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Novel Pseudo-Staphylococcal Cassette Chromosome *mec* Element (ΨSCC*mec*$_{57395}$) in Methicillin-Resistant *Staphylococcus pseudintermedius* CC45

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Genetic characterization of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) from Thailand and Israel revealed the presence of a predominant atypical clonal lineage which was not typeable by SmaI-PFGE and SCC*mec* typing. All the atypical isolates ($n = 34$) belonged to CC45 (30 ST45 and 2 ST179 isolates, 1 ST57 isolate, and 1 ST85 isolate). The isolates originated from healthy and diseased dogs and cats, as well as from the environment of one clinic. Cfr9I–pulsed-field gel electrophoresis (Cfr9I-PFGE) and *dru* typing permitted the further distinction of CC45 isolates from the two different countries. Microarray analysis identified genes that confer resistance to β-lactams (*mecA*; *blaZ*), aminoglycosides [*aac(6′)-Ie–aph(2′)-Ia*; *aph(3′)-III*; *ant(6)-Ia*], macrolides and lincosamides [*erm*(B)], tetracyclines [*tet*(M)], trimethoprim [*dfr*(G)], streptothricin (*sat4*), and chloramphenicol (cat_{pC221}). Fluoroquinolone resistance was attributed to specific amino acid substitutions, i.e., Ser84Leu in GyrA and Ser80Ile and Asp84Asn in GrlA. A novel pseudo-staphylococcal cassette chromosome (ΨSCC*mec*$_{57395}$) element was identified in MRSP strain 57395 (sequence type ST45) by whole-genome sequencing. The 12,282-bp ΨSCC*mec*$_{57395}$ element contained a class C1 *mec* gene complex but no *ccr* genes. In addition to the methicillin resistance gene *mecA*, ΨSCC*mec*$_{57395}$ also carried determinants of resistance to heavy metals, such as arsenic, cadmium, and copper. Bsu36I restriction analysis of the ΨSCC*mec*$_{57395}$ element amplified by long-range PCR revealed the presence of ΨSCC*mec*$_{57395}$ in the 33 additional isolates of MRSP CC45. The ΨSCC*mec*$_{57395}$ element represents a new class of SCC*mec* and has been identified in MRSP of CC45, which is a predominant clonal lineage in Israel and Thailand.

Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) strains have emerged in the animal health care environment during this past decade (1–6). Because they can colonize healthy animals and humans and survive and persist in clinical environments (7–13), MRSP strains became difficult to eradicate from hospitals and the community and are now established as one of the most common causes of canine dermatitis, as well as hospital-acquired infections, in companion animals (14, 15). They also occasionally cause severe infections in humans (16, 17). While one predominant MRSP clone belonging to sequence type ST71 has spread worldwide (18, 19), other clonal lineages have been reported to be predominant in some countries (20, 21). MRSP strains are particularly resistant to many different classes of antibiotics, such as β-lactams, aminoglycosides, fluoroquinolones, tetracyclines, lincosamides, macrolides, folate pathway inhibitors, and phenicols, thus limiting the therapeutic options (22, 23). The majority of the resistance has been associated with topoisomerase mutations, chromosomal transposons, and staphylococcal cassette chromosome *mec* (SCC*mec*) elements (24–26). Typical SCC*mec* elements contain a *mec* gene complex that carries *mecA*, which encodes an alternative penicillin-binding protein, PBP2a, which confers broad-spectrum beta-lactam resistance, and the cassette chromosome recombinases (Ccrs) that are responsible for site-specific integration and excision of the element (27). They are flanked by characteristic direct repeat (DR) sequences that define the transferrable unit (28–30).

To date, only 3 different SCC*mec* elements of MRSP have been sequenced, namely, SCC*mec* II-III in KM1381 and E140 (ST71), SCC*mec* VII-241 in KM241 (ST93 according to the new MLST scheme [31], formerly ST73 [25]), and SCC*mec* V in 06-3228 (ST68) and in K7 (ST233) (25, 26, 32, 33). SCC*mec* II-III (class A, *ccrA3/B3*) consists of a combination of SCC*mec* II from *S. epidermidis* and SCC*mec* III from *S. aureus*, but it lacks the cadmium resistance operon. SCC*mec* VII-241 is a new element that contains a novel recombinase operon (class A, *ccrA5/B3*) (25). SCC*mec* V is closely related to the SCC*mec* V (5C2&5) identified in *S. aureus* (26, 27, 34). Otherwise, the SCC*mec* elements in MRSP have been identified only by using the SCC*mec* typing method of Kondo et al. (35), which classifies SCC*mec* elements based on the combination of the *mec* class complex (classes A, B, and C) and recombinase genes types (*ccrA*, *ccrB*, *ccrC*) (27). However, several SCC*mec* elements remained unidentified in MRSP using this method (9, 11, 18, 20, 36, 37).

Advances in bacterial genome sequencing have revealed the vast diversity in structural organization and genetic content of SCC*mec* elements in *Staphylococcus* species, including different

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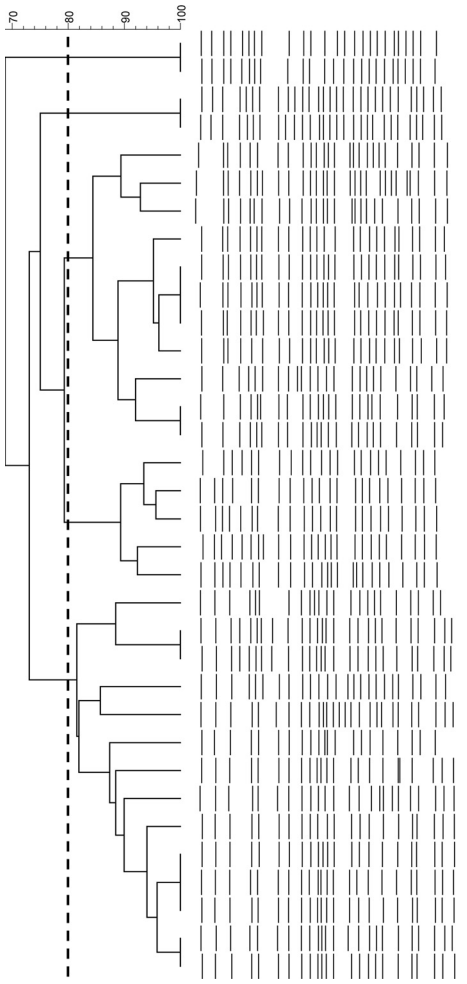

Strain	Source	Anamnese	Clinic	Country	PFGE	MLST	*spa*	SCC	*dru* type
58544	dog	dermatitis	I1	Israel	A	ST179	t09	ΨSCC*mec*$_{57395}$	11a
54339	dog	pyoderma	I1	Israel	A	ST179	t09	ΨSCC*mec*$_{57395}$	11a
S4	dog	nasal carriage	T1	Thailand	B	ST45	t09	ΨSCC*mec*$_{57395}$	11a
AH18	dog	perineal carriage	T2	Thailand	B	ST45	t09	ΨSCC*mec*$_{57395}$	11a
AK30	dog	pyoderma	T2	Thailand	C	ST45	neg	ΨSCC*mec*$_{57395}$	11ci
AJ7	dog	nasal carriage	T2	Thailand	C	ST45	neg	ΨSCC*mec*$_{57395}$	11y
AJ14	dog	nasal carriage	T2	Thailand	C	ST45	neg	ΨSCC*mec*$_{57395}$	11y
AJ28	dog	nasal carriage	T2	Thailand	C	ST45	neg	ΨSCC*mec*$_{57395}$	11cj
U4	dog	nasal carriage	T1	Thailand	C	ST45	neg	ΨSCC*mec*$_{57395}$	11cb
AK12	dog	nasal carriage	T2	Thailand	C	ST45	neg	ΨSCC*mec*$_{57395}$	11y
AI39	dog	nasal carriage	T2	Thailand	C	ST45	neg	ΨSCC*mec*$_{57395}$	11y
AI25	dog	nasal carriage	T2	Thailand	C	ST45	neg	ΨSCC*mec*$_{57395}$	11ci
AH23	dog	pyoderma	T2	Thailand	C	ST45	neg	ΨSCC*mec*$_{57395}$	11y
AP62	dog	nasal carriage	T2	Thailand	C	ST45	neg	ΨSCC*mec*$_{57395}$	11cb
AH10	dog	nasal carriage	T2	Thailand	C	ST45	neg	ΨSCC*mec*$_{57395}$	11cb
AI13	dog	pyoderma	T2	Thailand	D	ST45	neg	ΨSCC*mec*$_{57395}$	8af
57395	dog	rhinitis	I2	Israel	D	ST45	neg	ΨSCC*mec*$_{57395}$	11cj
54792	dog	eye secretion	I3	Israel	D	ST45	neg	ΨSCC*mec*$_{57395}$	11cj
AC1	dog	nasal carriage	T2	Thailand	D	ST45	neg	ΨSCC*mec*$_{57395}$	11cb
52672	dog	not defined	I4	Israel	D	ST45	neg	ΨSCC*mec*$_{57395}$	11cj
AK16	dog	nasal carriage	T1	Thailand	E	ST45	neg	ΨSCC*mec*$_{57395}$ [a)]	11cj
97890	cat	pyothorax	I7	Israel	E	ST45	neg	ΨSCC*mec*$_{57395}$	11cj
68407	dog	otitis	I5	Israel	E	ST85	t09	ΨSCC*mec*$_{57395}$	11a
AP65	dog	perineal carriage	T1	Thailand	E	ST45	neg	ΨSCC*mec*$_{57395}$	11cj
98645	dog	otitis	I6	Israel	E	ST45	neg	ΨSCC*mec*$_{57395}$ [a)]	11cj
DE5	environment	-	I1	Israel	E	ST57	neg	ΨSCC*mec*$_{57395}$	11cj
44546	dog	otitis	I2	Israel	E	ST45	neg	ΨSCC*mec*$_{57395}$ [a)]	11cj
99518	dog	pyoderma	I1	Israel	E	ST45	neg	ΨSCC*mec*$_{57395}$	11cj
57896	cat	pyoderma	I1	Israel	E	ST45	neg	ΨSCC*mec*$_{57395}$	10cm
DE3	environment	-	I1	Israel	E	ST45	neg	ΨSCC*mec*$_{57395}$	11cj
25995	cat	rhinitis	I2	Israel	E	ST45	neg	ΨSCC*mec*$_{57395}$	11cj
DE21	environment	-	I1	Israel	E	ST45	neg	ΨSCC*mec*$_{57395}$	11cj
98240	dog	otitis	I1	Israel	E	ST45	neg	ΨSCC*mec*$_{57395}$	11cj
DE12	environment	-	I1	Israel	E	ST45	neg	ΨSCC*mec*$_{57395}$	11cj

FIG 1 Phylogenetic tree constructed from the pulsed-field gel electrophoresis (PFGE) pattern of methicillin-resistant *S. pseudintermedius* CC45. The tree was generated by the unweighted-pair group method using average linkages (UPGMA) using Bionumerics 6.6 (Applied Maths, Kortrjk, Belgium) and comparison settings (Dice; optimization 1.5%, position tolerance 1.5%) as recommended by PulseNet International (www.pulsenetinternational.org). The dotted line indicates the cutoff value of ≥79%, determining clonality between the isolates according to Miragaia et al. (63). Capital letters indicate the origin of clinics (I, Israel; T, Thailand), and the numbers indicate the different clinics. Strains marked with a superscript "a)" harbored a variable region spanning ΨSCC*mec*$_{57395}$ and the core gene genome different from that of MRSP strain 57395; all other strains contained a variable region which exhibited a restriction profile similar to that of strain 57395. neg, no PCR amplification of the *spa* gene. *dru* types are marked as follows: 11cj, 5a-2d-4a-1b-2d-5b-3a-1b-3b-4e-3e; 10 cm, 5a-4a-1b-2d-5b-3a-1b-3b-4e-3e; 11ci, 5a-2d-4a-1b-2d-5b-4h-1b-3b-4e-3e; 11a, 5a-2d-4a-0-2d-5b-3a-2g-3b-4e-3e; 11cb, 5a-3c-4a-1b-2d-5b-3a-2g-3b-4e-3e; 11y, 5a-2d-4a-1b-2d-5b-3a-2g-3b-4e-3e; 8af, 5a-2d-5b-3a-2g-3b-4e-3e.

types of elements and variability of these types as a consequence of insertions and deletions, as well as composite elements and elements arranged in tandem (27, 35, 38–41). Indeed, SCC elements that carry *mecA* and *ccr* on separate elements arranged in tandem have been recently described in *S. aureus* and *S. haemolyticus* and have been classified as pseudo-(Ψ) SCC*mec*-SCC elements (27, 39, 40). Furthermore, there is evidence that the *ccr* genes can be lost independently from the *mecA* gene in *S. haemolyticus* (41, 42).

Dogs and cats in Israel and Thailand can be carriers of or can be infected with specific MRSP strains that were nontypeable by SmaI pulsed-field gel electrophoresis (PFGE) and SCC*mec* typing using the Kondo method (35). To identify the degree of identity of these particular MRSP isolates, we compared their antibiotic resistance and genetic profiles and identified the novel ΨSCC*mec*$_{57395}$ elements.

MATERIALS AND METHODS

Sampling and identification of bacterial strains. Samples were obtained by swabbing the nasal cavities and perineal skin of healthy dogs, infection sites of dogs and cats, and clinical environments. *S. pseudintermedius* isolates were cultivated on Trypticase soy agar plates containing 5% sheep blood (TSA-S) (Becton, Dickinson and Company, Franklin Lakes, NJ) at 37°C for 18 to 24 h. The isolates were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) analysis using a direct smear method and a 70% formic acid overlay for better resolution (Microflex LT; Bruker Daltonics GmbH, Bremen, Germany) and the interpretation criteria of the manufacturer, as well as by sequence analysis of the partial 16S rRNA gene (43). The isolates were kept at −80°C in Trypticase soy medium containing 30% glycerin. Bacterial strains, together with their sources, origins, and characteristics, are listed in Fig. 1.

Determination of antibiotic resistance profile. The MICs of 17 antibiotics (chloramphenicol, ciprofloxacin, clindamycin, dalfopristin-qui-

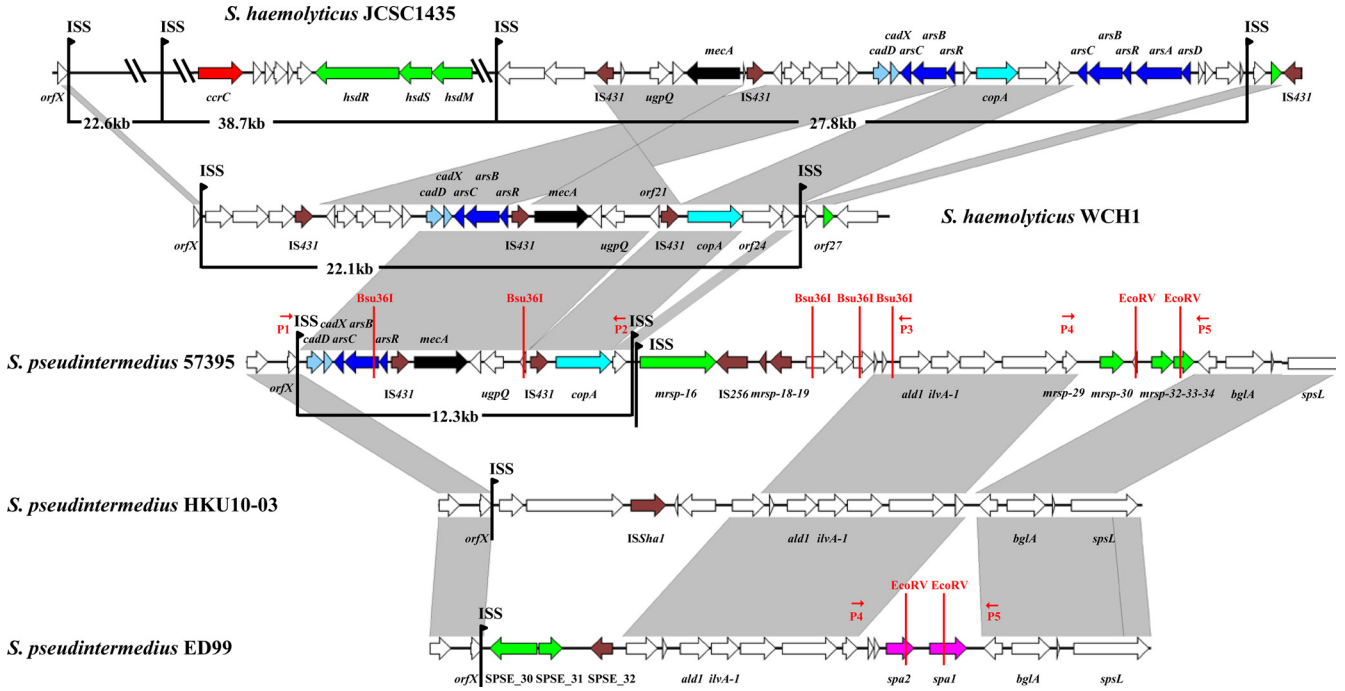

FIG 2 Structure of ΨSCC*mec* elements of *S. pseudintermedius* 57395 (GenBank accession no. HE984157), *S. haemolyticus* WCH1 (accession no. JQ764731), and *S. haeomlyticus* JCSC1435 (accession no. AP006716) (upper part) and comparison of the *orfX* downstream region of *S. pseudintermedius* 57395 with those of SCC-negative *S. pseudintermedius* strains HKU10-03 (accession no. CP002439) and ED99 (accession no. CP002478) (lower part). Gray areas indicate regions with more than 97% nucleotide sequence identity. Integration site sequences (ISS) and sizes of SCC elements are indicated by thick black lines. The positions and orientations of open reading frames (ORFs) are represented by arrows, *mecA* is shown in black, ORFs encoding heavy metal resistance determinants (*cadD*, *cadX*, *arsC*, *arsB*, *arsR*, *copA*) are shown in blue, *ccrC* recombinase is shown in light red, transposases are indicated in dark red (IS*431*, IS*256*, *mrsp-18*, *mrsp-19*, ISS*ha1*, SPSE_32), protein A genes are indicated in magenta (*spa1*, *spa2*), and ORFs associated with a restriction-modification system are indicated in green (*hsdR*, *hsdS*, *hsdM*, *mrsp-16*, *mrsp-30*, *mrsp-32*, *mrsp-33*, SPSE_30, SPSE_31). The primers used in this study for long-range PCR (small red arrows) (P1, orfX-R3; P2, attSCC-R; P3, ald1-R; P4, mrsp29-F; P5, mrsp34-F) and cleavage sites for restriction endonucleases Bsu36I and EcoRV are indicated in red. The figure was generated using the program Easyfig (62). Note that for *S. haemolyticus* JCSC1435, the ΨSCC*mec* is shown completely, and the region upstream of the ΨSCC*mec* is represented only partially; *orf21* (*S. haemolyticus* WCHI), *mrsp-18* (*S. pseudintermedius* 57395), and SPSE_32 (*S. pseudintermedius* ED99) are annotated as pseudogenes.

nupristin, erythromycin, fusidic acid, gentamicin, kanamycin, linezolid, mupirocin, oxacillin, penicillin, rifampin, streptomycin, tetracycline, trimethoprim, and vancomycin) were determined in Müller-Hinton broth by the microdilution technique (44) using custom-made Sensititre susceptibility plates (NLEUST; Trek Diagnostics Systems, East Grinstead, West Sussex, United Kingdom; MCS Diagnostics BV, JL Swalmen, The Netherlands). The resistance breakpoints were those proposed for coagulase-negative staphylococci (and for *S. aureus* with mupirocin) in the CLSI M100-S23 informal supplement (45), except for streptomycin, for which the breakpoint came from the French Society for Microbiology (www.sfm-microbiologie.org).

Antibiotic resistance genes were detected using a custom-made microarray (AMR+ve-3 array tubes; Alere Technologies GmbH, Jena, Germany) (46). The presence of the bifunctional gene *aac(6′)-Ie–aph(2′)-Ia* was verified by PCR (47).

DNA extraction. Genomic DNA was isolated using a peqGOLD bacterial DNA kit (PEQLAB Biotechnologie GMBH, Erlangen, Germany). For better cell lysis, the Tris-EDTA (TE) buffer of the kit was supplemented with 50 μg/ml lysostaphin (Sigma-Aldrich, St. Louis, MO), and the cells were incubated for 20 min at 37°C.

Multilocus sequence typing (MLST), *dru* typing, *spa* typing, analysis of the *spa* region, and Cfr9I-PFGE. MLST was performed by determining the nucleotide allele sequences of 7 genes (*ack*, *cpn60*, *fdh*, *pta*, *purA*, *sar*, and *tuf*), as described previously (31). Allelic profiles and ST numbers of *S. pseudintermedius* were determined using the MLST website (http://pubmlst.org/spseudintermedius/) of the University of Oxford (48). Sequences of each *mec*-associated direct repeat unit (*dru*) were determined as described previously (49). *dru* repeats and *dru* types were assigned using the *dru*-typing website (http://dru-typing.org/). *spa* types were determined by PCR and sequence analysis using primers SIspa-F (5′-AACCT GCGCCAAGTTTCGATGAAG) and SIspa-R (5′-CGTGGTTTGCTTTA GCTTCTTGGC) as described previously (50). The genomic region expected to contain *spa* genes was amplified by PCR using primers mrsp29-F (5′-GAGATAGTGGATTACATGGAG) and mrsp34-F (5′-TA TACCATTCGACGCATCAG) and was analyzed by EcoRV restriction digestion (Fig. 2). PFGE was carried out on DNA digested with SmaI and Cfr9I, as described previously (51). PFGE was run on a Chef DRIII apparatus (Bio-Rad, Hercules, CA) at 5.6 V/cm with the pulse time ramping from 5 to 40 s for 18 h and from 20 to 25 s for 5 h at 14°C (52).

Identification of ΨSCC*mec*$_{57395}$ elements among MRSP strains. The isolates were first screened for typical SCC*mec* elements by multiplex PCR, which identified the *mec* class complex and the recombinase genes (35). The nontypeable SCC*mec* element of MRSP strain 57395 was identified by whole-genome sequencing (see below). In the MRSP isolates carrying nontypeable SCC*mec*, the presence of elements similar to ΨSCC*mec* in strain 57395 was explored by long-range PCR followed by restriction analysis with Bsu36I. PCRs were performed using an Expand Long Template PCR system, which contains *Taq* and *Tgo* DNA polymerases (Roche Diagnostics, Rotkreuz, Switzerland). ΨSCC*mec*$_{57395}$ elements were amplified using primer orfX-R3 (5′-AGATGAAAAAGCACC CGAAAC), which anneals in the *orfX* gene, and primer attSCC-R (5′-AT ATGCTTCTGCGTATCG), which anneals in the 3′-end attachment site

of ΨSCC*mec*$_{57395}$ (Fig. 2), and an annealing temperature of 52°C and an extension time of 10 min. The ΨSCC*mec*$_{57395}$ element, including the 3′-end flanking region, was amplified using primer orfX-R3 and primer ald1-R (5′-CGTAAATCCAGAGCCTATAC), which anneals in the alanine dehydrogenase gene *ald1* situated in the core chromosome of *S. pseudintermedius* outside the 3′ end of ΨSCC*mec* (annealing temperature, 57°C; extension time, 16 min) (Fig. 2). PCRs were performed according to the manufacturer's recommendations.

Genome sequencing, SCC*mec* region assembly, and annotation. High-throughput sequencing of MRSP 57395 was performed with Roche 454 GS Titanium chemistry and protocols (GS Junior System, Roche Diagnostics). Sequence reads were assembled *de novo* using newbler 2.6 (http://www.vital-it.ch), yielding 90 contigs with an N50 (length-weighted media) of 125,268 bp, mean contig size of 30,086 bp, maximum contig length of 293,708 bp, and contig sum of 2,707,740 bp. To obtain the SCC*mec* sequence and flanking regions, contigs were analyzed for the presence of characteristic genes of SCC*mec* elements (*mecA*, *ugpQ*, *ccr*) and for open reading frames (ORFs) found downstream of the *orfX* gene in the genome of the SCC*mec*-negative *S. pseudintermedius* strains ED99 (GenBank accession no. CP002478) (53) and HKU10-03 (GenBank accession no. CP002439) (54). PCRs and sequencing using an ABI PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, CA) were performed to fill the gaps between the contigs and specify the orientations of the contigs. ORFs were defined with the help of prodigal software for gene finding in prokaryotes (55). Annotation of the ORFs was performed by BLAST homology and motif analysis using the Prosite and Pfam databases.

Putative integration site sequences for SCC (ISS) were identified by searching for the consensus sequence GA[AG]GC[AGT]TATCA[CT]AA[AG]T[AG][AG] (positions with possible alternative nucleotides are indicated in square brackets) (28).

To detect *ccr* genes in MRSP 57395, the translated contigs of the whole genome were analyzed for the presence of site-specific serine recombinase motifs derived from Prosite entry PS00397 (http://prosite.expasy.org). Prosite entry PS00397 specifies the residues in the active site of serine recombinases and is defined as Y-[LIVAC]-R-[VA]-**S**-[ST]-x (2)-Q, with the catalytic serine residue indicated in bold and amino acids acceptable for one given position listed between square brackets and x for any aa followed by the possible repetition range within the parentheses. Alignment of the recombinase motif of Ccr proteins revealed alternative residues at position 2 for CcrC and at position 4 for CcrA (see Fig. S1 in the supplemental material). The consensus pattern of PS00397 was subsequently modified into the sequence Y-[LIVACST]-R-[VAQ]-**S**-[ST]-x (2)-Q (newly added residues are underlined) and used as a scan motif with the ExPASy tool ScanProsite (http://prosite.expasy.org/scanprosite/) (56).

Nucleotide accession number. The nucleotide sequence of ΨSCC*mec*$_{57395}$ and flanking regions has been deposited into the GenBank/EMBL/DDBJ databases under accession number HE984157.

RESULTS AND DISCUSSION

Genetic characteristics and antibiotic resistance profile of the MRSP isolates. A total of 63 isolates from dogs in Thailand and 22 isolates from Israel (12 from dogs, 6 from cats, and 4 from a clinic environment) were characterized by SmaI PFGE and SCC*mec* typing. Among them, 36 MRSP isolates from Thailand and 17 from Israel were not typeable with both typing methods. All the atypical MRSP isolates belonged to CC45, with the predominant sequence type being ST45 (all CC45 isolates from Thailand and 13 from Israel), followed by ST179 ($n = 2$), ST57 ($n = 1$), and ST85 ($n = 1$) found in Israel only. Such atypical MRSP isolates have also been observed in the Netherlands (9). In addition to the atypical MRSP strains of the CC45, fewer MRSP strains belonging to other CCs were identified in Israel (3 ST180, 1 ST76, and 1 ST181) or in Thailand (8 ST112, 5 ST181, 3 ST182, 2 ST183, and 2 ST185 and one each of ST55, ST114, ST116, ST121, ST125, ST169, and ST178). Seventeen atypical MRSP isolates from each country were further characterized. They originated from 2 different veterinary clinics in Thailand and from 7 different clinics in Israel (Fig. 1). Cfr9I-PFGE classified the CC45 isolates into five specific clusters (A, B, C, D, and E) (Fig. 1). Whereas PFGE clusters A, B, and C contained isolates from either Israel (A) or Thailand (B and C), PFGE clusters D and E included isolates from both countries. Only 5 isolates could be further characterized by *spa* typing, revealing the presence of *spa* type t09 in 2 ST179 isolates, 2 ST45 isolates, and 1 ST85 isolate (Fig. 1). Diversity of the isolates was further assessed by *mec*-associated *dru* typing revealing 7 different *dru* types which permitted the further distinction of the ST45 isolates (Fig. 1). Whereas ST45 isolates from Israel contained predominantly *dru* type 11cj, isolates from Thailand contained additional and more diverse *dru* types (11a, 11ci, 11y, 11cb, and 8af). However, two ST45 isolates from Thailand shared a *dru* type (11cj) and PFGE profiles similar to those from Israel. The genetic profile which remained highly conserved between isolates from Israel, independently of the animal or clinic source, indicates the spread of a specific clone in this country, whereas the different *dru* types detected within ST45 isolates from the same clinic in Thailand revealed several lineages (Fig. 1). The higher *dru* diversity in MRSP ST45 from Thailand may be indicative of an ancient lineage or else of a more rapid evolutionary process than in MRSP strains from Israel.

All the isolates were susceptible to dalfopristin-quinupristin, fusidic acid, linezolid, mupirocin, rifampin, and vancomycin. They all exhibited resistance to oxacillin, penicillin, kanamycin, gentamicin, streptomycin, erythromycin, clindamycin, ciprofloxacin, tetracycline, and chloramphenicol. Five of them (isolates 58544, 54339, AH18, S4, and 68407) had an additional resistance to trimethoprim. In addition to the β-lactam resistance gene *mecA*, all the isolates contained the β-lactamase gene *blaZ*, the bifunctional aminoglycoside acetyltransferase-phosphotransferase gene *aac(6′)-Ie–aph(2′)-Ia*, the kanamycin and neomycin phosphotransferase gene *aph(3′)-III*, the streptomycin adenylnucleotidyltransferase gene *ant(6)-Ia*, the streptothricin acetyltransferase gene *sat4*, the macrolide, lincosamide, and streptogramin B 23S rRNA methylase gene *erm*(B), the tetracycline and minocycline resistance gene *tet*(M), and the chloramphenicol acetyltransferase gene *cat*$_{pC221}$. The trimethoprim-resistant isolates contained the dihydrofolate reductase gene *dfr*(G). Fluoroquinolone resistance was attributed to the amino acid substitution Ser84Leu in the topoisomerase GyrA in all isolates, as well as to the amino acid substitution Ser80Ile in GrlA in all isolates except 54339 and 58544, which displayed an Asp84Asn substitution.

Genome sequencing and identification of the novel ΨSCC*mec*$_{57395}$ element. Whole-genome sequencing of one MRSP isolate representative of the predominant ST45 (strain 57395) permitted for the first time the identification of a ΨSCC*mec* element in MRSP. The novel ΨSCC*mec*$_{57395}$ element was 12,282 bp in size and contained a class C1 *mec* gene complex but no *ccr* genes. It was integrated at the 3′ end of *orfX* and was delimited at both ends by DR with the classical ISS (ISS1 [GAAG CGTATCATAAATGA] and ISS2 [GAAGCATATCATAAGT AG]) and by characteristic inverted repeats (IR) (AATGATGCG GTTTTTA and TAAAAACCGCATCATT), features typically found after an element insertion catalyzed by CcrAB or CcrC (28–30)

(Fig. 2). A third copy of an ISS (ISS3 GAAGCATATCATAAA TGA) was found just upstream of ΨSCC*mec*$_{57395}$ (Fig. 2).

ΨSCC*mec*$_{57395}$ shared the highest homology with the ΨSCC*mec* element of *S. haemolyticus* WCH1 (accession no. JQ764731) (41) (Fig. 2). A ΨSCC*mec*$_{57395}$-like structure was present within the 22.1-kb ΨSCC*mec* element of *S. haemolyticus* WCH1 (Fig. 2). The ΨSCC*mec*$_{57395}$ of MRSP 57395 shared 98.8% nucleotide identity overall with this 14.1-kb fragment situated in the right part of the ΨSCC*mec* of *S. haemolyticus* WCH1 (positions 8368 to 22435 [accession no. JQ764731]). The differences were due to the absence of *orf21* and *orf24* as well as a 36-bp-shorter *arsC* gene in MRSP 57395 (Fig. 2).

The 5,940-bp class C1 *mec* gene complex of ΨSCC*mec*$_{57395}$ contained the *mecA* and *ugpQ* genes flanked by two copies of IS*431* (Fig. 2). One of the IS*431* elements was inserted into the *mecR1* gene, 17 bp downstream of the start codon, as found in the class C1 *mec* gene complex of the SCC*mec* X of *S. aureus* JCSC6945 (6,422 bp) (accession no. AB505630) (57) and of the ΨSCC*mec* element of *S. haemolyticus* WCH1 (6,302 bp) (accession no. JQ764731) (41). Unlike *S. aureus* JCSC6945, which contained 14 *mec*-associated direct repeat units (*dru*) between the *ugpQ* gene and IS*431*, *S. pseudintermedius* 57395 and *S. haemolyticus* WCH1 contained only 11 *dru* repeats and displayed *dru* types dt11cj and dt11a, respectively (49) (Fig. 1).

Since the transposition of the recombinase gene *ccrC* outside an SCC*mec* element has been demonstrated recently in *S. aureus* (38), the possible presence of *ccr* gene variants outside the ΨSCC*mec*$_{57395}$ element was determined by a search of the translated genome of MRSP 57395 for a site-specific serine recombinase motif that also recognized Ccr proteins (see Fig. S1 in the supplemental material). Two hits were obtained, and both were associated with resolvases that are unrelated to the mobilization of SCC*mec* (see Fig. S1). In *S. haemolyticus*, *mecA*-negative strains that contained a *ccrC* gene, as well as *mecA*-positive strains that lacked a *ccrAB* or *ccrC* gene, were described, illustrating that *mecA* and *ccr* gene complexes can exist as autonomous units in this species (41, 42). Furthermore, in methicillin-resistant *S. haemolyticus* JCSC1435, several sequentially integrated SCC elements were detected downstream of *orfX*, comprising one ΨSCC*mec* and one intact SCC that carried a *ccrC* gene (39) (Fig. 2). This array of ΨSCC/SCC elements in tandem may facilitate genomic diversity by the mobilization of different SCC units. The presence of a third copy of an ISS, which is situated 103 bp downstream of the ΨSCC*mec* in MRSP 57395, suggests a remnant of SCC since this DNA sequence region is also present downstream of *orfX* in an additional SCC*mec* published in GenBank (Fig. 2). Alterations in SCC*mec* structures due to recombination and deletions have also been reported in *S. aureus* (38, 58, 59).

Other than *mecA*, none of the antibiotic resistance genes carried by MRSP 57395 were located on ΨSCC*mec*$_{57395}$. However, ΨSCC*mec*$_{57395}$ contained accessory genes that code for resistance to the heavy metals arsenic, cadmium, and copper (Fig. 2). The structural organizations of these genes were similar in MRSP 57395 and *S. haemolyticus* WCH1, particularly the junction between the *mec* gene complex with *arsR* upstream and *copA* downstream, which had identical sequences (Fig. 2).

As previously shown for *S. aureus* (60), the genomes of *S. pseudintermedius* also contained variable regions downstream of the integration site of SCC*mec* elements (Fig. 2). In MRSP 57395, this variable region contains 2 genes that code for transposases, one of them associated with IS*256*, as well as 4 genes related to restriction-modification systems (ORFs *mrsp-16*, *mrsp-30*, *mrsp-32*, and *mrsp-33*) (Fig. 2). In SCC*mec*-negative *S. pseudintermedius* strain ED99, this variable region contained the *spa1* (50) and *spa2* genes (Fig. 2). No homologous sequence to protein A gene was found in the genome of strains 57395 and HKU10-03 (54) (accession no. CP002439) (Fig. 2), confirming observations of other studies that the *spa* genes are not always present in *S. pseudintermedius* (9, 20, 36, 37). The presence or absence of *spa* genes between ORFs *mrsp-29* and *mrsp-34* was confirmed in isolates of this study by PCR amplification and EcoRV restriction analysis (Fig. 2). All *spa*-negative strains (Fig. 1) exhibited the same restriction pattern as strain 57395, with sizes of 2,256 bp, 1,717 bp, and 690 bp, whereas the restriction pattern of the *spa*-positive strains (58544, 54339, S4, AH18, and 68407) corresponded to that of *spa*-positive strain ED99, with sizes of 2,156 bp, 1,485 bp, and 1,233 bp (Fig. 2). These findings identified the *spa* region in *S. pseudintermedius* as a potential integration site that can be occupied with other variable sequences in *spa*-negative strains.

Distribution of ΨSCC*mec*$_{57395}$ among MRSP strains from different origins. Restriction analysis with Bsu36I of PCR products amplified with primers orfX-R3 and attSCC-R generated 3 fragments with sizes identical to those obtained with strain 57395 (5,406 bp, 4,024 bp, and 3,207 bp), confirming the presence of genetic elements similar to ΨSCC*mec*$_{57395}$ in the 34 MRSP isolates from Israel and Thailand. Among them, 31 isolates also contained variable regions spanning the cassette and the core genome similar to those of strain 57395 as demonstrated by Bsu36I digestion of the PCR products obtained with primers orfX-R3 and ald1-R, which generated 6 fragments with sizes of 422 bp, 1,181 bp, 1,866 bp, 3,207 bp, 5,406 bp, and 10,571 bp (Fig. 2). The other three isolates revealed minor differences in the region spanning ΨSCC*mec*$_{57395}$ and the core genome (Fig. 1). No evidence for precursor SCCs in tandem could be obtained, indicating that the ΨSCC*mec*$_{57395}$ element in MRSP CC45 has lost recombination function and has evolved to be an immobilized part of the chromosome that is restricted from spreading by horizontal gene transfer.

The presence of the ΨSCC*mec*$_{57395}$ element in MRSP strains of the same ST lineage, but with variable PFGE profiles and *dru* types, supports the hypothesis that these MRSP CC45 strains likely arose from a common ancestor, which evolved into different fingerprint patterns and became established as the predominant MRSP strains in two different countries. The presence of similar clones in the two countries suggests that MRSP CC45 may have been introduced in these countries with imported dogs, as the trade of pet dogs between the two countries is not uncommon.

Like other MRSP strains (61), MRSP CC45 isolates contain antibiotic resistance genes and mutations that make them resistant to virtually all antibiotics licensed for veterinary medicine. These resistances, once again, illustrate the importance of the judicious use of antibiotics in veterinary medicine, the importance of antibiotic susceptibility testing, and the need for strict hygiene practices and surveillance of antibiotic resistance development in veterinary settings. Such efforts will limit the establishment of nosocomial multidrug-resistant pathogens and their spread to animals and humans.

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