ORIGINAL ARTICLE

COMPARATIVE PREVALENCE OF ANTIBIOTIC RESISTANCE, STAPHYLOCOCCAL CASSETTE CHROMOSOME MEC AND SUPERANTIGENIC TOXIN GENES IN COAGULASE-NEGATIVE STAPHYLOCOCCI

Yukiko Abe1), Dong-Liang Hu2), Katsuhiko Omoe3), Ikuo Fukuda4) and Akio Nakane2)

Abstract Coagulase-negative staphylococci (CNS) are increasingly recognized to cause clinically significant infections. To investigate relationship of antibiotic resistance profiles, staphylococcal cassette chromosome mec (SCCmec) and superantigenic toxin genes in CNS, a total of 79 clinical CNS isolates from patients with surgical operations were comprehensively determined for minimum inhibitory concentration (MIC) of antibiotics, and searched for SCCmec and staphylococcal superantigenic toxin genes by novel multiplex PCR. Among 79 CNS isolates, at least one SCCmec gene was detected in 55 isolates (69.6%). The most of SCCmec type was type III (36.7%), and other detected types were type IVa (20.3%), type II (19.0%), type V (16.5%), type I (15.2%), and type IVb (3.8%). The isolates with type I or IVa gene showed highly resistance to clindamycin and gentamicin, the isolates with type II showed more resistance to teicoplanin, the isolates with type III or type V showed more resistance to clindamycin. se and stt-1 genes were detected in none of the CNS isolates tested. These results suggested that there is high prevalence of SCCmec in clinical CNS isolates, and the SCCmec types are related to the resistances against specific antibiotics, but SCCmec do not related to superantigenic toxin genes in the CNS.

Key words: staphylococci; antibiotic resistance; staphylococcal cassette chromosome mec; multiplex PCR; superantigen.

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Received for publication, January 17, 2012
Accepted for publication, February 15, 2012
Introduction

Coagulase-negative staphylococci (CNS) are one of members of the normal microbial flora of the skin and mucous membranes of humans, and an important cause of nosocomial infections, particularly foreign device-related infections and infections among immunocompromised patients. CNS represent a serious concern in hospital-acquired infections including endocarditis and urinary tract diseases\(^1\). Methicillin resistance in staphylococci is caused by the expression of penicillin-binding protein PBP2a (PBP2\(^{\prime}\)), which is encoded by the \textit{mecA} gene located on a mobile genetic element called the staphylococcal cassette chromosome \textit{mec} (SCC\textit{mec})\(^2\). Several different \textit{SCCmec} types which are different in size (21 to 67 kbp) have been recognized, and characterized by a different set of \textit{cer} recombinase genes and \textit{mec} gene complex\(^3-5\). Some of the types were further classified into subtypes according to differences in their J region DNA\(^4,6\).

\textit{SCCmec} elements have been detected in CNS, which are isolated from patients, healthy humans, animals such as cattle and sheep, environment such as marine water and beach sand\(^7-10\). In addition to harboring \textit{SCCmec} which is involved in methicillin-resistance, a considerable number of CNS isolates were resistant to non-\(\beta\)-lactam antibiotics, such as kanamycin, tobramycin, gentamicin and erythromycin\(^11\). The \textit{SCCmec} has been identified exclusively among staphylococci, but its origin remains unknown. It has been suggested that \textit{mecA} of methicillin-resistant \textit{Staphylococcus aureus} (MRSA) originate from \textit{mecA} homologue in \textit{S. sciuri}\(^12\). The \textit{SCCmec} has been identified as having occurred in vivo\(^13\).

Staphylococci, especially MRSA, produce one or more specific staphylococcal superantigenic toxins including staphylococcal enterotoxins (SEs), enterotoxin-like superantigen and toxic shock syndrome toxin-1 (TSST-1)\(^14\). To date, 21 SEs have been identified based on sequence homologies\(^15,16\). Superantigenic toxins bypass normal antigen presentation and have strong T cell mitogenic activity by direct binding to the V\(\beta\) region of specific T cells and major histocompatibility complex class II molecules of antigen presenting cells. This leads to a massive release of proinflammatory cytokines such as tumor necrosis factor (TNF-\(\alpha\)), interleukin (IL)-2, IL-6 and interferon-\(\gamma\) (IFN-\(\gamma\)), which are responsible for physiopathology of toxic shock syndrome and contributes to the severity of \textit{S. aureus} sepsis\(^17\). Little information is available about the superantigen genes among populations of CNS isolates. It is still unclear whether CNS carry superantigenic toxin genes and how is prevalence of the superantigenic toxin genes in CNS isolates from clinical patients.

In the present study, in order to reveal prevalence and relationships among antibiotic resistance, \textit{SCCmec} and superantigenic toxin genes of CNS, we investigated minimum inhibitory concentrations (MIC) of antibiotics, \textit{SCCmec} types and superantigenic genes, and correlation of them in clinical CNS isolates collected from different patients.

Materials and Methods

Bacteria isolates and culture conditions

A total of 79 CNS clinical isolates tested in this study were collected from patients of the Department of Cardiovascular Surgery, Hirosaki University Hospital, Hirosaki, Aomori, Japan. The isolates were stocked in trypticase soy broth with 15% glycerol at \(-80^\circ\)C until use. The \textit{SCCmec} typing standard MRSA control strains, including \textit{SCCmec} type I (NCTC10442), type II (N315), type III (85/2082), type IVa (CA05), type IVb (8/6-3P), type IVc (MR108), type IVd (JCSC4469), and type V (WIS [WBG8318] -JCSC3624) (Table 1), were kindly provided by
Prevalence of Antibiotic Resistance and SCCmec in CNS

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Eleven reference strains for se and tst gene typing (N315; DDB/GenBand/EMBL BA000018, Mu50; BA000017, MW2; BA000033) were used in this study.

Antibiotic susceptibility testing

The minimum amount of antibiotic that inhibited the visible growth of an isolate or MIC was determined. A serial two-fold dilution of the antibiotics was prepared in Mueller-Hinton agar. A volume of 2 μl of bacterial isolate was inoculated on the surface of agar containing various dilutions of antibiotics and incubated at 37°C overnight. The lowest concentration of antibiotic that inhibited visible growth on surface of agar was taken as MIC. Ten antibiotic agents, that is, arbekacin sulfate (ABK), cefazolin sodium (CEZ), ciprofloxacin (CPFX), clindamycin phosphate (CLDM), gentamicin sulfate (GM), imipenem hydrate sodium (IPM), linezolid (LZD), teicoplanin (TEIC), sultamicillin tosilate (SBTPC), and vancomycin hydrochloride (VCM) were used for analyzing the susceptibility of CNS isolates.

DNA preparation

Template DNA for multiplex PCR was prepared as previously described. Each CNS isolate was cultured on tryptic soy agar for 24 h and then one or two bacterial colonies were suspended in 50 μl of sterile distilled water. The suspension was heated at 100°C for 10 min, and centrifuged at 20,000 g for 1 min. The supernatant was used as template DNA in multiplex PCR for detection of SCCmec and superantigenic toxin genes, or stored at −80°C until use.

Multiplex PCR for typing of SCCmec genes

SCCmec type- and subtype-unique and specific primers as well as the specific primers for meca genes (Table 1) were designed based on the comprehensive analyses and alignments

Table 1 Primers used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Oligonucleotide sequence</th>
<th>Product size (bp)</th>
<th>Specificity</th>
<th>Reaction mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I-F</td>
<td>GCTTTAAAGAGTGCTTTACAGG</td>
<td>613</td>
<td>SCCmec I</td>
<td>A</td>
</tr>
<tr>
<td>Type I-R</td>
<td>GTTCTCTCATATGATATGCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type II-F</td>
<td>CGTTGAAAGGATGGAAGCG</td>
<td>398</td>
<td>SCCmec II</td>
<td>A</td>
</tr>
<tr>
<td>Type II-R</td>
<td>CAAATTCTTAATTGACGACC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type III-F</td>
<td>CCAATTGTGTTGATGACGACC</td>
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<td>SCCmec III</td>
<td>A</td>
</tr>
<tr>
<td>Type III-R</td>
<td>CCAATTGTGTTGACGACC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type IVa-F</td>
<td>GCCTTATTGAGAAAGCG</td>
<td>776</td>
<td>SCCmec IVa</td>
<td>B</td>
</tr>
<tr>
<td>Type IVa-R</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type IVb-F</td>
<td>TCTGGAATTACTTCAGCAGC</td>
<td>493</td>
<td>SCCmec IVb</td>
<td>B</td>
</tr>
<tr>
<td>Type IVb-R</td>
<td>AAACATATTTGCTCTCCCTC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type IVc-F</td>
<td>AACAATTTTTGATTATCGAGACC</td>
<td>200</td>
<td>SCCmec IVc</td>
<td>B</td>
</tr>
<tr>
<td>Type IVc-R</td>
<td>AACAATTTTTGATTATCGAGACC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type IVd-F</td>
<td>CTCAAATACGGACCTACCATACA</td>
<td>881</td>
<td>SCCmec IVd</td>
<td>C</td>
</tr>
<tr>
<td>Type IVd-R</td>
<td>CTCAAATACGGACCTACCATACA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type V-F</td>
<td>GAACATTGTCTCTTTAAATGACCG</td>
<td>325</td>
<td>SCCmec V</td>
<td>C</td>
</tr>
<tr>
<td>Type V-R</td>
<td>GAACATTGTCTCTTTAAATGACCG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MecA147-F</td>
<td>GTGAGAATACATTACAGGAT</td>
<td>147</td>
<td>meca</td>
<td>A, B, C</td>
</tr>
<tr>
<td>MecA147-R</td>
<td>ATGCGCTATAGGATGAAAGGAT</td>
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</table>
of S. aureus and MRSA genomes and SCCmec sequences currently available in the GenBank database. The oligonucleotide primers used in this study were synthesized and purchased from Greiner Japan (Greiner bio-one, Tokyo, Japan). SCCmec multiplex PCR typing assay contained 9 pairs of primers including the unique and specific primers for SCCmec types and subtypes I, II, III, IVa, IVb, IVc, IVd, and V, and the primers for meca gene. Multiplex PCR assays were performed directly from bacterial suspensions obtained after the rapid DNA extraction method. An aliquot of 2 µl of the suspension was added to 23 µl of PCR mixture containing 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 2.5 mM MgCl₂, 0.2 mM of each deoxynucleoside triphosphate (GeneAmp dNTP, Applied Biosystems, Tokyo, Japan), various concentrations of the respective primers, and 1.0 unit of AmpliTaq Gold polymerase (Applied Biosystems). The amplification was performed in iCycler (Bio-Rad, Tokyo, Japan) beginning with an initial denaturation step at 94℃ for 5 min followed by 32 cycles of 94℃ for 1 min, 50℃ for 1 min, and 72℃ for 2 min, ending with a final extension step at 72℃ for 10 min and followed by a hold at 4℃. For the single target amplification, PCR was run in 23 µl of PCR mixture but containing 0.2 µM of each primer, with cycling parameters beginning with an initial denaturation step at 94℃ for 5 min followed by 31 cycles of 94℃ for 1 min, 50℃ for 1 min, and 72℃ for 2 min, ending with a final extension step at 72℃ for 10 min. The PCR products were visualized using a transilluminator after electrophoresis in a 2.5% agarose gel and stained by 0.5 µg/ml ethidium bromide.

**Results**

**Antibiotic resistance of CNS isolates from clinical specimen**

Seventy-nine CNS isolates obtained from clinical specimens were studied for the resistance to ten antibiotics (Table 2). Of them, all of the CNS isolates were susceptible to arbekacin (MIC ≤0.25 µg/ml), 75.9% of the isolates were susceptible to imipenem, 67.1% of them were susceptible to ciprofloxacin, 62.0% were susceptible to sultamicillin tosilate and 57.0% were susceptible to gentamicin (MIC ≤ 0.25 µg/ml). In contrast to these antibiotics, the CNS isolates showed high resistance (MIC was ≥4 µg/ml) to clindamicin (68.3%) and teicoplanin (50.7%).
Prevalence of Antibiotic Resistance and SCCmec in CNS isolates

To construct the multiplex PCR for detecting the SCCmec genes in the CNS isolates, uniplex PCR using each primer set with total DNA of reference MRSA strains was firstly performed. The sizes of PCR products obtained by the uniplex PCR were identical to the predicted SCCmec and meca genes from the design of the primers (Figure 1A). The combination of primer sets and reaction conditions for the multiplex PCR was optimized to ensure that all PCR products of target genes were satisfactorily amplified (Figure 1B). We ultimately constructed three optimized multiple primer sets. Reliable amplification of PCR products was observed in all multiplex PCR reactions using the three primer sets. The sizes of PCR products obtained from the positive control corresponded to their predicted sizes. A total

### Table 2 Antibiotic resistance of CNS isolates (%)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MIC (μg/mL)</th>
<th>≤0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>≥32</th>
</tr>
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<tbody>
<tr>
<td>Arbekacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefazolin</td>
<td></td>
<td>100.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>43.0</td>
<td>17.7</td>
<td>17.7</td>
<td>8.9</td>
<td>0</td>
<td>3.8</td>
<td>0</td>
<td>8.9</td>
</tr>
<tr>
<td>Clindamycin</td>
<td></td>
<td>67.1</td>
<td>2.5</td>
<td>2.5</td>
<td>1.3</td>
<td>13.9</td>
<td>7.6</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Gentamicin</td>
<td></td>
<td>0</td>
<td>1.3</td>
<td>8.9</td>
<td>21.5</td>
<td>39.2</td>
<td>11.4</td>
<td>0</td>
<td>17.7</td>
</tr>
<tr>
<td>Imipenem/Cilastatin</td>
<td></td>
<td>50.0</td>
<td>3.8</td>
<td>1.3</td>
<td>2.5</td>
<td>3.8</td>
<td>11.4</td>
<td>11.4</td>
<td>8.9</td>
</tr>
<tr>
<td>Linezolid</td>
<td></td>
<td>0</td>
<td>0</td>
<td>25.3</td>
<td>54.4</td>
<td>11.4</td>
<td>6.3</td>
<td>2.5</td>
<td>0</td>
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<tr>
<td>Teicoplanin</td>
<td></td>
<td>19.0</td>
<td>7.6</td>
<td>12.7</td>
<td>10.1</td>
<td>3.8</td>
<td>17.7</td>
<td>16.5</td>
<td>12.7</td>
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<tr>
<td>Sultamicillin tosilate</td>
<td></td>
<td>62.0</td>
<td>8.9</td>
<td>8.9</td>
<td>3.8</td>
<td>0</td>
<td>1.3</td>
<td>3.8</td>
<td>11.4</td>
</tr>
<tr>
<td>Vancomycin</td>
<td></td>
<td>12.7</td>
<td>32.9</td>
<td>24.1</td>
<td>10.1</td>
<td>15.2</td>
<td>5.1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 1 Multiplex PCR assay to optimize SCCmec types and subtypes I, II, III, IVa, IVb, IVc, IVd, and V, and simultaneously detect methicillin resistance (meca gene). (A) Single PCR. I, Type I, strain NCTC10442; II, Type II, strain N315; III, Type III, strain85/2082; IVa, Type IVa, strain CA05; IVb, Type IVb, strain8/6-3P; IVc, Type IVc, strain MR108; IVd, Type IVd, strain JCSC4469; V, Type V, strain WIS-JCSC3624; M, molecular size markers; NC, negative control. The arrow shows meca. (B) Multiplex PCR. A, primers specific for type I, II, III, and meca; B, primers specific for type IVa, IVb, IVc, and meca; C, primers specific for type IVd, V, and meca; M, molecular size marker. The arrow shows meca.

Prevalence of SCCmec genes in clinical CNS isolates

To construct the multiplex PCR for detecting the SCCmec genes in the CNS isolates, uniplex PCR using each primer set with total DNA of reference MRSA strains was firstly performed. The sizes of PCR products obtained by the uniplex PCR were identical to the predicted SCCmec and meca genes from the design of the primers (Figure 1A). The combination of primer sets and reaction conditions for the multiplex PCR was optimized to ensure that all PCR products of target genes were satisfactorily amplified (Figure 1B). We ultimately constructed three optimized multiple primer sets. Reliable amplification of PCR products was observed in all multiplex PCR reactions using the three primer sets. The sizes of PCR products obtained from the positive control corresponded to their predicted sizes. A total
of 79 CNS isolates from clinical specimens were subjected to *mecA* and SCC*mec* typing analysis. Among these CNS isolates, at least one SCC*mec* gene was detected in 55 isolates (69.6%), and more than one SCC*mec* gene were detected in 24 of the 55 isolates. SCC*mec* type of the isolates were classified into type III (36.7%), type IVa (20.3%), type II (19.0%), type V (16.5%), type I (15.2%) and IVb (3.8%), and the highest proportion of SCC*mec* type (29/79 strains) was type III (Figure 2A). SCC*mec* IVc and IVd were not detected in any of the isolates. Thirty-six (45.6%) of the CNS isolates harbored the *mecA* genes (*mecA*+). We further compared SCC*mec* types between *mecA*+ and *mecA*− isolates (Fig. 2B). For the *mecA*+ isolates, 58.3% of the isolates carried SCC*mec* type III, 44.4% carried SCC*mec* type IVa and 30.6% carried SCC*mec* type V genetic element, and showed considerably higher than those of the *mecA*− isolates (Figure 2B). In contrast, for the *mecA*− isolates, 48.8% of them were not detected any SCC*mec* element tested in this study. SCC*mec* type patterns were considerably different between *mecA*+ isolates and *mecA*− isolates.

**Figure 2** Detection of SCC*mec* genes of CNS isolates collected from clinical specimens. Total DNA of a CNS isolate was purified and 3 primer sets of SCC*mec* genes based on the molecular weight of its PCR product were used to anneal to unique regions and generate amplicons. The number of positive genes in each isolate was recorded and the percentage was calculated with the total isolates. (A) Percentage of SCC*mec* genetic elements in a total of 79 CNS isolates. (B) Percentage of SCC*mec* genetic elements in *mecA*− and *mecA*+ CNS isolates.
Prevalence of Antibiotic Resistance and SCC\textit{mec} in CNS

Association between \textit{meca} gene and antimicrobial resistance of CNS isolates

We further compared antibiotic resistance patterns between \textit{meca}⁻ and \textit{meca}⁺ CNS isolates. Although both of \textit{meca}⁻ and \textit{meca}⁺ CNS isolates were susceptible (MIC ≤0.25 μg/ml) to arbekacin, the \textit{meca}⁻ CNS isolates were more susceptible to cefazolin, ciprofloxacin, gentamicin and imipenem than \textit{meca}⁺ isolates. Specifically, rates of \textit{meca}⁻ isolates for which MIC of cefazolin, ciprofloxacin, gentamicin and imipenem showed ≤0.25 μg/ml were 53.5%, 81.4%, 72.1% and 83.7%, whereas those of \textit{meca}⁺ isolates were 30.6%, 50.0%, 38.9% and 66.7%, respectively. Furthermore, \textit{meca}⁺ CNS isolates showed considerably resistant to clindamycin and gentamicin, specifically the isolates of MIC ≥4 μg/ml were 80.6% and 52.9%, respectively.

Relationship of SCC\textit{mec} types and antibiotic resistance patterns of CNS isolates

To investigate the relationship between SCC\textit{mec} type and antibiotic resistance pattern, SCC\textit{mec} types and MIC of antibiotics of CNS isolates were compared and analyzed (Figure 3). The isolates harboring SCC\textit{mec} type I...
gene showed the high level resistance (the average of MICs >8 μg/ml) to clindamycin and gentamicin. The isolates harboring SCCmec type II were extremely resistant to teicoplanin (the average of MIC >12 μg/ml) compared with those of other antibiotics. For the isolates harboring SCCmec type III, MICs of clindamycin, gentamicin and teicoplanin showed the high level resistance (the average of MIC >8 μg/ml) than those of other seven antibiotics. The isolates harboring SCCmec type IVa gene showed high resistance (the average of MICs >8 μg/ml) to clindamycin, gentamicin and teicoplanin. MICs of clindamycin and teicoplanin for the isolates harboring SCCmec type V were considerably higher compared with those of other eight antibiotics.

Detection of superantigenic toxin genes in CNS isolates

To investigate the prevalence of superantigenic toxin genes, se and tst genes in a total of 79 clinical CNS isolates and 8 MRSA isolates (as positive control strains) were analyzed by multiplex PCR. The MRSA isolates were constantly detected se and/or tst genes. In contrast, any se or tst gene was not detected in all of the CNS isolates tested in this study.

Discussion

CNS are the main clinical isolates of bloodstream and central venous catheter related bloodstream infections among patients with hematological disorders. In this study, a total of 79 CNS isolates from patients with cardiovascular surgery were comprehensively determined for antibiotic resistance, SCCmec types and superantigenic toxin genes. Of them, 45.6% were meca+ isolates, and SCCmec type III, IVa and V were detected in 21 (58.3%), 16 (44.4%) and 11 (30.6%) isolates, respectively. SCCmec genes were also detected in meca- isolates at a high ratio of 51.2% (22/43 isolates) and showed SCCmec type II was 27.9%, type III was 18.6% and Type I was 16.3%. These results indicated that there are considerable differences of SCCmec type patterns between the meca+ and meca- CNS clinical isolates. Previous studies reported that 61.7% of MR CNS isolates from healthy Japanese children harbored SCCmec IVa, 52% of meca+ CNS isolates from hospitalized patients harbored SCCmec type III, and 27.9% of the isolates harbored SCCmec type V. Among the isolates from elderly patients in a long-term-care facility, SCCmec type IV was found in 33% and type V was found in 18% of the isolates. For MR CNS isolates detected from outpatients, 29% was type IVa, 26% was type V. Previous studies indicated that the majority of MR CNS isolates from humans other than hospitalized patients harbored SCCmec type IV or type V, but type IVa was also detected in comparatively many meca+ isolates in this study. On the other hand, in the present study, SCCmec elements were also detected from meca- CNS and rate of the isolates harboring SCCmec elements was 51.2%. The result suggested that SCCmec elements defective in meca may exist.

The relationships between SCCmec types and antimicrobial resistance have been studied on MRSA, but little on CNS isolates. Kadlec et al. reported that MRSA isolates harboring SCCmec type V (43.4%) were resistant to macrolide and lincosamide including clindamycin. MRSA isolates harboring SCCmec type V from bovine mastitis and swine were also significantly resistant to clindamycin. Nakaminami et al. reported that 90.8 % of the MRSA isolates from patients with impetigo, which are mainly composed of SCCmec type IV gene, were resistant to gentamicin. Our study analyzed the relation of SCCmec genes and the antibiotic resistance of CNS isolates and showed that CNS harboring SCCmec genes were more resistant.
to clindamycin, gentamicin and teicoplanin than other antibiotics tested in this study (Fig. 3). Mombach Pinheiro Machado et al. reported that 91.7% of CNS isolates harboring SCC\textit{mec} type I and 89.2% of SCC\textit{mec} type III were resistant to gentamicin, and 73.8% of the isolates with SCC\textit{mec} type III were resistant to clindamycin\textsuperscript{10}. Interestingly, our results showed that the isolates harboring SCC\textit{mec} type II gene were considerably resistant to teicoplanin than other 9 antibiotics, and the CNS harboring type V was considerably resistant to clindamycin, and type IVa was more resistant to gentamicin. The mechanism between SCC\textit{mec} types and antibiotic resistance profiles needs to be further investigated.

Staphylococcal superantigenic toxin genes in CNS have reported by some studies, but those results did not correspond. Previous studies reported that some CNS isolates from cheese had \textit{se} genes\textsuperscript{27}, while CNS isolates from foodstuffs and patients did not carry \textit{se} genes\textsuperscript{28}. Seventeen (26.2%) of CNS isolates from cheese had any of \textit{sea}, \textit{seb}, and \textit{sec}\textsuperscript{27}. In 102 bovine CNS isolates from milk, none of the genes, or \textit{sea} to \textit{see}, \textit{seg} to \textit{seq}, \textit{seu} and \textit{tst} were detected\textsuperscript{29}. Our results showed that \textit{se} and \textit{tst-1} genes were detected in none of the 79 CNS isolates from clinical specimen, suggesting that CNS does not carry superantigenic toxin genes. These results indicate that SCC\textit{mec} types of CNS are strongly associated to their antimicrobial resistance profiles, but SCC\textit{mec} do not directly related to superantigenic toxin genes.

**Acknowledgments**

This study was supported by Grants-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (19590438 to D.L.H.; 20390122 to A.N. and 21590475 to D.L.H), the Fund for the Promotion of International Scientific Research (B2 to D.L.H) and Grant for Hirosaki University Institutional Research (A.N.). We thank K. Hiramatsu and T. Ito, Juntendo University in Tokyo, Japan, for providing the SCC\textit{mec} typing standard MRSA control strains and M. Nakai for supporting this study.

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