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Note

Characterization of methicillin-resistant *Staphylococcus aureus* isolated from tertiary care hospitals in Tokyo, Japan

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ABSTRACT

The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) remains problematic in both hospital and community settings. Investigations of MRSA existing in the local area are necessary to understand the detailed epidemiology of healthcare-associated MRSA (HA-MRSA). In the present study, molecular epidemiological analysis was performed on 584 MRSA isolated from four hospitals in Tokyo, Japan. In the pulsed-field gel electrophoresis (PFGE) analysis, four epidemic pulsotypes (I to IV) were found. The isolates of the epidemic pulsotype I mainly consisted of the SCC*mec* type II, toxic shock syndrome toxin 1 gene (*tst*)-negative, *spa* type t002, and ST764 clones. The ST764 clone, which is a novel hybrid variant of the ST5 HA-MRSA lineage with the arginine catabolic mobile element (ACME), was first found in Niigata, Japan. However, no ACME genes were detected in the isolates of the epidemic pulsotype I. In contrast, the other isolates of the epidemic pulsotypes mainly consisted of the SCC*mec* type II, *tst*-positive, *spa* type t002, and ST5 clones, which are the most predominant clones of HA-MRSA in Japan. Resistance rates of non-β-lactams for the isolates of the epidemic pulsotype I were higher than those of the other epidemic pulsotypes. Our data showed that the novel ACME-negative ST764 clones are being distributed throughout multiple hospitals in Tokyo. The ST764 clones in Tokyo have the potential to acquire ACME in the future, because the ACME-positive ST764 clones have already been found in both hospital and community settings in other areas of Japan.

Colonization of methicillin-resistant *Staphylococcus aureus* (MRSA), which shows multidrug resistance, restricts therapeutic options and has become a serious problem worldwide. In Japan, the isolation rate of MRSA in healthcare settings is higher than that in other countries [1]. The characteristics of typical Japanese health-care associated MRSA (HA-MRSA) is the staphylococcal cassette chromosome *mec* (SCC*mec*) type II, *spa* type t002, and multi-locus sequence type (MLST) 5 strains, which is one of the worldwide MRSA clones [2]. Until 2000, MRSA was mainly confined to healthcare settings, but the emergence of community-acquired MRSA (CA-MRSA) has changed the global epidemiology of MRSA infections [3]. It is thought that CA-MRSA possesses unique features compared to HA-MRSA, which may be related to its enhanced virulence. Two such candidates are Panton-Valentine Leukocidin (PVL) and the arginine catabolic mobile element (ACME) found in ST8 CA-MRSA USA300, which is the most common clone in the United States [4]. Recently, ST764 MRSA, a novel hybrid variant of the ST5 HA-MRSA lineage with the characteristics of CA-MRSA, strain NN54 was first found in Niigata, Japan [5]. Despite the HA-MRSA clones, ST764 strains carry the ACME *arc* cluster adjacent to their type II SCC*mec* element. Additionally, SaPIm1/n1 carrying the toxic shock syndrome toxin 1 (TSST-1) gene, which has been

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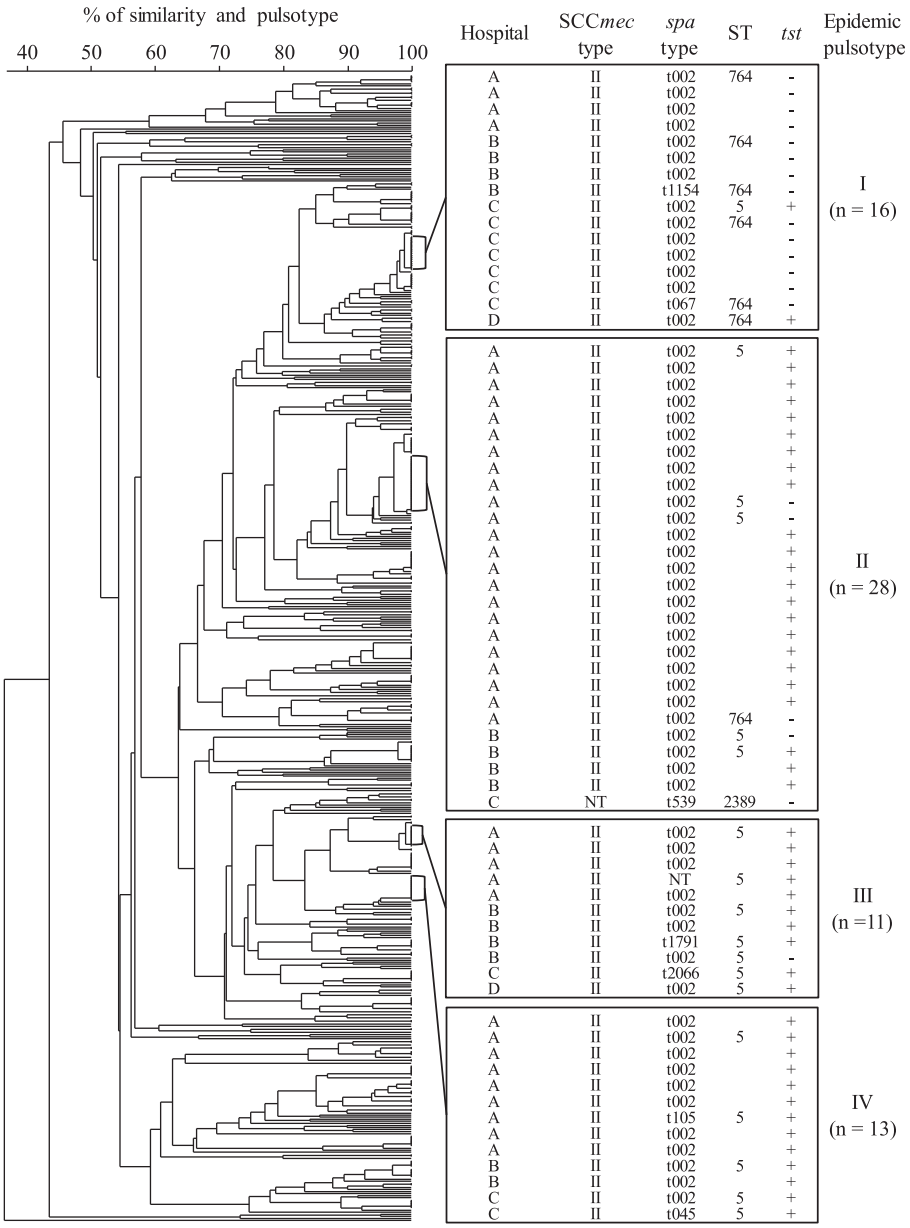

Fig. 1. Molecular epidemiological analysis of MRSA isolates from four hospitals ($n = 554$) based on PFGE, SCC*mec* type, *spa* type, and MLST. The open boxes indicate the isolates belonging to epidemic pulsotypes I, II, III, or IV.
NT, non-typeable.

frequently found in ST5 strains, is not found in strain NN54. In contrast, SaPInn54, carrying the staphylococcal enterotoxin B (SEB) gene, is located on the genome of strain NN54. Furthermore, the ACME-positive ST764 clones were isolated from outpatients in Hokkaido, Japan [6]. Therefore, dissemination of the ACME-positive ST764 clones is of concern in both healthcare and community settings in Japan.

The prevalence of MRSA remains problematic in both hospital and community settings. Investigations of MRSA existing in the local area are necessary to understand the detailed epidemiology of HA-MRSA. In the present study, we performed molecular epidemiological analysis on the epidemic isolates of MRSA isolated from four tertiary care hospitals in Tokyo, Japan.

In 2009, 584 MRSA isolates were collected from Hospitals A (271 isolates), B (121 isolates), C (125 isolates), and D (65 isolates). SCC*mec* typing was performed according to the method of Kondo et al. [7]. PCR assays for the detection of *mecA*, *tst* encoding TSST-1, *pvl* encoding PVL, and *seb* encoding SEB were performed as described previously [8]. The detection of the ACME *arcA* and *opp-3C* genes were performed according to the method of Takano et al. [5]. Differences in the detection rates of the virulence genes were tested by the χ^2 test using JMP software (SAS Institute Inc., NC, USA). *P* values <0.05 were considered statistically significant. PFGE analysis was performed as described previously [8]. Isolates exhibiting 100% similarity were determined to be identical pulsotypes [9]. The pulsotypes, including more than ten isolates, which were isolated from at least three hospitals, were defined as the epidemic pulsotype. *spa* typing was performed as described by Shopsin et al. [10]. MLST was performed as described by Enright et al. [11]. MICs were determined by the agar doubling-dilution method, according to the CLSI guidelines [12]. The following antimicrobial agents were used: ampicillin (Wako Pure Chemical