Diversity of SCCmec Elements in Methicillin-Resistant Coagulase-Negative Staphylococci Clinical Isolates

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Abstract

Background: Methicillin-resistant coagulase-negative staphylococci (MR-CoNS) are opportunistic pathogens and serve as a large reservoir of staphylococcal cassette chromosome mec (SCCmec). Characterization of SCCmec in MR-CoNS can generate useful information on the mobilization and evolution of this element.

Methodology/Principal Findings: Non-repetitive MR-CoNS clinical isolates (n = 84; 39 S. epidermidis, 19 S. haemolyticus, 9 S. hominis, 6 S. capitis, 4 S. warneri, 2 S. cohnii, 2 S. saprophyticus, 1 S. kloosii, 1 S. simulans and 1 S. massiliensis) were collected. All isolates could grow on plates with 4 mg/L cefoxitin and all had mecA as detected by PCR. Strain typing using RAPD and ERIC-PCR revealed that almost all isolates were of different strains. SCCmec typing was performed using multiplex PCR published previously. For isolates in which SCCmec could not be typed, the mec complex classes were determined by additional PCR and the ccr genes were amplified with published or newly-designed primers and then sequenced. SCCmec types were assigned for 63 isolates by multiplex PCR and were assigned for 14 other isolates by PCR targeting mec and ccr. Among 77 isolates with determined SCCmec types, 54 had a single type, including type III (n = 19), IV (n = 14), V (n = 10), II (n = 2), I (n = 1), VIII (n = 1) and five unnamed types (n = 7), while 23 isolates had two types, III+V (n = 12), II+V (n = 8), II+IV (n = 2) or IV+V (n = 1). The five unnamed types were assigned UT1 (class A mecA/ccrA1/ccrB1), UT2 (class C1 mecA/ccrA4/ccrB4), UT3 (class A mecA, ccrA5/ccrB3), UT4 (class C2 mec, ccrA2/ccrB2 plus ccrC1) and UT5 (class A mecA, ccrA1/ccrB1 plus ccrC1).

Conclusions/Significance: SCCmec types III, IV and V were prevalent in MR-CoNS and many isolates could harbor more than one type. Several new types of SCCmec were identified, highlighting the great genetic diversity and the need of developing classification schemes for SCCmec in MR-CoNS.

Introduction

Coagulase-negative staphylococci (CoNS) comprise a variety of Staphylococcus species and are opportunistic pathogens commonly associated with infections in patients with indwelling devices or being immunocompromised [1]. CoNS are usually resistant to methicillin [2] and in staphylococci, methicillin resistance is mainly due to the expression of the mecA gene, which specifies penicillin binding protein 2a (PBP2a), a transpeptidase with a low affinity for β-lactams [3,4]. mecA is carried by a mobile genetic element (MGE) termed the staphylococcal cassette chromosome mec (SCCmec) [5]. Generally, SCCmec contains two essential components, i.e. the mec gene complex and the ccr gene complex. The mec gene complex consists of mecA, the regulatory genes and associated insertion sequences and has been classified into six different classes, i.e. A, B, C1, C2, D and E (Table 1). Cassette chromosome recombinase (ccr) genes (ccrC or the pair of ccrA and ccrB) encode recombinases mediating integration and excision of SCCmec into and from the chromosome [6,7]. The ccr gene(s) and surrounding genes form the ccr gene complex. In addition to ccr and mec gene complexes, SCCmec contains a few other genes and various other MGE, e.g. insertion sequences, transposons and plasmids [7].

Eleven types (I to XI) of SCCmec have been assigned for Staphylococcus aureus based on the classes of the mec gene complex and the ccr gene types (www.sccmec.org/Pages/SCC_TypesEN.html) (Table 1). As methicillin resistance is prevalent in CoNS, methicillin-resistant CoNS (MR-CoNS) may serve as a large reservoir of SCCmec available for S. aureus to form methicillin-resistant S. aureus (MRSA) [6]. According to the available data [8,9,10,11,12,13,14,15,16,17,18,19], SCCmec elements are more diverse in MR-CoNS, with new variants of ccr genes continuing to be identified [11,18,19,20]. In addition, many SCCmec elements in MR-CoNS could not be typed using currently-available schemes based on multiplex PCR [6,19]. To obtain the information on SCCmec in local MR-CoNS, 84 clinical isolates were investigated.

Methods

Bacterial isolates

Non-repetitive MR-CoNS isolates were collected from clinical specimens in West China Hospital, Chengdu, western China, from
Table 1. SCCmec types.

| SCCmec type | mec class | ccr type | Species (locations and references) |
|-------------|-----------|----------|----------------------------------|
| I           | B         | A1B1     |                                  |
| II          | A         | A2B2     |                                  |
| III         | A         | A3B3     |                                  |
| IV          | B         | A2B2     |                                  |
| V           | C2        | C1       |                                  |
| VI          | B         | A4B4     |                                  |
| VII         | C1        | C1       |                                  |
| VIII        | A         | A4B4     |                                  |
| IX          | C2        | C1A1B1   |                                  |
| X           | C1        | A1B6     |                                  |
| XI          | E         | A1B3     |                                  |
| UT1         | A         | A1B4     | S. saprophyticus (China, this study) |
| UT2         | C1        | A4B4     | S. haemolyticus (China, this study) |
| UT3         | A         | A5B3     | S. hominis (China, this study) |
| UT4         | C2        | A2B2&C1  | S. epidermidis (China, this study; Algeria, Cambodia and Mali [8]) |
| UT5         | A         | A1B1&C1  | S. cohnii (Algeria [8]), S. hominis (China, this study) |
| UT5v        | A         | A1B1     | S. capitis (Finland [27]), S. hominis (Finland [27], Norway [28]) |
| UT6         | A         | A1       | S. epidermidis (Finland [27]; Mali [8]) |
| UT7         | B         | C1       | S. epidermidis (Algeria [8]), S. hominis (Mali [8]) |
| UT8         | C2        | A4B4&C1  | S. epidermidis (Algeria [8]) |
| UT9         | C1        | A5B3     | S. haemolyticus (China [11]) |
| UT10        | C1        | A2B2&C1  | S. haemolyticus (Norway [28]) |

1SCCmec types I to XI have been assigned for S. aureus according to http://www.sccmec.org/Pages/SCC_TypesEN.html. UT1 to UT10 types are assigned in this study. The types seen in this study are bolded. UT5v represents a variant of UT5.

2Class of mec: A, IS431-mecA-mecI-mecR; B, IS431-mecA-mecI-mecR (two IS431s in the same direction); C, IS431-mecA-mecI-mecR; E, IS431-mecA-mecI-mecR (two IS431s in the opposite direction); D, IS431-mecA-mecR1.

3ccr type: A, ccrA; B, ccrB; C1, ccrC1.

4originally designated ccrAtype and ccrBtype.

doi:10.1371/journal.pone.0020191.t001

March to May 2010. Species identification and antimicrobial susceptibility were determined using the MicroScan Walkaway 96 SI (Siemens Healthcare Diagnostic, Deerfield, IL) or Vitek II (bioMérieux, Durham, NC) automated microbiology system. These isolates could also grow on brain heart infusion plates (Oxoid, Hampshire, UK) containing 4 mg/L cefoxitin (Sigma, St Louis, MO). Species identification of 20 randomly-selected isolates was confirmed by partially sequencing 16S rRNA genes amplified with the universal primers 27F and 1492R [21].

**Strain typing**

MR-CoNS were typed using two rapid PCR-based methods, i.e., random amplification of polymorphic DNA (RAPD) with the 10-mer primer (5’-AGCGTCACTG-3’) and the Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR with the primer pair of ERIC 1R and ERIC 2 as described previously [22,23]. Isolates were considered of the same strain if they had identical RAPD patterns and identical ERIC-PCR profiles; otherwise, they would be assigned to different strains.

**SCCmec typing**

Genomic DNA of MR-CoNS isolates were prepared using a commercial kit (Tiangen, Beijing, China) and then used as template for PCR. Maxima Hot Start Taq (Fermentas, Burlington, ON, Canada) and ExTaQ premix (Takara, Dalian, China) were used for multiplex and singlex PCR, respectively. SCCmec typing was performed using the multiplex PCR scheme that was published previously [24]. For isolates in which SCCmec could not be typed by the multiplex PCR, classes of the mec complex and the ccr genes (ccrA1, ccrB, ccrC1 and ccrC2) were examined by additional PCR as described previously [24]. For those that primers targeting ccrA1, ccrB, ccrC1 and ccrC2 genes and amplicons were then sequenced. ccr genes were assigned based on >85% nucleotide identity with known allotypes [7]. For those that did not yield amplicons from PCR amplifying class A or B mec complex, the presence of the insertion sequence IS431 upstream and downstream of mecA was examined by PCR using a primer (IS431-F2 or IS431-R1, Table 2) located in either direction of IS431 paired with a primer in mecA. SCCmec types were assigned based on the mec complex classes and the ccr gene set according to the criteria set for S. aureus [7].

**Sequencing**

Amplicons were purified using a commercial kit (Omega, Norcross, GA) and then sequenced using an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster, CA) at the Beijing
variants have been deposited at GenBank under accession (DNASTAR Inc, Madison, WI) and similarity searches were using the SeqMan II program in the Lasergene package.

### Results

**MR-CoNS isolates were diverse in clonality**

A total of 84 MR-CoNS were collected from clinical specimens, including 39 *Staphylococcus epidermidis*, 19 *Staphylococcus haemolyticus*, 9 *Staphylococcus hominis*, 6 *Staphylococcus capitis*, 4 *Staphylococcus warneri*, 2 *Staphylococcus cohnii*, 2 *Staphylococcus saprophyticus*, 1 *Staphylococcus kloosii*, 1 *Staphylococcus simulans* and 1 *Staphylococcus massiliensis* (Table 3). Of note, *S. massiliensis* is a newly-recognized species [25]. To our knowledge, as *S. massiliensis* has only been reported once before, this study is therefore probably the second ever report of this species.

These 84 isolates had a quite diverse clonal background. For species other than *S. epidermidis* and *S. haemolyticus*, none of isolates had identical RAPD and ERIC profiles (data not shown) and these isolates were therefore of different strains. *S. epidermidis* isolates (n = 39) could be assigned to 34 strains with five isolates from two wards (3 from orthopedics and 2 from neurology) belonging to a single strain in light of their identical RAPD and ERIC profiles. A total of 17 strains could be identified for the 19 *S. haemolyticus* isolates. Two *S. haemolyticus* isolates from different wards (neuro-surgery and endocrinology) belonged to a single strain and two other isolates (from orthopedics and neurology) were also of a single strain. Although nosocomial dissemination of individual *S. epidermidis* and *S. haemolyticus* strains was identified, the vast majority of MR-CoNS isolates from different patients were of different strains and no strain could be identified as the predominant clone circulating locally. This suggests that most isolates might not have been acquired in the hospital.

**SCCmec types were determined by multiplex PCR in most MR-CoNS isolates**

All of the 84 MR-CoNS isolates had mecA as detected by PCR [24]. SCCmec types were assigned for 63 of 84 (75%) isolates by multiplex PCR. Among these 63 isolates, 40 had a single SCCmec type including type I (n = 1), II (n = 1), III (n = 15), IV (n = 13; IVa subtype, n = 12, and IVb subtype, n = 1) and V (n = 10), while 23

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**Table 2. Selected primers used for PCR.**

| Primer   | Sequence (5’-3’)1 | Target/loc | Reference |
|----------|------------------|------------|-----------|
| ccrA-UF1 | AATGTAAGTATTATGTGTYA | ccrA | [34] |
| ccrA-UR1 | GGTCATTCTTTTAAATGAT | ccrA | This study |
| ccrA-UF2 | AYTHACATGAYAATTTGAAAA | ccrA | This study |
| ccrA-UR2 | ACGDCACARTGTTAGGRRTT | ccrA | [11] |
| ccrA_up  | TGCACTTGATTGTGAGGAC | ccrA | [11] |
| ccrA_dw  | CAATTGACGTATTGTGTT | IS431 | This study |
| ccrB-UF1 | CGGATCAACGDAAACTCAAA | ccrB | [34] |
| ccrB-UR1 | CTTTACATTITGGAYWATTTC | ccrB | [11] |
| ccrB_up  | GTCTCTTACATGGAATCT | ccrB | [11] |
| ccrC-UF1 | GCAGGAAAGTCTTATTACA | ccrC1 | This study |
| ccrC-UR1 | TTATCATRATAATAAATCA | ccrC2 | [34] |
| 28-24    | GGAAACACAGGGCTGTGGA | ccrC2 | [34] |
| 28-26    | AGCTTTCACACGGCAGATTTC | ccrC2 | [34] |
| IS431-F2 | GGGCAGGGCTGTGGAATCT | IS431 | This study |
| IS431-R1 | CGCAGGATACGGCAGATTTC | IS431 | This study |
| mecA-R2  | TCGAAGGCTACTATCAAGG | mecA | This study |

1: A, G, or T; H: A, C or T; D: A, G or T; M: A or C; R: A or G; W: A or T; Y: C or T; V: A, C or G. doi:10.1371/journal.pone.0020191.t002

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**Table 3. SCCmec typing results.**

| Species          | No. | Origins (no.)1 | SCCmec type2 (no.) |
|------------------|-----|----------------|--------------------|
| *S. epidermidis* | 39  | Wound secretion (25), blood (8), ascites (2), CSF (2), drainage (1), urine (1) | I (1), II (1), III (8), IV (11), V (5), II+V (1), III+V (1), III+V (8), IV+V (1), UT4 (1), NT (1) |
| *S. haemolyticus* | 19  | Wound secretion (7), blood (2), prostatic fluid (4), ear secretion (1), CSF (1), urine (2), urethral meatus secretion (1), sputum (1) | II (1), III (4), V (3), II+V (6), III+V (1), UT2 (2), NT (2) |
| *S. hominis*     | 9   | Blood (6), wound secretion (2), CSF (1) | III (3), V (1), VIII (1), II+V (1), UT3 (1), UT5 (1), NT (1) |
| *S. capitis*     | 6   | Wound secretion (2), blood (2), prostatic fluid (1), urine (1) | III (1), IV (1), III+IV (1), III+V (3) |
| *S. warneri*     | 4   | CSF (3), wound secretion (1) | IV (2), III (1), NT (1) |
| *S. cohnii*      | 2   | Blood (1), wound secretion (1) | III (2) |
| *S. saprophyticus* | 2   | Blood (2) | UT1 (1), NT (1) |
| *S. kloosii*     | 1   | Wound secretion (1) | NT (1) |
| *S. simulans*    | 1   | Sputum (1) | UT2 (1) |
| *S. massiliensis* | 1   | Wound secretion (1) | V (1) |
| Total            | 84  | Wound secretion (40), blood (21), CSF (7), urine (4), prostatic fluid (5), ear secretion (1), ascites (2), sputum (2), drainage (1), urethral meatus secretion (1) | I (1), II (2), III (19), IV (14), V (10), VIII (1), II+V (8), III+V (2), III+V (12), IV+V (1), UT1 (1), UT2 (3), UT3 (1), UT4 (1), UT5 (1), NT (7) |

1:CSF, cerebral spinal fluid;
2:SCCmec types (the ccr type and class of the mec complex) is available in Table 1.
3:The isolate was positive to PCR specific for two IV subtypes, IVa and IVb, in addition to V. doi:10.1371/journal.pone.0020191.t003

Genomics Institute (Beijing, China). Sequences were assembled using the SeqMan II program in the Lasergene package (DNASTAR Inc, Madison, WI) and similarity searches were carried out using BLAST programs (http://www.ncbi.nlm.nih.gov/BLAST/).

**Nucleotide accession numbers.** The sequences of new *ect* variants have been deposited at GenBank under accession numbers HQ848693 to HQ848707.
had two types including II+V (n = 8), III+IV (n = 2), III+V (n = 12) and IV+V (n = 1), containing two IV subtypes, IVa and IVb).

Five unnamed types were identified
The remaining 21 isolates in which SCCmec types could not be determined by multiplex PCR, additional PCR amplifying the mec complexes and ccr genes and sequencing were used to determine SCCmec types. SCCmec types could be assigned for 14 of the 21 MR-CoNS (Table 4), including Type II for 1 isolate (S. epidermidis), Type III for 4 (2 S. haemolyticus, 1 S. hominis and 1 S. cohnii), Type IV for 1 (S. epidermidis), Type VIII for 1 (S. hominis) and five unnamed types for 7. Of note, Type VIII SCCmec was originally found in MRSA [26] and has rarely been reported in MR-CoNS except UT1 to UT5 seen in this study. Several unnamed types of SCCmec have been reported by other investigators [8,11,27,28], designated UT6, UT7, UT8, UT9 and UT10 here (Table 1).

ccr genes could not be obtained from the remaining 7 isolates despite repeated attempts and SCCmec types in these isolates were therefore undetermined. Among the 7 isolates, 4 isolates (3 S. haemolyticus and 1 S. kloosii) had class C1 mec, 1 (S. epidermidis) had class C2 mec and 1 (S. hominis) had class A mec but the class of mec could not be determined for the remaining 1 (S. warneri).

A diversity of ccr variants were present
As mentioned above, among the 21 isolates in which SCCmec types could not be determined by multiplex PCR, ccr genes were obtained from 14 isolates and then sequenced (Table 4). Variants of 10 ccr allotypes, ccrA1, ccrA2, ccrA3, ccrA4, ccrA5, ccrB1, ccrB2, ccrB3, ccrB4 and ccrC1, were identified. The combinations of these ccr allotypes laid a foundation of nine different SCCmec types seen here (Table 4). In particular, the combination of ccrA1/ccrB4 had not been seen before.

### Table 4. Closest matches of ccrA and ccrB genes in the 14 isolates in which SCCmec types were determined by PCR amplifying the mec complexes and ccr genes.

| Isolate | Species       | SCCmec type | ccr variant | Closest match in S. aureus mec (identity %); strain | Closest match in CoNS ccr (identity %); species strain |
|---------|---------------|-------------|-------------|----------------------------------------------------|----------------------------------------------------|
| WCG53   | S. epidermidis | II          | ccrA2       | ccrA2 (95.5); M06/0078                              | ccrA2 (95.5); S. epidermidis RP62A                  |
| WCF34   | S. haemolyticus| III         | ccrA3       | ccrB2 (97.0); JCSC1968                              | ccrB2 (97.5); S. epidermidis CS8                   |
| WCG74   | S. hominis    | III         | ccrA3       | ccrA3 (100); JK6D008                                | ccrA3 (100); S. pseudointermius KM1381             |
| WCH47   | S. hominis    | III         | ccrA3       | ccrB3 (99.9); JK6D008                                | ccrB3 (99.9); S. pseudointermius KM1381             |
| WCH62   | S. cohnii     | III         | ccrA3       | ccrB3 (98.2); JK6D008                                | ccrB3 (98.2); S. cohnii WC26                       |
| WCH62   | S. cohnii     | III         | ccrA3       | ccrB3 (100); TW20                                   | ccrB3 (99.9); S. pseudointermius KM1381             |
| WCG25   | S. hominis    | VIII        | ccrA4       | ccrB2 (99.7); JCSC6668                              | ccrB2 (99.7); S. epidermidis RP62A                  |
| WCH02   | S. saprophyticus| UT1        | ccrA1       | ccrA1 (99.5); COL                                   | ccrA1 (99.7); S. hominis GIFU12263                  |
| WCF77   | S. haemolyticus| UT2        | ccrA4       | ccrB4 (94.1); CHE482                                | ccrB4 (90.5); S. epidermidis ATCC 12228            |
| WCG38   | S. haemolyticus| UT2        | ccrA4       | ccrB4 (94.0); CHE482                                | ccrB4 (94.0); S. pseudointermius KM1381             |
| WCH55   | S. simulans   | UT2         | ccrA4       | ccrB4 (93.8); BK2078                                | ccrB4 (93.8); S. epidermidis ATCC 12228            |
| WCG08   | S. haemolyticus| UT3        | ccrA5       | ccrA5 (79.2); COL                                   | ccrA5 (85.3); S. pseudointermius KM241             |
| WCF80   | S. epidermidis | UT4         | ccrA2       | ccrA2 (98.6); N315                                  | ccrA2 (98.1); S. epidermidis RP62A                  |
| WCI20   | S. hominis    | UT5         | ccrA1       | ccrB2 (98.6); JCSC1968                              | ccrB2 (99.5); S. epidermidis ATCC 12228            |

doi:10.1371/journal.pone.0020191.t004
Discussion

Genetic diversity of SCCmec in MR-CoNS imposes a great challenge for SCCmec typing

In total, SCCmec types were determined for most isolates (77/84) using multiplex PCR and single PCR targeting ccr and the mec gene complex. SCCmec in MR-CoNS exhibited substantial genetic diversity with new types continuously being identified. Consistent with previous reports [8,11,14,27,29,30], type I and VIII SCCmec were rare and type VI, VII, IX, X and XI have not been identified in MR-CoNS yet, while type II, III, IV and V were relatively common. In this study, type III was the most common type being present in 33 of 84 MR-CoNS isolates either alone or combined with other types, followed by type V (31/84) and then by IV (17/84).

The distribution of different types of SCCmec in MR-CoNS varied depending on the host species and possibly on the geographical locations. It has been proposed that type IV has been preferentially associated with S. epidermidis [8,29,31] and type V dominates in S. haemolyticus [8]. Indeed, most (13 of 17) type IV SCCmec were seen in S. epidermidis here. Type III SCCmec was in particular widely distributed and has been found in S. aureus and a variety of CoNS species, although the mechanism responsible for this wide distribution has not been well understood. In this study, type III was the dominant type of SCCmec in S. epidermidis (17/39), S. capitis (5/6) and S. cohnii (2/2), while in S. haemolyticus (n = 19) the most common type was the combination of type II and V (6/19).

A substantial proportion of MR-CoNS isolates (n = 23) were determined to have two SCCmec elements by multiplex PCR. This is no surprise as the co-existence of two SCCmec elements appears to be common in MR-CoNS [29]. It is likely that the two SCCmec elements actually constitute a composite rather than two independent units. One of the limitations of the multiplex and single PCR-based schemes is the inability to differ composites from separate elements. Consistent with previous reports [15,28,29,32], SCCmec types in a few MR-CoNS isolates could not be assigned by currently-available PCR-based methods. In most cases of “non-typeable” SCCmec, ccr genes could not be amplified although the class of the mec gene complex had been determined. ccr genes might be unrecognized types, be deleted or contain mutations in the primer-targeting regions in these “non-typeable” SCCmec [6]. The frequent identification of co-existed SCCmec and the presence of “non-typeable” elements represent great challenges for SCCmec typing in MR-CoNS.

A classification scheme for SCCmec typing in MR-CoNS is required

The presence of a few unnamed types identified previously and in this study suggests that more studies on MR-CoNS are required to reveal the reservoir of SCCmec and also highlights the need of establishing classification schemes for SCCmec in MR-CoNS. An obvious option is to extend the current MRSA-focused SCCmec classification scheme to those in MR-CoNS and therefore transforms it into a scheme for SCCmec not based on the species of host isolates. Alternatively, a new classification scheme specific for SCCmec in MR-CoNS should be developed based on the MRSA-focused scheme.

Dynamics of SCCmec within CoNS and possible horizontal transfer of SCCmec between CoNS and S. aureus

None of S. haemolyticus isolates belonging to the same strain carried the same type of SCCmec elements, while the five S. epidermidis isolates of the same strain carried type III (n = 1), V (n = 2), III+IV (n = 1) or III+V (n = 1) SCCmec. Isolates carrying different SCCmec elements were of the same strain and isolates carrying the same type of SCCmec elements belonged to different strains. The discrepancy between strain background and the SCCmec type carried might suggest frequent gain and loss of SCCmec elements by CoNS.

Generally, ccrA and ccrB genes identified in local MR-CoNS were not always closest to the counterparts in MR-CoNS isolated elsewhere but could be closer to those in MRSA (Table 4). This may illustrate that SCCmec in MR-CoNS remains under-characterized but also suggests possible horizontal transfers of SCCmec between MR-CoNS and MRSA.

Interestingly, ccrA4 and ccrB4 genes in S. hominis strain WCG25 were identical to the ccrA4-2 and ccrB4-2 in MRSA strain CHE482, which circulated among intravenous drug users in Zurich, Switzerland [33]. ccrA4-2 had 86.4% and 83.2% nucleotide identity to ccrA4 genes in MRSA strains HDE288 and C10682, respectively, while ccrB4-2 had 93.2% and 91.8% identity to ccrB4 in HDE288 and C10682, respectively. HDE288 and C10682 were the representative strains of Type VI and Type VIII SCCmec, respectively. In light of the relatively low identity with ccrA/B4 in other MRSA strains and their absence in MRSA outside Switzerland, ccrA/B4-2 might have an origin outside S. aureus. The presence of ccrA/B4-2 in S. hominis found here suggests a possible origin of the two genes. Of note, S. saprophyticus strain WCH02 had also ccrB4-2 but had ccrA1 instead of ccrA4-2. This suggests that homologous recombination between different types of SCCmec might have occurred and then resulted in a novel combination of ccrA1 and ccrB genes.

Many of ccrA/B genes identified here were new variants with nucleotide differences from all known ones available in GenBank, although all of which could be assigned to known allotypes based on the cut-off value, 85% identity. Among the variants identified, a ccrA5 gene with relatively low identity (≤85.3%) to known ccrA5 variants was present in S. hominis strain WCG08. ccrA5 was mainly seen in CoNS but was also recently found in S. aureus strain Msida_536245_Z661 (GU066221). The ccrA5 genes in WCG08, S. pseudintermedius strain KM241 (AM904731), S. haemolyticus H9 (EU934095) and S. cohnii WC28 (GU370073) displayed less than 90% identity between each other, suggesting that the diversity of ccrA5 genes in CoNS might be species-specific.

In summary, for most MR-CoNS (63/84; 75%) SCCmec types were assigned by the multiplex PCR [24], which was originally developed for SCCmec typing in MRSA, suggesting that this scheme could be a suitable starter method for SCCmec typing for MR-CoNS. For two thirds (n = 14) of the remaining 21 isolates, SCCmec types were assigned by PCR targeting the mec and ccr complexes. According to our experience, multiple pairs of primers for ccr genes should be used to maximize the possibility of obtaining ccr amplicons. The common types of SCCmec in MR-CoNS were II, III, IV and V, either alone or in various combinations and Type III was in particular common and widely distributed in a variety of species. Only SCCmec types seen in MRSA have been assigned a number (I to XI), which left many SCCmec types in MR-CoNS unnamed. Five of such unnamed types were encountered locally and were tentatively assigned UT1 (class A mec, ccrA1/ccrB4), UT2 (class C1 mec, ccrA4/ccrB8), UT3 (class A mec, ccrA5/ccrB3), UT4 (class C2 mec, ccrA2/ccrB2 plus ccrG) and UT5 (class A mec, ccrA1/ccrB1 plus ccrG). UT1 and UT2 were new types, while UT3, UT4 and UT5 have been seen in MR-CoNS before. The growing number of unnamed types reported highlights the need of developing classification system for SCCmec types in MR-CoNS, either incorporating
them in the current system for those in MRSA or establishing a new system.

Acknowledgments

We thank Yanyu Gao and Rujia Yu for their technical assistance.

Author Contributions

Conceived and designed the experiments: ZZ XL. Performed the experiments: ZZ. Analyzed the data: ZZ CP. Contributed reagents/materials/analysis tools: ZZ CP. Wrote the paper: ZZ.

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