

First report of Pantone–Valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus* ST88 harbouring Φ Sa2usa isolated from refractory breast abscesses in Japan

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Abstract

A methicillin-susceptible *Staphylococcus aureus* with Pantone–Valentine leukocidin (PVL) genes was isolated from refractory breast abscesses of 12-year-old girl in Japan, and classified into ST88, *spa*-t1245 and *coa*-IIIa. This strain harboured PVL phage Φ Sa2usa, which is usually found in ST8 community-acquired methicillin-resistant *S. aureus* clone USA300.

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Keywords: Pantone–Valentine leukocidin, refractory abscess, *Staphylococcus aureus*, ST88, Φ Sa2usa

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Staphylococcus aureus causes various infections in community as well as healthcare settings. Pantone–Valentine leukocidin (PVL), a two-component toxin encoded by two genes *lukF-PV* and *lukS-PV* carried on lysogenic bacteriophages [1], has been demonstrated to have a significant role in the pathogenesis of *S. aureus*, especially community-acquired methicillin-resistant *S. aureus* (MRSA) [2]. However, PVL is also associated with increased

virulence of methicillin-susceptible *S. aureus* (MSSA), as represented by ST121 clone [3]. Here we report the detection of PVL-positive ST88 MSSA in Japan.

A 12-year-old previously healthy girl was admitted to our hospital for exacerbated redness and swelling of her left breast. Enhanced computed tomography and echography findings revealed that she had multiple abscesses in her left breast, of which the largest was 5 × 3 × 3 cm (Fig. 1). After incision and drainage of her abscesses, cefazolin sodium (70 mg/kg/day) was administered intravenously for 12 days. However, the condition of the breast abscesses was not improved. Accordingly, reoperation with extensive drainage under general anaesthesia was conducted. After administration of cefazolin sodium for another 9 days, the breast abscesses had mostly regressed and the patient was discharged with oral cefaclor for 7 days at home. She has been fully recovered without recurrence for 6 months. One month before this admission, she had been treated for skin abscess in her left femur caused by MSSA. Because of her recurrent abscess episodes, we suspected an immunodeficiency. However, laboratory tests revealed no neutropenia, and neutrophils showed normal phagocytosis and generated enough superoxide anion. Lymphocyte subset, serum immunoglobulin and complement level were also normal. The patient and her family members had no medical history and had never been abroad.

An MSSA strain designated NK1119 was isolated from the abscesses. The strain NK1119 was classified into ST88, *spa*-type t1245, *agr* group III and coagulase genotype IIIa. This strain was resistant to ampicillin (MIC, 128 mg/L) and tetracycline (MIC, 64 mg/L) having *bla_Z*, *tet(K)* and *tet(L)*, but susceptible to all the other 17 antimicrobials examined including cefazolin. Further genetic analysis revealed the presence of genes for PVL, enterotoxins (*sec*, *seh*, *sei*, *sel*), haemolysins (*hla*, *h1b*, *hlg*, *hlg2*), core/accessory adhesins (*clfA*, *sdrD*, etc.), and chemotaxis inhibitory protein (*chp*), whereas other enterotoxins, exfoliative toxins, toxic shock syndrome toxin-1 (TSST-1) and arginine catabolic mobile element were negative. The PVL genes of NK1119 were assigned to haplotype group R by sequence analysis [4], and PVL phage was identified as Φ Sa2usa by the PCR scheme as described previously [4,5]. For further confirmation, we determined sequences at both ends of PVL prophage: a 5189-bp sequence containing *lukF-PV/lukS-PV* and ϕ SLT ORF484-like protein (lysin) gene, and a 1279-bp sequence with a partial integrase gene (GenBank Accession nos KX130855 and KX130854, respectively). These sequences showed 99%–100% identity to those of Φ Sa2usa in the genome of ST8 strains (e.g. USA300_FPR3757, GenBank Accession no. CP000255) [6]. For the present case, cefazolin and cefaclor were administered because anti-staphylococcal penicillins are

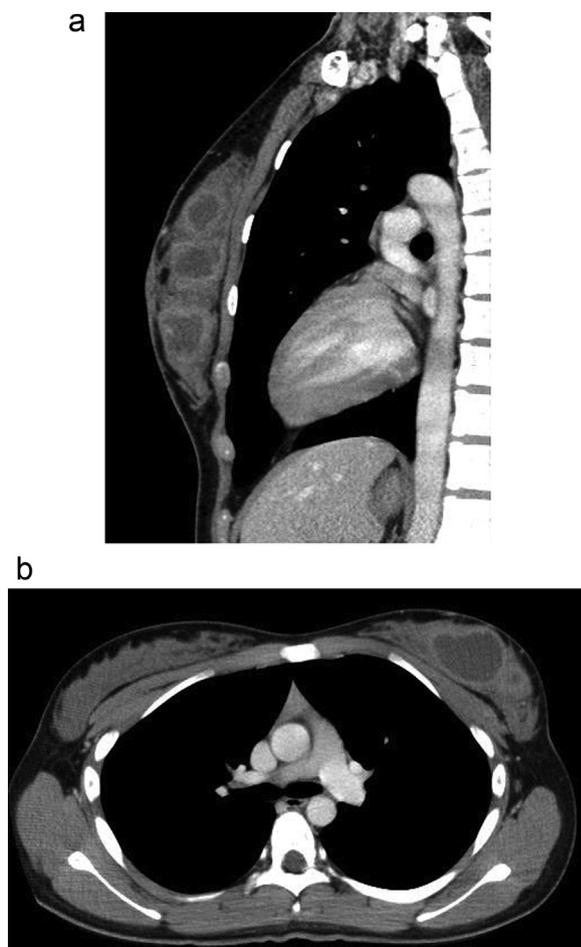


FIG. 1. Enhanced computed tomography scan of the patient's chest. Sagittal image (a) showed three abscesses in her left breast. Axial image (b) indicated the largest one located at the top.

not available in Japan, and the causative MSSA isolate was susceptible to these drugs. However, considering the patient's recent history of skin abscess due to MSSA suggestive of PVL production, an antimicrobial agent reducing toxin production, such as clindamycin, would also be effective, and may be recommended for similar cases.

ST88 has been a relatively rare lineage distributed globally among MRSA and MSSA with and without PVL [7]. In Asia, ST88 *S. aureus* from clinical specimens has been reported in Bangladesh, Myanmar, Nepal, and somewhat frequently in China [8]. In Japan, PVL-negative ST88 MRSA isolates producing exfoliative toxin A were isolated from bullous impetigo as well as the nasal cavity of a paediatric patient [9–12]. There is only one report of a PVL-positive ST88 MSSA isolate from an outpatient with suppurative arthritis [13]. Hence, our present report described the first case of a severe infection with ST88 MSSA with PVL in Japan. Although the reason for the refractory symptoms in the present case was unknown, it was noted that

the strain NK1119 possessed Φ Sa2usa, which is usually found in ST8 community-acquired MRSA clone (USA300), which is highly virulent and spreading in the USA [5,6]. In contrast, ST88 strains have been described as having other PVL phages with a PVL gene of haplotype group H2 [4,14]. In Japan, ST8 MRSA appears to have been distributed in the community gradually since the late 2000s [15–17]. Therefore, it is conceivable that Φ Sa2usa might have been transmitted from the emerging ST8 MRSA to ST88 *S. aureus*, which is distributed in the community. Further genetic analysis may elucidate the mechanisms by which the strain NK1119 acquired Φ Sa2usa with increased virulence. This report reminds clinicians of the presence of a novel PVL-positive MSSA in Japan.

Conflict of Interest

The authors have no conflicts of interest to declare.

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