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深部感染症から分離された

黄色ブドウ球菌の臨床的・分子疫学的解析

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First report of Panton–Valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus* ST88 harbouring ΦSa2usa isolated from refractory breast abscesses in Japan

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Abstract

A methicillin-susceptible *Staphylococcus aureus* with Panton–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 ΦSa2usa, which is usually found in ST8 community-acquired methicillin-resistant *S. aureus* clone USA300.

Keywords: Panton–Valentine leukocidin, refractory abscess, *Staphylococcus aureus*, ST88, ΦSa2usa
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Staphylococcus aureus causes various infections in community as well as healthcare settings. Panton–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 administrated 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 *blaZ*, *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*, *hlb*, *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 ΦSa2usa 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 phiSLT 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 ΦSa2usa in the genome of ST8 strains (e.g. USA300_FPR3757, GenBank Accession no. CP000255) [6]. For the present case, cefazolin and cefaclor were administrated because anti-staphylococcal penicillins are

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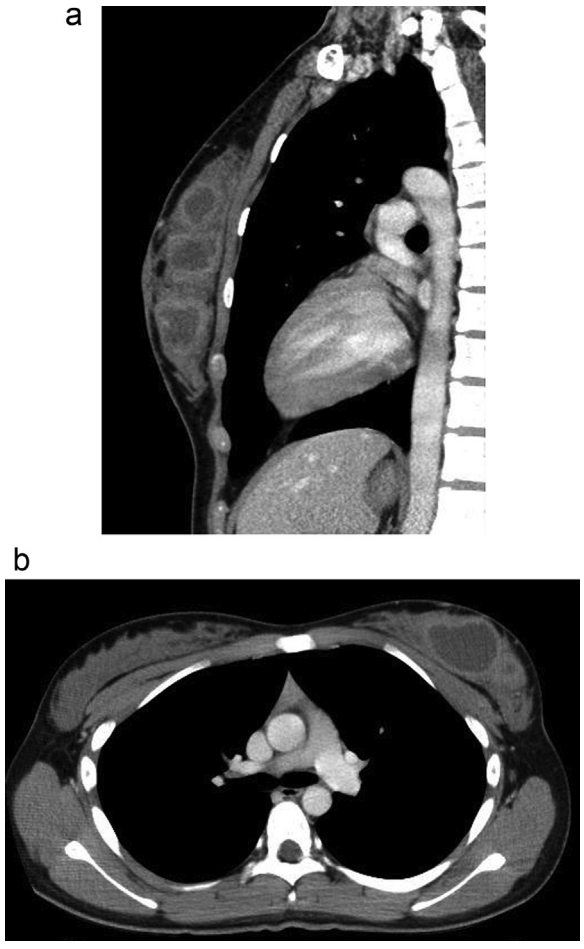

FIG. 1. Enhanced computed tomography scan of the patient's chest. Sagittal image (a) showed three abscesses in her left breast. Axial image (b) indicated the largest one located at the top.

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 ΦSa2usa, 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 ΦSa2usa 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 ΦSa2usa 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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Septic arthritis caused by an emerging ST121 methicillin-susceptible, PVL-negative *Staphylococcus aureus* harbouring a variant of bone sialoprotein-binding protein gene

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Abstract

ST121/*agr*-IV methicillin-susceptible *Staphylococcus aureus* was isolated from a patient of septic arthritis (synovial fluid, blood, skin and nasal cavity). Although the Panton-Valentine leukocidin (PVL) gene was negative, this isolate harboured a gene encoding a variant of bone sialoprotein-binding protein with a shortened SD-repeat region.

Keywords: Bone sialoprotein-binding protein, septic arthritis, ST121, *Staphylococcus aureus*

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ST121 *S. aureus,* which mostly harbours Panton-Valentine leucocidin (PVL), is a highly virulent clone and is distributed globally [1,2]. However, PVL-negative ST121 isolate is also considered an emerging cause of severe diseases [3]. Here we report isolation of ST121 PVL-negative methicillin-susceptible *S. aureus* (MSSA) from septic arthritis, and identification of a novel variant of bone sialoprotein-binding protein (Bbp) [4].

A 12-year-old Japanese boy abruptly developed severe right shoulder pain and high-grade fever, and was brought to our hospital for care. At the age of 4 he had been diagnosed as having polyarticular juvenile idiopathic arthritis, and contracture of his systemic joints had progressed. He had recently been treated with prednisolone, methotrexate, and human monoclonal anti–tumor necrosis factor alpha antibody. On admission, his temperature was 39.9°C and systolic blood pressure was 92 mm Hg. The right shoulder joint was warm, swollen and tender with a restricted range of movement. Head and neck examination revealed postauricular eczema with bleeding. Blood test revealed increased white blood cell count (11 600/mm^3, 77% neutrophils) and C-reactive protein (15.54 mg/dL). Magnetic resonance imaging of the right shoulder revealed a moderate effusion in the right shoulder joint and external to the joint. Aspiration of the shoulder joint yielded purulent synovial fluid containing 160 000 leukocytes/mm^3 and Gram-positive cocci. Acute septic arthritis of the right shoulder was diagnosed. Arthroscopic lavage of the joint was performed. Cefazolin and vancomycin were administrated as the initial therapy; thereafter, cefazolin was administered for 6 weeks according to the antibiotic sensitivity of the bacteria isolated from synovial fluid and venous blood. There was no evidence of infective endocarditis. He fully recovered, without recurrence for a year.

From synovial fluid, venous blood, exudate of postauricular eczema and swabs of nasal cavity of the patient, MSSA was isolated and designated YM514-j/v/e/n, respectively. They were classified into ST121, *spa*-t5072, *agr*-IV, *coa*-Va and showed identical genetic traits, having *bbp* which encodes Bbp, an Sdr family adhesin [4] (Tables 1 and 2), while findings were negative for the PVL gene. These findings indicated that the four isolates were of the same clone, suggesting occurrence of endogenous infection in the patient. Nucleotide sequences of *bbp* determined for YM514-j and YM514-v were identical, encoding a 1088 aa protein (GenBank accession no. KY095832), which is 83 aa shorter than the prototype Bbp [4]. Alignment of the Bbp revealed that YM514 Bbp has an SD-repeat region comprising 92 aa, which is shorter than other reported Bbp by 62 to 84 aa (Supplementary Fig. 1). This repeat region spans the bacterium's cell wall and acts as a stalk connecting with ligand-binding region of this protein by analogy with other Sdr proteins [5,6].

TABLE 1. Genotype of *Staphylococcus aureus* isolated from patient with septic arthritis

Isolate	Specimen	Genotype (ST, *spa, agr, coa*)
YM514-j	Synovial fluid	ST121, *spa*-t5072, *agr*-IV, *coa*-Va
YM514-v	Blood	ST121, *spa*-t5072, *agr*-IV, *coa*-Va
YM514-e	Postauricular eczema	ST121, *spa*-t5072, *agr*-IV, *coa*-Va
YM514-n	Nasal cavity	ST121, *spa*-t5072, *agr*-IV, *coa*-Va

ST, sequence type.

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TABLE 2. Molecular characteristics of *Staphylococcus aureus* isolated from patient with septic arthritis

Characteristic	Value
Detected genes	
Leukocidin	*lukDE*
Haemolysin	*hla, hlb, hld, hlg, hlg2*
Enterotoxin(-like)	*seg, sei, sem, sen, seo, selu, selx, sely, selw*
Adhesin	*icaA, icaD, cna, eno, fnbA, fnbB, ebpS-v,*[a] *clfA, clfB, fib, sdrC, sdrE, bbp*
Exoenzyme	*V8* (*sspA*), *sspB, aur, acpA, sak, efb, splA, splB, splC*
Other virulence factors	*isaA, isaB, vWbp*[b]
Regulatory elements	*agrA, cvfA, mgr, rot, sarA, saeR-saeS, trap, vraR,* DeoR family, GntR family, LysR family, MarR family
Aminoglycoside resistance	*aac(6′)-Ie-aph(2″)-Ia*
Mutation in quinolone resistance–determining region	*gyrA*: Ser 84 Leu; *grlA*: Ser 80 Phe
Drug resistance	Gentamicin, levofloxacin

[a]Variant of elastin-binding protein with internal deletion [9].
[b]Von Willebrand factor binding protein gene.

Although its relation to virulence is not clear, the SD-region variant possibly could affect the stability of Bbp on the bacterial cell surface. Despite the low prevalence in *S. aureus, bbp* is often associated with bloodstream infections and osteomyelitis [7,8]. However, *bbp*-positive ST121/*agr*-IV *S. aureus* has been mostly PVL positive and/or methicillin resistant [1,3,9,10]. Accordingly, PVL-negative MSSA (ST121/*agr*-IV) harbouring *bbp*, as detected in the present study, may be an emerging virulent clone to be noted.

Conflict of Interest

None declared.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.nmni.2017.05.010.

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