 1), suggests a local predominance of the ST71 clone as previously described for various western European settings (Bannoehr et al., 2007; Descloux et al., 2008; Perreten et al., 2010; Ruscher et al., 2010, 2009).

While SCC*mec* IV is the most common and geographically most widespread SCC*mec* type in *S. aureus*, it seems to be much rarer in MRSP being present in 25 out of 180 published genomes. In our study, we identified three subtypes all of which have previously been found also in *S. aureus*. One of these subtypes, SCC*mec* IVg (SA40) predominates among published genomes accounting for 24 out of 25 SCC*mec* IV elements in MRSP (Table 4) and was the only SCC*mec* IV variant found in an Australian study (Worthing et al., 2018).

SCC*mec* V/VT elements are also common in *S. aureus*, especially in community- or livestock-associated (LA) strains. They have also been observed in MRSP but are not as common among published sequences (40 out of 180 *S. pseudintermedius* sequences harbouring *mecA*, Table 4). Some studies from China, Korea and Japan have shown that SCC*mec* V is common (Feng et al., 2012; Moon et al., 2012; Onuma et al., 2012; Wang et al., 2012). One of the few SCC*mec* V/VT elements observed herein also originated from a case submitted from China. In North America, SCC*mec* V has been reported as most abundant SCC*mec* type being linked to the dominant ST68 lineage (Perreten et al., 2010). One element observed herein yielded a hybridisation pattern matching SCC [*mec* VT + *cas* + Cu] $_{(VET0460R)}$ that has been observed in a CC398 livestock-associated (LA-) MRSA, VET0460R, GenBank JIKU. This element includes a gene, *cas1*$_{(MSHR1132)}$, encoding a CRISPR-associated endonuclease 1 identical to that one previously sequenced in *Staphylococcus argenteus* (GenBank FR821777.2; 62,418 to 63,323) and a SCC-borne copper resistance gene. Another SCC*mec* VT element observed herein also harboured *cas1*, the heavy metal resistance gene *czrC* (that is very common in LA-MRSA) and additional *ccr* recombinase genes. Ten published sequences apparently comprise the same element, with SL/154, BioSample accession number SAMN06054141 being prototypical.

One isolate was identified with a pseudo-SCC*mec* element that included a class C *mec* complex and determinants for arsenic, cadmium and copper resistance. Theoretically it could also be a SCC*mec* XII element (Wu et al., 2015) whose *ccr* genes are not recognised by the current arrays. However, an apparently identical element is present in the MRSP sequence of strain 57395, GenBank HE984157 and its published sequences rule out a presence of *ccr* genes (Perreten et al., 2013; Worthing et al., 2018). It is likely that this element emerged from a SCC*mec* V/heavy metal resistance composite element by loss of *ccrC*. Pseudo-SCC*mec* elements are rather common among MRSP (Worthing et al., 2018); 26 published sequences contain such elements and 22 of them have class C *mec* complexes.

Finally, one isolate was found with a *mec* complex that included *mecA/R/I* and *ugpQ* but that lacked *xylR,* PSM-*mec* and *cstB*-SCC1. This *mec* complex is not yet formally named. It can be found in various coagulase-negatives (*Staphylococcus hominis* ZBW5 GenBank AKGC, *S. hominis* M0480 GenBank KK013382.1 and *Staphylococcus epidermidis* VCU120 GenBank AHLC) and it had been observed in one *S. aureus* strain (WA59 07-16590, GenBank KT316803). Contrarily to these genome sequences, heavy metal resistance genes were not present in the MRSP isolate. In the present MRSP isolate, the recombinase gene *ccrC* was detected, while the other sequences with this *mec* complex harboured *ccrAB1* + *ccrAB4* (KK013382.1), *ccrAB1* + *ccrAB4* + *ccrC* (AKGC), *ccrB4* (AHLC) or no *ccr* genes at all (KT316803).

In only 8 out of 23 cases subtyped herein, SCC*mec* elements were found that previously have been identified in *S. aureus*. This might indicate that the evolution of SCC*mec* elements in both species proceeds largely independently and in parallel, with only a limited number of cross-species transfers. Data also suggest that the majority of MRSP is still caused by one single strain and comparison to previously published reports and sequences suggest that this was the ST71-SCC*mec* III strain that also predominates elsewhere in Western Europe. This is quite a different picture than in *S. aureus*/MRSA. In *S. aureus*/MRSA SCC*mec* III strains are geographically widespread, they used to be common but are increasingly surpassed by strains mainly those with smaller SCC*mec* IV or V elements (Aires de Sousa et al., 2008; Conceicao et al., 2007; D'Souza et al., 2010; Monecke et al., 2018). It will be interesting to see whether MRSP with its smaller and less complex type of KM1381-like SCC*mec* III elements will follow the same trajectory.

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Conflicts of interest

Elke Müller, Annett Reissig, Sascha Braun, Ralf Ehricht and Stefan Monecke were previously employees of Abbott (Alere Technologies GmbH), the company that manufactures the microarrays used for this study; Peter Slickers is employee of Abbott (Alere Technologies GmbH), the company that manufactures the microarrays used for this study. The other authors declare no conflicting interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2019.04.002.

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