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Bullous Impetigo in Children Infected with Methicillin-Resistant *Staphylococcus aureus* Alone or in Combination with Methicillin-Susceptible *S. aureus*: Analysis of Genetic Characteristics, Including Assessment of Exfoliative Toxin Gene Carriage[▽]

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Among bullous impetigo isolates, exfoliative toxin (ET) gene carriage was found in 61.5% of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates versus 90.6% of methicillin-susceptible *S. aureus* (MSSA) isolates. MRSA-only cases were ETB or ETA positive, while MRSA/MSSA coinfection cases were ET negative for MRSA but ETA positive for MSSA. Collagen adhesin may facilitate some MRSA infections.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a major cause of nosocomial infections since the 1960s (2). Since 1997 to 1999, community-associated MRSA (CA-MRSA), which spreads in the community and often produces Panton-Valentine leucocidin (PVL), has also become a major concern worldwide (3, 17). PVL-positive CA-MRSA includes several major clones, such as USA300 (ST8) (16, 17).

In Japan, bullous impetigo, a localized blistering skin disease, is a common disease of children (6, 9). The causative agent has traditionally been considered to be methicillin-susceptible *S. aureus* (MSSA) producing exfoliative toxin (ET) (9). ET from clinical isolates is serologically divided into three types, ETA, ETB, and ETD, which are serine proteases that specifically bind and cleave desmoglein 1, a cadherin-type intercellular adhesion molecule in desmosomes (1, 9, 10, 13). The ETA gene (*eta*), ETB gene (*etb*), and ETD gene (*etd*) are carried by phage (18), plasmid (19), and a pathogenicity island (PI) (20), respectively.

Previous studies have shown that CA-MRSA (ST8, ST88, ST89, and ST91) is also frequently isolated from bullous impetigo (15, 21). Such studies, on the other hand, have shown that considerable numbers of MRSA strains from bullous impetigo were ET gene negative (15). The reason ET-negative MRSA is isolated from bullous impetigo is unknown. In this study, we isolated both MRSA and MSSA from bullous impetigo in children and examined their genotypes and ET gene carriage.

MRSA and MSSA were isolated from 145 children, ages between 7 months and 11 years, suffering from bullous impetigo in Niigata, Osaka, and Tokyo (swab samples from affected part of bullous impetigo). Nasal MRSA strains (14 strains) were isolated from healthy children of ages 2 months to 15 years. Hospital-acquired MRSA (HA-MRSA) isolates ($n = 235$) from adult inpatients were also studied. Multilocus sequence typing (MLST) was performed using seven housekeeping genes, as previously described (5). The sequence type (ST) was obtained from the MLST website (http://www.mlst.net/), and the ST data were further analyzed using the eBURST software program (http://eburst.mlst.net/) to determine the clonal complex (CC) to which each ST belonged. Staphylococcal cassette chromosome *mec* (SCC*mec*) types were analyzed by PCR as previously described (4, 7, 8, 12, 23). Standard strains used for SCC*mec* typing included strains 10442 (for SCC*mec*I), N315 (for SCC*mec*II), JCSC3063 (for SCC*mec*IIb), 85/2082 (for SCC*mec*III), JCSC1968 (for SCC*mec*IVa), JCSC1978 (for SCC*mec*IVb), JCSC4788 (for SCC*mec*IVc), JCSC4469 (for SCC*mec*IVd), and WIS (for SCC*mec*V). Virulence genes were detected by PCR using previously reported primers. The targeted genes were the three ET genes (*eta*, *etb*, and *etd* [encoding ETD]), the collagen adhesin (CNA) gene (*cna*), the bone sialoprotein adhesin (BBP) gene (*bbp*), the PVL gene, and superantigen (staphylococcal enterotoxin [SE]) genes (14). Statistical comparison was performed using Fisher's exact test and Bonferroni's correction. A P value of <0.05 was considered significant. Bonferroni's correction requires a significance level of $P < 0.0167$.

Of 145 children with bullous impetigo, 134 (92.4%) were positive for *S. aureus*. Of those, 26 (26/134; 19.4%) were positive for MRSA. Sixteen MRSA cases were MRSA-only infection, and 9 MRSA cases were MRSA/MSSA coinfection; in the remaining MRSA case, the presence of MSSA was not determined. One hundred eight *S. aureus*-positive cases were MSSA-only infection. ET gene carriage was 61.5% (16/26) for MRSA and 90.6% (106/117 [108 plus 9]) for MSSA ($P < 0.001$).

The characteristics of MRSA and MSSA strains are summarized in Table 1. Among ET-positive MRSA strains ($n = 16$), 12 isolates (75.0%) were ETB positive, 4 isolates (25.0%) were ETA positive, and none were ETD positive; ETB was domi-

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TABLE 1. Isolation of *S. aureus* from bullous impetigo in three distinct patterns and isolate characteristics

S. aureus from bullous impetigo (n[a])	Type			Presence of[c]:			
	ST	CC	SCC*mec*	ETA	ETB	ETD	CNA
MRSA only[b] (16)							
Group 1 (9)	89	509	IIb	−	+	−	+ (3)
Group 2 (4)	88	88	IVx[d]	+	−	−	−
Group 3 (3)	91	509	IVa	−	+	−	+ (1)
MRSA plus MSSA[b] (9)							
Group 1 (6)							
MRSA	89	509	IIb	−	−	−	+ (3)
MSSA	121	121		+	−	−	−
Group 2 (3)							
MRSA	8	8	IVx[d]	−	−	−	−
MSSA	15	15		+	−	−	−
MSSA only[b] (108)							
Group 1 (89)	(121)[e]	(121)[e]		+	−	−	+/−[f]
Group 2 (6)	(1442/1443)[g]	(509/509)[g]		−	+	−	+/−[h]
Group 3 (2)	25	25		−	−	+	+/−
Group 4 (11)	ND[i]	ND		−	−	−	+/−[j]
Other[k] (1)	30	30	IVc	−	−	−	+ (2)

[a] n, no. of cases.
[b] ET positivity was compared by using Fisher's exact test and Bonferroni's correction ($P = 1.00$ for MRSA only versus MRSA plus MSSA; $P = 0.60$ for MRSA plus MSSA versus MSSA only; $P = 0.36$ for MRSA only versus MSSA only).
[c] Numbers in parentheses represent the numbers of repetitions of region B in the CNA gene (*cna*).
[d] Type IV with unknown subtypes (other than IVa, IVb, IVc, or IVd).
[e] ST (CC) types of three MSSA strains, which were randomly chosen.
[f] CNA positive, 32.6%.
[g] ST (CC) types of three MSSA strains, which were randomly chosen. Of three, two were ST1442 (CC509) and one was ST1443 (CC509).
[h] CNA positive, 3 of 6.
[i] ND, not determined.
[j] CNA positive, 27.3%.
[k] MRSA isolated but MSSA not examined.

nant for MRSA. In contrast, for ET-positive MSSA ($n = 106$), 98 isolates (92.5%) were ETA positive, 6 isolates (5.7%) were ETB positive, and 2 isolates (1.9%) were ETD positive; ETA was dominant for MSSA.

For MRSA-only cases ($n = 16$), MRSA isolates were positive for ETB (STs ST89 and ST91) or ETA (ST88); ST89 was the most common ST for MRSA from bullous impetigo. As for MRSA-plus-MSSA cases ($n = 9$), MRSA was ET negative while MSSA was ETA positive, and two combinations were observed: ST89 MRSA plus ST121 MSSA ($n = 6$) and ST8 MRSA plus ST15 MSSA ($n = 3$). ST121 seemed to be the most common ST for ET-positive MSSA-alone cases. MRSA isolates, except ST30 MRSA ($n = 1$), were PVL negative. SCC*mec* types of MRSA strains were IV (or IIb), consistent with SCC*mec* types of CA-MRSA (16, 22).

Regarding adhesin, 69.2% of MRSA strains (18/26), including MRSA with the most common ST (ST89), were positive for CNA, while CNA-positive rates for MSSA were 32.5% (38/117) ($P < 0.008$). Moreover, CNA-positive rates for nasal MRSA of healthy carriers and HA-MRSA from inpatients were also lower (28.6% [4/14], $P < 0.02$, and 0.4% [1/235], $P < 0.00001$, respectively), indicating that CNA may facilitate bullous impetigo infections with some MRSA strains, such as ST89, ST91, and ST30 MRSA strains.

CNA consists of region A (possessing collagen-binding activity), region B repeats (contributing as a "stalk" allowing presentation of region A at the bacterial surface for ligand interaction), and three other bacterial cell-associated regions (W, M, and C) (24). To examine the number of repetitions of region B (length of "stalk"), PCR primers (CNABN-F1, 5′-TGACAAAAATGGCAAGACTA; and CNABN-R1, 5′-TTGGTAATTCTTTTAGAGG) were designed; PCR products are 866 bp long for one repetition, 1,427 bp for two repetitions, and 1,988 bp for three repetitions. The number of repetitions of region B varied depending on the ST of MRSA and was three for ST89 (a major ST of MRSA from bullous impetigo), two for ST30, and one for ST91 (Table 1). We speculate that ST89, ST30, or ST91 MRSA infects skin and soft tissues through binding to collagens exposed by scratching and that among them, ST89 MRSA possessing CNA with a longer "stalk" has been selected for bullous impetigo (most probably due to its higher ability to reach collagen).

In addition to PVL-positive ST30 MRSA in this study, we recently isolated one more PVL-positive, ET-negative MRSA, with the genotype ST1335 (a single locus variant of ST30)/SCC*mec*IVc, from bullous impetigo (however, again, no data were available as to coinfection with MSSA). Since we have had only two PVL-positive MRSA cases, the association of PVL with bullous impetigo seemed low. ST30 and its variant ST MRSA strains were positive for both CNA and BBP.

The reason ET-negative, CNA-negative ST8 MRSA was isolated from bullous impetigo is not known; however, it is noteworthy that such ST8 MRSA was positive for *S. aureus* PI (SaPIm1/n1) carrying the superantigen genes *tst*, *sec*, and *sel* (11), in contrast to MRSA from bullous impetigo with other STs (ST30, ST88, ST89, and ST91). In Japan, such ST8 MRSA

was associated not only with bullous impetigo but also with invasive infections in children, such as epidural abscesses (unpublished data).

In this study, clinical information on these children was obtained for only limited cases (10 cases for the MRSA-only group, 3 for the MRSA-plus-MSSA group, and 11 for the MSSA-only group). As for the degree of illness, spread of bullous impetigo to the whole body surface was observed in one case in the MRSA-only group; and as for complications, upper respiratory infection was observed in one case in the MRSA-plus-MSSA group. Patients were treated with cefdinir, cefditoren, or fosfomycin for 5 days. In the MRSA-only group, initial therapy failed in 4 of 10 cases. Those patients were further treated with minocycline or fosfomycin for an additional 5 days. In the MRSA-plus-MSSA group and the MSSA-only group, all 3 and 11 cases recovered with initial therapy, indicating that infection with MRSA required longer therapy than infection with MSSA alone ($P = 0.035$).

In conclusion, in this study, all infection groups (MRSA only, MRSA plus MSSA, and MSSA only) were associated with the presence of ET. MRSA with the genotype ST89/SCC*mec*IIb/*etb*/*cna*, ST88/SCC*mec*IVx/*eta*, or ST91/SCC*mec*IVa/*etb*/*cna* was associated with bullous impetigo alone. Moreover, MRSA with the genotype ST89/SCC*mec*IIb/*cna* or ST8/SCC*mec*IVx/SaPIm1/n1 was also detected among bullous impetigo isolates but in combination with MSSA with the genotype ST15/*eta* or ST121/*eta*, resulting in decreased ET gene carriage rates in MRSA. CNA may facilitate some MRSA infections in bullous impetigo, such as infections with ST89 MRSA, ST91 MRSA, and PVL-positive ST30 (or ST1335) MRSA. SaPIm1/n1 was found only in ST8 MRSA (which was negative for both ET and CNA).

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