JOURNAL OF CLINICAL MICROBIOLOGY, May 2008, p. 1818–1823 Vol. 46, No. 5
0095-1137/08/$08.00+0 doi:10.1128/JCM.02255-07

NOTES

Characterization of New Staphylococcal Cassette Chromosome *mec* (SCC*mec*) and Topoisomerase Genes in Fluoroquinolone- and Methicillin-Resistant *Staphylococcus pseudintermedius*▽

Sybill Descloux, Alexandra Rossano, and Vincent Perreten*

Institute of Veterinary Bacteriology, University of Bern, CH-3001 Bern, Switzerland

Received 21 November 2007/Returned for modification 13 January 2008/Accepted 20 February 2008

Fluoroquinolone- and methicillin-resistant *Staphylococcus pseudintermedius* isolates harbor two new staphylococcal cassette chromosome *mec* (SCC*mec*) elements that belong to class A, allotype 3 (SCC*mec* II-III), and to the new allotype 5 (SCC*mec* VII). Analysis of the complete nucleotide sequences of the topoisomerase loci *gyrB*/*gyrA* and *grlB*/*grlA* revealed mutations involved in fluoroquinolone resistance.

Staphylococcus pseudintermedius is an opportunistic pathogen that primarily causes skin and nosocomial infections in dogs and cats but can also occasionally affect humans (1, 29). Fluoroquinolones and cephalosporins are widely used to treat staphylococcal infections in veterinary medicine (23). The frequent use of these antibiotics may augment the risk of rapidly selecting for bacteria resistant to both classes of antibiotics. In the past year, multidrug-resistant *S. pseudintermedius* strains have been isolated with increasing frequency from infection sites of dogs at our diagnostic unit. The antibiotic resistance mechanisms of these *S. pseudintermedius* strains were characterized with an emphasis on resistance to methicillin and fluoroquinolone.

Strain identification and antibiotic resistance profile. The isolated strains ($n = 15$) were cultured on tryptone soy agar plates containing 5% sheep blood (Oxoid Ltd., Basingstoke, England) and were identified by catalase activity, Gram staining, and sequencing of the *sodA* gene as described previously (24, 26). All isolates harbored the leukocidin gene *lukS* (25) but not the Panton-Valentine leukocidin gene *lukS-PV* (28), as determined by PCR using the primers listed in Table 2. Antibiotic susceptibility was determined by broth microdilution using a custom Sensititre susceptibility plate, model NLV57 (Trek Diagnostics Systems, East Grinstead, England; MCS Diagnostics BV, Swalmen, The Netherlands), according to CLSI (formerly NCCLS) guidelines (4). Resistance genes were detected using a microarray (22) (Table 1). In addition to fluoroquinolone and oxacillin (methicillin) resistance, the isolates also displayed resistance to other antibiotics (Table 1).

Characterization of mutations in topoisomerase genes. The mechanism of resistance to fluoroquinolones was investigated by sequence analysis of the topoisomerase II (*gyrA* and *gyrB*) and IV (*grlA* and *grlB*) genes, since mutations in these genes have been shown to confer resistance to fluoroquinolones on *Staphylococcus aureus* (8, 11, 13, 14, 18, 27, 30). Fragments of *gyrA*, *gyrB*, *grlA*, and *grlB* of type strains *S. pseudintermedius* CCUG49543^T (also known as LMG 22219^T [10]) (CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden) and *Staphylococcus intermedius* DSM20373^T (also known as NCTC 11048^T) (DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) were amplified by PCR and sequenced on an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA) using oligonucleotide primers (Table 2) designed from conserved regions found after alignment of DNA sequences of *grlB*/*grlA* and *gyrB*/*gyrA* from *S. aureus* (GenBank accession no. L25288 and BA000018), *Staphylococcus epidermidis* (GenBank accession no. CP000029 and AE015929), and *Staphylococcus haemolyticus* (GenBank accession no. AY341071, AY341072, AY341073, and AY341074) using MultAlin (7). The nucleotide sequences flanking the amplified fragments were determined by genome walking using the Universal Vectorette system according to the manufacturer's protocol (Sigma-Genosys, St. Louis, MO) and genomic DNA digested with Sau3A, EcoRI, HindIII, and ApoI. The nucleotide sequences of the *grlB*/*grlA* and *gyrB*/*gyrA* loci of *S. pseudintermedius* CCUG49543 share 89% and 94% identity, respectively, with those of *S. intermedius* DSM20373. The entire *gyrB*/*gyrA* and *grlB*/*grlA* loci of fluoroquinolone-resistant and fluoroquinolone-susceptible *S. pseudintermedius* strains were then amplified using the Expand Long Template PCR system (Roche Applied Science, Indianapolis, IN) (annealing at 54°C for 30 s and extension at 68°C for 5 min) with specific primer pairs gyrB-si-PF0–gyrA-si-RV and grlB-si-PF–grlA-si-R0 and were sequenced. Nucleotide sequence comparison revealed high levels of nucleotide polymorphism in the gyrase and topoisomerase IV genes. Most of the mutations are silent (Fig. 1). Mutations that cause amino acid substitutions in the topoisomerases were found in both resistant and susceptible strains and cannot, therefore, be responsible for resistance. However, other amino acid mutations

* Corresponding author. Mailing address: Institute of Veterinary Bacteriology, University of Bern, Länggass-Strasse 122, Postfach, CH-3001 Bern, Switzerland. Phone: 41 31 631 2430. Fax: 41 31 631 2634. E-mail: vincent.perreten@vbi.unibe.ch.

▽ Published ahead of print on 27 February 2008.

TABLE 1. Antibiotic resistance profiles, *lukS* gene, SCC*mec* type, and clonal relatedness of 15 *Staphylococcus pseudintermedius* isolates from dogs and of *S. pseudintermedius* type strain CCUG49543^T

Strain	Source	MIC (μg/ml) for the following antibiotic[a] (resistance breakpoint[b])																	Antibiotic resistance genes detected[c]	Leukocidin	SCC *mec* type	*gyr/grl* group	ST	*agr* type
		AMK (≥64)	AMX (≥8/4)	CEF (≥32)	CHL (≥32)	ENRO (≥4)	CLI (≥4)	ERY (≥8)	GEN (≥16)	KAN (≥64)	LZD (−)	NIT (≥128)	OXA (≥4)	PEN (≥0.25)	STR (≥32)	SXT (≥4/76)	Q/D (≥4)	TET (≥16)	VAN (≥16)						
CCUG49543^T (LMG22219^T)	Type strain	≤16	≤2/1	≤2	8	≤0.25	**>8**	**>16**	≤2	≤1,000	≤0.5	≤16	0.5	**0.25**	**>32**	≤0.5/9.5	≤0.5	**32**	≤1	*ant*(6′)-*Ia*, *aph*(3′)-*III*, *blaZ*, *erm*(B), *sat4*, *tet*(M)	*lukS*			ST63	IV
KM241	Otitis externa	≤16	≤2/1	≤2	≤4	**4**	≤0.5	≤0.25	≤2	≤1,000	≤0.5	≤16	**16**	≤0.12	≤4	≤0.5/9.5	≤0.5	**32**	≤1	*mecA*, *tet*(M)	*lukS*	VII	1	ST73	II
KM336	Osteitis after surgery	≤16	≤2/1	≤2	**32**	**16**	**>8**	**>16**	4	≤1,000	≤0.5	≤16	**16**	**2**	**>32**	**4/76**	≤0.5	≤1	≤1	*aac*(6′)-*Ie*, *ant*(6′)-*Ia*, *aph*(3′)-*III*, *blaZ*, *cat*$_{pC221}$, *erm*(B), *mecA*, *sat4*, *dfr*(G)	*lukS*	II-III	5	ST71	III
KM337	Otitis externa	≤16	≤2/1	≤2	≤4	≤0.25	≤0.5	≤0.25	≤2	≤1,000	≤0.5	≤16	≤0.25	≤0.12	≤4	≤0.5/9.5	≤0.5	≤1	≤1		*lukS*			ST74	II
KM571	Infected wound after surgery	≤16	8/4	≤2	8	**>16**	**>8**	**>16**	**16**	≤1,000	2	≤16	**>16**	**>8**	**>32**	**8/152**	1	**>32**	2	*aac*(6′)-*Ie*, *ant*(6′)-*Ia*, *aph*(3′)-*III*, *blaZ*, *erm*(B), *mecA*, *sat4*, *tet*(K), *dfr*(G)	*lukS*	II-III	5	ST71	III
KM631	Osteitis after surgery	≤16	4/2	8	**64**	**16**	**>8**	**>16**	**32**	≤1,000	≤0.5	≤16	**>16**	**>8**	**>32**	**8/152**	1	**>32**	≤1	*ant*(6′)-*Ia*, *aph*(3′)-*III*, *blaZ*, *cat*$_{pC221}$, *erm*(B), *mecA*, *sat4*, *tet*(K), *dfr*(G)	*lukS*	II-III	5	ST71	III
KM1087	Vaginitis	≤16	≤2/1	≤2	≤4	≤0.25	≤0.5	≤0.25	≤2	≤1,000	≤0.5	≤16	≤0.25	≤0.12	≤4	≤0.5/9.5	≤0.5	**32**	≤1	*blaZ*, *tet*(M)	*lukS*			ST75	I
KM1250	Infected wound	≤16	≤2/1	≤2	8	≤0.25	≤0.5	≤0.25	≤2	≤1,000	1	≤16	≤0.25	≤0.12	≤4	≤0.5/9.5	≤0.5	≤1	≤1		*lukS*			ST41	II
KM1381	Fistula after surgery	≤16	≤2/1	≤2	**64**	**16**	**>8**	**>16**	**16**	≤1,000	≤0.5	≤16	**>16**	≤0.12	**>32**	**4/76**	≤0.5	**>32**	≤1	*aph*(3′)-*III*, *aph*(2′)-*Ia*, *cat*$_{pC221}$, *erm*(B), *mecA*, *sat4*, *tet*(K), *dfr*(G)	*lukS*	II-III	5	ST71	III
KM1395	Otitis externa	≤16	≤2/1	≤2	8	**>16**	**>8**	**>16**	**32**	≤1,000	1	≤16	**>16**	≤0.12	**>32**	**8/152**	1	**>32**	≤1	*aac*(6′)-*Ie*, *ant*(6′)-*Ia*, *aph*(3′)-*III*, *blaZ*, *erm*(B), *mecA*, *sat4*, *tet*(K), *dfr*(G)	*lukS*	II-III	4	ST71	III
KM1542	Seroma	≤16	4/2	≤2	**64**	**>16**	**>8**	**>16**	**500**	≤1,000	1	≤16	**2**	**0.5**	**>32**	**8/152**	1	**>32**	≤1	*aac*(6′)-*Ie*, *ant*(6′)-*Ia*, *aph*(3′)-*III*, *blaZ*, *cat*$_{pC221}$, *erm*(B), *mecA*, *sat4*, *tet*(K), *dfr*(G)	*lukS*	II-III	5	ST71	III
KM1591	Pyoderma	≤16	≤2/1	≤2	≤4	**8**	**>8**	**>16**	≤2	≤1,000	≤0.5	≤16	≤0.25	≤0.12	**>32**	≤0.5/9.5	≤0.5	**32**	≤1	*ant*(6′)-*Ia*, *aph*(3′)-*III*, *erm*(B), *sat4*, *tet*(M)	*lukS*		2	ST51	I
KM1832	Osteitis after surgery	≤16	≤2/1	≤2	**32**	**16**	**>8**	**>16**	**16**	≤1,000	≤0.5	≤16	**>16**	**0.25**	**>32**	**4/76**	≤0.5	**>32**	≤1	*aac*(6′)-*Ie*, *ant*(6′)-*Ia*, *aph*(3′)-*III*, *blaZ*, *cat*$_{pC221}$, *erm*(B), *mecA*, *sat4*, *tet*(K), *dfr*(G)	*lukS*	II-III	5	ST71	III
KM1896	Gingivitis	≤16	≤2/1	≤2	**32**	**16**	**>8**	**>16**	**16**	≤1,000	≤0.5	≤16	**>16**	≤0.12	**>32**	**4/76**	1	**>32**	≤1	*ant*(6′)-*Ia*, *aph*(3′)-*III*, *cat*$_{pC221}$, *erm*(B), *mecA*, *sat4*, *tet*(K), *dfr*(G)	*lukS*	II-III	5	ST71	III
SD91	Ear	≤16	≤2/1	≤2	**64**	**16**	**>8**	**>16**	**500**	≤1,000	≤0.5	≤16	**4**	**>8**	**>32**	**4/76**	≤0.5	**>32**	≤1	*ant*(6′)-*Ia*, *aph*(3′)-*III*, *blaZ*, *cat*$_{pC221}$, *erm*(B), *mecA*, *sat4*, *tet*(K), *dfr*(G)	*lukS*	II-III	5	ST71	III
SD1071	Nasal cavities	≤16	≤2/1	≤2	≤4	**8**	2[d]	**>16**	≤2	≤1,000	1	≤16	≤0.25	≤0.12	**>32**	≤0.5/9.5	≤0.5	**32**	≤1	*ant*(6′)-*Ia*, *aph*(3′)-*III*, *blaZ*, *erm*(B), *sat4*, *tet*(M)	*lukS*		3	ST76	IV

[a] AMK, amikacin; AMX, amoxicillin; CEF, cephalotin; CHL, chloramphenicol; CLI, clindamycin; ENRO, enrofloxacin; ERY, erythromycin; GEN, gentamicin; KAN, kanamycin; LZD, linezolid; NIT, nitrofurantoin; OXA, oxacillin; PEN, penicillin; STR, streptomycin; SXT, trimethoprim-sulfamethoxazole; Q/D, quinupristin-dalfopristin; TET, tetracycline; VAN, vancomycin.

[b] The MIC breakpoints (in micrograms per milliliter) determining resistance were those recommended for *S. aureus* in CLSI supplement M100-S16 (6) except for enrofloxacin and streptomycin, for which breakpoints of CLSI from supplement M31-S1 for bacteria from animals (3) and from the French Society for Microbiology (www.sfm.asso.fr) were used. MICs above resistance breakpoints are boldfaced.

[c] Antibiotic resistance genes and their functions are as follows: *erm*(B), macrolides, lincosamides, and streptogramin B methylase; *blaZ*, β-lactamase; *cat*$_{pC221}$, chloramphenicol acetyltransferase; *dfr*(G), dihydrofolate reductase (trimethoprim resistance); *tet*(K), tetracycline efflux; *aac*(6′)-*Ie* and *aph*(3′)-*III*, aminoglycoside acetyltransferase and phosphotransferase; *ant*(6′)-*Ia*, streptomycin adenylyltransferase; *sat4*, streptothricin acetyltransferase; *mecA*, penicillin-binding protein PBP2′.

[d] Inducible clindamycin resistance as determined according to CLSI guidelines (5).

TABLE 2. Oligonucleotides used for PCR amplification of leukocidin genes, SCC*mec*, topoisomerase IV, and gyrase genes

Gene	Primer name	Sequence	Primer design reference or source
Gyrase and topoisomerase IV			
grlA	grlA-cons-F	GGTNCGTTTAAGTCAAGA[a]	This study
	grlA-cons-R	CCTTCNACNATATGCATNCG[a]	
grlB	grlB-sa-F2	TATNCGNGAAGGTTTAAC[a]	This study
	grlB-cons-R	GTTTCNGGNTTCATNGTNGTTTCCCA[a]	
grlB-grlA	grlB-si-PF	GCTATTTAGATTAGGCTTGC	This study
	grlA-si-R0	TGTGACCAAATCAAGTCG	
gyrA	gyrA-cons-F	ACNGATTTACGTGATGAAAC[a]	This study
	gyrA-cons-R	ACGTCTNAAACGCATNTCTAAA[a]	
gyrB	gyrB-sa-F4	GTTGATATTCAAGAAAANATGGG[a]	This study
	gyrB-sa-R2	AGACCCCCCGGCAGAGTC[a]	
gyrB-gyrA	gyrB-si-PF0	GATGACGTCTTAAGTGAGTTGG	This study
	gyrA-si-RV	ATTGGCGATAAGTTGTCAAAGG	
SCC*mec*			
ccrA	ccrA-F	AACGTGTCATTGCSACAC	This study
	ccrA-R	GGGCGTAAGATTTATCAAGCTT	
mecI	mecI-F	CCCTTTTTATACAATCTCGTT	31
	mecI-R	ATATCATCTGCAGAATGGG	
orfX	ORFX1r	AACGTTTAGGCCCATACACCA	9
IR-L SRI*mec* III	SccmecIR-F	CTTATCAGTTGATGATGCG	This study
lukS	lukSi-F1	ATGGTAAAAAATAAATTATTAGCCG	This study
	lukSi-R0	TTAATTATGCCCCTTTACTTTAATTTC	
lukS-PV	luk-PV-1	ATCATTAGGTAAAATGTCTGGACATGATCCA	17
	luk-PV-2	GCATCAASTGTATTGGATAGCAAAAGC	

[a] Primers designed from conserved regions found after alignment of DNA sequences of *grlB*/*grlA* and *gyrB*/*gyrA* from *S. aureus* (GenBank accession no. L25288 and BA000018), *S. epidermidis* (GenBank accession no. CP000029 and AE015929), and *S. haemolyticus* (GenBank accession no. AY341071, AY341072, AY341073, and AY341074).

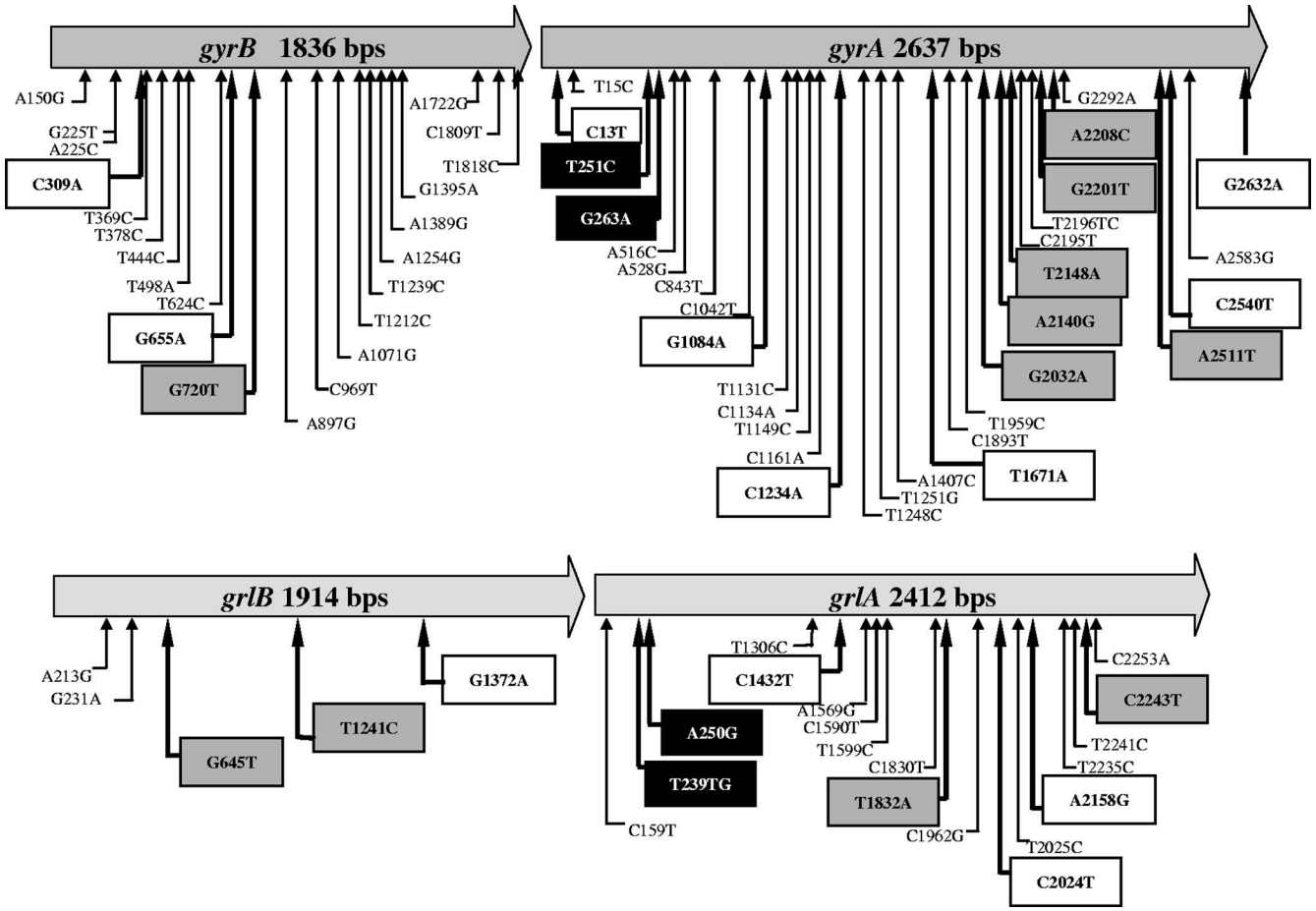

FIG. 1. Consensus nucleotide mutations present in the topoisomerase IV and gyrase genes of 12 fluoroquinolone-resistant and 4 fluoroquinolone-susceptible strains. Silent mutations (not boxed) are indicated with thin arrows. Open boxes, mutations that cause amino acid substitutions in both fluoroquinolone-resistant and fluoroquinolone-susceptible strains; solid boxes, mutations that cause amino acid substitutions at the same positions as those described for fluoroquinolone-resistant *S. aureus*; shaded boxes, additional mutations that cause amino acid substitutions in fluoroquinolone-resistant *S. pseudintermedius*.

were found only in fluoroquinolone-resistant strains (Table 3). Some of these amino acid changes occurred at the same positions as those reported for fluoroquinolone-resistant *S. aureus*, *S. intermedius*, and *Staphylococcus schleiferi* strains, including positions 251 (Ser84Leu) and 263 (Glu88Gly) on *gyrA* and positions 239 (Ser80Ile) and 250 (Asp84Asn) on *grlA* (8, 13–15, 18, 27, 30) (Table 3). *S. pseudintermedius* strains harboring at least one of these mutations showed decreased susceptibility to enrofloxacin (MICs, 4 to 8 μg/ml). Higher MICs (≥16 μg/ml) were observed when two additional mutations (Thr678Ala and Glu714Lys) were present in *gyrA* (Table 3). Additional amino acid substitutions were found in the topoisomerase genes, but their roles in fluoroquinolone resistance remain to be determined (Fig. 1).

Characterization of two new SCC*mec* elements. Methicillin resistance is mediated by the *mecA* gene, which is located on a large staphylococcal cassette chromosome *mec* (SCC*mec*) element. SCC*mec* elements are characterized by the completeness of the methicillin resistance regulon containing *mecA*, by the allotype of the recombinase genes *ccrA* and *ccrB*, and by the general genetic structure (2, 12, 20). SCC*mec* are delineated by two inverted repeats, IR-L and IR-R (Fig. 2). Six different SCC*mec* (I to VI) have been described to date for *Staphylococcus* (2, 12, 16, 20, 21). In the course of our study, SCC*mec* of *S. pseudintermedius* could not be classified using PCR methods previously developed to determine the SCC*mec* class (19, 31) and were therefore sequenced. First, the regions spanning *orfX* to *mecI* and *mecI* to *ccrA* of all methicillin-resistant *S. pseudintermedius* strains were amplified using the Expand Long Template PCR system (Roche Applied Science, Indianapolis, IN) with primers ccrA4-F1 and mecI-R (fragment A) and mecI-F and ORFX1r (9) (fragment B) (annealing at 50°C for 30 s; extension at 68°C for 15 min) (Table 2; Fig. 2). Restriction analysis of fragments A and B, digested with HindIII and PstI, respectively, revealed two types of SCC*mec*. The SCC*mec* of KM241 had a unique profile, whereas all the other profiles were identical to that of KM1381. The fragments were sequenced using a primer-walking strategy. To complete the entire nucleotide sequence of the cassette, the 5′ end of the SCC*mec* of KM1381 situated upstream of *ccrA* was amplified using primer ccrA-R and primer SccmecIR-F, which is specific to *S. aureus* SCC*mec* III IR-L (GenBank accession no. AB037671). The 5′-end sequence of the SCC*mec* of KM241 was determined using the Universal Vectorette system (Sigma-Genosys Co., St. Louis, MO). Sequence analysis of the two entire cassettes revealed two new SCC*mec*, SCC*mec* II-III in KM1381 and SCC*mec* VII in KM241 (Fig. 2). SCC*mec* II-III consists of a combination of *S. aureus* SCC*mec* III (accession no. AB037671) (100% nucleotide identity from IR-L to ORF12) and *S. epidermidis* SCC*mec* II (GenBank accession no. CP000029) (98.9% nucleotide identity from ORF13 to IR-R) (Fig. 2). SCC*mec* VII contains new recombinase genes, *ccrA5* and *ccrB5*, classifying it as a new allotype, allotype 5. The amino acid sequences of *ccrA5* and *ccrB5* showed 75.6% identity overall to *S. aureus* CcrA, allotype 3 (GenBank accession no. BAA88754), and 92.3% identity overall to *S. aureus* CcrB, allotype 3 (GenBank accession no. BAA88755). The rest of the SCC*mec* VII downstream of the *ccrA5* and *ccrB5* loci until IS*431* showed 99% nucleotide identity to *S. aureus* SCC*mec* III (GenBank accession no. AB037671) (Fig. 2) but differed from

TABLE 3. Nucleotide mutations that cause amino acid substitutions in gyrase and topoisomerase IV in fluoroquinolone-resistant *S. pseudintermedius*

Strain	Presence or absence of the indicated mutation in the following gene:													MIC (μg/ml) for enrofloxacin	*gyr*/*grl* group
	grlA			*grlB*			*gyrA*						*gyrB* T720G (Phe240Leu)		
	G239T (Ser80Ile)[a]	G250A (Asp84Asn)[a]	A1832T (Gln611Leu)	T2243C (Val748Ala)	T645G (Asp215Glu)	T1241C (Leu414Ser)	C251T (Ser84Leu)[a]	A263G (Glu88Gly)[a]	A2032G (Thr678Ala)	G2140A (Glu714Lys)	T2201G (Leu734Arg)	C2208A (Asn736Lys)	T2511A (Asp837Glu)		
Fluoroquinolone susceptible															
CCUG 49543[T]	−	−	−	−	−	−	−	−	−	−	−	−	−	≤0.25	−
KM337	−	−	−	−	−	−	−	−	−	−	−	−	−	≤0.25	−
KM1087	−	−	−	−	−	−	−	−	−	−	−	−	−	≤0.25	−
KM1250	−	−	−	−	−	−	−	−	−	−	−	−	−	≤0.25	−
Fluoroquinolone resistant															
KM241	+	−	+	−	−	−	−	+	−	−	−	−	+	4	1
KM1591	−	+	−	−	−	+	−	−	−	−	+	+	−	8	2
SD1071	−	−	−	+	−	−	−	−	−	−	−	−	−	8	3
KM1395	−	−	−	−	+	−	−	−	+	+	−	+	−	>16	4
SD91	+	−	−	−	−	−	+	−	+	+	−	−	−	16	5
KM1381	+	−	−	−	−	−	+	−	+	+	−	−	−	16	5
KM631	+	−	−	−	−	−	+	−	+	+	−	−	−	16	5
KM1832	+	−	−	−	−	−	+	−	+	+	−	−	−	16	5
KM1896	+	−	−	−	−	−	+	−	+	+	−	−	−	16	5
KM336	+	−	−	−	−	−	+	−	+	+	−	−	−	>16	5
KM571	+	−	−	−	−	−	+	−	+	+	−	−	−	>16	5
KM1542	+	−	−	−	−	−	+	−	+	+	−	−	−	>16	5

[a] Amino acid substitution known to confer fluoroquinolone resistance in *S. aureus* (8, 13, 14, 18, 27, 30).

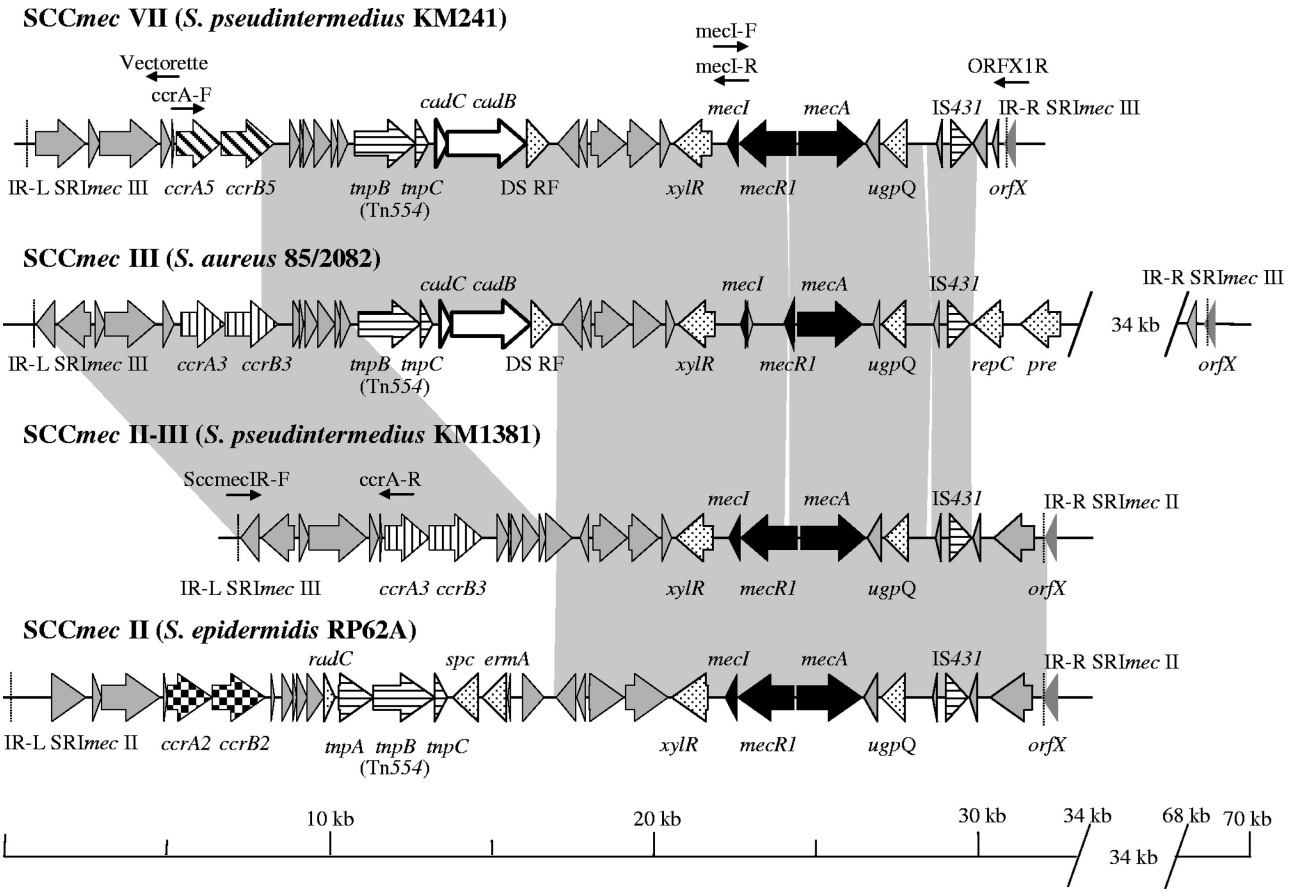

FIG. 2. Alignment of *S. pseudintermedius* SCC*mec* II-III and SCC*mec* VII of with *S. aureus* SCC*mec* III and *S. epidermidis* SCC*mec* II. Solid arrows represent the *mecA* regulon. Diagonally lined, vertically lined, and checked arrows represent the different cassette chromosome recombinases A and B. Horizontally lined arrows, transposases; open arrows, cadmium resistance genes; shaded arrows, hypothetical proteins. Cassettes are delimited by inverted repeats IR-L and IR-R (dashed lines).

SCC*mec* III by a complete *mecA* regulon. Both SCC*mec* II-III and SCC*mec* VII are new cassettes, which belong to class A, allotype 3, and class A, allotype 5, respectively.

Differentiation of strains by ST. Sequence typing (ST) of five gene loci (the 16S rRNA gene, *tuf*, *cpn60*, *pta*, and *agrD*) and examination of the allelic variation of *agrD* (1) showed that all methicillin-resistant strains containing SCC*mec* II-III were clonally related. They belong to ST71, *agr* type III (Table 1), which is the predominant clonal group in North and Central Europe (1). They also contain the same mutations in the *gyrB*/*gyrA* and *grlB*/*grlA* genes (*gyr*/*grl* group 5), except for strain KM1395. *S. pseudintermedius* strain KM241 (SCC*mec* VII) belongs to a new group (ST73, *agr* IV). The methicillin- and fluoroquinolone-susceptible strains belong to the distinct ST group ST41, *agr* II, and to new ST groups ST74, *agr* II, and ST75, *agr* I (Table 1).

New SCC*mec* with new recombinase genes in *S. pseudintermedius* represent a new reservoir of the *mecA* gene for methicillin-sensitive *Staphylococcus* species. Until now, mainly SCC*mec* III has been detected in *S. pseudintermedius* (26). Additionally, sequence analysis of topoisomerase genes has allowed us to determine, for the first time for *S. pseudintermedius*, mutations that play a role in fluoroquinolone resistance.

The presence of multidrug-resistant *Staphylococcus* of animal origin is a further demonstration that the use of antibiotics in veterinary medicine selects for resistant strains. Guidelines regarding the use and choice of antibiotics should be followed in veterinary medicine to suppress the rapid, nationwide dissemination of multidrug-resistant *S. pseudintermedius* clones.

Nucleotide sequence accession numbers. Nucleotide sequences were deposited in the EMBL/GenBank/DDBJ databases. SCC*mec* II-III and SCC*mec* VII were assigned accession no. AM904732 and AM904731. The *gyrB*/*gyrA* and *grlB*/*grlA* loci of *S. pseudintermedius* CCUG49543^T and KM1381 and of *S. intermedius* DSM20373^T were assigned accession no. AM262968 and AM262971, AM262969 and AM262972, and AM262967 and AM262970, respectively.

We thank the personnel of the diagnostic unit of the Institute of Veterinary Bacteriology of the University of Bern who isolated the strains. We also thank J. Ross Fitzgerald for helpful advice on sequence typing.

REFERENCES

1. **Bannoehr, J., N. L. Ben Zakour, A. S. Waller, L. Guardabassi, K. L. Thoday, A. H. M. van den Broek, and J. R. Fitzgerald.** 2007. Population genetic structure of the *Staphylococcus intermedius* group: insights into *agr* diversification and the emergence of methicillin-resistant strains. J. Bacteriol. **189:** 8685–8692.
2. **Chongtrakool, P., T. Ito, X. X. Ma, Y. Kondo, S. Trakulsomboon, C. Tiensasitorn, M. Jamklang, T. Chavalit, J. H. Song, and K. Hiramatsu.** 2006.

Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 11 Asian countries: a proposal for a new nomenclature for SCC*mec* elements. Antimicrob. Agents Chemother. **50:**1001–1012.

3. **Clinical and Laboratory Standards Institute.** 2004. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; informational supplement M31–S1, vol. 24, no. 17. Clinical and Laboratory Standards Institute, Wayne, PA.
4. **Clinical and Laboratory Standards Institute.** 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed., vol. 26, no. 2. Approved standard M7–A7. Clinical and Laboratory Standards Institute, Wayne, PA.
5. **Clinical and Laboratory Standards Institute.** 2006. Performance standards for antimicrobial disk susceptibility tests, 9th ed., vol. 26, no. 1. Approved standard M2–A9. Clinical and Laboratory Standards Institute, Wayne, PA.
6. **Clinical and Laboratory Standards Institute.** 2006. Performance standards for antimicrobial susceptibility testing; sixteenth informational supplement M100–S16, vol. 26, no. 3. Clinical and Laboratory Standards Institute, Wayne, PA.
7. **Corpet, F.** 1988. Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res. **16:**10881–10890.
8. **Couzinet, S., J. Yugueros, C. Barras, N. Visomblin, P. Francois, B. Lacroix, G. Vernet, D. Lew, A. Troesch, J. Schrenzel, and C. Jay.** 2005. Evaluation of a high-density oligonucleotide array for characterization of *grlA*, *grlB*, *gyrA* and *gyrB* mutations in fluoroquinolone resistant *Staphylococcus aureus* isolates. J. Microbiol. Methods **60:**275–279.
9. **Cuny, C., and W. Witte.** 2005. PCR for the identification of methicillin-resistant *Staphylococcus aureus* (MRSA) strains using a single primer pair specific for SCC*mec* elements and the neighbouring chromosome-borne *orfX*. Clin. Microbiol. Infect. **11:**834–837.
10. **Devriese, L. A., M. Vancanneyt, M. Baele, M. Vaneechoutte, E. De Graef, C. Snauwaert, I. Cleenwerck, P. Dawyndt, J. Swings, A. Decostere, and F. Haesebrouck.** 2005. *Staphylococcus pseudintermedius* sp. nov., a coagulase-positive species from animals. Int. J. Syst. Evol. Microbiol. **55:**1569–1573.
11. **Ferrero, L., B. Cameron, B. Manse, D. Lagneaux, J. Crouzet, A. Famechon, and F. Blanche.** 1994. Cloning and primary structure of *Staphylococcus aureus* DNA topoisomerase IV: a primary target of fluoroquinolones. Mol. Microbiol. **13:**641–653.
12. **Hanssen, A. M., and J. U. Ericson Sollid.** 2006. SCC*mec* in staphylococci: genes on the move. FEMS Immunol. Med. Microbiol. **46:**8–20.
13. **Horii, T., Y. Suzuki, A. Monji, M. Morita, H. Muramatsu, Y. Kondo, M. Doi, A. Takeshita, T. Kanno, and M. Maekawa.** 2003. Detection of mutations in quinolone resistance-determining regions in levofloxacin- and methicillin-resistant *Staphylococcus aureus*: effects of the mutations on fluoroquinolone MICs. Diagn. Microbiol. Infect. Dis. **46:**139–145.
14. **Iihara, H., T. Suzuki, Y. Kawamura, K. Ohkusu, Y. Inoue, W. Zhang, M. M. Shah, Y. Atagiri, Y. Hashi, and T. Zaki.** 2006. Emerging multiple mutations and high-level fluoroquinolone resistance in methicillin-resistant *Staphylococcus aureus* isolated from ocular infections. Diagn. Microbiol. Infect. Dis. **56:**297–303.
15. **Intorre, L., M. Vanni, D. Di Bello, C. Pretti, V. Meucci, R. Tognetti, G. Soldani, G. Cardini, and O. Jousson.** 2007. Antimicrobial susceptibility and mechanism of resistance to fluoroquinolones in *Staphylococcus intermedius* and *Staphylococcus schleiferi*. J. Vet. Pharmacol. Ther. **30:**464–469.
16. **Ito, T., X. X. Ma, F. Takeuchi, K. Okuma, H. Yuzawa, and K. Hiramatsu.** 2004. Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, *ccrC*. Antimicrob. Agents Chemother. **48:**2637–2651.
17. **Lina, G., Y. Piémont, F. Godail-Gamot, M. Bes, M.-O. Peter, V. Gauduchon, F. Vandenesch, and J. Etienne.** 1999. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin. Infect. Dis. **29:**1128–1132.
18. **Noguchi, N., T. Okihara, Y. Namiki, Y. Kumaki, Y. Yamanaka, M. Koyama, K. Wakasugi, and M. Sasatsu.** 2005. Susceptibility and resistance genes to fluoroquinolones in methicillin-resistant *Staphylococcus aureus* isolated in 2002. Int. J. Antimicrob. Agents **25:**374–379.
19. **Oliveira, D. C., and H. de Lencastre.** 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **46:**2155–2161.
20. **Oliveira, D. C., C. Milheiriço, and H. de Lencastre.** 2006. Redefining a structural variant of staphylococcal cassette chromosome *mec*, SCC*mec* type VI. Antimicrob. Agents Chemother. **50:**3457–3459.
21. **Oliveira, D. C., C. Milheiriço, S. Vinga, and H. de Lencastre.** 2006. Assessment of allelic variation in the *ccrAB* locus in methicillin-resistant *Staphylococcus aureus* clones. J. Antimicrob. Chemother. **58:**23–30.
22. **Perreten, V., L. Vorlet-Fawer, P. Slickers, R. Ehricht, P. Kuhnert, and J. Frey.** 2005. Microarray-based detection of 90 antibiotic resistance genes of gram-positive bacteria. J. Clin. Microbiol. **43:**2291–2302.
23. **Piriz, S., J. Valle, E. M. Mateos, R. de la Fuente, D. Cid, J. A. Ruiz-Santaquiteria, and S. Vadillo.** 1996. *In vitro* activity of fifteen antimicrobial agents against methicillin-resistant and methicillin-susceptible *Staphylococcus intermedius*. J. Vet. Pharmacol. Ther. **19:**118–123.
24. **Poyart, C., G. Quesne, C. Boumaila, and P. Trieu-Cuot.** 2001. Rapid and accurate species-level identification of coagulase-negative staphylococci by using the *sodA* gene as a target. J. Clin. Microbiol. **39:**4296–4301.
25. **Prévost, G., T. Bouakham, Y. Piémont, and H. Monteil.** 1995. Characterisation of a synergohymenotropic toxin produced by *Staphylococcus intermedius*. FEBS Lett. **376:**135–140.
26. **Sasaki, T., K. Kikuchi, Y. Tanaka, N. Takahashi, S. Kamata, and K. Hiramatsu.** 2007. Methicillin-resistant *Staphylococcus pseudintermedius* in a veterinary teaching hospital. J. Clin. Microbiol. **45:**1118–1125.
27. **Sierra, J. M., J. G. Cabeza, M. Ruiz Chaler, T. Montero, J. Hernandez, J. Mensa, M. Llagostera, and J. Vila.** 2005. The selection of resistance to and the mutagenicity of different fluoroquinolones in *Staphylococcus aureus* and *Streptococcus pneumoniae*. Clin. Microbiol. Infect. **11:**750–758.
28. **Tristan, A., T. Ferry, G. Durand, O. Dauwalder, M. Bes, G. Lina, F. Vandenesch, and J. Etienne.** 2007. Virulence determinants in community and hospital methicillin-resistant *Staphylococcus aureus*. J. Hosp. Infect. **65**(S2)**:**105–109.
29. **Van Hoovels, L., A. Vankeerberghen, A. Boel, K. Van Vaerenbergh, and H. De Beenhouwer.** 2006. First case of *Staphylococcus pseudintermedius* infection in a human. J. Clin. Microbiol. **44:**4609–4612.
30. **Vickers, A. A., A. J. O'Neill, and I. Chopra.** 2007. Emergence and maintenance of resistance to fluoroquinolones and coumarins in *Staphylococcus aureus*: predictions from *in vitro* studies. J. Antimicrob. Chemother. **60:**269–273.
31. **Zhang, K., J.-A. McClure, S. Elsayed, T. Louie, and J. M. Conly.** 2005. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **43:**5026–5033.