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NOTE

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Emergence of the community-acquired methicillin-resistant *Staphylococcus aureus* USA300 clone in Japan

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Abstract We isolated methicillin-resistant *Staphylococcus aureus* (MRSA) from a 3-month-old Indian girl who was born in the United States, moved to Japan, and suffered from subcutaneous abscesses in 2007. The MRSA (strain NN36) belonged to multilocus sequence type (ST) 8, exhibited *agr*1, staphylococcal cassette chromosome *mec* (SCC*mec*) type IVa, and coagulase type III, and was positive for Panton-Valentine leukocidin (PVL) and the arginine catabolic mobile element (ACME), just like the USA300 clone, which is the predominant community-acquired MRSA (CA-MRSA) in the United States. Strain NN36 shared an identical pulsed-field gel electrophoresis (PFGE) pattern with the USA300 clone. Although the USA300 clone is of *spa*1, strain NN36 possessed *spa*985. Strain NN36 was resistant to erythromycin and kanamycin, in addition to β-lactam agents (e.g., oxacillin). The data suggest that the USA300 clone has emerged in Japan. Because the USA300 clone has recently spread to European countries, surveillance of the USA300 clone should be actively performed in Japan.

Key words Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) · Panton-Valentine leukocidin (PVL) · Arginine catabolic mobile element (ACME) · Abscess

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Since the late 1990s, community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has emerged worldwide as a life-threatening pathogen in the community.[1] CA-MRSA is primarily associated with skin soft tissue infection (SSTI) in otherwise healthy children or adolescents, such as athletes, in the community.[1,2] It is also associated with severe systemic infections such as sepsis and necrotizing pneumonia.[1] CA-MRSA is genetically heterogeneous, and includes a variety of clones such as multilocus sequence type (ST) 1 (USA400 clone) and ST8 (USA300 clone), which has emerged as a major clone in the United States;[3] ST80, which has emerged as a major clone in Europe;[4] and ST30, which has spread worldwide.[4–6] The USA300 clone has the ability to replace preexisting MRSA clones, and now is the predominant CA-MRSA clone in the United States.[3] Many strains of the USA300 clone are multiple drug-resistant.[3,7] The USA300 clone has recently spread to several European countries.[8] In this study, we describe the first isolation of the USA300 clone in Japan.

The patient was a 3-month-old Indian girl. She was born in a hospital in South Alameda, California, United States, stayed there for 1 month, and then moved to Tokyo with her parents (28-year-old father and 27-year-old mother). On May 15, 2007, abscess formation was noted in the right cervical region, and she was brought to the Department of Pediatrics, Tokyo Metropolitan Hiroo General Hospital. As fever (37.7°C) and an increase in the inflammatory response (white blood cell [WBC] count, 13 700/ml; C-reactive protein [CRP], 2.1 mg/dl) were observed, she was admitted on May 18. Physical findings on admission included a subcutaneous abscess measuring 10 × 10 mm in diameter in the right temporal region and a subcutaneous abscess measuring 30 × 20 mm in diameter, with flare and pressure pain, in the right cervical region (Fig. 1). As empiric therapy, cefazolin (CEZ) at 80 mg/kg per day was started. Three days after admission, pyretolysis was achieved; however, hemorrhage from the subcutaneous abscess in the right temporal region was noted, and gentamicin (GM) was administered. Six days after admission, incision and drainage were performed, and a drain was inserted; however, fever (38°C or higher) persisted thereafter, and MRSA was detected from

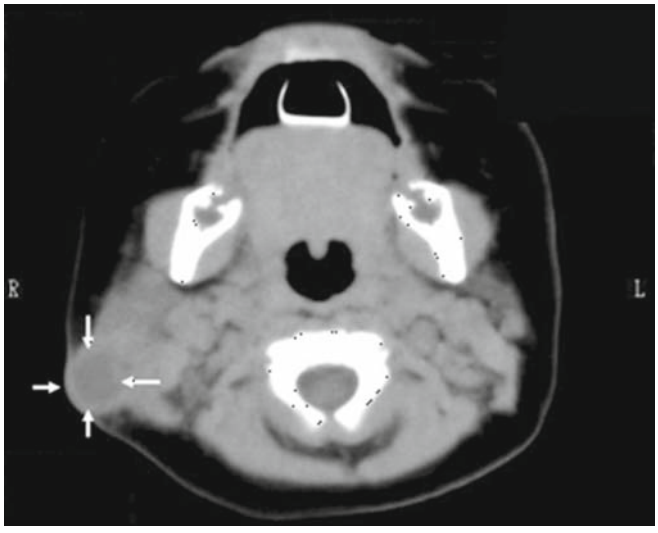

Fig. 1. Computed tomography (CT) showing a subcutaneous abscess in the right cervical region. *Arrows* point to the subcutaneous abscess

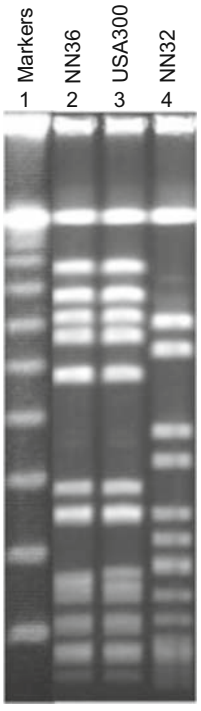

Fig. 2. Pulsed-field gel electrophoresis (PFGE) analysis of methicillin-resistant *Staphylococcus aureus* (MRSA) ST8 (strain *NN36*) from a 3-month-old infant in Japan (*lane 2*), in comparison with Panton-Valentine leucocidin (PVL)-positive community-acquired (CA)-MRSA ST8 from the United States (the *USA300* clone [USA300-0114]; *lane 3*) and PVL-positive CA-MRSA ST30 (strain *NN32*) from a fatal case of pediatric pneumonia in Japan *(lane 4*). *M*, molecular size standard

temporal and cervical subcutaneous abscesses (blood culture showed a negative reaction). CEZ was therefore switched to vancomycin (VCM) at 10 mg/kg per dose, which was administered at 6-h intervals from 7 days after admission; pyretolysis was achieved immediately. The subsequent course was favorable and VCM administration was completed 23 days after admission. As her general condition was good, the girl was discharged 26 days after admission (June 12).

Molecular characterization of MRSA was performed as described previously.[5] Briefly, multilocus sequence typing (MLST) was performed using seven housekeeping genes, and an allelic profile (allele no.) was obtained from the MLST website (http://www.mlst.net/). The *spa* type was analyzed by PCR, and determined using a public *spa* type data base (http://tools.egenomics.com/) or (http://spaserver.ridom.de/). Detection of the accessory gene regulator (*agr*) allele group was done by PCR with the reported primers.[9] The staphylococcal cassette chromosome *mec* (SCC*mec*) types (I to V) were analyzed by PCR, using reference strains.[10,11] Coagulase typing was conducted using a staphylococcal coagulase antiserum kit (Denka Seiken, Tokyo, Japan) in accordance with the manufacturer's instructions. PCR analysis of the Panton-Valentine leukocidin (PVL) gene was performed with the reported primers. The arginine catabolic mobile element (ACME) was determined by detection of the *arcA* gene by PCR.[7] For pulsed-field gel electrophoresis (PFGE) analysis, bacterial DNA was digested with SmaI, and the digested DNA was applied to PFGE (1.2% agarose). A lambda ladder (Bio-Rad Laboratories, Tokyo, Japan) was used as the molecular size standard. Susceptibility testing of bacterial strains was done by the agar dilution method with Mueller-Hinton agar according to previous procedures.[12] Thirty-five antimicrobial agents were used. They included oxacillin (OXA) and ampicillin (ABPC) for penicillins; CEZ, cefaclor (CCL), cefotiam (CTM), cefotaxime (CTX), ceftazidime (CAZ), and cefpirome (CPR) for cephems; latamoxef (LMOX) and flomoxef (FMOX) for oxacephems; imipenem (IPM), panipenem (PAPM), meropenem (MEPM), and biapenem (BIPM) for carbapenems; GM, kanamycin (KM), streptomycin, and arbekacin for aminoglycosides; VCM and teicoplanin for glycopepdides; linezolid for oxazolidinones; tetracycline (TC) and minocycline for tetracyclines; erythromycin (EM) for macrolides; clindamycin (CLDM) for lincosamides; norfloxacin (NFLX), ciprofloxacin (CPFX), and levofloxacin for fluoroquinolones; and chloramphenicol, fosfomycin, trimethoprim, sulfamethoxazole, fusidic acid, rifampicin, and mupirocin (MUP). Breakpoints for drug resistance were those described by the Clinical and Laboratory Standards Institute (CLSI).[12] Inducible resistance to CLDM was examined as described.[12] Plasmid DNA of MRSA strains was prepared using the Plasmid Midi Kit (Qiagen Sciences, Tokyo, Japan). The USA300 type strain (SmaI PFGE pattern number, USA300-0114),[3] which was kindly provided by L.K. McDougal and L.L. McDonald (Center for Disease Control and Prevention), was used as a reference strain.

The isolated MRSA (strain NN36) shared an identical PFGE pattern with the USA300 type strain (Fig. 2). Just like the USA300 clone,[3,7] strain NN36 belonged to ST8, exhibited *agr*1, SCC*mec*IVa, coagulase type III,[5] and was positive for PVL and ACME. Although the USA300 clone is of *spa*1 (alternatively t008), strain NN36 possessed *spa*985 (alternatively t711), indicating a slight divergence.

The MIC value of OXA for strain NN36 was low (8 μg/ml). The MICs (μg/ml) of other β-lactam agents for strain NN36 were 16 for ABPC, 2 for CEZ, 16 for CCL, 2 for CTM,

8 for CTX, 32 for CAZ, 1 for CPR, 32 for LMOX, 2 for FMOX, 0.06 for IPM, 0.12 for PAPM, 0.5 for MEPM, and 0.12 for BIPM.

Regarding non-β-lactam antimicrobial agents, strain NN36 was resistant to EM and KM, and carried two plasmids of 52 kb and 1.5 kb in size. The USA300 type strain was resistant to EM, KM, TC, and NFLX (intermediate for CPFX), and carried three plasmids of 47 kb, 6.9 kb, and 5.8 kb in size. Because the other USA300 clone (strain FPR3757) had three plasmids, a 37-kb plasmid (encoding for EM, CLDM, and MUP resistance), a 4.4-kb plasmid (encoding for TC resistance), and a 3.1-kb cryptic plasmid,[7] most probably strain NN36 lacked a small (around 4- or 5-kb) TC-encoding plasmid. Moreover, in contrast to the USA300 strain FPR3757, strain NN36 (and the USA300 type strain) was susceptible to CLDM and MUP.

In Japan, PVL-positive ST30 MRSA is associated with (e.g.) bullous impetigo in a child (strain isolated, NN1[13]), pelvic abscesses in an athlete (strain isolated, NN12[13]), and fatal pneumonia (not associated with influenza) in a child (strain isolated, NN32[14]). No PVL-positive ST1, ST8, or ST80 MRSA strains have been found in Japan. The present study demonstrates the first isolation of the USA300 clone (PVL-positive ST8 MRSA) in Japan. Because (1) the patient was born in the United States and moved to Japan, (2) the parents had no history of SSTI within the past year, (3) the patient was always with her mother and had never been in other facilities, such as a nursery school, and (4) no MRSA was isolated from the parents, the USA300 clone (strain NN36) could have originated in the United States (most probably a hospital in South Alameda, California). Because the USA300 clone is growing as a worldwide CA-MRSA,[8] is becoming a multiple drug-resistant clone,[3,7,8] and is associated with severe invasive infections, such as (influenza-associated) epidemic community-acquired pneumonia[15] and sepsis,[1] surveillance of the USA300 clone should be actively performed in Japan, similar to surveillance in the United States and Europe.

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