Meticillin-resistant \textit{Staphylococcus aureus} clones with distinct clinical and microbiological features in a Korean community

Meticillin-resistant \textit{Staphylococcus aureus} (MRSA) is well established as a major pathogen in healthcare-associated infections, but in recent years it has also become increasingly common in community-acquired infections. Community-acquired MRSA (CA-MRSA) strains are characterized by the predominance of staphylococcal chromosomal cassette mec (SCC\textit{mec}) type IV, the lack of multi-drug resistance, and the presence of specific toxins, including Panton-Valentine leukocidin (PVL) and occasionally exfoliative toxins. CA-MRSA has been shown to arise from diverse clones rather than from the worldwide spread of specific clones, and in Asian countries the most predominant PVL-positive CA-MRSA clone is sequence type (ST)30 (Takizawa et al., 2005; Hsu et al., 2006; Wijaya et al., 2006).

In Korea, healthcare-associated MRSA (HA-MRSA) has risen dramatically over the last decade and currently accounts for up to 70\% of \textit{S. aureus} infections in most tertiary care hospitals (Kim et al., 2003; Cha et al., 2005), represented by two predominant clones, ST239 and ST5. To date, there has been no report of CA-MRSA infections in Korea, but since 2003, sporadic cases of staphylococcal scalded skin syndrome (SSSS), and skin and soft tissue infections not attributable to HA-MRSA have been recorded in Kyungnam Province, Korea. This finding prompted us to investigate the clinical and microbiological characteristics of the isolates from such infections.

From November 2004 to August 2005, 21 CA-MRSA isolates were collected from outpatients in Kyungnam Province, Korea (Table 1). Two isolates from patients with SSSS in 2003 were also included in the strain set. CA-MRSA was defined as an MRSA isolate recovered from a clinical culture from a patient who had no history of hospitalization or admission to a long-term care facility within one year before the MRSA-culture date. MRSA was

\begin{table}
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\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
Isolate no. & Sex/age (years) & Clinical diagnosis & ST & \textit{spa} type & \textit{SCC\textit{mec}} et gene & PFGE type & OXA MIC (\textmu g ml\textsuperscript{-1}) & Antimicrobial resistance & \textit{erm} gene \\
\hline
04CST011 & F/2 & Impetigo & 5 & t002 & IV & – & A & 64 & OXA, GEM & – \\
05CST087 & F/66 & Otitis media & 5 & t002 & II variant & – & B & 256 & OXA, TET, CLI, ERM, CIP & \textit{erm\textit{A}} \\
05CST090 & M/39 & Skin abscess & 72 & t324 & IVA & – & C & 64 & OXA, ERM & \textit{erm\textit{C}} \\
05CST108 & F/28 & Breast abscess & 72 & t324 & IVA & – & C & 64 & OXA & – \\
05CST125 & F/1 & Perianal abscess & 72 & t324 & IVA & – & C & 64 & OXA, ERM & \textit{erm\textit{C}} \\
05CST115 & F/34 & Breast abscess & 72 & t664 & IVA & – & C & 128 & OXA, ERM & \textit{erm\textit{C}} \\
05CST088 & F/54 & Vaginitis & 72 & t664 & IVA & – & C & 64 & OXA, ERM & \textit{erm\textit{C}} \\
04CST015 & F/45 & Skin abscess & 72 & t664 & IVA & – & C & 64 & OXA, ERM & \textit{erm\textit{C}} \\
05CST001 & M/1 & Impetigo & 72 & t664 & IVA & – & C & 128 & OXA, ERM & \textit{erm\textit{C}} \\
05CST045 & M/58 & Otitis media & 72 & t664 & IVA & – & C & 32 & OXA & – \\
05CST077 & M/35 & Pneumonia & 72 & t664 & IVA & – & C & 64 & OXA, ERM & \textit{erm\textit{C}} \\
05CST082 & F/1 & Otitis media & 72 & t664 & IVA & – & C & 64 & OXA, ERM & \textit{erm\textit{C}} \\
04CST047 & M/2 & Skin infection & 72 & t664 & IVA & – & C & 32 & OXA, GEM, ERM & \textit{erm\textit{C}} \\
03CST001 & M/5 & SSSS & 89 & t375 & II variant & \textit{etb} & D & 32 & OXA, GEM, CLI, ERM & \textit{erm\textit{A}} \\
03CST002 & M/4 & SSSS & 89 & t375 & II variant & \textit{etb} & D & 32 & OXA, GEM, CLI, ERM & \textit{erm\textit{A}} \\
04CST044 & F/4 & Skin infection & 89 & t375 & II variant & \textit{etb} & D & 64 & OXA, GEM, CLI, ERM & \textit{erm\textit{A}} \\
05CST003 & M/7 & SSSS & 89 & t375 & II variant & – & D & 64 & OXA, GEM, CLI, ERM & \textit{erm\textit{A}} \\
05CST013 & F/2 & SSSS & 89 & t375 & II variant & \textit{etb} & D & 128 & OXA, GEM, CLI, ERM & \textit{erm\textit{A}} \\
05CST043 & M/3 & SSSS & 89 & t375 & II variant & \textit{etb} & D & 16 & OXA, CLI, ERM & \textit{erm\textit{A}} \\
05CST124 & M/3 & SSSS & 89 & t375 & II variant & \textit{etb} & D & 32 & OXA, CLI, ERM & \textit{erm\textit{A}} \\
05CST057 & F/3 & SSSS & 89 & t375 & II variant & \textit{etb} & D & 8 & OXA, CLI, ERM & \textit{erm\textit{A}} \\
05CST139 & F/2 & SSSS & 89 & t375 & II variant & \textit{etb} & D & 32 & OXA, CLI, ERM & \textit{erm\textit{A}} \\
05CST002 & F/2 & SSSS & 89 & t375 & II variant & \textit{etb} & D & 32 & OXA, GEM, TET, CLI, ERM & \textit{erm\textit{A}} \\
\hline
\end{tabular}
\caption{Clinical and microbiological characteristics of CA-MRSA infections in Kyungnam province, Korea}
\end{table}

F, Female; M, male; CIP, ciprofloxacin; CLI, clindamycin; ERM, erythromycin; GEM, gentamicin; OXA, oxacillin; TET, tetracycline.
confirmed by PCR amplification of the nuc and mecA genes (Pérez-Roth et al., 2001). Isolates were investigated for multilocus ST (Enright et al., 2000), spa gene type (Shopsin et al., 1999), SCCmec cassette group (Oliveira & de Lencastre, 2002; Chongtrakool et al., 2006) and by PFGE of SmaI digests. The presence of toxin genes eta, etb and pvl (Becker et al., 1998; Jarraud et al., 2002), and ermA and ermC (Sutcliffe et al., 1996), was investigated by PCR with specific primers. The antimicrobial susceptibility of the isolates was determined by agar dilution according to the National Committee for Clinical Laboratory Standards guidelines (NCCLS, 2003). Inducible clindamycin resistance was determined using the D-test (Schreckenberger et al., 2004).

The 23 CA-MRSA isolates were classified into 3 STs, 4 spa types, 4 PFGE types and 2 SCCmec types with variants (Table 1); the pvl gene was not detected in any of the isolates. Ten ST89 MRSA isolates carried the SCCmec type II variant and were indistinguishable in PFGE pattern (type D). These isolates carried cr2, class A mec, pUB110, ds and mecC, but not kdp. SCCmec elements carrying type 2 cr and class A mec are typical features of SCCmec type II. The etb gene was detected in 9 of the 10 ST89 isolates. A total of 11 ST72 MRSA isolates were SCCmec type IVA and PFGE type C but were subdivided into 2 spa types, t324 (3 isolates) and t664 (8 isolates). Two isolates were of ST5 but the structures of SCCmec were different from those of ST3 HA-MRSA isolates; one ST5 CA-MRSA isolate carried SCCmec type IV and the other was a SCCmec type II variant with an identical pattern to the ST89 strains.

SSSS was the most common disease presentation, accounting for 9 of the 23 (37.5%) cases, followed by skin and soft tissue infections (impetigo and abscesses) in a further 9 patients (Table 1). ST89 was restricted to SSSS isolates, whereas ST72 was associated with a wide range of clinical manifestations in children and adults.

The MIC of oxacillin for all isolates ranged from 8 to 256 µg ml⁻¹, and resistance rates to erythromycin, clindamycin, gentamicin and tetracycline were 87.0, 47.8, 34.8 and 8.7%, respectively. A total of 6 of the 11 isolates with SCCmec type II variant were resistant to gentamicin through the integrated pUB110, with the remainder showing intermediate susceptibility (MIC 8 µg ml⁻¹). Among the 20 erythromycin-resistant isolates, 11 isolates with SCCmec type II variant constitutively expressed ermA and were also resistant to clindamycin phenotypically, and 9 carried ermC but were susceptible to clindamycin and were positive in the D-test.

To our knowledge, this is the first report of the emergence of CA-MRSA infections in Korea. Three distinct CA-MRSA clones were identified in Kyungnam Province: ST89 SCCmec type II variant is a newly recognized genotype, while ST72 and ST5 strains are genetically identical or closely similar to HA-MRSA strains in Korea (Cha et al., 2005; Ko et al., 2005). The presence of PVL toxin and carriage of SCCmec type IV are important features of CA-MRSA strains (Vandenesch et al., 2003). However, our isolates did not carry the PVL toxin. Until now, sporadic cases of SSSS caused by ST89 MRSA have been reported in Kyungnam Province and other communities in Korea, but this ST has not been detected in isolates from healthcare-associated infections, suggesting that this strain did not originate in hospitals and spill over into the community. Analysis of Japanese CA-MRSA isolates from patients with bullous impetigo showed the predominance of ST89 and ST91, a single locus variant of ST89 (Piao et al., 2005; Takizawa et al., 2005). The Japanese strains carried the etb gene and two unclassified SCCmec types, but not the pvl gene, suggesting that the ST89 CA-MRSA clone in Japan and Korea have similar genetic backgrounds. One possible explanation for the similarities between the Japanese clones and our strains is clonal spread between the two countries.

ST72 strains were previously found in a minority of HA-MRSA strains in Korea (Cha et al., 2005; Ko et al., 2005). They showed identical genetic backgrounds in terms of spa type, SCCmec type and PFGE patterns to those examined here from the community, indicating that they emerged from hospital reservoirs and are now widely distributed in the community. A similar migration from hospitals to the community appears to have occurred with strains of ST5 (Cha et al., 2005; Ko et al., 2005). The PVL-positive ST30 CA-MRSA clone was not detected in this survey despite its high prevalence in Asia.

Acknowledgements

This study was supported by a grant from National Institute of Health (2005), Korea Center for Disease Control and Prevention, Republic of Korea.

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