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A New Local Variant (ST764) of the Globally Disseminated ST5 Lineage of Hospital-Associated Methicillin-Resistant *Staphylococcus aureus* (MRSA) Carrying the Virulence Determinants of Community-Associated MRSA

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The ST5 lineage of methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most globally disseminated hospital-associated MRSA (HA-MRSA) lineages. We isolated a new local variant (designated ST764) over at least 5 years that causes invasive infections, including necrotizing fasciitis, and is carried by medical students, as well as household members. Analysis of the genome sequence of one isolate compared to that of the reference ST5 strain revealed that ST764 had acquired virulence traits similar to those of community-associated MRSA (CA-MRSA) through the acquisition of two new mobile genetic elements, ACMEII and SaPInn54, which carried ACME *arcA* and the staphylococcal enterotoxin B gene (*seb*), respectively, and through enhanced expression of cytolytic peptide genes, although ST764 was negative for Panton-Valentine leukocidin. Other differences between ST764 and ST5 included the acquisition of an ACMEII-related cassette (cJR1), prophage $\varphi 2_{NN54}$, and streptococcal Tn*5251* and decreased numbers of copies of Tn*554*. As for superantigen genes, although the two possessed *seg*, *sei*, *sem*, *sen*, and *seo*, ST764 lacked *tst*, *sec*, *sel*, and *sep*. The data suggest that ST764 MRSA is a novel hybrid variant of ST5 HA-MRSA with the characteristics of CA-MRSA and that the evolution of ST764 includes multiple steps, e.g., acquisition of novel or nonstaphylococcal mobile elements.

Methicillin-resistant *Staphylococcus aureus* (MRSA) was reported in 1961 and has continued to be a life-threatening multiple-drug-resistant bacterium in hospitals (1). MRSA is generated from methicillin-susceptible *S. aureus* (MSSA) by the acquisition of staphylococcal cassette chromosome *mec* (SCC*mec*) at the 3′ end (i.e., the 15-bp SCC*mec* insertion site, *att*) of *orfX* (2, 3). It is thought that this has occurred only a limited number of times, resulting in MRSA epidemics in hospital settings (4–6).

Among these epidemic MRSA strains, ST5 is one of the most globally disseminated lineages (4–6). The ST5/SCC*mec*II lineage was previously referred to as the New York/Japan clone (5–8). This type of MRSA became dominant in Tokyo in 1992 (9). The dominant Japanese MRSA clone was originally characterized by type II coagulase, a ribotype pattern similar to N315, a toxin type positive for toxic shock syndrome toxin 1 (TSST-1) and staphylococcal enterotoxin C (SEC), and multiple-antibiotic resistance, including ciprofloxacin, and emerged through replacement of the preexisting dominant MRSA strain in 1982, which was characterized by type IV coagulase.

In the United States, a dominant MRSA clone (the New York clone), which was characterized by *mecA* polymorph I, Tn*554* pattern A, and pulsed-field gel electrophoresis (PFGE) pattern A (clonal type I:A:A), was isolated in 1994 (10) and 1998 (11). In 2000, the two major MRSA clones in Japan and the United States were shown to be the same, and transcontinental spread from Japan to the United States was suggested (12). The MRSA clone was then recognized as the New York/Japan clone, with genotype ST5/SCC*mec*II (5, 6, 8), and also as a global clone spreading to, for example, North and Central America, Asia, and Europe (13–17).

Since 1981 (18) or 1997 to 1999 (19), another class of MRSA (called community-associated MRSA [CA-MRSA]), which spreads in community settings, has also been noted; since then, the term "hospital-associated MRSA (HA-MRSA)" has been used for MRSA isolated in the nosocomial environment.

HA-MRSA is multidrug resistant, and infections occur most frequently among inpatients, for example, those who have undergone invasive medical procedures or those aged 50 to 60 years and older (20, 21). In contrast, CA-MRSA is resistant to β-lactam agents only or to some agents in restricted classes, although some recently successful CA-MRSA strains, such as USA300, have become resistant to multiple antibiotics, including levofloxacin (22, 23). Moreover, CA-MRSA infections occur through skin-to-skin contact in healthy individuals, especially children and adolescents, and are associated mainly with skin and soft tissue infections (SSTIs), including pyogenic skin infection, but occasionally with invasive infections, such as bacteremia (and sepsis) and necrotizing pneumonia (1, 21, 24, 25).

It is thought that CA-MRSA possesses unique features compared to HA-MRSA, possibly related to enhanced virulence. One

Received 2 June 2012 Returned for modification 22 July 2012
Accepted 1 January 2013
Published ahead of print 14 January 2013
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Supplemental material for this article may be found at http://dx.doi.org/10.1128/AAC.01147-12.

doi:10.1128/AAC.01147-12

such candidate is the arginine catabolic mobile element (ACME), carrying the *arc* and *opp-3* regions, found in ST8 CA-MRSA USA300, the most common clone in the United States (22, 26). USA300 has an array (combination) of ACME and SCC*mec*IVa (a subtype [a] of SCC*mec*IV, a typical SCC*mec* type of CA-MRSA), which is supposed to be responsible for the community spread and colonization of MRSA (USA300) (27). ACME includes three types: type I (ACMEI), carrying both *arcA* and *opp-3* regions; ACMEII, carrying only the *arcA* region; and ACMEIII, carrying only the *opp-3* region (27, 28).

The enhanced virulence candidates of CA-MRSA also include the cytolytic peptide (or phenol-soluble modulin [PSM]) genes (e.g., *psm*α [encoding PSMα] or *hld* [encoding δ-hemolysin]), which can be expressed at higher levels than those of HA-MRSA (26, 29). All analyzed *S. aureus* genome sequences contain the genes encoding δ-hemolysin, PSMα, and PSMβ; of those, PSMα and δ-hemolysin induce potent cytolytic activity in human cells (29). The enhanced levels of PSMs are supposed to be involved in the pathogenesis of CA-MRSA infections, such as bacteremia and abscesses (26).

CA-MRSA often produces Panton-Valentine leukocidin (PVL) (26); USA300 (22) is an example. PVL causes apoptosis and necrosis in human polymorphonuclear cells or monocytes (30). In this study, we isolated a new local variant (designated ST764) of the ST5 lineage from hospitalized patients, medical students, and family members and characterized the ST764 variant by comparative genomics using a reference ST5 strain.

MATERIALS AND METHODS

Bacterial strains. MRSA strain NN54 was isolated from a case of necrotizing faciitis occurring in a 60-year-old inpatient with a history of chronic kidney failure, hemodialysis, diabetes, and hospitalization and was cultured from blood, a biopsy specimen of the lesion, and abscesses. MRSA NN37 was isolated from the blood of a 54-year-old inpatient with bacteremia on the day of admission in Niigata, Japan, in 2005. He had no history of hospitalization, surgery, dialysis, or indwelling percutaneous medical devices or catheters in the past 1 year.

MRSA carriage in fifth-year medical students undergoing clinical practice in Niigata University Hospital was investigated for 5 years from 2006 through 2010. Two of 261 students were positive for MRSA; MRSA NN35A and NN35B were isolated from their hands in 2006 and 2009, respectively.

Independently of the above study on patients and medical students, nasal MRSA carriage by healthy family members in Niigata was investigated, and three MRSA strains, NN41F1G, NN41F1F, and NN41F1D, were isolated from three members, a 56-year-old grandfather, 34-year-old father, and 6-year-old daughter, of the same household in 2007.

The control strains used in this study include N315 (ST5 [31]), Mu50 (ST5 [31]), I6 (ST5 [32]), I8 (ST5 [32]), I10 (ST5 [this study]), BK2464 (ST5 [4]), USA300-0114 (ST8 [33]), NN36 (ST8 [34]), NN47 (ST8 [35]), 549 (ST8 [36]), PM1 (ST59 [37]), and COL (ST250 [5, 6]).

Molecular typing of MRSA. Molecular characterization of MRSA was performed as described previously (37). Sequence type (ST) typing was conducted using seven housekeeping genes (38), and the ST was obtained from the MLST (multilocus sequence typing) website (http://www.mlst.net/). The *spa* type was analyzed by sequencing the PCR product of the *spa* gene (39) and determined using a public *spa*-type database (http://tools.egenomics.com/) or Ridom SpaServer (http://www.spaserver.ridom.de/). Typing of *agr* was carried out by PCR with previously reported primers (40, 41). The SCC*mec* types I to V (2, 42) were analyzed by PCR, as described by Kondo et al. (42), using reference strains. Coagulase (Coa) typing was conducted using a staphylococcal coagulase antiserum kit (Denka Seiken, Tokyo, Japan). Virulence genes were analyzed by PCR following previously published methods (27, 37, 43–46). The target genes in PCR included 49 genes: genes from three leukocidins (*lukS*-PV–*lukF*-PV, *lukE-lukD*, and *lukM*), 5 hemolysin genes (*hla*, *hlb*, *hlg*, *hlgv*, and *hld*), a peptide cytolysin gene (*psm*α), 18 staphylococcal enterotoxin (SE) genes (*tst*, *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq*, and *ser*), a putative staphylococcal enterotoxin gene (*seu*), 3 exfoliative toxin genes (*eta*, *etb*, and *etd*), a staphylococcal superantigen-like gene cluster (*ssl*), the epidermal cell differentiation inhibitor gene (*edin*), 14 adhesin genes (*icaA*, *icaD*, *eno*, *fib*, *fnbA*, *fnbB*, *ebpS*, *clfA*, *clfB*, *sdrC*, *sdrD*, *sdrE*, *cna*, and *bbp*), and ACME *arcA* and ACME *opp-3C* genes (27).

SEB assay. The amount of staphylococcal enterotoxin B (SEB) in the supernatants of bacterial cultures at 2.0×10^9 CFU/ml was examined using an SET-RPLA kit (Denka Seiken) according to the instructions of the manufacturer.

Susceptibility testing. Susceptibility testing of bacterial strains was carried out using the agar dilution method with Mueller-Hinton agar, according to previous procedures, by the Clinical and Laboratory Standards Institute (47).

Genome analysis. The MRSA NN54 genome was analyzed by pyrosequencing using an FLX genome sequencer system with the assembler software GS De Novo Assembler version 2.0 (Roche Diagnostics, Branford, CT). In this study, 291,924 reads yielded 131 Mb of raw sequences (ca. 47 times the genome). The constructed contigs were mapped on the 2,814,816-bp complete genome of MRSA strain N315 (GenBank accession number FN433596) using In Silico MolecularCloning software version 4.2 (In Silico Biology, Yokohama, Japan). The gene or open reading frame (*orf*) was searched for using In Silico MolecularCloning software version 4.2. Homology analysis was performed using BLAST (http://blast.ncbi.nlm.nih.gov/).

Assay of mRNA expression levels. The mRNA expression levels of the cytolytic peptide genes (*psm*α and *hld*), the ACME *arcA* gene, and the 16S rRNA genes were examined by reverse transcription (RT)-PCR assay with PCR primers reported previously (27, 45), essentially according to the experimental strategy described previously (48). Band intensity was determined using image-processing and analysis software, NIH Image (NIH, Bethesda, MD).

Statistical analysis. Data were evaluated by analysis of variance with repeated measurement. The level of significance was defined as a P value of <0.05.

Nucleotide sequence accession number. The NN54 genome was deposited in GenBank under accession number BAFI01000000.

RESULTS AND DISCUSSION

Isolation and genotying of ST764 MRSA. The seven MRSA strains isolated in Niigata from 2005 to 2009 from invasive infections in patients, the hands of medical students undergoing clinical practice, and the nasal mucosa of healthy household members were typed, and the results showed that all were an ST764 variant of ST5/SCC*mec*II lineage (Table 1); ST764 is a single-locus variant of ST5. All seven strains were negative for PVL, similar to ST5; positive for the ACME *arcA* and *seb* genes, in contrast to ST5; and negative for SaPIm1/n1, in contrast to the ST5 Japanese type (but similar to the U.S. type strain BK2464). All ST764 strains were resistant to multiple antibiotics, including levofloxacin and fosfomycin, similar to ST5 (31, 32).

Of two ST764 isolates from patients, strain NN37 met the Centers for Disease Control and Prevention (CDC) criteria for the CA-MRSA definition (20), while strain NN54 met the HA-MRSA definition. Regarding colonization, 5th-year medical students probably have a risk for MRSA colonization; however, although the ST5 MRSA is the predominant HA-MRSA in hospitals in Niigata (similar to Tokyo), no ST5 MRSA was isolated from the nares and hands of 5th-year medical students ($n = 261$), suggesting that ST764 MRSA may possess higher potential for colonization than

TABLE 1 Molecular characterization of ST764 MRSA strains compared to ST5/SCC*mec*II MRSA in Japan

Characteristic	Value[a]	
	ST764 variants ($n = 7$)	ST5/SCC*mec*II control strains ($n = 5$)
Type		
ST	764	5
spa	2 (t002)	2 (t002)
agr	2	2
SCC*mec*	II	II
Coagulase	II	II
Virulence gene		
Leukocidin genes		
luk$_{PV}$*SF*	−	−
lukE-lukD	+	+
Hemolysins		
hla	+	+
hlg, *hlgv*	+ (5/7)	+
hlb	(+)[b]	(+)[b]
Peptide cytolysin		
psmα, *hld*	+	+
Enterotoxin		
SaPIm1/n1 (*tst*, *sec*, *sel*)	−	+
SaPInn54 (*seb*)	+	−
egc (*seg*, *sei*, *sem*, *sen*, *seo*)	+	+
sep	−	+ (2/5)
Adhesin		
c12ag[c]	+	+
ACME		
arcA	+	−
opp-3C	−	−
Drug resistance (non-β-lactams)[d]	ERY, CLI, GEN, KAN, TET, FOS, LVX	ERY, CLI (3/5), GEN (4/5), KAN, TET, FOS (2/5), LVX, VAN (1/5)[e]

[a] ST764 ($n = 7$) included NN54, NN37, NN35A, NN35B, NN41F1G, NN41F1F, and NN41F1D; ST5/SCC*mec*II ($n = 5$) included type strains N315 and Mu50 and clinical isolates I6, I8, and I10. +, positive; −, negative. Unique characteristics of ST764 are shaded.

[b] Split *hlb* gene.

[c] *c12ag*, core 12 adhesin genes shared by all strains: *icaA* and *icaD* (for biofilm formation); *eno* (for laminin-adhesin); *fnbA* and *fnbB* (for fibronectin-adhesin); *ebpS* (for elastin-adhesin); and *clfA*, *clfB*, *fib*, *sdrC*, *sdrD*, and *sdrE* (for fibrinogen-adhesin).

[d] ERY, erythromycin; CLI, clindamycin; GEN, gentamicin; KAN, kanamycin; TET, tetracycline; FOS, fosfomycin; LVX, levofloxacin; VAN, vancomycin.

[e] Strain Mu50 was vancomycin intermediate (31).

ST5 MRSA. In the present screening test for household colonization in Niigata, ST764 MRSA was the second most frequent isolate, following ST8 CA-MRSA (data not shown).

Comparative genomics of ST764 MRSA (strain NN54). The NN54 genome was estimated to be at least 2.8 Mb, showing approximately 99.7% homology with the N315 genome, albeit with marked divergence in mobile DNA structures (Fig. 1). There were four insertions in the NN54 genome (Fig. 1). One insert (23.5 kb) consisted of cassette JR1 (cJR1) and ACMEII, inserted in tandem between *orfX* (the *att* site) and SCC*mec*II (of N315); the entire structure is described below. The second insert was 13.5 kb, carried the *seb* gene, and was inserted into the noncoding region, located downstream of transfer-messenger RNA (tmRNA); this insert was designated SaPInn54 (Fig. 1), and the entire structure is described below.

The third insert was the 44.5-kb prophage φ2 (designated φ2$_{NN54}$), which was inserted into the hypothetical protein gene (SA1320) (Fig. 1). The prophage φ2 group includes two types (49): one carrying the PVL gene (e.g., φSa2$_{USA300}$ of USA300) (22) and the other without the PVL gene. NN54 carried the latter type. Compared to φ2 group phages, φ2$_{NN54}$ showed the highest homology (85.3%) to φSa2$_{USA300}$.

The fourth insert was the *tetM*-carrying Tn*5251* of *Streptococcus pneumoniae*, which was inserted into the noncoding region, located downstream of the hypothetical protein gene (SA0368). The *tetM*-carrying Tn*5251* (Tn*916*-like genetic element) is part of the composite conjugative transposon of *S. pneumoniae* (50). Tn*5251* is inserted into Tn*5252* to form the composite element Tn*5253* (50); however, NN54 lacked Tn*5252*, consistent with the previous notion that Tn*5251* behaves as an independent, fully functional conjugative transposon (51).

NN54 carried the pN315-like sequence, which is similar to the penicillinase plasmid (pN315) of N315 (31), albeit with some divergence; NN54 (pN315-like) carried the truncated β-lactamase gene (Δ*blaZ*) and fosfomycin resistance protein gene (*fosD*), for example.

As for deletions, although N315 possessed four copies (*a*, *b1*, *b2*, and *c*) of the erythromycin resistance-encoding Tn*554*, three copies (*b1*, *b2*, and *c*) were deleted in the NN54 genome; NN54 carried only a Tn*554* copy, corresponding to Tn*554a*. Moreover, ST764 lacked superantigen genes *tst*, *sec*, and *sel* compared to N315; this was because ST764 carried SaPInn54-2, which lacked *tst*, *sec*, and *sel*, while N315 carried SaPIn1 with *tst*, *sec*, and *sel* (homology between SaPInn54-2 and SaPIn1 was 62.3%) (Fig. 1). ST764 also lacked *sep*; ST764 carried φ3$_{NN54}$ without *sep*, while N315 carried φN315 with *sep* (homology between φ3$_{NN54}$ and φN315 was 86.5%) (Fig. 1).

Comparative genomic analysis between NN54 and N315 demonstrated further divergences, e.g., a gene mutation causing *ebhA-ebhB* fusion (for a putative adhesin). More detailed comparative genomic analysis between ST764 and ST5 (including predicted gene products) is illustrated in Table S1 in the supplemental material.

Structure of cJR1-ACMEII-SCC*mec*II. NN54 carried the three-cassette array cJR1-ACMEII-SCC*mec*II, each demarcated by the 15-bp *att* direct-repeat sequences, as shown in Fig. 2 (middle).

ACMEII carried a 6.2-kb *arc* cluster very similar (99.9% homologous) to that of ACMEI (of USA300) but lacked the *opp-3* cluster (unlike USA300) (Fig. 2, bottom). Moreover, ACMEII carried the intact transposase gene (*tnp*) of IS*431*, while IS*431-tnp* in ACMEI had a frameshift mutation.

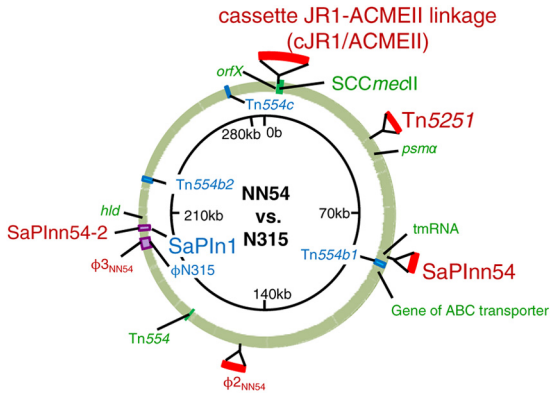

FIG 1 Comparative genomics of ST764 MRSA strain NN54. The NN54 genome contigs were mapped on the N315 (ST5) genome (shown as a circle). Information on the NN54 and N315 genomes is presented outside and inside the genome circle, respectively. Colored regions in NN54: green, highly homologous to N315; blue, deletion; red, insertion; purple, SaPI or prophage diversity.

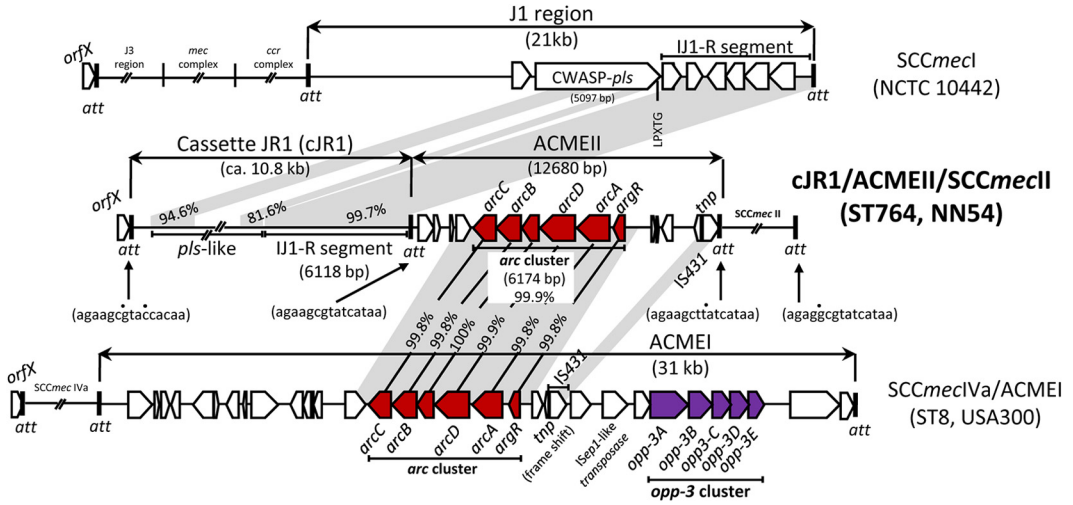

FIG 2 Structure of the cJR1-ACMEII-SCC*mec*II region on the NN54 genome. A novel *att*-mediated array (cJR1-ACMEII-SCC*mec*II) (middle) was compared to the J1 region of SCC*mec*I (top) and ACMEI (bottom); the GenBank accession numbers are AB687558, AB033763, and CP000255, respectively. Homologous regions are shaded. Dots above 15-bp *att* sequences show a divergent nucleotide (from *orfX att*).

ACME of CA-MRSA USA300, carrying ACME *arcA* and *opp-3*, exhibits multiple functions and is considered to enhance the growth and survival of USA300; USA300 with ACME outcompeted an isogenic strain without ACME in pathogenicity and fitness in a rabbit infection model (26, 27). It is also thought that ACME enhances colonization and survival on the skin (26). This model is also supported by previous observations that arginine deiminase, encoded by *arcA*, e.g., inhibits human peripheral blood mononuclear cell proliferation (22, 52) and protects bacteria against damage caused by acidic environments through the production of NH_3 (53) and that oligopeptide permease (Opp), encoded by the *opp-1* and *opp-2* operons in *S. aureus*, is a member of the ABC transporter family and displays multiple functions, including bacterial growth in animal infection models (54). In contrast to the above-mentioned ACMEI, the role of ACMEII, which lacks the *opp* region of ACMEI, remains less understood.

As for cJR1, the right-side 6.1-kb region showed high homology (99.7%) to the right-side J1-joining region of SCC*mec*I (Fig. 2, top). Although the SCC*mec*I J1 region contained the 5,097-kb large *orf* (*pls*), encoding a cell-wall-anchored surface protein (CWASP) with the LPXTG motif (55), the *pls*-homologous sequence (*pls*-like) in cJR1 had a deletion; due to sequence repetitions, the *pls*-like sequence remained incomplete. cJR1 possessed no *tnp* gene.

The function of cJR1 remains unknown; however, it carries part of the J1 region, containing the putative surface protein gene (*pls*-like) and may act as an adhesin and contribute to students', as well as household members', carriage, in combination with ACMEII. Another example, in which the J1 region of SCC*mec* encodes a putative surface protein (a possible adhesin), is ST8 CA-MRSA/J, a successful CA-MRSA clone in Japan (56, 57). CA-MRSA/J is characterized by (i) genotype ST8/*spa*606(t1767)/*agr*1/CoaII; (ii) SCC*mec*IV of a novel subtype, which is expressed as IV.new.1.1 (now designated IVl), reflecting the differences in the three nonessential component regions of SCC*mec* (J1, J2, and J3), according to the guidelines (2); (iii) mosaic SaPIj50 with the *tst*, *sec*, and *sel* genes; and (iv) high expression of the *psm*α and *hld* genes, similar to USA300. CA-MRSA/J is negative for PVL and ACME.

In NN54, the cJR1-ACMEII array seems to have been inserted as a unit at the *orfX att* site. During the course of this study, Shore et al. described ACMEII in MRSA with ST22/SCC*mec*IVh (58); in their strain (M08/0126), the ACMEII-ΔJ1SCC*mec*I array is inserted at the *att* site between *orfX* and SCC*mec*IVh, resulting in the structure *orfX*-ACMEII-ΔJ1SCC*mec*I-SCC*mec*IVh (therefore, in opposite orientation in this study, *orfX*-cJR1-ACMEII-SCC*mec*II).

Structure of SaPInn54. The entire structure of SaPInn54 is shown in Fig. 3 (middle). SaPIs are actually phage-related chromosomal islands and represent phage satellites producing phage-like infectious particles, and the integrase gene (*int*) and replication initiator gene (*rep*) play roles in SaPI transfer (59). The SaPI core region (9.5 kb), carrying the *rep* and *int* genes, of SaPInn54 was very similar to the corresponding region of SaPImw2, which is present in CA-MRSA USA400 strain MW2. They also shared the same 15-bp *att* sequence and insertion site on the chromosome (Fig. 3, top and middle); however, the virulence gene-carrying regions of SaPInn54 and SaPImw2 were divergent. SaPInn54 carried the *seb* gene, while SaPImw2 carried the *sec* and *sel* genes (although the two SaPIs shared similar *ear* genes, encoding a putative penicillin-binding protein [PBP]).

The 2.0-kb *ear*- and *seb*-carrying segment of SaPInn54 was very similar to that of SaPI3 in ST250 MRSA COL (Fig. 3, bottom) and ST59 MRSA PM1 (data not shown). The SaPI core region (carrying the *rep* and *int* genes) and *att* of SaPInn54 and SaPI3 were divergent (Fig. 3, bottom).

The *seb* genes of SaPInn54 and SaPI3 were not the same: homology was 97.4% at the nucleotide level and 96.6% at the amino acid level; mature SEB1, produced from SaPI3, and SEB2, produced from SaPInn54, differed by 5 amino acids (see Fig. S1 in the supplemental material). SEB1 was identical to purified SEB protein (Protein Data Bank [PDB] code ISE4A); therefore, SEB2 represents a new SEB variant. SEB production levels, immunologically determined, were 14.3 μg/ml for NN54, 14.3 μg/ml for COL, and 31.3 μg/ml for PM1.

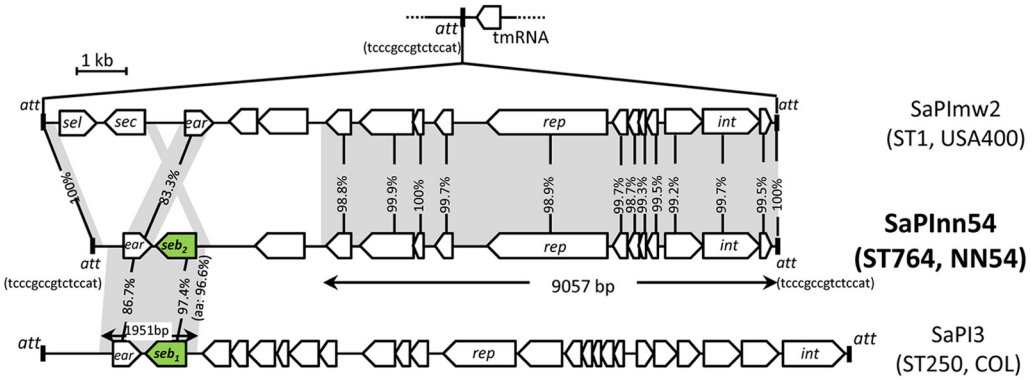

FIG 3 Structure of SaPInn54 carrying the SEB gene on the NN54 genome. A novel mosaic, SaPInn54 (middle), was compared to SaPImw2 (top) and SaPI3 (bottom); the GenBank accession numbers are AB690437, NC_003923, and SaPI3 CP000046, respectively. Homologous regions are shaded.

SEB is a superantigen (60) and a potential biological weapon (category B bioterrorism agent) (61). Since SEB is also considered to play a role in immune evasion upon staphylococcal infection (62), SEB may contribute to community infection, similar to successful ST59 CA-MRSA with SaPI3 (e.g., strain PM1) in Taiwan (37).

mRNA expression levels of virulence genes. As shown in Fig. 4, for the PSMα and δ-hemolysin genes (*psm*α and *hld*, respectively), mRNA expression levels of ST764 strains were similar to those of USA300 but significantly higher than those of ST5 ($P < 0.05$), suggesting that ST764 MRSA possessed enhanced cytolytic peptide genes (with high expression) of CA-MRSA. Similar to USA300 (26), high levels of PSMα (and δ-hemolysin) in ST764 MRSA may contribute to bacteremia.

For the ACME *arcA* gene, the mRNA expression levels of ST764 MRSA (carrying ACMEII) and ST8 CA-MRSA USA300 (carrying ACMEI) were similar (Fig. 4). Further experiments in animal infection models are needed to evaluate the virulence of ST764 MRSA.

Origin of ST764 MRSA. The origin of ST764 MRSA is not known; however, most probably it evolved from ST5/SCC*mec*II MRSA in Japan (Niigata), because its ST is a novel ST5 variant, and the unique genetic features of ST764, as described in this study, have not been reported previously.

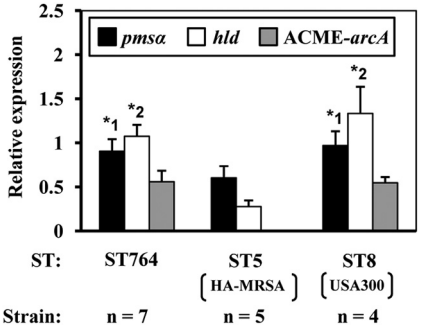

FIG 4 Levels of mRNA expression for cytolytic peptide genes (*psm*α and *hld*) and the ACME *arcA* gene of ST764 MRSA compared to those of ST5/SCC*mec*II MRSA and USA300. The ST764 and ST5 strains are described in Table 1. The ST8 strains included the type strain USA300-0114 and three Japanese isolates NN36, NN47, and 549. For each strain, mRNA expression levels were measured in triplicate. The experiment was independently repeated three times. The *psm*α and *hld* mRNA expression levels in ST764 and ST8 were similar, but their levels (*1 and *2) were significantly higher than in ST5 ($P < 0.05$). ACME *arcA* mRNA expression levels in ST764 and ST8 were similar (ST5 lacked the ACME *arcA* gene).

Conclusions. In summary, ST764 MRSA, a novel hybrid variant of the ST5 HA-MRSA lineage with the characteristics of CA-MRSA, has emerged in Niigata, Japan, causing invasive infections (necrotizing fasciitis and bacteremia) and carried by medical students in hospitals and also producing carriers in the community. The evolution of ST764 MRSA includes multiple steps involving the insertion of genetic structures, cJR1/ACMEII, SaPInn54 (with *seb2*), $\varphi 2_{NN54}$ (lacking the PVL gene), and streptococcal Tn*5251* (carrying *tetM*), and the acquisition of enhanced expression of the *psm*α and *hld* genes, similar to CA-MRSA. Further divergence was observed in SaPI (at *groEL*), φ3, plasmid pN315, and several chromosomal genes.

ACKNOWLEDGMENTS

We thank H. de Lencastre for the BK2464 and COL strains, L. K. McDougal and L. L. McDonald for the USA300-0114 strain, and K. Hiramatsu for the N315 and Mu50 strains.

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