IJID Regions 8 (2023) 16–18

Contents lists available at ScienceDirect

IJID Regions

journal homepage: www.elsevier.com/locate/ijregi

Case Report

Acute osteomyelitis/septic pulmonary embolism associated with familial infections caused by PVL-positive ST6562 MRSA-IVa, a presumptive variant of USA300 clone

Nao Harada[a], Atsuo Togashi[a], Meiji Soe Aung[b], Jun Kunizaki[a], Kazutaka Nogami[a], Yoshinobu Nagaoka[a], Akira Ishii[a], Ima Kosukegawa[c], Wakiko Aisaka[d], Satoshi Nakamura[e], Tomohiro Wakabayashi[e], Takeshi Tsugawa[a], Nobumichi Kobayashi[b,*]

[a] Department of Pediatrics, Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan
[b] Department of Hygiene, Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan
[c] Department of Orthopedic Surgery, Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan
[d] Department of Intensive Care Medicine, Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan
[e] Department of Pediatrics, Hakodate Municipal Hospital, Hakodate, Hokkaido, Japan

ARTICLE INFO

Keywords:
Osteomyelitis
Pulmonary embolism
MRSA
ST6562
USA300 clone
PVL

ABSTRACT

Panton–Valentine leukocidin (PVL)-positive methicillin-resistant *Staphylococcus aureus* (MRSA) occasionally causes severe invasive infections. A 10-year-old immunocompetent boy in Hokkaido, the northern main island of Japan, was admitted with acute osteomyelitis of the right ilium, complicated by septic thrombophlebitis of the right common iliac vein and septic pulmonary embolism. As MRSA was isolated from blood and sputum samples of the patient, linezolid and vancomycin were initially used for treatment, and later clindamycin was added based on PCR-positive results for PVL genes. During his hospitalization, the patient was complicated by abscesses around the right ilium and septic arthritis of the right hip, which required surgical drainage. Prior to his admission, his youngest sister had developed a right breast abscess, and another sister and his mother developed contagious impetigo and hordeolum, respectively, during his hospitalization. These infections in the patient and his family members were caused by an identical PVL-positive MRSA strain belonging to ST6562, a single-locus variant of ST8. Due to the genetically close characteristics, this ST6562 MRSA was considered a genetic variant of the USA300 CA-MRSA clone (ST8-MRSA-IVa) predominating in the United States. The ST6562 MRSA-IVa is suggested to have occurred in Japan, associated with potential spread of the USA300 clone.

1. Introduction

Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is a cause of skin and soft tissue infections and also severe invasive infections, and is distributed worldwide. Among the globally prevalent CA-MRSA, the USA300 clone has predominated in the United States during the past two decades [1], and is characterized by genotype ST8 (CC8)/*spa* t008, carrying SCC*mec*-IVa, ACME (arginine catabolic mobile element; type I), and Panton–Valentine leukocidin (PVL) encoded by two genes (*lukS-PV-lukF-PV*) on a temperate phage [2]. PVL is a major virulence factor related to the pathogenesis of severe disease caused by CA-MRSA [3], and thus the potential spread of the USA300 clone outside the United States is also considered a public health concern [4]. This report describes a case of refractory invasive infection and intra-familial infections caused by USA300 clone-like strains in Hokkaido, the northern main island of Japan.

2. Case report

A 10-year-old previously healthy boy was admitted to Sapporo Medical University Hospital with acute osteomyelitis of the right ilium, and pyomyositis and abscesses around the right ilium. He also had concomitant septic thrombophlebitis of the right common iliac vein, complicated by septic pulmonary embolism (Figure 1a, b). Blood and sputum cultures were positive for MRSA. He was treated with linezolid and then switched to vancomycin. As the MRSA isolates were found to be positive for PVL

* Corresponding author: Nobumichi Kobayashi, Department of Hygiene, Sapporo Medical University School of Medicine, S-1 W-17, Chuo-ku, Sapporo 060-8556, Japan. Tel: +81-11-611-2111 ext. 27330. Fax: +81-11-612-1660.
E-mail address: nkobayas@sapmed.ac.jp (N. Kobayashi).

https://doi.org/10.1016/j.ijregi.2023.05.006
Received 7 April 2023; Received in revised form 16 May 2023; Accepted 17 May 2023

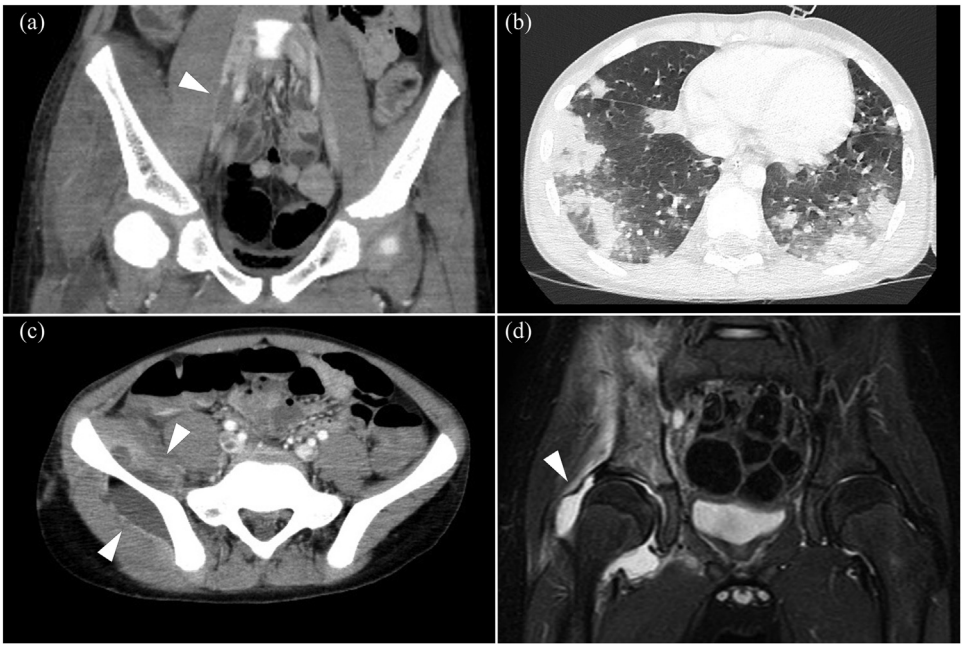

Figure 1. (a) CT of the pelvis, coronal view, revealing non-occlusive thrombosis of the right common iliac vein (arrowhead). (b) CT of the lungs, axial view, showing multiple consolidations and nodules consistent with septic pulmonary embolism. (c) CT of the pelvis, axial view, demonstrating abscesses around the right ilium (arrowheads). (d) MRI of the hip, coronal view, showing septic arthritis of the right hip (arrowhead).

genes by PCR, clindamycin was added to the treatment, due to its efficacy in inhibiting toxin production and its bone penetration properties. During treatment, the patient was complicated by sacral osteomyelitis, abscesses around the right ilium (Figure 1c), and septic arthritis of the right hip (Figure 1d). Therefore, repeated surgical drainage was performed, and the pus culture was also positive for MRSA.

The patient did not have atopic dermatitis and there was no evidence of immunodeficiency. In the convalescent phase, his blood neutrophil count was within the normal range, and neutrophil superoxide production, serum immunoglobulin levels, and complement levels were normal.

He lived with his parents and two younger sisters. One month prior to his admission, his youngest sister, 6 months old, developed a right breast abscess caused by PVL-positive MRSA that required puncture and drainage. During his hospitalization, she developed right otitis externa, facial cellulitis, and a subcutaneous abscess in the peri-auricular area due to MRSA. The abscess required incision and drainage. In addition, the older sister, 8 years old, developed contagious impetigo and his mother developed hordeolum. PVL-positive MRSA was isolated from their lesions. His father was not a carrier of MRSA in his nasal cavity. There was no family history of suspected immunodeficiency prior to these episodes, and only the older sister had atopic dermatitis. The patient was discharged after 75 days of inpatient treatment. Before he was discharged, his family was given intranasal mupirocin ointment and chlorhexidine bathing for sterilization, for 3 days. He continued to receive treatment with oral clindamycin for about 6 months after discharge, and had no further relapses.

The MRSA isolates recovered from clinical specimens obtained from the patient, his two sisters, and mother were analyzed genetically for their genotypes and presence of virulence factors, and also for antimicrobial susceptibility, as described previously [5]. All of the isolates were revealed to be a genetically identical strain: the MRSA belonged to ST6562 (allelic profile 3-3-1-1-4-739-3), *spa* t1188 (repeat unit profile 11-19-12-21-34-24-34-22-25), coagulase genotype IIIa, *agr* type I, and had SCC*mec*-IVa and PVL genes, while being negative for ACME-*arcA*, enterotoxin genes (*sea-see, seg-seu, sey*), exfoliative toxin genes, and the TSST-1 gene (Supplementary Material Table S1). All of the isolates were resistant to oxacillin, cefoxitin, ampicillin, and levofloxacin, having mutations in QRDR (quinolone resistance determining region) in GyrA (S84L) and ParC (S80Y), but were susceptible to most of the other antimicrobials tested (Supplementary Material Table S2). A representative strain (HK-S-0045) from the blood of the patient and an isolate from the mother's eyelid hordeolum lesion carried *aac(6′)-Ia-aph(2″)-Ie* and *blaZ*, also showing resistance to gentamicin, which was distinct from the MRSA isolates from the two sisters. By PCR and sequencing analysis, the PVL genes were assigned to haplotype R1 and found to be located on a PVL phage ΦSa2usa. The prevalence of virulence factor genes and adhesin genes, along with other genetic characteristics in the ST6562 MRSA isolates from the four subjects, was similar to that of the USA300 clone, except for the absence of *sek, seq*, and ACME (Supplementary Material Table S1).

3. Discussion

The MRSA strains isolated in this study were found to be genetically closely related to the USA300 clone: ST6562 is a single locus variant of ST8 (allelic profile 3-3-1-1-4-4-3) and *spa* t1188 has a similar repeat unit profile to that of t008 (11-19-12-21-17-34-24-34-22-25), having the same type of SCC*mec* (IVa) and PVL genes (haplotype R1) carried by the same PVL phage ΦSa2usa. However, the present ST6562 isolates lacked ACME, while the USA300 clone characteristically possesses this genetic element. These findings suggest that the ST6562 MRSA is a genetic variant of the USA300 clone.

Before the present MRSA was isolated in 2021, we first identified ST6562 in MRSA colonizing the oral cavity of a 66-year-old female in 2020 [6] and subsequently identified it in an isolate from the skin of the hand of an 18-year-old male in 2021 [7], also in Hokkaido, Japan. These colonizing ST6562 belonged to the same genotypes (*spa*-t1188/*coa*-IIIa/SCC*mec*-IVa) as the isolates presented in this report, while having PVL genes and ACME type I as seen in the USA300 clone [2]; moreover, the former isolate harbored resistance genes *aph(3′)-IIIa* and *msrA*.

As of March 2023, only eight ST6562 MRSA isolates from Japan had been submitted to the PubMLST database (https://pubmlst.org/organisms/staphylococcus-aureus); these were reported between 2020 and 2022 and were derived from pus, skin swab, eye, and urine. This ST has also been described for a PVL-positive MRSA having SCC*mec*-IVa in an epidemiological study in Japan [8]. Accordingly, ST6562 MRSA appears to have emerged recently and spread in Japan, and is considered to have been subjected to genetic evolution, including the loss of

ACME or acquisition of antimicrobial resistance genes, as observed in the present study and our previous study [6].

Some distinct variants of the USA300 clone have been reported recently in Japan, including a strain with an altered *ccrB2* [9] and a Latin American variant (USA300-LV/J) [10]. Similarly, the occurrence of USA300 variants with different genetic traits from those of the USA300 clone, e.g., ST923 (SLV of ST8) and ACME-negative isolates, have been reported in Switzerland and Belgium [11,12]. These reports, along with the present findings, suggest that USA300 variants have been occurring and persisting regionally, followed by the global transmission of the original USA300 clone [4]. As the virulence and antimicrobial resistance traits might change during the genetic alteration of this clone, genetic and phenotypic characterization of the regionally disseminated strains derived from the USA300 clone is of particular importance.

Although the prevalence of ST8 MRSA with SCC*mec*-IVa (USA300 clone) in Japan has appeared to remain at a low level (e.g., 1.4% in clinical isolates from outpatients [13]), an increasing trend has been described recently in epidemiological studies [8,14], reaching 5% among blood isolates in a study in Hokkaido [15]. Therefore, it is suggested that the occurrence of ST6562 might be related to the spread of the USA300 clone.

This report describes a severe invasive infection in a boy and intrafamilial infections caused by PVL-positive ST6562 MRSA-IVa, a presumptive variant of the USA300 CA-MRSA clone. More attention regarding the USA300 clone and its variants may be required because of their potential spread in Japan.

Declarations

Funding: This study was supported in part by JSPS KAKENHI Grant Number JP20H03933.

Ethical approval: Not required.

Conflict of interest: None declared.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2023.05.006.

References

[1] Carrel M, Perencevich EN, David MZ. USA300 Methicillin-Resistant *Staphylococcus aureus*, United States, 2000-2013. *Emerg Infect Dis* 2015;**21**:1973–80. doi:10.3201/eid2111.150452.

[2] Diep BA, Gill SR, Chang RF, Phan TH, Chen JH, Davidson MG, et al. Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. *Lancet* 2006;**367**:731–9. doi:10.1016/S0140-6736(06)68231-7.

[3] Hussain K, Bandyopadhyay A, Roberts N, Mughal N, Moore LSP, Fuller LC. Panton-Valentine leucocidin-producing *Staphylococcus aureus*: a clinical review. *Clin Exp Dermatol* 2022;**47**:2150–8. doi:10.1111/ced.15392.

[4] Nimmo GR. USA300 abroad: global spread of a virulent strain of community-associated methicillin-resistant *Staphy lococcus aureus*. *Clin Microbiol Infect* 2012;**18**:725–34. doi:10.1111/j.1469-0691.2012.03822.x.

[5] Aung MS, Urushibara N, Kawaguchiya M, Sumi A, Shinagawa M, Takahashi S, et al. Clonal Diversity and Genetic Characteristics of Methicillin-Resistant *Staphylococcus aureus* Isolates from a Tertiary Care Hospital in Japan. *Microb Drug Resist* 2019;**25**:1164–75. doi:10.1089/mdr.2018.0468.

[6] Hirose M, Aung MS, Fukuda A, Yahata S, Fujita Y, Saitoh M, et al. Antimicrobial Resistance and Molecular Epidemiological Characteristics of Methicillin-Resistant and Susceptible Staphylococcal Isolates from Oral Cavity of Dental Patients and Staff in Northern Japan. *Antibiotics (Basel)* 2021;**10**:1316. doi:10.3390/antibiotics10111316.

[7] Hirose M, Aung MS, Fujita Y, Kato T, Hirose Y, Yahata S, et al. Genetic Characterization of Staphylococcus aureus, *Staphylococcus argenteus*, and Coagulase-Negative Staphylococci Colonizing Oral Cavity and Hand of Healthy Adults in Northern Japan. *Pathogens* 2022;**11**:849. doi:10.3390/pathogens11080849.

[8] Yamaguchi T, Nakamura I, Sato T, Ono D, Sato A, Sonoda S, et al. Changes in the Genotypic Characteristics of Community-Acquired Methicillin-Resistant *Staphylococcus aureus* Collected in 244 Medical Facilities in Japan between 2010 and 2018: a Nationwide Surveillance. *Microbiol Spectr* 2022;**10**:e0227221. doi:10.1128/spectrum.02272-21.

[9] Takadama S, Nakaminami H, Takii T, Noguchi N. Identification and detection of USA300 methicillin-resistant *Staphylococcus aureus* clones with a partial deletion in the *ccrB2* gene on the type IV SCC*mec* element. *Diagn Microbiol Infect Dis* 2019;**94**:86–7. doi:10.1016/j.diagmicrobio.2018.11.016.

[10] Takadama S, Nakaminami H, Kaneko H, Noguchi N. A novel community-acquired MRSA clone, USA300-LV/J, uniquely evolved in Japan. *J Antimicrob Chemother* 2020;**75**:3131–4. doi:10.1093/jac/dkaa313.

[11] Seidl K, Leimer N, Palheiros Marques M, Furrer A, Senn G, Holzmann-Bürgel A, et al. USA300 methicillin-resistant *Staphylococcus aureus* in Zurich, Switzerland between 2001 and 2013. *Int J Med Microbiol* 2014;**304**:1118–22. doi:10.1016/j.ijmm.2014.08.005.

[12] Argudín MA, Deplano A, Nonhoff C, Yin N, Michel C, Martiny D, et al. Epidemiology of the *Staphylococcus aureus* CA-MRSA USA300 in Belgium. *Eur J Clin Microbiol Infect Dis* 2021;**40**:2335–47. doi:10.1007/s10096-021-04286-3.

[13] Aung MS, Kawaguchiya M, Urushibara N, Sumi A, Ito M, Kudo K, et al. Molecular Characterization of Methicillin-Resistant *Staphylococcus aureus* from Outpatients in Northern Japan: Increasing Tendency of ST5/ST764 MRSA-IIa with Arginine Catabolic Mobile Element. *Microb Drug Resist* 2017;**23**:616–25. doi:10.1089/mdr.2016.0176.

[14] Takadama S, Nakaminami H, Sato A, Shoshi M, Fujii T, Noguchi N. Dissemination of Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* USA300 clone in multiple hospitals in Tokyo, Japan. *Clin Microbiol Infect* 2018;**24**:1211 e1-1211.e7. doi:10.1016/j.cmi.2018.02.012.

[15] Aung MS, Urushibara N, Kawaguchiya M, Hirose M, Ito M, Habadera S, et al. Clonal diversity of methicillin-resistant *Staphylococcus aureus* (MRSA) from bloodstream infections in northern Japan: Identification of spermidine N-acetyltransferase gene (*speG*) in staphylococcal cassette chromosomes (SCCs) associated with type II and IV SCCmec. *J Glob Antimicrob Resist* 2021;**24**:207–14. doi:10.1016/j.jgar.2020.12.008.