

A Comparison of Adult and Pediatric Methicillin-Resistant *Staphylococcus aureus* Isolates Collected from Patients at a University Hospital in Korea

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In this study, we compared the phenotypic and genotypic characteristics of 138 MRSA isolates obtained from adult and pediatric patients (adult, 50; children, 88). The resistance rates against gentamicin, clindamycin, and ciprofloxacin were much higher in the adult MRSA isolates than in the pediatric MRSA isolates. The *ermC* gene, which is responsible for inducible clindamycin resistance, was detected in 52(59.1%) of the 88 pediatric MRSA isolates but in only 5(10.0%) of the 50 adult MRSA isolates. MRSA isolates of clonal type ST5 with an integration of SCCmec type II/II variants was the most predominant clone among the adult isolates, while clonal type ST72 with an integration of SCCmec IV/IVA was the most predominant clone among the pediatric MRSA isolates. Staphylococcal enterotoxin A and toxic shock syndrome toxin-1 were prevalent among the adult MRSA isolates but not among the pediatric MRSA isolates. The results of this study demonstrated remarkable differences between adult and pediatric MRSA isolates in terms of their antimicrobial susceptibility profiles, SCCmec type, multilocus sequence type, staphylococcal toxin genes, and erythromycin resistance genes.

Keywords: MRSA, MLST, SCCmec type, staphylococcal toxin, erythromycin resistance gene

Staphylococcus aureus is a major gram-positive pathogen that is capable of causing several kinds of infectious diseases, such as skin and soft tissue infection, pneumonia, and sepsis. The prevalence of invasive *S. aureus* infections dramatically decreased after the introduction of penicillin, which was followed by the introduction of penicillinase-stable penicillins. However, the introduction of these antibiotics has also contributed to the emergence of methicillin-resistant *S. aureus* (MRSA) strains and increasing numbers of MRSA have been isolated worldwide in hospitals (hospital associated-MRSA, HA-MRSA). In recent years, colonization by and infection with MRSA in children and adults who have little or no access to the healthcare system, commonly referred to as community-associated MRSA (CA-MRSA), have been reported with increasing frequency and the characteristics of CA-MRSA are distinct from the strains isolated from hospital-acquired infections (Ma *et al.*, 2002; Okuma *et al.*, 2002; Eady and Cove, 2003; Ko *et al.*, 2005b; Diep *et al.*, 2006). In addition to the increasing frequency of CA-MRSA, reports of skin and soft tissue infections caused by MRSA in children have also increased (Lina *et al.*, 1999b; Eady and Cove, 2003). Although there have been a number of reports comparing the characteristics of HA-MRSA and CA-MRSA, there has been little research comparing the characteristics of adult and pediatric MRSA isolates. Adult and children differ as

hosts for MRSA due to the following factors: 1) children may encounter novel MRSA strains either by different colonization of the skin or by the difference in host defense; 2) the risk factors for MRSA colonization may differ between children and adults because children may be exposed to different environments, such as daycare centers and schools, and because children have different behavioral habits than adults; and 3) antimicrobial drug selection pressure may vary due to the difference in antimicrobial drugs that are used to treat infections in children and adults (David *et al.*, 2006). Therefore, the possibility of differences in MRSA strains between adults and children is very high and these characteristics should be defined in order to provide a valuable guideline for clinical practice.

In this study, we compared the phenotypic and genotypic characteristics of adult and pediatric MRSA isolates collected from adult and pediatric patients hospitalized in a university hospital. A total of 138 isolates were studied and the antimicrobial susceptibility, SCCmec type, multilocus sequence type, staphylococcal toxin genes, and macrolide-resistance genes were compared.

Materials and Methods

Bacterial isolates

Fifty non-duplicate MRSA strains were isolated from adult patients hospitalized between August 2003 and November 2003 at a university hospital in Korea. Eighty-eight MRSA strains were isolated from pediatric patients hospitalized at the same university hospital, between August 2003 and December

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2005 (2003, 17 isolates; 2004, 38 isolates and 2005, 33 isolates). All MRSA isolates were screened for the presence of the *mecA* gene using PCR as previously described (Cha *et al.*, 2005).

Antimicrobial susceptibility test

The antimicrobial susceptibility test was performed using the agar dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, presently Clinical and Laboratory Standards Institute) (NCCLS, 2004). *S. aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as quality control strains. The antimicrobial agents were used ampicillin (USB corporation, USA), teicoplanin (Sigma, Germany), vancomycin (Sigma), gentamicin (Duchefa Biochemie B.V., Netherlands), tobramycin (Amresco Inc., USA), erythromycin (BM, Germany), clindamycin (Duchefa Biochemie B.V.), tetracycline (IBI Shelton Scientific Inc., USA), trimethoprim (ICN Biomedicals, Germany), sulfamethoxazole (MP Biomedicals, France), chloramphenicol (USB corporation), trimethoprim-sulfamethoxazole (at a ratio of 1:19; ICN Biomedicals), rifampin (Yuhan Pharmaceutical, Korea), and ciprofloxacin (Fluka, Switzerland).

DNA isolation

Chromosomal DNA was prepared using the Wizard Genomic DNA Preparation Kit (Promega, USA) according to the manufacturer's instructions. The method, however, was modified for *S. aureus* by the inclusion of 0.5 mg/ml of lysostaphin and 0.3 mg/ml of RNase at the cell lysis step. The extracted chromosomal DNA was used as a template for the PCR experiments.

SCCmec typing

To identify the SCCmec structural types of MRSA isolates,

SCCmec typing was performed using the multiplex PCR method developed by Oliveira and de Lencastre (2002). Amplification was performed on a DNA thermal cycler according to the following program: 15 min at 95°C; followed by 35 cycles of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C; followed by an extension step of 10 min at 72°C. The *ccr* gene complex type, the localization of the IS1272-*mecR1* member of the class B *mec* gene complex, and the localization of the *mecI-mecR1* member of the class A *mec* gene complex were examined by a PCR assay using primers described in previous reports (Ito *et al.*, 2001; Okuma *et al.*, 2002).

Multilocus sequence typing (MLST)

MLST was carried out according to the method described by Enright *et al.* (2000). PCR products were purified with the Wizard PCR-prep DNA purification system (Promega, USA) and the products were then directly sequenced in an ABI Prism 3100 Analyzer (Applied Biosystems, Germany). Allele numbers were assigned using the MLST website (<http://www.mlst.net>) and sequence types (STs) were determined using a browsable database (<http://saureus.mlst.net/>).

The detection of staphylococcal toxin genes

Sequences specific for staphylococcal enterotoxin genes (*sea* to *see*), the toxic shock syndrome toxin gene (*tsst-1*), exfoliative toxin genes (*eta* and *etb*) and Panton-Valentine Leukocidin (PVL) genes (*lukS-PV-lukF-PV*) were detected by PCR as described previously (Becker *et al.*, 1998; Lina *et al.*, 1999b; Ladhani *et al.*, 2001).

Disk diffusion test (D-test) and detection of erythromycin resistance genes

Isolates exhibiting erythromycin resistance and clindamycin susceptibility were subjected to detection of inducible clinda-

Table 1. The antimicrobial susceptibility of MRSA strains isolated from adult and pediatric patients

Antibiotics	No. of resistant strains (%)		P value ^a
	Adults	Children	
Ampicillin	50 (100.0)	88 (100.0)	N.D.
Gentamicin	48 (96.0)	41 (46.6)	<0.0001
Tobramycin	42 (84.0)	76 (86.4)	0.7046
Erythromycin	46 (92.0)	67 (76.1)	0.0200
Clindamycin	41 (82.0)	14 (15.9)	<0.0001
Tetracycline	18 (36.0)	23 (26.1)	0.2229
Ciprofloxacin	41 (82.0)	7 (8.0)	<0.0001
Trimethoprim/Sulfamethoxazole	12 (24.0)	0	1.994E-06 [*]
Sulfamethoxazole	11 (22.0)	35 (39.8)	0.0333
Trimethoprim	8 (16.0)	0	2.024E-04 [*]
Rifampin	7 (14.0)	1 (1.3)	0.0035 [*]
Chloramphenicol	0	1 (1.3)	N.D.
Teicoplanin	0	0	N.D.
Vancomycin	0	0	N.D.

N.D.: Not done

^aThe chi-square test or Fisher's exact test was performed and statistical analysis was not performed when the number of isolates was less than 5.

mycin resistance by the agar disk diffusion (D test) method, in accordance with the recommendations of the NCCLS (2003). The erythromycin resistance gene was detected using PCR as previously described (Ardic *et al.*, 2005).

Statistical analyses

Statistical analyses were performed using the χ^2 test or Fisher's exact test. Statistical significance was assigned to two-sided P values of <0.05. Statistical analyses were performed using SPSS version 12 for Windows (SPSS Inc, USA).

Results

Antimicrobial susceptibility testing

The results of the antimicrobial susceptibility testing showed that the resistance rate against ampicillin, tobramycin, and erythromycin was high in both the adult and pediatric MRSA isolates (Table 1). The resistance rate against trimethoprim, rifampin, chloramphenicol, teicoplanin, and vancomycin was low in both types of MRSA isolates. However, the resistance rates against gentamicin, erythromycin, clindamycin, ciprofloxacin, trimethoprim/sulfamethoxazole, trimethoprim, rifampin, and sulfamethoxazole were significantly different ($P < 0.05$) between the MRSA isolates from the

adult and pediatric patients. The resistance rates against these antibiotics were significantly higher in the MRSA isolates from adult patients than in the MRSA isolates from the pediatric patients, with the exception of sulfamethoxazole.

Detection of inducible clindamycin resistance using a D-test and the detection of macrolide resistance genes by PCR

Fifty-seven of 138 MRSA isolates showed the erythromycin-resistant and clindamycin-susceptible phenotype, and these isolates were subjected to D-testing in order to detect inducible clindamycin resistance. All of the 57 isolates scored positive on the D-test, indicating the presence of inducible clindamycin resistance. The macrolide resistance genes were subsequently detected by PCR in all 138 MRSA isolates (Table 2). The *ermC* gene was detected in all of the 57 MRSA isolates that scored positive for inducible clindamycin on the D-test, 52 of which were pediatric MRSA isolates (Table 2 and 4). The *ermA* gene was detected in all 55 MRSA isolates that were resistant to both erythromycin and clindamycin and three of the strains carried both *ermA* and *ermC*.

SCCmec typing

The SCCmec type of each of the 138 MRSA isolates was

Table 2. Erythromycin resistance genes and susceptibility to erythromycin and clindamycin in MRSA isolates from adult and pediatric patients

Resistance genes	No. of isolates (%)		MIC range (ug/ml)	
	Adults	Children	Erythromycin	Clindamycin
<i>ermA</i>	38 (76.0)	14 (15.9)	>512	>512
<i>ermA</i> and <i>ermC</i>	3 (6.0)		>512	>512
<i>ermC</i>	5 (10.0)	52 (59.1)	>512	0.125-0.25
None	4 (8.0)	22 (25.0)	0.125-0.5	<0.125-0.5
Total no. of isolates	50 (100)	88 (100)		

Table 3. SCCmec types of MRSA strains isolated from adult and pediatric patients

SCCmec type	Multiplex band pattern ^a	<i>ccr</i> complex type	<i>mec</i> complex type	No. of isolates (%)		P value ^c
				Adults	Pediatrics	
II	C, B, D, G	2	A	7 (14)	3 (3)	0.0359*
IIvar-1	C, B, D	2	A	22 (44)		<0.0001
IIvar-2	C, D, G	2	A		9 (10)	0.0262*
IIvar-3	D, N ^b	2	A		1 (1)	N.D.
III	C, E, H, F	3	A	7 (14)		6.165E-04*
IIIA	C, E, F	3	A	1 (2)	1 (1)	N.D.
IV	D	2	B		14 (15)	0.0029
IVA	D, G	2	B	9 (18)	60 (68)	<0.0001
IVvar	D, F	2	B	4 (8)		N.D.
Total no. of isolates (%)				50 (100)	88 (100)	

N.D.: Not done

^aLocus described by the previous study (Oliveira and de Lencastre, 2002). B, *kdp* operon; C, *mecI* gene; D, *dcs* gene; E, region between pl258 and Tn554; F, region between Tn554 and *orfX*; G, left junction between IS431 and pUB110; H, left junction between IS431 and pT181.

^bN, novel band first found in this study; The nucleotide sequences of this band corresponded to the nucleotide sequences of the *mecI* region [from nucleotides 49428 to 42636 with a 39-bp deletion (from nucleotides 42549 to 42588), GenBank accession no. D86934].

^cThe chi-square test or Fisher's exact test was performed and statistical analysis was not performed when the number of isolates was less than 5.

Table 4. MLST, SCCmec type, erythromycin resistance genes, and staphylococcal toxin genes in MRSA isolates distributed among adult and pediatric patients

Clonal complex	Sequence type	SCCmec type	Erythromycin resistance genes	Toxin genes	No. of isolates (%)		P value ^d
					Adults	Children	
CC5	ST5	II/IIvariant	<i>ermA</i>	<i>sec, tsst-1</i>	29 (58.0) ^a	6 (6.8) ^b	<0.0001
CC8	ST239	III/IIIA	<i>ermA</i>	<i>sea</i>	8 (16.0)	0	2.024E-04*
		IIIA	<i>ermA</i>	-	0	1 (1.1)	N.D.
		IVvar	<i>ermA, ermC</i>	<i>sea</i>	3 (6.0)	0	N.D.
CC72	ST345	IVvar	<i>ermA</i>	<i>sea</i>	1 (2.0)	0	N.D.
		ST72	IVA	<i>ermC</i>	-	4 (8.0)	42 (47.7)
CC1	ST1	IV/IVA	<i>ermC</i>	<i>sea</i>	0	4 (4.5)	N.D.
		IV/IVA	<i>ermC</i>	-	1 (2.0)	6 (6.8)	0.4217*
CC89	ST89	IV/IVA	-	<i>sea</i>	0	1 (1.1)	N.D.
		IV/IVA	-	-	2 (4.0)	2 (2.2)	N.D.
		IIvariant	<i>ermA</i>	<i>etb</i>	0	7 (8.0) ^c	0.0484*
CC30	ST30	IV	-	<i>pvl</i>	0	1 (1.1)	N.D.
Total no. of isolates					50 (100)	88 (100)	

N.D.: Not done

^a4 of 29 strains, presence of *sec* alone; 3 of 29 strains, presence of *tsst-1* alone; 22 of 29 strains, presence of both *sec* and *tsst-1*. 7 of 29 strains, SCCmec type II; 22 of 29 strains, SCCmec type IIvar-1.^b1 of 6 strains, presence of *tsst-1* alone; 5 of 6 strains, presence of both *sec* and *tsst-1*. 3 of 6 strains, SCCmec type II; 3 of 6 strains, SCCmec type IIvar-2.^c6 of 7 strains, SCCmec type IIvar-2; 1 of 7 strains, SCCmec type IIvar-3.^dThe chi-square test or Fisher's exact test was performed and statistical analysis was not performed when the number of isolates was less than 5.

determined using multiplex PCR. Most MRSA isolates were found to possess one of the previously reported SCCmec types (i.e., type II, III, IIIA, IV, or IVA), but 36 of the MRSA isolates deviated from the known patterns. The *ccr* gene and the *mec* gene complex were examined for further clarification of these untypeable SCCmec patterns. The SCCmec variants were tentatively classified and named based on the results, as shown in Table 3. SCCmec type IIvar-1 differs from the SCCmec Type II variant in that the 381 bp band corresponding to the pUB110 insertion (locus G) absent in SCCmec type IIvar-1 and SCCmec type IIvar-2 differs from type II by the absence of the 284 bp band corresponding to the *kdp* operon (locus B). SCCmec type IIvar-3 showed two bands of 342 bp (locus D) and 170 bp (a new band [N]). Further sequencing analysis of the new band revealed that the nucleotide sequences of this band corresponded to the nucleotide sequences of the *mecI* region [from nucleotides 49,428 to 42,636 with a 39 bp deletion (from nucleotides 42,549 to 42,588), GenBank accession no. D86934]. Although locus B, which is known to be specific for SCCmec type II, was not found in IIvar-2 and IIvar-3, the coexistence of *ccr2* and the class A *mec* complex indicated that these cassettes most strongly resembled the type II variant. As shown in Table 3, there was a significant difference ($P < 0.05$) in the SCCmec type between the adult MRSA and pediatric MRSA isolates. SCCmec type IIvar-1 was the most frequently detected SCCmec [22 out of 50 isolates (44.0%)] in the adult MRSA, while type IVA was the most frequently detected SCCmec in the MRSA isolates obtained from the pediatric patients [60 of 88 isolates (68.2%)].

MLST

We also determined the sequence type (ST) for all 138 MRSA isolates by MLST and identified eight sequence types (Table 4). While ST5 was the sequence type most frequently detected [29 out of 50 isolates (58.0%)] among the MRSA isolates obtained from adult patient, ST72 was the most frequently detected sequence type among the MRSA isolates obtained from pediatric patient [60 out of 88 isolates (68.2%); 14 of 17 isolates collected in 2003, 26 of 38 isolates collected in 2004, and 20 of 33 isolates collected in 2005]. ST89 and ST30 were detected only in the MRSA strains isolated from pediatric patients.

The detection of staphylococcal toxin genes

The *sea*, *sec*, *tsst-1*, *sec* and *tsst-1*, *etb*, and *pvl* genes were detected in 17, 4, 4, 27, 7, and 1 of the 138 MRSA isolates, respectively (Table 4). In total, 60 strains (43.5%) were positive for one or more of these staphylococcal toxin genes. Among the 50 adult MRSA isolates, 41 (82.0%) were toxin gene positive, whereas only 18 of the 88 pediatric MRSA isolates (20.5%) were toxin gene positive (Table 4). The two most frequently found toxins in the adult MRSA isolates were *sec* and *tsst-1* [29 out of 50 isolates (58.0%)]. The *etb* and *pvl* genes were only found in the pediatric MRSA isolates.

Discussion

In the present study, we compared the phenotypic and genotypic characteristics of MRSA strains isolated from adult and pediatric patients. The results revealed remarkable dif-

ferences between the two MRSA groups in terms of their antimicrobial susceptibility, SCCmec type, multilocus sequence type, and the presence of staphylococcal toxin genes. These findings suggest that adults and children face a different reservoir of MRSA isolates and a different source of antimicrobial drug selection pressure.

The resistance rates against most antimicrobial agents, except for ampicillin, sulfamethoxazole and tobramycin, were lower in the MRSA isolates obtained from pediatric patients than in the MRSA isolates obtained from adult patients. The resistance rates to ciprofloxacin and gentamicin were remarkably lower in the MRSA isolates obtained from pediatric patients than in the MRSA isolates obtained from adult patients. Most of the MRSA isolates from adult patients showed resistance to more than six of the non- β -lactam drugs tested, including ampicillin, gentamicin, tobramycin, erythromycin, clindamycin, and ciprofloxacin. On the other hand, the pediatric MRSA strains were resistant to a maximum of three antimicrobial drugs (ampicillin, tobramycin, and erythromycin).

Clindamycin, a lincosamide antibiotic, has long been considered to be an optional drug in the treatment of infections caused by both MSSA and MRSA strains. The expression of inducible clindamycin resistance, however, could limit the effectiveness of this drug. Phenotypically, inducible clindamycin-resistant strains appeared to be resistant to erythromycin and susceptible to clindamycin on routine antimicrobial susceptibility testing (Weisblum, 1985). Inducible resistance, however, can be expressed during a double disk diffusion test (D-test) (Leclercq, 2002; Lewis and Jorgensen, 2005) in which an erythromycin disk will induce clindamycin resistance. In this study, all 57 of the MRSA isolates with the erythromycin-resistance/clindamycin-susceptible phenotype showed inducible clindamycin resistance, as evidenced by the results of a D-test, thus indicating the possibility of treatment failure. Moreover, this inducible clindamycin resistance was found in 52 of the 88 pediatric MRSA isolates (59.1%). This high rate of inducible clindamycin resistance among pediatric MRSA isolates suggests that clindamycin should be used with caution in children. The genetic determinants responsible for the inducible clindamycin resistance phenotype were determined by PCR that was performed using previously reported primers specific to the genes responsible for macrolide-lincosamide-streptogramin B (MLS_B) resistance. All 57 MRSA strains with the inducible clindamycin resistance phenotype were found to carry the *ermC* gene. Fifty-five of the MRSA strains that were resistant to both erythromycin and clindamycin carried the *ermA* gene and three of these strains carried both the *ermA* and *ermC* genes. Our results were similar to those of two previous studies (Lina *et al.*, 1999a; Schmitz *et al.*, 2000) in that the *ermC* gene was the predominant genetic determinant in strains with an inducible MLS_B phenotype, while the predominant genetic determinant in strains expressing a constitutive MLS_B phenotype was *ermA*.

The major MRSA clonal types distributed among the adult and pediatric patients were different. The ST5, ST239, ST254, and ST345 clonal types were found primarily in the MRSA isolates from adult patients, while the ST72, ST1, and ST89 clonal types were discovered mainly among the MRSA isolates from pediatric patients. The most frequently

distributed MRSA clonal type among the adult patients was ST5, whereas the most predominant MRSA clonal type among the pediatric patients was ST72 (followed by ST1). MRSA strains with the ST5 clonal type were found to carry either SCCmec type II or II variant, while the MRSA strains with the ST72 clonal type carried SCCmec Type IV or IVA. The predominance of the ST5 MRSA clone, with the features of multi-drug resistance and the integration of the SCCmec Type II element, in Korea and Japan has been reported by several previous studies (Cha *et al.*, 2005; Ko *et al.*, 2005a and b) and the characteristics of the ST5 clone have been determined in previous studies (Ko *et al.*, 2005a, 2005b). However, the predominance of the ST72 clone, featured by the integration of the SCCmec type IV or IVA among pediatric patient has not yet been reported. Until now, the only known pediatric MRSA reported was ST5 with the integration of the SCCmec Type IV element.

MRSA clones of type ST5, ST239, ST254, and ST345, which were primarily found in the adult patients, were associated with the *ermA* gene, but the MRSA clones of type ST72 and ST1, which were primarily distributed among the pediatric patients, were associated with the *ermC* gene. Another interesting finding of this study was that a certain MRSA genotype was linked to a certain staphylococcal toxin gene: the ST5 MRSA isolates, with an integration of the SCCmec Type II or II variant, were associated with the *sec* and/or *tsst-1* toxin genes, whereas the MRSA isolates of type ST239, ST254, ST345, and ST1, carrying the SCCmec Type III, IV, or IVvar were associated with the *sea* toxin gene. These results were very similar to the findings of Kim *et al.* (2006) who reported the association of specific SCCmec types with particular toxin gene combinations. According to a previous study (Kim *et al.*, 2006), 77.3% of the SCCmec Type II strains had the *sec* and *tsst-1* genes, 48.8% of the SCCmec Type III strains harbored the *sea* and *see* genes, and 46.9% of the SCCmec IV strains carried the *sea* and *seb* genes. The ST89 MRSA clone, with the integration of the SCCmec type II variant, was identified in seven of the pediatric patients and was found to carry the *etb* toxin gene, which is known to cause staphylococcal scalded skin syndrome in children. This MRSA clonal type has been reported in a previous survey (Hisata *et al.*, 2005) in which 12 of 44 MRSA strains isolated from healthy Japanese children carried the *etb* gene and all six strains whose MLSTs were tested belonged to the ST89 clonal type. To our knowledge, the ST89 MRSA clone carrying the *etb* gene has only been identified in Japan and Korea.

In conclusion, the ST5 clone with the integration of SCCmec Type II or IIvar-1 was the major MRSA isolate distributed among adult patients at a university hospital in Korea. The ST5 clone was associated with multidrug resistance (ampicillin, gentamicin, tobramycin, erythromycin, clindamycin, and ciprofloxacin), the erythromycin resistance gene *ermA*, and the staphylococcal toxin gene *sea/tsst-1*. The MRSA isolate most frequently distributed among the pediatric patients was the ST72 clone with the integration of the SCCmec Type IV or IVA. This clone mainly expresses inducible clindamycin resistance due to the presence of the *ermC* gene. These findings show that the difference between adult and pediatric patients in terms of the major type of MRSA clone

may be useful in the development of control policies and treatment guidelines for clinical practice.

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