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Diversity of Staphylococcal Cassette Chromosome *mec* Structures in Methicillin-Resistant *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* Strains among Outpatients from Four Countries▽

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In staphylococci, methicillin (meticillin) resistance (MR) is mediated by the acquisition of the *mecA* gene, which is carried on the size and composition variable staphylococcal cassette chromosome *mec* (SCC*mec*). MR has been extensively studied in *Staphylococcus aureus*, but little is known about MR coagulase-negative staphylococci (MR-CoNS). Here, we describe the diversity of SCC*mec* structures in MR-CoNS from outpatients living in countries with contrasting environments: Algeria, Mali, Moldova, and Cambodia. Their MR-CoNS nasal carriage rates were 29, 17, 11, and 31%, respectively. Ninety-six MR-CoNS strains, comprising 75 (78%) *Staphylococcus epidermidis* strains, 19 (20%) *Staphylococcus haemolyticus* strains, 1 (1%) *Staphylococcus hominis* strain, and 1 (1%) *Staphylococcus cohnii* strain, were analyzed. Eighteen different SCC*mec* types were observed, with 28 identified as type IV (29%), 25 as type V (26%), and 1 as type III (1%). Fifteen strains (44%) were untypeable for their SCC*mec*. Thirty-four percent of MR-CoNS strains contained multiple *ccr* copies. Type IV and V SCC*mec* were preferentially associated with *S. epidermidis* and *S. haemolyticus*, respectively. MR-CoNS constitute a widespread and highly diversified MR reservoir in the community.

Methicillin (meticillin)-resistant (MR) staphylococci cause a wide variety of infections and raise high concerns, because often few therapeutic options are available. Among the staphylococci, *Staphylococcus aureus* is much more virulent than the other species, which are grouped together under the generic name of coagulase-negative staphylococci (CoNS). CoNS usually are much more resistant to antibiotics than *S. aureus* (11), but in most cases they cause infections only in patients who carry indwelling devices and/or are immunocompromised (20). CoNS are believed to constitute a reservoir of resistance genes for *S. aureus* (16). The nares are an ecological niche for staphylococci (8), as 20 to 30% of humans are colonized by *S. aureus* (31, 48), although the few available data on CoNS carriage range from 46 to 65% of hospital patients (1, 42). The horizontal transfer of resistance genes from CoNS to *S. aureus* has been clearly demonstrated for fusidic acid, gentamicin, and mupirocin (2, 13, 21, 45, 46) and at least once for MR (47). MR in staphylococci is driven by the acquisition of the *mecA* gene, which encodes PBP2A, a transpeptidase with a low affinity for β-lactams (18, 33). *mecA* is part of the *mec* complex, which includes its repressor genes *mecI* and *mecR1*. Ten *mec* complex subclasses, divided into six main classes (A to E), have been described so far on the basis of the polymorphism of *mecI* and *mecR1* (25, 28, 29, 44). The *mec* complex is carried by a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*) (26), a genomic island of variable size (range, 21 to 67 kb). This island is integrated at the 3′ extremity of *orfX* (23), a gene of unknown function located near the chromosomal origin of replication. In addition to *mecA*, SCC*mec* also carries a set of cassette chromosome recombinase (*ccr*) genes encoding recombinases responsible for both its chromosomal integration and excision (26). Six *ccr* allotypes have been identified so far (10, 22, 24, 26, 38). The definition of an SCC*mec* type is based on the combination of a *mec* complex class and a *ccr* allotype (22). In *S. aureus*, six main types of SCC*mec* (I to VI) and several subtypes have been described (9, 22, 24, 32, 38). Much less is known about the genetics of MR in CoNS. Recent reports suggest that in CoNS, SCC*mec* structures are more diverse and include either *mec-ccr* combinations as-yet undescribed for *S. aureus* (35) or more

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TABLE 1. Primers used in this study

PCR	Primer name	5′–3′ Sequence	Gene(s)	RT-PCR probe	Reference or source
16S rRNA for species identification	A2	AGAGTTTGATCATGGCTCAG	*rrs*		39
	A10	AAACTCAAATGAATTGACGG	*rrs*		39
	S15	GGGCGGTGTGTACAAGGCC	*rrs*		39
	S10	CCGTCAATTCATTTGAGTTT	*rrs*		39
mec complex typing	mA7	ATATACCAAACCCGACAACTACA	*mecA*		30
	mI6	CATAACTTCCCATTCTGCAGATG	*mecI*		30
	IS7	ATGCTTAATGATAGCATCCGAATG	IS*1272*		30
	IS2	TGAGGTTATTCAGATATTTCGATGT	IS*431*R		30
	IS2L	GAACCGCAGGTCTCTTCAGATC	IS*431*L		Thisstudy
ccr complex typing	mA1	TGCTATCCACCCTCAAACAGG	*mecA*		30
	mA2	AACGTTGTAACCACCCCAAGA	*mecA*		30
	α1	AACCTATATCATCAATCAGTACGT	*ccrA1*		30
	α2	TAAAGGCATCAATGCACAAACACT	*ccrA2*		30
	α3	AGCTCAAAAGCAAGCAATAGAAT	*ccrA3*		30
	βc	ATTGCCTTGATAATAGCCTTCT	*ccrB1*,*ccrB2*, *ccrB3*		30
	γF	CGTCTATTACAAGATGTTAAGGATAAT	*ccrC*		30
	ccrCU1	TTACCTTTGACCAATATCACATC	*ccrC*		Thisstudy
	α4U	GCGACGAATCAAATGTCCTTACTG	*ccrA4*		Thisstudy
	β4U	ATCGCTCCAGTGTCTATACTTCGC	*ccrB4*		Thisstudy

than one *ccr* allotype (17). However, the epidemiological magnitude of these combinations has not yet been assessed. Country-to-country variations in the antibiotic susceptibility of staphylococci have been observed (1, 7, 27, 31, 50), but they may result from differences in the epidemiological and microbiological methods of investigation used. Here, we undertook a multiple-country study based on the same protocol designed to describe the nasal MR-CoNS reservoir in outpatients. We found that the prevalences of MR-CoNS carriage significantly differed according to geographical area, and that their SCC*mec* elements were much more diverse than those so far described for *S. aureus*.

MATERIALS AND METHODS

Populations and bacterial strains. The present study was performed on a subset of nasal samples gathered during a large epidemiological study devoted to the analysis of *S. aureus* nasal carriage in a community of countries with different environments (41 and S. Mesli, L. Armand-Lefevre, S. Benchouk, H. Hassaine, K. Megueni, M. Benkalfat, J. C. Lucet, A. Andremont, and R. Ruimy, presented at the 26ème Réunion Interdisciplinaire de Chimiothérapie Anti-Infectieuse (RICAI), Paris, France, 7 to 8 December 2006). Nasal swabs were obtained using a standard procedure by an investigator trained in the coordinating center of the study. Swabs were taken within 8 h of the admission of 330, 338, 448, and 442 consecutive patients into the respective emergency wards of the major hospitals in the following four towns: Tlemcen, Algeria (June to October 2005); Chisinau, Moldova (June to October 2005); Bamako, Mali (March to August 2005); and Phnom Penh, Cambodia (June to October 2006). Swabs were rapidly discharged into 1.5 ml of brain heart infusion broth with 10% glycerol, stored at −80°C, and transported frozen to the coordinating center of the study, where 70 samples per site were randomly selected. Fifty microliters of the selected samples were plated on Chapman agar (Oxoid, Basingstoke, United Kingdom) for 48 h at 37°C. Four different mannitol-negative colonies were randomly chosen from each plate, subcultured, and screened by triplex real-time PCR (RT-PCR) using primers hybridizing a specific *rrs* region (located in the 16S RNA gene) for the *Staphylococcus* genus, the *femA* gene (specific for *S. aureus*) and the *mecA* gene (MR), as described previously (40). *mecA* gene-positive and *femA* gene-negative isolates (i.e., putative MR-CoNS) were further characterized by the disc diffusion method for susceptibility to 18 antibiotics: benzylpenicillin, oxacillin, cefoxitin, moxalactam, kanamycin, tobramycin, gentamicin, erythromycin, lincomycin, pristinamycin, levofloxacin, vancomycin, teicoplanin, tetracyline, cotrimoxazole, rifampin (rifampicin), fusidic acid, and fosfomycin, as recommended by the French Society for Microbiology (www.sfm.asso.fr). In the same isolates, species were identified by sequencing 1,300 bp located within the *rrs* gene, as described previously (39). The sequences were aligned using BioEdit 5.0.6 (http://www.mbio.ncsu.edu/BioEdit/bioedit) with those of 38 *Staphylococcus* species obtained from GenBank and corresponding to the type strain of each species when it was available. Phylogenetic analysis was carried out using the neighbor-joining algorithm (Kimura 2-parameter distance estimation) as implemented in MEGA 4.0. This identification targeting *rrs* was sufficiently accurate in terms of *Staphylococcus* species found here, as described previously (3, 14). Coresistances to non-β-lactam antibiotics were scored in each strain, as described previously (36). One phenotype per patient was considered for further analysis.

SCC*mec* fragments were characterized in all nonreplicate isolates by typing *ccr* and *mec* complexes. *ccr* complexes were typed by multiplex PCR (M-PCR), as described previously (30), except that three primers were changed as follows: (i) γR was replaced a new primer, ccrCU1 (Table 1), which generates amplicons of 607 bp with the primer γF (517 bp for the original pair γF/γR), thus allowing the detection of all known *ccrC* allotypes; and (ii) primers α4.2 and β4.2 were replaced by two new primers, α4U and β4U (Table 1), respectively, which amplify a 1,304-bp fragment and target *ccrAB4*, to detect recently published *ccrAB4* alleles (12). *mec* complexes were typed by M-PCR as described previously (30), except that a fifth newly designed primer, IS2L (Table 1), was added in order to detect the class C1 *mec* complexes described for *Staphylococcus haemolyticus* (28). All PCRs were performed in 50-μl mixtures containing 1× *Taq* DNA polymerase buffer (Roche), 2.5 mM $MgCl_2$, 0.5 pmol of each primer, 200 μM of each deoxynucleoside triphosphate, 2.5 U of DNA *Taq* polymerase (Roche), and 2 μl of bacterial extract, and the mixtures were subjected to a denaturation step of 4 min at 94°C; 30 cycles of 30 s at 94°C, 1 min of annealing at either 57°C (*ccr* M-PCR) or 60°C (*mec* M-PCR), and 2 min of extension at 72°C; and a final elongation step of 2 min at 72°C in the GeneAmp PCR system 2700 (Applied Biosystem, Courtaboeuf, France). PCR products were visualized after migration in 1.7% agar 0.5× Tris-acetate-EDTA (TAE) gel using SybrSafe (Invitrogen, Cergy-Pontoise, France) as the double-stranded DNA marker.

From the seven strains harboring three *ccr* allotypes (see below), we selected three strains (A186-1, M327-2, and C327-2) whose *mec-ccr* contents were representative of the combinations found in the four others. These contents were as follows: class A *mec* complex, *ccrAB3*, *ccrAB4*, and *ccrC*; class B *mec* complex, *ccrAB2*, *ccrAB4*, and *ccrC*; and class C2 *mec* complex, *ccrAB2*, *ccrAB4*, and *ccrC*). In the three strains, we PCR amplified the long fragments located between *mecA* and *ccr* to ascertain whether or not they were located in the *mecA* environment by following the protocol given below. The 25 strains harboring SCC*mec* type V also were tested for *ccrC* positioning toward *mecA*. Primer mA1 or mA2 was used as an anchor on *mecA*, together with each of the *ccr* primers described in Table 1. The inferred location of all *ccr* genes, upstream/downstream and sense/antisense in relation to *mecA*, then was assessed in each strain by the size of the amplicons resulting from the above-described PCRs. The size of the fragments joining the *ccr* allotypes in a given strain also was tested. Long-range PCR experiments were performed using the GeneAmp XL PCR kit (Applied Biosystems). Reaction mixtures contained 1× XL buffer II, 1 mM $MgOAc_2$, 20 pmol of each selected primer (mA1 or mA2), one *ccr* primer (to test all of the putative positions of *ccr* toward *mecA*), 1 U of r*Tth* DNA polymerase XL, 0.8 mM of each

TABLE 2. Characteristics of the populations studied and prevalence of nasal carriage of CoNS and MR-CoNS

Characteristic	Result by country				*P* value
	Algeria	Mali	Moldova	Cambodia	
No. of subjects	70	70	70	70	
Avg age (yr)	42	52	39	32	<0.0001
Sex ratio (male/female)	1.1	1.8	1.2	1.8	NS[a]
MR-CoNS carriage prevalence (%)	28.6	17.1	11.4	31.0	<0.05
No. of MR-CoNS strains	33	19	12	32	
Antibiotic resistance prevalence (%)					
Kanamycin	59	79	58	56	NS
Tobramycin	47	58	58	44	NS
Gentamicin	29	58	58	38	NS
Erythromycin	32	53	67	53	NS
Lincomycin	9	0	58	37	<0.0001
Pristinamycin	0	0	33	9	<0.001
Ofloxacin	18	58	58	41	<0.05
Fusidic acid	50	16	0	6	<0.001
Fosfomycin	6	0	0	0	NS
Rifampin	12	5	32	12	NS
Cotrimoxazole	35	74	76	65	NS
Tetracycline	41	63	83	56	NS
Coresistance score	28	39	42	33	<0.05

[a] NS, not significant.

deoxynucleoside triphosphate, and 2 μl of bacterial extract in a final volume of 50 μl. The mixtures were subjected to a first denaturation step of 4 min at 94°C; 10 cycles of 15 s of denaturation at 94°C, 30 s of annealing at 55°C, and 8 min of extension at 68°C; 20 cycles of 15 s of denaturation at 94°C, 30 s of annealing at 55°C, and an 8-min (with an increment of 15 s at each cycle) extension at 68°C; and a final elongation step of 10 min at 72°C. PCR products were visualized by a migration step in a 0.8% agar 0.5× TAE gel with SybrSafe as the double-stranded DNA marker. Control strains for long-range PCR are described below.

Reference strains. The following strains were used as references: *S. aureus* strains COL (SCC*mec* Type I), BK2464 (SCC*mec* Type II), ANS46c (SCC*mec* Type III), HU25 (SCC*mec* Type IIIA), and HDE288 (SCC*mec* Type VI), kindly provided by Herminia de Lencastre, and *S. aureus* strain WCH100 (SCC*mec* Type V), kindly provided by Michele Bes.

Statistical analysis. Epi-Info v3.2.2 (Centers for Disease Control and Prevention, Atlanta, GA) was used for statistical analysis. Associations between species, antibiotic profiles, country, *mec* complex class, *ccr* complex type, and SCC*mec* type were investigated using the chi-squared test. Continuous variables were compared by the analysis of variance test. $P < 0.05$ was considered significant.

RESULTS

The mean (range) ages of the subjects from Algeria, Moldova, Mali, and Cambodia were 42 (17 to 79), 39 (16 to 69), 52 (17 to 80), and 32 (16 to 71) years ($P < 0.0001$), respectively, and their respective male/female ratios were 1.1, 1.2, 1.8, and 1.8 (Table 2). The overall prevalence of MR-CoNS carriage (a patient was considered an MR-CoNS carrier when at least one MR-CoNS isolate was isolated from a nasal sample) was 22%, and it ranged from 31% (Cambodia), 29% (Algeria), and 17% (Mali) to 11% (Moldova, $P < 0.05$). This prevalence was higher in men than women (27% for men and 18% for women; $P < 0.05$). The mean (range) age was not significantly different between noncarriers and carriers (39 [4 to 80] and 42 [14 to 79] years, respectively).

Antibiotic susceptibility testing. In all, 1,120 mannitol-negative strains were screened, of which 120 were *mecA*-CoNS positive by RT-PCR. After duplicates had been excluded on the basis of species identification and antibiotic susceptibility patterns, there were 96 (8.6%) separate strains from 62 subjects (20, 8, 12, and 21 from Algeria, Moldova, Mali, and Cambodia, respectively). All of these strains expressed phenotypic MR. Ninety-two (96%) also were resistant to at least one of the non-β-lactam antibiotics tested, as follows: 57 (62%) were resistant to kanamycin, 38 (41%) to tobramycin and gentamicin, 44 (48%) to erythromycin, 45 (49%) to cotrimoxazole, 51 (55%) to tetracycline, 16 (17%) to rifampin, and 2 (2%) to fosfomycin. There were no significant differences in this respect between countries. In contrast, significant between-country differences (Table 2) were observed for resistance to fluoroquinolones (the lowest resistance was in Algeria), lincomycin (the highest resistance was in Moldova), cotrimoxazole (the highest resistances was in Cambodia and Mali), and fusidic acid (the highest resistance was in Algeria). Coresistance scores also were significantly different for the four countries, with mean scores of 24.2, 29.7, 32.7, and 39.9% for Algeria, Cambodia, Mali, and Moldova, respectively ($P < 0.05$) (Table 2). However, coresistances were not significantly different between *S. haemolyticus* and *S. epidermidis*, the two most frequently identified species (34.2 and 28.9%, respectively).

Species identification. The phylogenetic position of isolates (data not shown) within the genus *Staphylococcus* showed that 75 (78%) of the 96 strains isolated were identified as *S. epidermidis*, 19 (20%) as *S. haemolyticus*, 1 as *Staphylococcus cohnii*, and 1 as *Staphylococcus hominis* (1%). Differences in species distribution between countries were not significant (data not shown).

***mec* complex typing.** Ten (10.4%), 47 (49.0%), 4 (4.2%), and 35 (36.4%) strains exhibited class A, B, C1, and C2 *mec* complexes, respectively. *S. haemolyticus* was combined with class C *mec* complexes significantly more frequently than *S. epidermidis* ($P < 0.001$), as classes C1 and C2 were found in 16/19 *S. haemolyticus* strains. However, there were no significant differences in *mec* complex distribution between countries (data not shown).

***ccr* complex typing.** As many as 120 *ccr* complexes were detected in 86 out of 96 MR-CoNS strains (90%), including 1

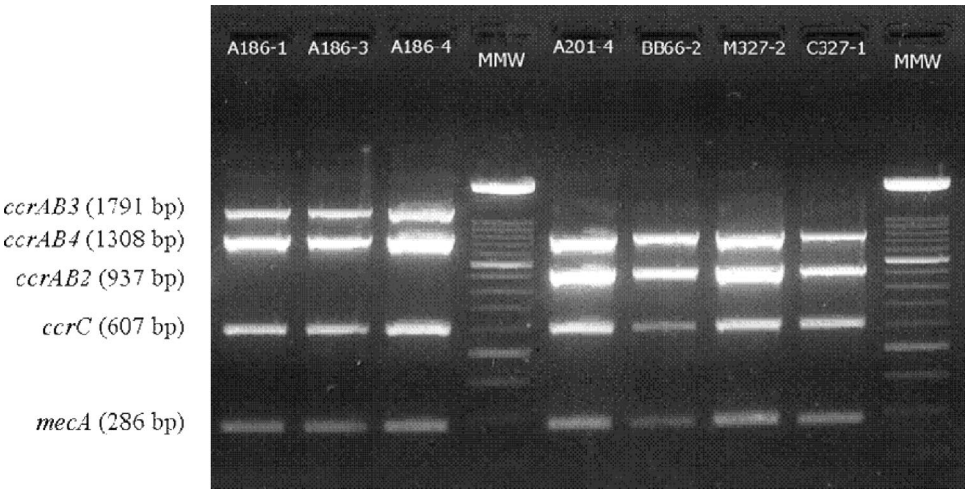

FIG. 1. Migration of PCR products obtained by the *ccr* typing by M-PCR used in this study (30) for the strains harboring three distinct *ccr* allotypes. Strains A186-1, A186-3, and A186-4 harbored three *ccr* allotypes: *ccrAB3*, *ccrAB4*, and *ccrC*. Strains A201-4, BB66-2, M327-2, and C327-1 harbored *ccrAB4*, *ccrAB2*, and *ccrC*. All strains were *mecA* positive. MMW, mass molecular weight (1 kb plus DNA ladder; Invitrogen).

ccrAB1, 52 *ccrAB2*, 10 *ccrAB3*, 7 *ccrAB4*, and 50 *ccrC* complexes (mean, 1.4 *ccr* complexes per strain; 34% of the strains had more than one complex). Four different combinations of two *ccr* complexes were observed (*ccrAB2* and *ccrAB3*, *ccrAB2* and *ccrAB4*, *ccrAB3* and *ccrC*, and *ccrAB4* and *ccrC*), and two combinations of three complexes were observed (*ccrAB2*, *ccrAB4*, and *ccrC* and *ccrAB3*, *ccrAB4*, and *ccrC*) (Fig. 1). *ccrAB2* was significantly more prevalent in *S. epidermidis* than in *S. haemolyticus* (51/75 and 1/18, respectively; $P < 0.0001$). There were no significant between-country differences in *ccr* complex distribution (data not shown). No *ccr* gene was detected with the primers used in 10 (10%) of the confirmed positive *mecA* strains.

SCC*mec* typing. Only 54 (56%) of the 96 strains could be assigned to known SCC*mec* types (Table 3), including 28 to type IV (27 *S. epidermidis* and 1 *S. haemolyticus*), 25 to type V (13 *S. epidermidis* and 12 *S. haemolyticus*), and 1 to type III (*S. epidermidis*). The remaining 42 strains (44%) had *mec-ccr* combinations that did not fit into the current classification scheme. They included 28 strains (29%) with more than one *ccr* complex (21 of them [20.8%] with two complexes and 7 [7.3%] with three complexes), 10 (10%) with untypeable *ccr*, 2 (2%) with already observed but not yet assigned *mec*-single-*ccr* combinations (class A *mec* and *ccrAB1* and class A *mec* and *ccrC* [17, 35]), and 2 (2%) new class B *mec* and *ccrC* combinations.

***ccr* allotype positions relative to *mecA*.** The following strains were tested for *ccr* allotype positions: A186-1 (class A *mec* complex; *ccrAB3*, *ccrAB4*, and *ccrC*), M327-2 (class B *mec* complex; *ccrAB2*, *ccrAB4*, and *ccrC*), and C327-1 (class C2 *mec* complex; *ccrAB2*, *ccrAB4*, and *ccrC*). The full positioning of *ccr* allotypes in relation to *mecA* could be determined only in strain A186-1

TABLE 3. Types of SCC*mec* according to Ito et al. (22)

mec complex class	*ccr* complex(es)	SCC*mec* type	Species (no. of isolates)	Country (no. of isolates)
A	AB1	NT	*S. cohnii* (1)	Algeria (1)
A	C	NT	*S. epidermidis* (1)	Mali (1)
A	AB2, AB3	NT	*S. epidermidis* (1)	Cambodia (1)
A	AB3, C	III	*S. epidermidis* (1)	Mali (1)
A	AB3, AB4, C	NT	*S. epidermidis* (3)	Algeria (3)
A	NT[a]	NT	*S. epidermidis* (2), *S. haemolyticus* (1)	Algeria (3)
B	AB2	IV	*S. epidermidis* (27), *S. haemolyticus* (1)	Algeria (11), Cambodia (10), Moldova (4), Mali (3)
B	C	NT	*S. epidermidis* (1), *S. hominis* (1)	Algeria (1), Mali (1)
B	AB2, AB3	NT	*S. epidermidis* (6)	Cambodia (5), Moldova (1)
B	AB2, C	NT	*S. epidermidis* (5)	Mali (3), Cambodia (1), Moldova (1)
B	AB2, AB4, C	NT	*S. epidermidis* (3)	Algeria (1), Cambodia (1), Moldova (1)
B	NT	NT	*S. epidermidis* (2), *S. haemolyticus* (1)	Algeria (3)
C1	NT	NT	*S. haemolyticus* (4)	Algeria (2), Cambodia (1), Mali (1)
C2	C	V	*S. epidermidis* (9), *S. haemolyticus* (4)	Cambodia (7), Mali (3), Moldova (2), Algeria (1)
C2	AB2, C	NT	*S. epidermidis* (8)	Algeria (5), Moldova (2), Cambodia (1)
C2	AB4, C	NT	*S. epidermidis* (1)	Algeria (1)
C2	C, C	NT	*S. haemolyticus* (8), *S. epidermidis* (4)	Cambodia (5), Mali (5), Algeria (1), Moldova (1)
C2	AB2, AB4, C	NT	*S. epidermidis* (1)	Cambodia (1)

[a] NT, not typeable.

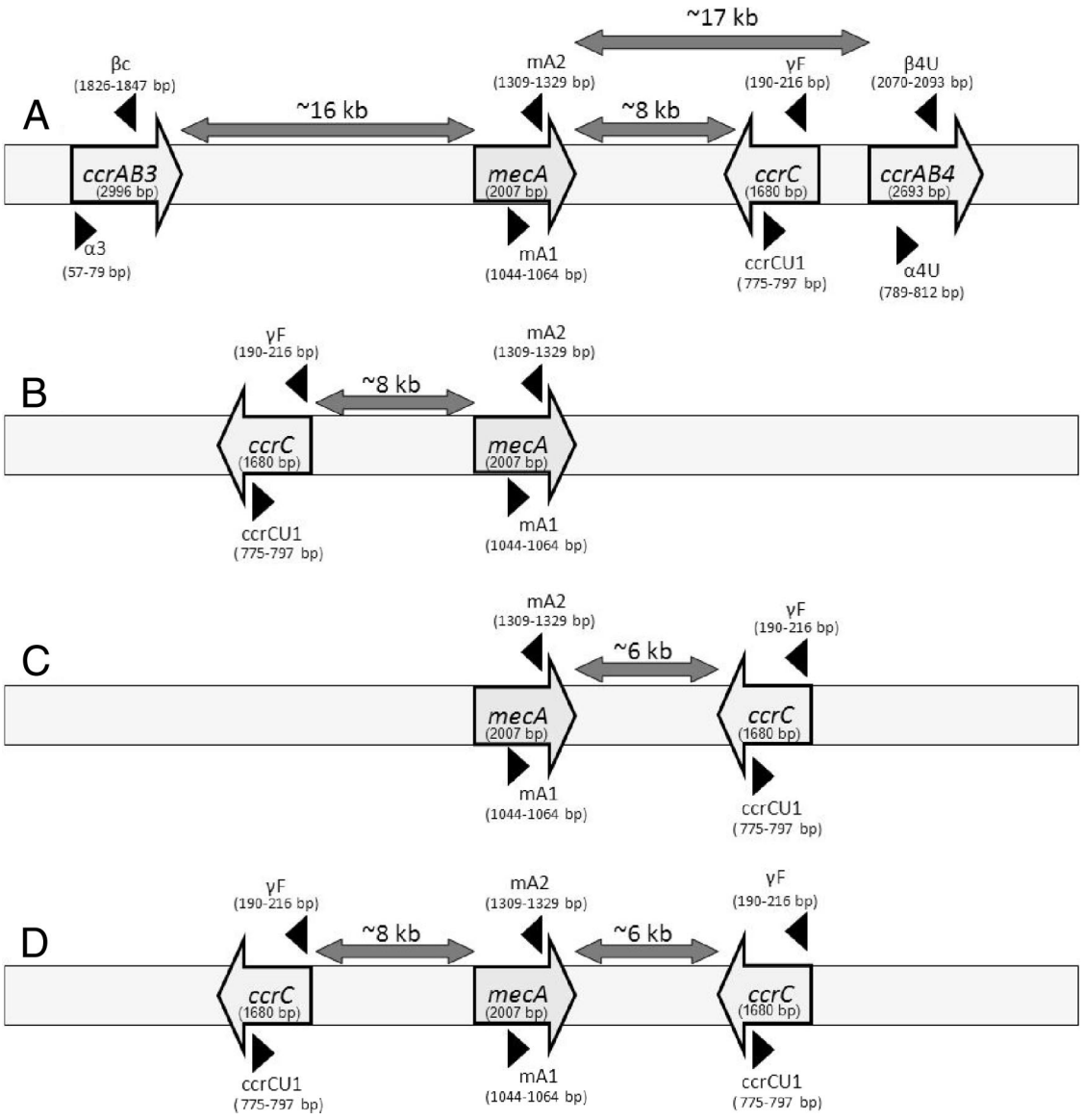

FIG. 2. Schematic representation of structural variations in type V SCC*mec* deduced from long-range PCR results. (A) Strain A186-1, harboring *ccrAB3* ~16 kb upstream from *mecA*, *ccrC* ~8 kb downstream from *mecA*, and *ccrAB4* ~17 kb downstream from *mecA*; (B) *ccrC* located ~8 kb upstream from *mecA*, as described for type V SCC*mec* (24); (C) *ccrC* located ~6 kb downstream from *mecA*; and (D) two *ccrC* allotypes located ~8 kb upstream and ~6 kb downstream from *mecA*.

(Fig. 2). A fragment amplified between primers α3 and mA2 was around ~20 kb, so that the deduced distance between the end of *ccrB3* and the beginning of *mecA* was ~16 kb, with *ccrAB3* being located upstream of *mecA*. A fragment amplified between primers mA1 and γF was ~10 kb, so that *ccrC* was deduced to be located ~8 kb downstream from *mecA.* A fragment amplified between mA1 and β4U was ~20 kb, so that the *ccrAB4* complex was deduced to be located ~17 kb downstream of *mecA*. In strain M327-2, *ccrAB2* was deduced to be located ~5 kb upstream from *mecA*, as described for type IV SCC*mec* (32) and *ccrC*, ~8 kb downstream from *mecA. ccrAB4* could not be located. In strain C327-1, *ccrAB2* and *ccrAB4* could not be located either, but surprisingly, we found two copies of *ccrC* located ~8 kb upstream from *mecA* (as described for type V SCC*mec* [24]) and ~6 kb downstream from *mecA*, respectively. Therefore, to assess the proportion of MR-CoNS strains harboring several copies of *ccrC*, we tested the 25 type V SCC*mec* strains harboring MR-CoNS in our collection and found that 9 had *ccrC* copies ~8 kb upstream from *mecA*, 2 had *ccrC* copies ~6 kb downstream from *mecA*, and 12 had 2 *ccrC* copies both ~8 kb upstream and ~6 kb downstream of *mecA*. We were unable to amplify the remaining two strains.

DISCUSSION

As far as we know, the present study is the first multicountry report on the epidemiology and SCC*mec* characterization of nasal MR-CoNS in outpatients. Rates of MR-CoNS carriage

ranged from 11 to 31%, with significant variations between the four countries studied. However, the species distribution, mostly limited to *S. epidermidis* and *S. haemolyticus*, the most prevalent species colonizing the human nares (4, 8), did not change significantly with the geographical origin of the subjects. Our results showed that nasal MR-CoNS in the community may constitute a diversified reservoir of resistance genes not only for MR but also for many other antibiotics, as shown by the fact that many other resistance traits were present in MR-CoNS.

We did not find any difference between *S. epidermidis* and *S. haemolyticus* in terms of coresistances, and these two species were homogeneously distributed among the four countries. In pathogens such as *Streptococcus pneumoniae*, country-to-country variations in resistance rates are closely related to overall antibiotic use (6). Our results suggest that this also applies to commensals such as CoNS. However, since the extent of antibiotic use is not known in the countries studied here, this hypothesis could not be tested.

The variations we observed also might be due to differences in the populations of patients attending each hospital. Although the male/female ratios of patients were similar among the four countries, their mean ages varied significantly. As strictly the same sampling and analysis methods were used in each country, we are confident that the differences and similarities observed between them were meaningful. The study was designed to ensure that the patients included were representative of community populations, as they were hospitalized under emergency conditions and sampled very soon afterwards to keep the risk of the hospital acquisition of resistant strains to a minimum. In addition, the local investigator in each country had been trained in the main investigator's laboratory to guarantee as much homogeneity as possible and to enable comparisons. Nevertheless, we cannot exclude the possibility that different recruitment biases existed between countries, thus explaining some of the differences observed. Also, we cannot exclude that some MR-CoNS strains had been acquired during a previous healthcare stay.

The structural biodiversity of the SCC*mec* regions was striking. We found, mostly in *S. epidermidis*, 15 *mec-ccr* combinations, some of which had already been observed in this species (17, 35) but not in *S. aureus*. We also found that 34 MR-CoNS strains (35.4%) harbored two distinct *ccr* allotypes, including 12 with two copies of *ccrC*. In addition, seven (7.3%) strains had three *ccr* allotypes. *S. aureus* strains with multiple *ccr* genes have seldom been described (5, 12, 19, 22), possibly because the methods used to type MR *S. aureus* (34, 37, 51) could not detect *ccr* duplication. In MR-CoNS strains, recent studies (10, 17) showed that multiple *ccr*-carrying strains were not infrequent; nevertheless, their prevalence was not evaluated, and to the best of our knowledge it was assessed here for the first time.

The presence of multiple *ccr* genes in some MR-CoNS strains suggested the presence of non-*mec* SCC elements in addition to SCC*mec*. Composite SCC already have been described; for instance, type III SCC*mec* was found to carry what is known as an SCC mercury element driven by the *ccrC3* allotype (22). A composite SCC*mec*, type V(T), harboring *ccrAB2* and *ccrC* in community-acquired MR *S. aureus* (5) has been described, as well as a new SCC*mec* also harboring *ccrAB2* and *ccrC* together with structures similar to those found in various known SCC elements (19). Our results suggest that SCC structures are composites, because SCC*mec* results from intra- and interspecies SCC exchanges probably mediated by the expression of *ccr* genes.

Long-range PCR experiments disclosed further information on the SCC*mec* backbone. The three strains tested carried partly structured *SCCmec* elements like SCC*mec* types III and IV. In strain A186-1, *ccrAB3* was located ~16 kb upstream from *mecA*, as was the case for type III SCC*mec* (22), and in strain M327-2, *ccrAB2* was located ~5 kb upstream from *mecA*, as was the case for all type IV SCC*mec* sequenced to date. Similarities between SCC elements found in *S. epidermidis* and *S. aureus* had been observed already (15, 49) and support the hypothesis of SCC transfers between these species.

Furthermore, we found that *ccrAB2* complexes were more prevalent in *S. epidermidis*, and *ccrC* complexes were more prevalent in *S. haemolyticus*. Compared to *ccr* complexes, *mec* complexes were much less diverse, but we observed some species specificity, for instance, class C *mec* complexes (C1 and C2) predominated in *S. haemolyticus*, whereas class B *mec* complexes predominated in *S. epidermidis*. Class B *mec-ccrAB2* (i.e., type IV SCC*mec*) complexes were found preferentially in *S. epidermidis*, and class C2 *mec-ccrC* (i.e., type V SCC*mec*) complexes were found in *S. haemolyticus*. Thus, *S. epidermidis* and *S. haemolyticus* appeared to be major reservoirs of type IV and V SCC*mec*, respectively, whatever the country.

We found a high prevalence of new combinations of previously known *mec* complexes and *ccr* allotypes that formed SCC*mec* structures that were not typeable by the current classification scheme (22). The structural diversity of SCC*mec* also was recently described by others (12, 17). Since SCC*mec* was first described (26) and its classification introduced (22), as many as 10 *mec* complexes (19, 22, 25, 28, 43, 44) and six *ccr* allotypes (9, 10, 22, 24, 38) have been reported from staphylococci. However, our observation in the present study, that one *mec* complex was combined with one, two, or three different *ccr* allotypes, shows that *mec* and *ccr* combinations are much more diverse than was previously thought. In the future, descriptions of new types of SCC*mec*, in both *S. aureus* and CoNS, should include both quantitative data (e.g., the number of *ccr* copies) and qualitative data on their *mec* and *ccr* content. Our results underscore the importance, and also the complexity, of CoNS as a reservoir for MR genes.

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