Meticillin-resistant *Staphylococcus aureus* clones with distinct clinical and microbiological features in a Korean community

Meticillin-resistant *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*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 *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

Table 1. Clinical and microbiological characteristics of CA-MRSA infections in Kyungnam province, Korea

Isolate no.	Clinical characteristic		Microbiological characteristic							
	Sex/age (years)	Clinical diagnosis	ST	<i>spa</i> type	SCC <i>mec</i>	<i>et</i> gene	PFGE type	OXA MIC ($\mu\text{g ml}^{-1}$)	Antimicrobial resistance	<i>erm</i> gene
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	<i>ermA</i>
05CST090	M/39	Skin abscess	72	t324	IVA	–	C	64	OXA, ERM	<i>ermC</i>
05CST108	F/28	Breast abscess	72	t324	IVA	–	C	64	OXA	–
05CST125	F/1	Perianal abscess	72	t324	IVA	–	C	64	OXA, ERM	<i>ermC</i>
05CST115	F/34	Breast abscess	72	t664	IVA	–	C	128	OXA, ERM	<i>ermC</i>
05CST088	F/54	Vaginitis	72	t664	IVA	–	C	64	OXA, ERM	<i>ermC</i>
04CST015	F/45	Skin abscess	72	t664	IVA	–	C	64	OXA, ERM	<i>ermC</i>
05CST001	M/1	Impetigo	72	t664	IVA	–	C	128	OXA, ERM	<i>ermC</i>
05CST045	M/58	Otitis media	72	t664	IVA	–	C	32	OXA	–
05CST077	M/35	Pneumonia	72	t664	IVA	–	C	64	OXA, ERM	<i>ermC</i>
05CST082	F/1	Otitis media	72	t664	IVA	–	C	64	OXA, ERM	<i>ermC</i>
04CST047	M/2	Skin infection	72	t664	IVA	–	C	32	OXA, GEM, ERM	<i>ermC</i>
03CST001	M/5	SSSS	89	t375	II variant	<i>etb</i>	D	32	OXA, GEM, CLI, ERM	<i>ermA</i>
03CST002	M/4	SSSS	89	t375	II variant	<i>etb</i>	D	32	OXA, GEM, CLI, ERM	<i>ermA</i>
04CST044	F/4	Skin infection	89	t375	II variant	<i>etb</i>	D	64	OXA, GEM, CLI, ERM	<i>ermA</i>
05CST003	M/7	SSSS	89	t375	II variant	–	D	64	OXA, GEM, CLI, ERM	<i>ermA</i>
05CST013	F/2	SSSS	89	t375	II variant	<i>etb</i>	D	128	OXA, GEM, CLI, ERM	<i>ermA</i>
05CST043	M/3	SSSS	89	t375	II variant	<i>etb</i>	D	16	OXA, CLI, ERM	<i>ermA</i>
05CST124	M/3	SSSS	89	t375	II variant	<i>etb</i>	D	32	OXA, CLI, ERM	<i>ermA</i>
05CST057	F/3	SSSS	89	t375	II variant	<i>etb</i>	D	8	OXA, CLI, ERM	<i>ermA</i>
05CST139	F/2	SSSS	89	t375	II variant	<i>etb</i>	D	32	OXA, CLI, ERM	<i>ermA</i>
05CST002	F/2	SSSS	89	t375	II variant	<i>etb</i>	D	32	OXA, GEM, TET, CLI, ERM	<i>ermA</i>

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 *Sma*I 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 *ccr2*, class A *mec*, *pUB110*, *dcs* and *medI*, but not *kdp*. SCCmec elements carrying type 2 *ccr* 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 ST5 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 $\mu\text{g ml}^{-1}$, 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 $\mu\text{g ml}^{-1}$). 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 Korea and Japan have very 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.

Sang Hyuk Ma¹, Yeong Seon Lee², Seung Hoon Lee¹, Han Kil Kim³, Jong Sook Jin⁴, Eun Kyung Shin² and Je Chul Lee⁴

¹Department of Pediatrics, Changwon Fatima Hospital, Changwon, Korea

²Division of Antimicrobial Resistance, Center for Infectious Diseases, National Institute of Health, Korea Center for Disease Control and Prevention, Seoul, Korea

³Department of Clinical Pathology, Changwon Fatima Hospital, Changwon, Korea

⁴Department of Microbiology, Kyungpook National University School of Medicine, Daegu 700-422, Korea

Correspondence

Je Chul Lee
leejc@knu.ac.kr

Becker, K., Roth, R. & Peters, G. (1998). Rapid and specific detection of toxigenic *Staphylococcus aureus*: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. *J Clin Microbiol* **36**, 2548–2553.

Cha, H. Y., Moon, D. C., Choi, C. H., Oh, J. Y., Jeong, Y. S., Lee, Y. C., Seol, S. Y., Cho, D. T., Chang, H. H. & other authors (2005). Prevalence of the ST239 clone of methicillin-resistant *Staphylococcus aureus* and differences in antimicrobial susceptibilities of ST239 and ST5 clones identified in a Korean hospital. *J Clin Microbiol* **43**, 3610–3614.

Chongtrakool, P., Ito, T., Ma, X. X., Kondo, Y., Trakulsomboon, S., Tiensasitorn, C., Jamklang, M., Chavalit, T., Song, J. H. & other authors (2006). Staphylococcal cassette chromosome *mec* (SCCmec) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 11 Asian countries: a proposal for a new nomenclature for SCCmec elements. *Antimicrob Agents Chemother* **50**, 1001–1012.

Enright, M. C., Day, N. P., Davies, C. E., Peacock, S. J. & Spratt, B. G. (2000). Multilocus sequence typing for characterization of

methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* **38**, 1008–1015.

Hsu, L. Y., Koh, Y. L., Chlebicka, N. L., Tan, T. Y., Krishnan, P., Lin, R. T., Tee, N., Barkham, T. & Koh, T. H. (2006). Establishment of ST30 as the predominant clonal type among community-associated methicillin-resistant *Staphylococcus aureus* isolates in Singapore. *J Clin Microbiol* **44**, 1090–1093.

Jarraud, S., Mougél, C., Thioulouse, J., Lina, G., Meugnier, H., Forey, F., Nesme, X., Etienne, J. & Vandenesch, F. (2002). Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease. *Infect Immun* **70**, 631–641.

Kim, H. B., Park, W. B., Lee, K. D., Choi, Y. J., Park, S. W., Oh, M., Kim, E. & Choe, K. W. (2003). Nationwide surveillance for *Staphylococcus aureus* with reduced susceptibility to vancomycin in Korea. *J Clin Microbiol* **41**, 2279–2281.

Ko, K. S., Kim, Y.-S., Song, J. H., Yeom, J. S., Lee, H., Jung, S. I., Jeong, D. R., Kim, S. W., Chang, H. H. & other authors (2005). Genotypic diversity of methicillin-resistant *Staphylococcus aureus* isolates in Korean hospitals. *Antimicrob Agents Chemother* **49**, 3583–3585.

NCCLS (2003). *Methods For Dilution Antimicrobial Susceptibility Tests For Bacteria*

That Grow Aerobically, approved standard M7–A6. Wayne, PA: National Committee for Clinical Laboratory Standards.

Oliveira, D. C. & de Lencastre, H. (2002). Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* **46**, 2155–2161.

Pérez-Roth, E., Claverie-Martin, F., Villa, J. & Méndez-Álvarez, S. (2001). Multiplex PCR for simultaneous identification of *Staphylococcus aureus* and detection of methicillin and mupirocin resistance. *J Clin Microbiol* **39**, 4037–4041.

Piao, C., Karasawa, T., Totsuka, K., Uchiyama, T. & Kikuchi, K. (2005). Prospective surveillance of community-onset and healthcare-associated methicillin-resistant *Staphylococcus aureus* isolated from a university-affiliated hospital in Japan. *Microbiol Immunol* **49**, 959–970.

Schreckenberger, P. C., Ilendo, E. & Ristow, K. L. (2004). Incidence of constitutive and inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci in a community and a tertiary care hospital. *J Clin Microbiol* **42**, 2777–2779.

Shopsin, B., Gomez, M., Montgomery, S. O., Smith, D. H., Waddington, M., Dodge, D. E., Bost, D. A., Riehman, M., Naidich, S. &

Kreiswirth, B. N. (1999). Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J Clin Microbiol* **37**, 3556–3563.

Sutcliffe, J., Grebe, T., Tait-Kamradt, A. & Wondrack, L. (1996). Detection of erythromycin-resistant determinants by PCR. *Antimicrob Agents Chemother* **40**, 2562–2566.

Takizawa, Y., Taneike, I., Nakagawa, S., Oishi, T., Nitahara, Y., Iwakura, N., Ozaki, K., Takano, M., Nakayama, T. & Yamamoto, T. (2005). A Panton-Valentine leucocidin (PVL)-positive community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) strain, another such strain carrying a multiple-drug resistance plasmid, and other more-typical PVL-negative MRSA strains found in Japan. *J Clin Microbiol* **43**, 3356–3363.

Vandenesch, F., Naimi, T., Enright, M. C., Lina, G., Nimmo, G. R., Heffernan, H., Liassine, N., Bes, M., Greenland, T. & other authors (2003). Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. *Emerg Infect Dis* **9**, 978–984.

Wijaya, L., Hsu, L. Y. & Kurup, A. (2006). Community-associated methicillin-resistant *Staphylococcus aureus*: overview and local situation. *Ann Acad Med Singapore* **35**, 479–486.