A survey of community-associated methicillin-resistant Staphylococcus aureus in Korea

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Objectives: Methicillin-resistant Staphylococcus aureus (MRSA), originally restricted to hospitals, has emerged as a significant pathogen in the community. Although MRSA accounts for over 60% of S. aureus in tertiary hospitals in Korea, little is known about the epidemiology of community-associated MRSA (CA-MRSA).

Methods: From January to July 2005, a hospital laboratory-based survey was conducted in seven community-based or tertiary hospitals. The medical records and Health Insurance Review Agency databases were reviewed and MRSA isolated from patients without apparent risk factors was defined as CA-MRSA. Susceptibilities to 12 antibiotics were tested by the disc diffusion method. SCCmec typing, Panton–Valentine leucocidin (PVL) gene detection and multilocus sequence typing (MLST) were performed according to published protocols.

Results: Of 3251 S. aureus, 1900 (58.4%) were MRSA. CA-MRSA accounted for 112 (5.9%) of the MRSA. Of the 112 CA-MRSA isolates, 27 and 33 were found to be pathogens and colonizers, respectively. Fifty-two isolates from the patients with chronic otitis media were classified as ‘undetermined’. Most of the 27 CA-MRSA patients had skin and soft tissue infections or acute ear infections. None of the patients died during the study period. Among 72 isolates tested, 64% were multidrug-resistant. SCCmec type IVa was the most common type among the colonizers and pathogens. On MLST analysis, ST72 was predominant, but ST5 and ST239 were prevalent in the ‘undetermined’ group. None possessed the PVL gene.

Conclusions: Despite MRSA-endemic hospital settings, CA-MRSA infections are not common in Korea. A new clone of CA-MRSA, ST72-SCCmec type IVa without the PVL gene, is the most common form.

Keywords: S. aureus, CA-MRSA, Panton–Valentine leucocidin, epidemiology
Community-associated MRSA in Korea

Introduction

*Staphylococcus aureus* is one of the most important pathogens, causing severe morbidity and fatal infections. The rapid evolution of antibiotic resistance in *S. aureus*, in particular methicillin-resistant *S. aureus* (MRSA), is of considerable concern. Whereas MRSA has been an important nosocomial pathogen since the 1960s, cases of MRSA infections in the community were at first rare in patients lacking the usual risk factors for MRSA acquisition. However, in the past decade, new strains of MRSA causing serious infections such as suppurative skin infections and severe necrotizing pneumonia in young, otherwise healthy, individuals have emerged in the community. Unlike healthcare-associated MRSA (HA-MRSA), these isolates possess not only a novel genetic element conferring methicillin resistance but frequently also a gene encoding Panton–Valentine leukocidin (PVL). Furthermore, community-associated MRSA (CA-MRSA) is now an established pathogen in many communities in the United States and in other countries.11–16

Although MRSA accounts for more than 60% of *S. aureus* nosocomial isolates in Korea, there have been no reports of MRSA outbreaks in the community except for a cluster of cases of staphylococcal scalded skin syndrome. In addition, the epidemiology of CA-MRSA in Korea has not been described to date. To determine the prevalence, and clinical and microbiological characteristics of CA-MRSA, we designed a prospective sentinel hospital laboratory-based survey in Korea.

Materials and methods

Data collection

This study was conducted in four community-based and three tertiary-care hospitals from January to June 2005. All MRSA isolates from all body sites of the patients were monitored by an infectious disease physician at each hospital. MRSA isolates from cultures of specimens obtained in outpatient clinics, emergency rooms or within 72 h of admission were enrolled for further classification. Duplicate isolates from a single individual were excluded.

CA-MRSA was defined as an isolate recovered from a clinical culture of a patient lacking apparent risk factors for MRSA acquisition.13,7,8 Established risk factors included: (i) a history of hospitalization (excluding that of normal newborns), surgery, dialysis or residence in a long-term care facility within 1 year from the date of MRSA culture; (ii) the presence of a permanent indwelling catheter or percutaneous medical device at the time of culture; or (iii) previous isolation of MRSA. We reviewed the medical records of patients with suspected CA-MRSA isolates using a standardized record form, to identify risk factors as well as to abstract other relevant clinical information. In addition, we attempted to review the records of these patients in Health Insurance Review Agency (HIRA) databases to ascertain whether they might have risk factors that were missed in the medical records; for example, previous hospitalization or operation within 1 year. After that, suspected cases were re-classified as confirmed CA-MRSA. The remaining isolates were deemed to be community-onset HA-MRSA.

The clinical significance of each CA-MRSA isolate was determined by the infectious disease physician in each hospital based on specimen type and clinical symptoms/signs. The criteria for the sites and types of infection were those recommended by the Centers for Disease Control and Prevention.19

To compare the clinical and the molecular characteristics of CA-MRSA and HA-MRSA, we selected HA-MRSA isolates at random during the study period. We then retrospectively reviewed the medical records of the patients from whom HA-MRSA were isolated.

Bacterial isolates

All participating hospitals sent the CA-MRSA and HA-MRSA isolates to the laboratory of Seoul National University Bundang Hospital (SNUBH). Upon receipt, these were subcultured onto blood agar to ensure purity. Initial identification was based on colony morphology and agglutination tests with PS LATEX (Eiken Chemical, Tokyo, Japan). When necessary, further confirmatory tests were performed using a Vitek system (bioMerieux, Durham, NC, USA). All isolates received were immediately stored at −70°C until required.

For quality control of the antimicrobial susceptibility test, we used *S. aureus* ATCC 25923. The staphylococcal cassette chromosome mec (SCCmec) typing standard MRSA control strains (I, II, III, IVa, IVb, IVc, IVd and V) were obtained from Dr Teruyo Ito of Juntendo University (Tokyo, Japan). Additional SCCmec reference strains (Ia, Ila and IIfa) were kindly provided by Dr Kwan Soo Ko of the Asian-Pacific Research Foundation for Infectious Diseases (Seoul, Korea).

Molecular typing studies

SCCmec typing was performed by a multiplex PCR method, which identifies types I to IV and their variants.20 Non-typeable types, showing unexpected fragments or lacking some fragments in the multiplex PCR and so not corresponding to previously defined SCCmec types, were defined as subtypes such as II variant or III variant by the presence or absence of various PCR products. The presence of the lukS-PV and lukF-PV genes coding for PVL in the MRSA isolates was determined by PCR as described previously.21 Multilocus sequence typing (MLST) was performed as described previously. Alleles of the seven loci were assigned by comparing the sequences of each locus to those in the *S. aureus* MLST database (http://www.mlst.net). Sequence types (ST) were assigned according to the pattern of the seven alleles, and clonal complexes (CC) were subsequently defined by the eBURSTv3 program available on the MLST web site.

Antimicrobial susceptibility testing

An *in vitro* antimicrobial susceptibility test was carried out by the disc diffusion method, in accordance with the CLSI guidelines. The following 12 antibiotics were tested: oxacillin, penicillin, erythromycin, clindamycin, vancomycin, teicoplanin, tetracycline, trimethoprim/sulfamethoxazole, rifampicin, gentamicin, chloramphenicol and ciprofloxacin. The breakpoints for resistance were those recommended by the CLSI. The double disc diffusion assay was performed to determine inducible clindamycin resistance.

The resistance rate was calculated as the number of intermediate and resistant strains over the total number of strains. Multidrug resistance was defined as resistance to penicillin and oxacillin plus three or more antibiotics.

Results

Survey

A total of 3251 *S. aureus* isolates were identified at seven hospitals over a period of 6 months. Of these, 1900 (58.4%) were...
MRSA. Although 365 of the MRSA were isolated from clinical samples obtained from outpatients (including those in the emergency rooms) or inpatients within 72 h of admission, 253 of these were excluded after reviewing medical records and the HIRA databases. Therefore, CA-MRSA accounted for 5.9% (112) of the MRSA isolates (Figure 1).

Of these 112 CA-MRSA isolates, 27 and 33 were identified as pathogens and colonizers, respectively. Because 52 CA-MRSA isolates from the patients with chronic otitis media (COM) could not be defined as either pathogens or colonizers due to clinical uncertainty, these isolates were classified as ‘undetermined’. Exposure to antibiotics within 1 year of the MRSA culture date was documented in 30 patients, according to the HIRA databases.

Seventy-two (64%) of the 112 CA-MRSA isolates were available during the study period and another 40 isolates were not stored for laboratory tests. Seventy-two HA-MRSA isolates that were randomly selected for comparison were also evaluated.

Clinical characteristics of the CA-MRSA and HA-MRSA infections

Most of the 27 CA-MRSA cases (Table 1) were categorized as having skin and soft tissue infections (9 cases) or acute ear infections (9 cases). Five patients, who were 1-year-old or less, had been diagnosed with acute bacterial conjunctivitis. There were even four invasive infections, including three of primary bacteraemia and one of septic arthritis. The median age of the 27 patients was 30 years (range 0–84). Chronic diseases were identified in six patients and exposure to antibiotics within 1 year before MRSA culture date in three cases. None died of the CA-MRSA infection over the study period. The clinical characteristics of the patients with CA-MRSA diseases did not differ greatly from those colonized by CA-MRSA. The CA-MRSA infections did not occur predominantly in young children.

The HA-MRSA group had a greater frequency of infections or colonizations among patients over 60 years old, but the CA-MRSA group had a lower frequency of infections or colonizations among patients of the same age (37/67 versus 17/60). The number of patients with underlying chronic illnesses was also higher in the HA-MRSA group (Table 1).

Molecular characteristics of the CA-MRSA and HA-MRSA isolates

PCR amplification of the PVL toxin genes was negative in all 72 CA-MRSA isolates. The most common SCCmec type was type IVa, which was found in 31 isolates (43%). SCCmec types II and III and their variants were found in 22 and 18 isolates, respectively; they were found mainly in the ‘undetermined’ group. Four isolates designated as II or III variant did not belong to any specific SCCmec types based on other methods.24,25 PVL genes were also not detected in any of 72 HA-MRSA isolates that were randomly selected. Fifty-nine (82%) of the HA-MRSA isolates were SCCmec types II and III and their variants.

In the MLST analysis, 72 CA-MRSA were assigned to nine STs. The most prevalent ST was ST72 (25, 35%), which was most common among the pathogens and colonizers. All the isolates belonging to ST72, except one, were not multidrug-resistant. ST5 and ST239, reported to be circulating in Korean hospitals,26,27 were prevalent in the ‘undetermined’ group. Cluster analysis by the eBURST program revealed two major singletons, two major clonal complexes and two minor singletons (Table 2). Of the diverse genotypes based on STs and SCCmec types, ST72-SCCmec type IVa was the most common in the Korean CA-MRSA isolates. On the other hand, most of the HA-MRSA were ST5 (29 isolates) or ST239 (33 isolates).
CC1 and ST72, the major genotypes in the groups of pathogens or colonizers in CA-MRSA isolates, were rare in the HA-MRSA isolates.

**Antibiotic susceptibilities**

Antimicrobial susceptibility tests of 12 antimicrobial agents were performed for 72 CA-MRSA and 72 HA-MRSA isolates. The antimicrobial susceptibilities of CA-MRSA and HA-MRSA isolates were described based on ST (Table 3).

Table 1. Clinical characteristics of patients from whom community-associated (n = 112) and hospital-associated methicillin-resistant Staphylococcus aureus (n = 72) were isolated

<table>
<thead>
<tr>
<th></th>
<th>CA-MRSA</th>
<th></th>
<th>HA-MRSA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pathogen</td>
<td>colonizer</td>
<td>undetermined</td>
<td>pathogen</td>
</tr>
<tr>
<td>Age, median (range)</td>
<td>30 (0–84)</td>
<td>34 (0–82)</td>
<td>47 (1–81)</td>
<td>52 (0–83)</td>
</tr>
<tr>
<td>&lt;1 year</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1–15 years</td>
<td>5</td>
<td>11</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>15–60 years</td>
<td>8</td>
<td>9</td>
<td>32</td>
<td>13</td>
</tr>
<tr>
<td>&gt;60 years</td>
<td>8</td>
<td>9</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Number of patients with underlying chronic illness</td>
<td>6</td>
<td>10</td>
<td>3</td>
<td>28</td>
</tr>
</tbody>
</table>

**Specimen types**

<table>
<thead>
<tr>
<th></th>
<th>CA-MRSA</th>
<th></th>
<th>HA-MRSA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pus or wound discharge</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>ear discharge</td>
<td>9</td>
<td>1</td>
<td>52</td>
<td>0</td>
</tr>
<tr>
<td>urine</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>respiratory specimen</td>
<td>0</td>
<td>17</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>eye discharge</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>blood</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>others</td>
<td>1 (joint fluid)</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Antimicrobial use within 1 year</td>
<td>3</td>
<td>7</td>
<td>20</td>
<td>NA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>NA, not available; risk factors for acquisition of MRSA or recent history of antibiotic use were not evaluated for HA-MRSA.

CC1 and ST72, the major genotypes in the groups of pathogens or colonizers in CA-MRSA isolates, were rare in the HA-MRSA isolates.

Among 60 isolates (83%) that showed resistance to erythromycin in the 72 CA-MRSA isolates, 38 had constitutive macrolide-lincosamide-streptogramin B (MLS<sub>B</sub>) resistance phenotypes and 22 had inducible MLS<sub>B</sub> phenotypes in the double-disc synergy test. The patterns of susceptibility of the 72 CA-MRSA isolates obtained from the medical records were not very different from those determined in the SNUBH laboratory. Most of the MRSA isolates, except the ST239 clone of HA-MRSA isolates, were susceptible to rifampicin, among other agents.

Table 2. Distribution of the sequence types and SCC<sub>mec</sub> types of the community-associated methicillin-resistant S. aureus isolates in Korea, according to clinical significance

<table>
<thead>
<tr>
<th></th>
<th>CC1</th>
<th></th>
<th>CC5</th>
<th></th>
<th>Singletons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST1</td>
<td>ST493&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ST573&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ST5</td>
<td>NT&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pathogen (19)</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Colonizer (24)</td>
<td>2</td>
<td></td>
<td></td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Undetermined (29)</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Total (72)</td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>SCC&lt;sub&gt;mec&lt;/sub&gt; type</td>
<td>IVa(5)</td>
<td>IVa(1)</td>
<td>IIIv(1)</td>
<td>II(4)</td>
<td>II(1)</td>
</tr>
</tbody>
</table>

<sup>a</sup>ST493 and ST573, and NT are single-locus variants of ST1 and ST5, respectively.
past few years in other countries. In addition, we documented a novel clone of CA-MRSA in Korea, but the epidemiology of CA-MRSA differed in several characteristics from those of other countries as discussed below.

CA-MRSA infection was not common. Its frequency may have been underestimated due to the fact that this was a hospital-based survey, not a population-based study. However, the absence to date of any CA-MRSA outbreak or of reports of serious outcomes caused by CA-MRSA suggest that the low prevalence seen in this study may be a true reflection of the situation in Korea. A population-based survey would be desirable to accurately monitor the current status of CA-MRSA infection.

Skin and soft tissue infections were common, but did not predominate. It is possible that patients with non-purulent cellulitis were not included in our study. Even when culture of needle aspirates was used for microbiological diagnosis, likely pathogens were identified in <30% of cases, and most of these were Gram-positive microorganisms. As a result, such cases were usually treated with empirical antibiotics if purulent discharges were not seen. Although the role of the PVL toxin in the pathogenesis of S. aureus skin and soft tissue infections has not been fully elucidated, the absence of CA-MRSA isolates carrying the PVL genes in our study may partly account for the mild to moderate frequency of CA-MRSA infections. This could be in accordance with the predominance among ocular infections of conjunctivitis without serious complications.

None of the CA-MRSA isolates contained the PVL genes, which are reported to be highly linked epidemiologic markers for CA-MRSA strains. Other studies conducted in Korea have also reported the rarity of the PVL genes in S. aureus isolates. There appears to be something unusual about our strains. Considering the high frequency of antibiotic prescriptions in Korea (22.97 defined daily doses/1000 person-days) based on HIRA statistical data (Dong-Suk Kim, Nam-Soon Kim and Sun-Mee Jang, unpublished data), the selective pressure due to antibiotic use in the community would be expected to influence the dissemination of a virulence gene and the clonal expansion of a PVL-negative strain.

Antibiotic resistance rates were higher than those in other reports, and the multidrug resistance rate was 64%. Erythromycin resistance (83%) or multidrug resistance rates were much higher than those (24.4% and 20.1%, respectively) of methicillin-susceptible S. aureus (MSSA) isolates from non-tertiary hospitals (Hong Bin Kim, Chong Moon Sa, Jae Il Yoo et al., unpublished data), which suggest that the evolution of CA-MRSA from MSSA in the community is not a plausible explanation for the situation in Korea. Furthermore, the prevalence of clindamycin resistance, including inducible resistance, was much higher (83%) than that (∼40%) in MSSA isolates from tertiary hospitals. The widespread use of antibiotics may have contributed to the remarkably high resistance rates of CA-MRSA in Korea. On the other hand, the high antibiotic resistance rates could be due to the circulation of multiple clones of MRSA in the community in Korea, because most ST72-SCCmec IVa clones were resistant to fewer than three antimicrobial agents including β-lactams. However, the antibiotic resistance rates of SCCmec type IVa were still higher than those in other countries. Although

Table 3. Antimicrobial susceptibilities of community-associated (n = 72) and hospital-associated methicillin-resistant S. aureus (n = 72) isolates based on sequence type; no. (%) of susceptible isolates

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>CA-MRSA [n (%)]</th>
<th>HA-MRSA [n (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST72 (n = 25)</td>
<td>ST5 (n = 20)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>1 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>15 (60)</td>
<td>5 (100)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>18 (72)</td>
<td>3 (60)</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>23 (92)</td>
<td>5 (100)</td>
</tr>
<tr>
<td>TMP/SXT</td>
<td>24 (96)</td>
<td>5 (100)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>23 (92)</td>
<td>5 (100)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>14 (56)</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>25 (100)</td>
<td>5 (100)</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>25 (100)</td>
<td>5 (100)</td>
</tr>
</tbody>
</table>

TMP/SXT, trimethoprim/sulfamethoxazole.
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clindamycin treatment is recommended in invasive CA-MRSA infections, clindamycin may not be sufficiently effective in Korea. Previous systemic antibiotic exposure was documented in 5 (20%) of 25 ST72 isolates and 16 (43%) of 37 ST5 or ST239 isolates.

It was a very unusual finding that 55% of CA-MRSA were isolated from patients with COM in this study. The types of clinical specimens from which all MRSA, including duplicated ones, originated during the study period consisted of respiratory specimen (51%), pus or wound discharge (22%), blood (6%), urine (5%), ear discharge (3%), eye discharge (0.3%) and others (13%); data not shown. Even though only 3% of all the specimens were from ear discharge, many of them were classified as CA-MRSA due to the lack of previously known risk factors for acquisition of MRSA. Therefore, we classified the MRSA isolates from patients with COM as ‘undetermined’. They were similar to HA-MRSA in terms of antibiotic susceptibility patterns, SCCmec types and MLST profiles. Although the patients with COM did not have the healthcare-associated risk factors defined in this study, they were usually treated with topical antibiotics during outpatient visits.

The MRSA isolates from COM were closely related to the HA-MRSA clones prevailing in tertiary hospitals as previously reported from the results of SCCmec typing and MLST. Even when recent systemic antibiotic exposure was included as a risk factor, 32 of 52 COM isolates were classified as CA-MRSA. The possibility of close contact with a person with risk factors could not be excluded, but not only systemic or topical antimicrobial use but also frequent visits to outpatient clinics should be assumed to be risk factors for MRSA acquisition. Even though the role of increased antibiotic use in selecting MRSA strains in the community is unknown, MRSA has been reported to be an increasing problem in ear infections in some countries. Additional careful surveys are needed to monitor and characterize CA-MRSA in ear infections. The new strain of CA-MRSA constitutes a different group based on the results of SCCmec types and MLST analysis.

ST72 was the most prevalent clone among the CA-MRSA isolates with regard to MLST, which distinguishes them from CA-MRSA isolates in other countries. In addition, ST1 and ST493, which were isolated in a primary obstetric clinic, were isolated from patients of 1-year-old or less who were treated for acute bacterial conjunctivitis. These findings suggest that a number of strains of CA-MRSA are circulating in the community and in primary clinics. It is possible that simultaneous co-evolution of CA-MRSA is taking place in different areas, rather than the dissemination around the world of a single CA-MRSA clone.

Even though CA-MRSA infections are not yet common in Korea, vigilant surveillance and microbiological monitoring is called for to follow the trend. A novel clone of CA-MRSA (ST72-SCCmec IVa) without the PVL genes is circulating in the community, and further research on the genetic evolution of this CA-MRSA clone is warranted.

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Transparency declarations

None to declare.

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