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Antimicrobial Susceptibility and Clonal Relatedness between Community- and Hospital-Acquired Methicillin-Resistant *Staphylococcus aureus* from Blood Cultures

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We compared the antimicrobial resistance and clonal relationships among the community-acquired (CA) and hospital-acquired (HA) methicillin-resistant *Staphylococcus aureus* (MRSA) strains that were isolated from blood cultures in a university hospital over a 4-year period. A total of 131 MRSA isolates, including 28 CA-MRSA and 103 HA-MRSA strains, were identified; antimicrobial susceptibility testing indicated that the CA-MRSA isolates were more susceptible to erythromycin (21% vs 6%; P=0.02), clindamycin (46% vs 12%; P<0.01), ciprofloxacin (43% vs 11%; P<0.01), and gentamicin (43% vs 6%; P<0.01) than were the HA-MRSA isolates. Pulsed-field gel electrophoresis (PFGE) typing and antimicrobial resistance profiles separated the 20 CA-MRSA isolates into 14 and 10 different patterns, respectively, and the 53 HA-MRSA isolates were separated into 24 and 7 different patterns, respectively. Twenty-one (40%) of the 53 HA-MRSA isolates belonged to two predominant PFGE types, and most of them showed multi-drug resistant patterns. Four (20%) of the 20 CA-MRSA and 10 (19%) of the 53 HA-MRSA isolates fell into two common PFGE patterns, and each of them showed the same multi-drug resistant pattern. This study suggests that, although the CA-MRSA blood isolates showed diverse PFGE and antimicrobial resistance patterns, some of these isolates may have originated from the HA-MRSA strains.

Keywords: MRSA, community-acquired, antimicrobial resistance, clonal relationship

Since it was first identified in 1961, methicillin-resistant *Staphylococcus aureus* (MRSA) has been considered a major nosocomial pathogen (Barret *et al.*, 1968; Jorgensen *et al.*, 1999). MRSA accounts for more than 70% of the *S. aureus* isolates obtained from tertiary hospitals in Korea (Lee *et al.*, 1998). The risk factors for hospital-acquired (HA) MRSA include recent hospitalization or surgery, residence in a long-term care facility, dialysis, and indwelling percutaneous medical devices and catheters (de Sousa *et al.*, 2003). However, community-acquired (CA) MRSA colonization and infections have been reported with increasing frequency in recent years (Moreno *et al.*, 1995; Herold *et al.*, 1998; Gorak *et al.*, 1999; Suggs *et al.*, 1999). The available data suggest that CA-MRSA strains are more likely to cause skin or soft tissue infection, to occur in younger persons, and to be more susceptible to most of the other non-β-lactam antibiotics than are the HA-MRSA strains. Although in western countries, the CA-MRSA strains have been reported to differ from the HA-MRSA strains in terms of their antimicrobial susceptibility and genetic backgrounds (Fergie and Purcell, 2001; Fey *et al.*, 2003), these CA-MRSA strains have rarely been investigated in Korea.

S. aureus continues to be an important human pathogen that is capable of causing bacteremia in hospitals and community settings, and it has been associated with mortality rates ranging from 15% to 60% (Cosgrove *et al.*, 2003). Because concerns exist regarding the emergence or the increasing frequency of CA-MRSA strains that cause bacteremia, therapeutic options for the treatment of CA-MRSA bacteremia are now needed. However, there are limited data documenting the antimicrobial resistance of Korean CA-MRSA blood isolates. It is also not clear whether

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the bloodstream CA-MRSA strains were originally nosocomial strains that escaped from the hospital environment or whether these strains represent novel acquisition of a resistance-related gene.

To investigate the antimicrobial resistance and clonal relationships among the bloodstream CA-MRSA isolates and the HA-MRSA isolates, we analyzed all of the MRSA isolates that were recovered from blood cultures at our institution during a 4-year period.

Materials and Methods

Bacterial isolates

We analyzed a total of 131 MRSA blood isolates that were taken from patients who were admitted to Chonnam National University Hospital, a 1,000-bed, tertiary-care facility, between January 2000 and December 2003. One representative isolate from each patient for whom MRSA was recovered from one or more blood culture was included in this study. Blood cultures were processed in a BACTEC 9240 system (Becton Dickinson, USA). The standard microbiological methods for identification of *S. aureus* included Gram staining, the catalase test, and the latex agglutination test with using Staphaurex Plus (Remel, USA). If necessary, further confirmatory tests were done with a Microscan (Dade Microscan Walkaway 96, USA). Methicillin resistance was confirmed by disk diffusion testing with an oxacillin disk (bioMerieux) in Muller-Hinton agar and by following the NCCLS recommendations (NCCLS, 2004).

The MRSA isolates were classified as being CA or HA according to the following criteria (Chaves *et al.*, 2005; Chen *et al.*, 2005). CA isolates showed evidence of infection at the time of presentation, and the first blood specimen that was found to be positive for MRSA was collected less than 48 hours after admission. HA isolates showed no evidence of infection at the time of presentation, and the first blood specimen that was found to be positive for MRSA was collected more than 48 hours after admission.

Antimicrobial susceptibility tests

Antimicrobial susceptibility was determined by performing disk diffusion testing on Muller-Hinton agar according to the guidelines published by the National Committee for Clinical Laboratory Standards (NCCLS,

Table 1. Distribution of the community- and hospital-acquired MRSA blood isolates according to the year

Year	No.	No. (%) of MRSA		*P*-value
		Community-acquired (N=28)	Hospital-acquired (N=103)	
2000	28	3 (10.7)	25 (89.3)	0.008*
2001	30	3 (10.0)	27 (90.0)	
2002	37	10 (27.0)	27 (73.0)	
2003	36	12 (33.3)	24 (66.7)	
Total	131	28 (21.4)	103 (78.6)	

*according to linear-by-linear association.

Table 2. Antimicrobial susceptibility profiles of the community- and hospital-acquired MRSA blood isolates

Antibiotics	No. (%) of MRSA				*P*-value
	Community-acquired (N=28)		Hospital-acquired (N=103)		
	Susceptible	Resistant	Susceptible	Resistant	
Erythromycin	6 (21.4)	22 (78.6)	6 (5.8)	97 (94.2)	0.021
Clindamycin	13 (46.4)	15 (53.6)	12 (11.7)	91 (88.3)	0.000
Ciprofloxacin	12 (42.9)	16 (57.1)	11 (10.7)	92 (89.3)	0.000
Cotrimoxazole	23 (82.1)	5 (17.9)	83 (80.6)	20 (19.4)	0.547
Chloramphenicol	25 (89.3)	3 (10.7)	98 (95.1)	5 (4.9)	0.229
Tetracycline	10 (35.7)	18 (64.3)	26 (25.2)	77 (74.8)	0.193
Gentamicin	12 (42.9)	16 (57.1)	6 (5.8)	97 (94.2)	0.000

Table 3. Relationships between PFGE typings and antimicrobial resistance patterns among the community- and hospital-acquired MRSA blood isolates

PFGE type	Antibiotic resistance pattern	No. (%) of MRSA		Total
		Community-acquired (N=20)	Hospital-acquired (N=53)	
P1	OxEm	1		1
	Ox	3		3
	OxClCi		1	1
	OxEmGm		1	1
P2	OxEm(Cl)CiGmTc	3	2	5
P3	OxEmClTsCiGmTc	1	8	9
	OxEm	1		1
P4	OxEmClCiGmTc		9	9
	OxEmClCiGm		3	3
	OxEmClCmCiGmTc		1	1
P5	OxEmClCiGmTc		4	4
P6	OxEmClTsCiGmTc		3	3
P7	OxEmClCiGmTc		2	2
P8	OxEmClCiGm		2	2
P9	OxEmClCiGmTc		1	1
	OxEmClCmCiGmTc		1	1
P10	OxEmClCiGmTc		1	1
P11	OxEmClCiGmTc		1	1
P12	OxEmClCiGmTc		1	1
P13	OxEmClCiGmTc		1	1
P14	OxEmClCiGmTc		1	1
P15	OxEmClCiGmTc		1	1
P16	OxEmClCiGmTc		1	1
P17	OxEmClCiGmTc		1	1
P18	OxEmClCiGmTc		1	1
P19	OxEmClCiGmTc		1	1
P20	OxEmClCiGm		1	1
P21	OxEmClCiGm		1	1
P22	OxEmClTsCiGmTc		1	1
P23	OxEmClTsCiGmTc		1	1
P24	OxEm		1	1
P25	OxEmClCiGmTc	1		1
P26	OxEmClCiGmTc	1		1
P27	OxEmClCiGmTc	1		1
P28	OxEmClTsCiGmTc	1		1
P29	OxEmClTsCiGmTc	1		1
P30	OxEmTsCmTc	1		1
P31	OxEmClCiTc	1		1
P32	OxEmClGm	1		1
P33	OxEmClCm	1		1
P34	OxEmClCi	1		1
P35	OxTc	1		1

Ox, oxacillin; Em, erythromycin; Cl, clindamycin; Ts, trimethoprim/sulfamethoxazole; Cm, chloramphenicol; Ci, ciprofloxacin; Gm, gentamicin; Tc, tetracycline

2004). The antimicrobial agents we tested included oxacillin, penicillin, erythromycin, clindamycin, trimethoprim/sulfamethoxazole, vancomycin, teicoplanin, chloramphenicol, ciprofloxacin, gentamicin, and tetracycline. Strains with zones of inhibition that fell into the category of intermediate susceptibility to a particular antibiotic were considered resistant strains.

Pulsed-field gel electrophoresis (PFGE)

MRSA was typed by performing PFGE using the *Sma*I restriction enzyme, as previously described (LHI, 1999). The fragments were resolved on a 1% gel using a Genepath system (Bio-Rad, USA) PFGE apparatus at 6 V/cm for 22 h, with the switching times ramped from 5 to 35 sec at 14°C. A lambda DNA-PFGE molecular-sized standard (Amersham Pharmacia Biotech, United Kingdom) and an ATCC control strain were included in each gel. The PFGE patterns were interpreted according to the criteria of Tenover *et al.* (Tenover *et al.*, 1995).

Statistical Analysis

Statistical analysis was performed with SPSS 12.0 software. The categorical data were compared using a chi-squared test or Fisher's exact test. Statistical significance was defined as a *P*-value of <0.05.

Results

Prevalence of CA-MRSA and HA-MRSA isolates from blood cultures

Among a total of 131 MRSA blood isolates, 28 isolates (21.4%) were CA-MRSA and 103 isolates (78.6%) were HA-MRSA. The prevalence of CA-MRSA increased according to the year (P=0.008) (Table 1).

Results of antimicrobial susceptibility testing

The CA-MRSA blood isolates were more likely to be susceptible to multiple antimicrobial agents than were the HA-MRSA isolates (Table 2). The CA-MRSA isolates were more susceptible to clindamycin (46.4% vs 11.7%; P<0.001), erythromycin (21.4% vs 5.8%; P=0.021), ciprofloxacin (42.9% vs 10.7%; P<0.001), and gentamicin (42.9% vs 5.8%; P<0.001) than were the HA-MRSA isolates. Both the CA-MRSA and HA-MRSA blood isolates were highly susceptible to cotrimoxazole (82.1% vs 80.6%; P=0.547) and chloramphenicol (89.3% vs 95.1%; P=0.229). None of the isolates were resistant to vancomycin or teicoplanin. Three of 28 CA-MRSA isolates (10.7%) and 1 of 103 HA-MRSA isolates (1.0%) were resistant only to penicillin and oxacillin. In contrast, 17 of the CA-MRSA isolates (60.7%) were multi-drug resistant (resistant to more than three non-β-lactam antibiotics), whereas 95 of the HA-MRSA isolates (92.2%) showed multi-drug resistance.

PFGE analysis

Of the 131 blood isolates, 20 CA-MRSA isolates and 53 HA-MRSA isolates were available for analysis using the PFGE method (Table 3). Among the 20 CA-MRSA isolates, 14 different PFGE types were identified (patterns P1 through P3, and patterns P25 through P35). Three PFGE patterns (patterns P1 to P3) were shared by at least 2 CA-MRSA isolates (range: 2 to 4 isolates), and the other 11 patterns were unique. Three of the major PFGE types of the CA-MRSA isolates (patterns P1 to P3) were shared with 9 CA-MRSA isolates (45%) and 12 HA-MRSA isolates (23%) (Fig. 1 and Table 3). Fifty-three HA-MRSA isolates showed 24 PFGE banding patterns (patterns P1 through P24). Within the 53 HA-MRSA isolates, there were 2 major genotypes (PFGE types P4 and P3). PFGE type P4 and type P3 represented 13 (24.5%) and 8 (15.1%) of the HA-MRSA isolates, respectively. These two predominant clones were present throughout the 4-year period. The remaining 32 HA-MRSA isolates showed 22 different PFGE banding patterns.

Antimicrobial resistance profiles and PFGE patterns

The PFGE types and the respective antimicrobial resistance profiles of CA-MRSA and HA-MRSA are listed in Table 3. The antimicrobial resistance patterns

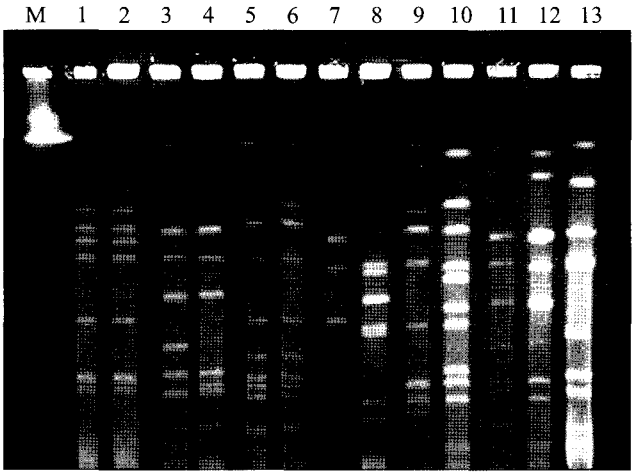

Fig. 1. Representative pulsed-field gel electrophoresis patterns using *Sma*I for the selected community- and hospital-acquired MRSA blood isolates. Three PFGE types were shared by the CA-MRSA isolates and HA-MRSA isolates (type P1: lanes 1 and 2; type P2: lanes 3 and 4; type P3: lanes 5 and 6). The remaining CA-MRSA and HA-MRSA blood isolates showed different PFGE banding patterns. Lane M = lambda molecular weight marker; lanes 1, 3, 5, 7, 8 and 9 = CA-MRSA; lanes 2, 4, 6, 10, 11, 12 and 13 = HA-MRSA.

allowed the grouping of 73 isolates into 12 different antibiotic resistance profiles. Ten and 7 antimicrobial resistance patterns were identified in 20 CA-MRSA isolates and 53 HA-MRSA isolates, respectively. Among these, 5 antimicrobial resistance patterns were shared by the CA-MRSA isolates and HA-MRSA isolates, and most of these patterns showed multi-drug resistance. By combining the antimicrobial resistance patterns and the PFGE patterns, 9 different profiles were shared by at least 2 isolates. Among these 9 profiles, the 2 PFGE types [PFGE type P2 with antimicrobial resistance pattern OxEm(Cl)CiGmTc (5 isolates, 6.8%) and PFGE type P3 with antimicrobial resistance pattern OxEmClTsCiGmTc (9 isolates, 12.3%)] were present in both the CA-MRSA isolates and HA-MRSA isolates. Four of 20 CA-MRSA isolates (20%) and 10 of 53 HA-MRSA isolates (19%) fell into two common PFGE patterns, and members of each of the groups showed the same multi-drug resistant patterns. Additionally, two CA-MRSA isolates had an identical PFGE banding pattern (P3) with the endemic HA-MRSA strains isolated in this hospital, and this was found throughout the 4-year period.

Discussion

The possible rising incidence of CA-MRSA infection has several implications for public health and also for the clinical diagnosis and treatment of patients. The epidemiology, microbiology, and resistance of CA-MRSA require further research and clarification due to the substantial burden of CA-MRSA disease and in order to prevent its increasing incidence. Recent studies have shown that the MRSA portion of community-acquired *S. aureus* bacteremia ranges from 4% to 34% (Akram and Glatt, 1998; Chi *et al.*, 2004). In our hospital, 21.4% of the MRSA blood isolates taken during the study period were categorized as CA-MRSA. We also found that the prevalence of CA-MRSA increased significantly according to the year.

Molecular epidemiologic studies of HA-MRSA isolates have demonstrated that only a few clones are responsible for the epidemic spread of these organisms (Dominquez *et al.*, 1994; de Sousa *et al.*, 2003; Da Silva Coimbra *et al.*, 2003). The clonal spread of multi-drug resistant strains has been reported between geographically separated hospitals (Witte *et al.*, 1994; Teixeira *et al.*, 1995; van Belkum *et al.*, 1997), and even between different countries and continents (Sanches *et al.*, 1995; Aires de Sausa *et al.*, 1998; Mato *et al.*, 1998; Sá-Leão *et al.*, 1999). Several of these pandemic multi-drug resistant MRSA clones have already been identified (Enright *et al.*, 2002; Oliveira *et al.*, 2002). Our study shows that 78.6% of the MRSA bacteremia cases in our hospital were of a nosocomial origin, and 21 (40%) of 53 HA-MRSA isolates belonged to two predominant PFGE types, with most of them showing multi-drug resistant patterns. These two PFGE types were present throughout the 4-year period, which reflects the two endemic clones found in this hospital.

In our study, PFGE typing separated 20 CA-MRSA isolates into 14 different patterns, and 53 HA-MRSA isolates were separated into different 24 patterns; this indicated that the CA-MRSA strains showed more divergent patterns than did the HA-MRSA strains. In contrast to the HA-MRSA strains, the CA-MRSA strains have been reported to present multiple patterns as determined by PFGE analysis, and they possess a type IV staphylococcal cassette chromosome *mec* (SCC*mec*) (Eady and Cove, 2003). However, a recent study on the prevalence of SCC*mec* type IV in the HA-MRSA isolates showed that 95% of the bloodstream HA-MRSA isolates were identified as being of SCC*mec* type IV (de A Trindade *et al.*, 2005). Ma *et al.* (2005) have recently shown that CA-MRSA isolates from patients with staphylococcal scaled skin syndrome in the Chang-won province of Korea contained a type variant IIA (SCC*mec*) element (Ma *et al.*, 2005). These data suggest that the CA-MRSA found in Korea is genetically different from the CA-MRSA found in other regions of the world.

With the increasing percentage of MRSA seen in nosocomial infections, the dissemination of MRSA into the community can be anticipated. However, a previous study using PFGE typing disclosed that the CA-MRSA isolates are highly related to one another and are divergent from the HA-MRSA isolates (Fey *et al.*, 2003). In that study, nearly all isolates were obtained from skin and soft tissue specimens. In our study, we analyzed blood MRSA isolates because of the reliability of their clinical significance and the potential utility of an investigation for defining the epidemiology of invasive MRSA infections. CA-MRSA isolates in Taiwan shared major PFGE patterns with the HA-MRSA isolates, and SCC*mec* type IV was identified in 25% and 40% of CA and HA strains, respectively. Furthermore, SCC*mec* type III was carried in 9% of the CA-MRSA strains without identified risk factors (Chen *et al.*, 2005). In this study, we found that three PFGE patterns were shared by 9 CA-MRSA isolates (45%) and 12 HA-MRSA isolates (23%). Among them, 4 CA-MRSA isolates (20%) shared an identical PFGE type and an identical multi-drug resistance pattern with the HA-MRSA strains. More specifically, 2 CA-MRSA isolates showed 2 identical PFGE banding patterns with the endemic HA-MRSA strains in this hospital, and this

banding pattern was present throughout the 4-year period. Although SCC*mec* typing was not performed in this study, the antimicrobial resistance and clonal relationship between the CA-MRSA and HA-MRSA isolates from our hospital suggest that the CA-MRSA blood isolates may have escaped from the hospital environment and disseminated into the community.

Bacteremia was the most common manifestation and accounted for 71% (n=20) of all 28 episodes of CA-MRSA infection, followed by soft tissue infection (n=4), pneumonia (n=2), urinary tract infection (n=1), and cholangitis (n=1). The mean age of presentation was 42.8±25.2 years, and 6 (21%) episodes occurred in children (data not shown). Patients with community-acquired infections can be further classified as having healthcare-related infections if they had documented evidence of hospitalization within the 6 months prior to the collection of the culture-positive blood specimen, if they had undergone peritoneal dialysis or hemodialysis within the previous 6 months, or if they had used a vascular device at home immediately before admission to the hospital for their current infection (Chaves *et al.*, 2005). Those infections unrelated to the healthcare system have been considered to be strictly community- acquired. However, it is difficult to differentiate true community-related infection from healthcare-related infection because of the easy access to medical care and the high consumption of antibiotics in Korea. Most patients who are admitted to the emergency departments of tertiary care facilities have a history of visiting private clinics or admission to other hospitals, and also often show recent antibiotic use due to their present illness. In our study, the commonly identified risk factors in patients with CA-MRSA infections included being cared for at another facility in the previous 6 months (46%), diabetes mellitus (36%), dialysis (32%), any surgical procedure in the previous 6 months (14%), and cardiovascular diseases (11%). Only 5 MRSA isolates from the cases, including 2 cases of soft tissue infection (n=2), 2 bacteremia cases (n=2), and 1 case of pneumonia (n=1), could be strictly categorized as CA-MRSA. Of the 5 CA-MRSA strains, 1 CA-MRSA strain shared a PFGE pattern (P3) with the HA-MRSA strains, and it was resistant only to erythromycin, penicillin, and oxacillin. Therefore, agreement of the PFGE typing and the antimicrobial resistance patterns of CA-MRSA and HA-MRSA in this study can be explained, in part, by the inclusion of healthcare- associated MRSA into the CA-MRSA category. Further investigation and comparison with CA-MRSA isolates from other hospitals or regions should be implemented to determine the origin of the CA-MRSA.

Despite appropriate antimicrobial treatment, CA-MRSA bacteremia has been associated with a higher mortality rate than that of CA-MSSA (community-acquired methicillin-susceptible *Staphylococcus aureus*) bacteremia. Most CA-MRSA isolates, which normally are genetically different from the nosocomial isolates, have been found to be relatively susceptible to the non-beta-lactam antibiotics. In this study, the CA-MRSA isolates were more susceptible to the non-beta-lactam antibiotics, which include erythromycin, clindamycin, ciprofloxacin, and gentamicin. However, our study also suggests that there was substantial variability among the CA-MRSA strains; some were resistant to one or more antibiotics and some had the resistance patterns of the nosocomial isolates, which most often reflects the spread of nosocomial MRSA in the community. Clinicians routinely prescribe penicillinase-stable penicillin or first-generation cephalosporins for the empirical treatment of community-acquired staphylococcal infections. However, this report and others (Weber, 2005) have indicated that CA-MRSA isolates are more susceptible to non-beta-lactam antibiotics such as clinamycin, ciprofloxacin, and gentamicin. Therefore, clinicians should give careful consideration to each case and should be more cautious when selecting empirical antibiotics for treating staphylococcal infections in the community.

The prevalence of CA-MRSA in Korea is unknown, but this and other reports indicate that the incidence of CA-MRSA is likely to increase in the future. Therefore, continuous surveillance programs for determining the extent of the CA-MRSA dissemination are needed to aid physicians in selecting empirically appropriate antibiotics for the treatment of staphylococcal infections in the community.

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