Monomicrobial Fournier’s gangrene caused by Panton-Valentine leukocidin-negative methicillin-susceptible Staphylococcus aureus ST8 in Japan: A case report

Hirofumi Yamagishi, Kenichi Kawahori, Noriaki Ohkiba, Nozomi Murooka and Takeshi Inazawa

Abstract:
Methicillin-resistant Staphylococcus aureus USA300, belonging to sequence type (ST) 8, is a rare cause of necrotizing fasciitis in the USA. We herein report a case of monomicrobial Fournier’s gangrene caused by an ST8, methicillin-susceptible Staphylococcus aureus (designated ksw1). Whole-genome sequencing and analyses for virulence determinants revealed that, unlike USA300, ksw1 lacked virulence genes, such as Panton-Valentine leukocidin and SCCmec, while harboring the toxic shock syndrome toxin-1 gene. These genomic features correlate with ST8 CA-MRSA/J, which is the major genotype of ST8 in Japan.

Key words: Fournier’s gangrene, methicillin-susceptible Staphylococcus aureus ST8, Panton-Valentine leukocidin, toxic shock syndrome toxin-1

(Intern Med Advance Publication) (DOI: 10.2169/internalmedicine.4334-19)

Introduction

Fournier’s gangrene is characterized by necrotizing fasciitis of the perineal, perianal or genital regions (1, 2). Diabetes mellitus is well known to be a particularly important risk factor for Fournier’s gangrene (2, 3). Fournier’s gangrene is typically caused by polymicrobial infection (2). It has been increasingly reported that monomicrobial infection of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) can cause necrotizing fasciitis (4).

In the USA, the most predominant CA-MRSA strain, USA300, belongs to multilocus sequence type (ST) 8 and typically harbors virulence genes, such as the Panton-Valentine leukocidin genes lukF-PV and lukS-PV (lukSF), and staphylococcal cassette chromosome mec (SCCmec) type IV carrying penicillin and cephem resistance gene mecA (4).

In Japan, the strain ST8 has not been well characterized (5) despite several studies showing evidence of USA 300 infection (6-10). We herein report a case of monomicrobial Fournier’s gangrene caused by a strain of methicillin-susceptible S. aureus ST8 (designated ksw1) that lacked the Panton-Valentine leukocidin gene while harboring the toxic shock syndrome toxin-1 gene.

Case Presentation

A 66-year-old man presented with a 1-month history of left buttock pain. His medical records indicated type 2 diabetes mellitus that had not been treated for 30 years. The patient had never been abroad. A clinical examination revealed tachycardia (100 beats per minute) with a normal blood pressure (105/55 mmHg) and slight pyrexia (38.1 °C). His body mass index was 18.64 kg/m² (height, 167 cm; body weight, 52.0 kg). A physical examination revealed an open wound with purulent discharge on his left buttock (Figurea). A laboratory examination indicated leucocytosis (white blood cell count, 23,820/μL), elevated level of C-reactive protein (CRP, 25.08 mg/dL), mildly elevated level of muscle-derived enzymes (aspartate aminotransferase, 90 U/L; lactate dehydrogenase, 433 U/L; creatine kinase, 322 U/L), normal level of serum creatinine (creatinine, 0.59 mg/dL) and hyperglycemia (blood glucose, Department of Endocrinology and Metabolism, Kashiwa Municipal Hospital, Japan

Received: December 12, 2019; Accepted: February 16, 2020; Advance Publication by J-STAGE: April 16, 2020
Correspondence to Dr. Hirofumi Yamagishi, crocchetta.dl.riso@gmail.com
The isolated strain ksw1 was found to share its sequence type 8 (ST8) with USA300, the most predominant CA-MRSA strain in the USA and known to cause skin and soft tissue infection. Epidemiological studies have demonstrated an association between CA-MRSA and the presence of lukPVSF genes and SCCmec type IV (17-19). Panton-Valentine leukocidin-negative methicillin-susceptible S. aureus ST8 in a patient with type 2 diabetes mellitus. The clinical course of necrotizing fasciitis caused by CA-MRSA is reported to be subacute, with symptoms presenting an average of six days before admission (4). In the present case, left buttock pain had been noticed one month before admission, and a residual lesion in the left scrotum developed over two weeks after the first debridement. Therefore, the clinical course of the present case is considerably persistent.

We encountered a case of monomicrobial Fournier’s gangrene caused by Panton-Valentine leukocidin-negative methicillin-susceptible S. aureus ST8 in a patient with type 2 diabetes mellitus. The clinical course of necrotizing fasciitis caused by CA-MRSA is reported to be subacute, with symptoms presenting an average of six days before admission (4). In the present case, left buttock pain had been noticed one month before admission, and a residual lesion in the left scrotum developed over two weeks after the first debridement. Therefore, the clinical course of the present case is considerably persistent.
Table 1. The Comparison of Multilocus Sequence Types and Virulence Factors between Ksw1, ST8 CA-MRSA/J and USA300. 1 Computational Prediction.

| Type, virulence gene, drug resistance | ksw1 | ST8 CA-MRSA/J -MM50 | USA300 -FPR3757 |
|--------------------------------------|------|---------------------|-----------------|
| ST                                   | 8    | 8                   | 8               |
| spa                                  | 1 (622) | 1 (t1767)         | 1 (t008)        |
| SCCmec virulence genes               |      |                     |                 |
| leukocidins                           |      |                     |                 |
| lukSF                                 | -    | -                   | +               |
| lukED                                 | +    | +                   | +               |
| phenol-soluble modulin               | +    | +                   | +               |
| hld                                  | +    | +                   | +               |
| hla                                  | +    | +                   | +               |
| hib                                  | +    | +                   | +               |
| hlg                                  | +    | +                   | +               |
| tsst-1                                | +    | +                   | -               |
| enterotoxins                         | sec, sel, sep | sec, sel, sep | sek, seq |
| adhesins                             | ebpS, sdrC, sdrD, sdrE, icaA, icaB, icaC, icaD, icaR, clfB | ebpS, sdrC, sdrD, sdrE, icaA, icaB, icaC, icaD, icaR, clfB | ebpS, sdrC, sdrD, sdrE, icaA, icaB, icaC, icaD, icaR, clfB |
| others                               | sak, snc | sak, snc           | sak, snc, cph  |
| drug resistance                      | PCG, ABPC | PCG, DMPPC, EM,G 1 | TC, CPFX, MUP  |

PCG: benzylpenicillin, ABPC: ampicillin, DMPPC: meticillin, EM: erythromycin, GM: gentamicin, CLDM: clindamycin, TC: tetracycline, CPFX: ciprofloxacin, MUP: mupirocin

Valentine leukokcidin is a cytotoxin that induces apoptosis of human neutrophils, and intradermal injection of purified Panton-Valentine leukokcidin to rabbits reportedly induces tissue necrosis (20, 21). Therefore, Panton-Valentine leukokcidin has been considered to be responsible for the enhanced virulence of CA-MRSA strains. However, several experimental studies in animal models of skin and soft tissue infection have shown a minimal effect of Panton-Valentine leukokcidin (22-24). Furthermore, five cases of invasive infection reported in South Korea were found to be caused by Panton-Valentine leukokcidin-negative CA-MRSA (25). This evidence negates the significant role of Panton-Valentine leukokcidin as a virulence factor in skin and soft tissue infections caused by CA-MRSA. Interestingly, our observations also indicate that Panton-Valentine leukokcidin is dispensable in causing necrotizing fasciitis.

The virulence factor composition of ksw1 differed considerably from that of the USA300 strain. Besides lacking Panton-Valentine leukokcidin, ksw1 was found to harbor the toxic shock syndrome toxin-1 gene. Regarding the virulence factor composition, ksw1 showed high similarities to ST8 CA-MRSA/J, a major genotype of ST8 CA-MRSA in Japan (16). Furthermore, the spa type of ksw1, 1 (622) includes variant strains of ST8 CA-MRSA/J (16). More detailed studies involving comparative genomics, especially of the gene structure including SCCmec, are desired.

The authors state that they have no Conflict of Interest (COI).

This research was conducted in Kashiwa Municipal Hospital, Chiba, Japan

References
1. Sorensen MD, Krieger JN, Rivara FP, et al. Fournier’s gangrene: population based epidemiology and outcomes. J Urol 181: 2120-2126, 2009.
2. Eke N. Fournier’s gangrene: a review of 1726 cases. Br J Surg 87: 718-728, 2000.
3. Nisbet AA, Thompson IM. Impact of diabetes mellitus on the presentation and outcomes of Fournier’s gangrene. Urology 60: 775-779, 2002.
4. Miller LG, Perdue-Remington F, Rieg G, et al. Necrotizing fasciitis caused by community-associated meticillin-resistant Staphylococcus aureus in Los Angeles. NEJM 352: 1445-1453, 2005.
5. Kawaguchiya M, Urushihara N, Ghosh S, et al. Genetic diversity of emerging Panton-Valentine leukokcidin/arginine catabolic mobile element (ACME)-positive ST8 SCCmec-IVA meticillin-resistant Staphylococcus aureus (MRSA) strains and ACME-positive CC5 (ST5/ST764) MRSA in northern Japan. JMM 62: 1852-1863, 2013.
6. Shibuya Y, Hara M, Higuchi W, et al. Emergence of the
community-acquired methicillin-resistant *Staphylococcus aureus* USA300 clone in Japan. J Infect Chemother 14: 439-441, 2008.
7. Higashiyama M, Ito T, Han X, et al. Epidural abscess caused by community-associated methicillin-resistant *Staphylococcus aureus* strain USA300 in Japan. J Infect Chemother 16: 345-349, 2010.
8. Higuchi W, Takano T, Iwao Y, et al. Emergence of the community-acquired methicillin-resistant *Staphylococcus aureus* USA300 clone in a Japanese child, demonstrating multiple divergent strains in Japan. J Infect Chemother 16: 292-297, 2010.
9. Yabe S, Takano T, Higuchi W, et al. Spread of the community-acquired methicillin-resistant *Staphylococcus aureus* USA300 among family members in Japan. J Infect Chemother 16: 372-374, 2010.
10. Nagao M, Iimura Y, Suzuki M, et al. First outbreak of methicillin-resistant *Staphylococcus aureus* USA300 harboring the Panton-Valentine leukocidin genes among Japanese health care workers and hospitalized patients. AJIC 38: e37-e39, 2010.
11. Enright MC, Day NP, Davis CE, et al. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 38: 1008-1015, 2000.
12. Bartels MD, Petersen A, Worning P, et al. Comparing whole-genome sequencing with Sanger sequencing for spa typing of methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 52: 4305-4308, 2014.
13. Wattam AR, Brettin T, Davis JJ, et al. Assembly, annotation, and comparative genomics in PATRIC, the all bacterial bioinformatics resource center. Methods Mol Biol 1704: 79-101, 2018.
14. Kaya H, Hasman H, Larsen J, et al. SCCmeCFinder, a web-based tool for typing of staphylococcal cassette chromosome mec in *Staphylococcus aureus* using whole-genome sequence data. mSphere 2018.
15. Diep BA, Gill SR, Chang RF, et al. Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. Lancet 367: 731-739, 2006.
16. Iwao Y, Ishii R, Tomita Y, et al. The emerging ST8 methicillin-resistant *Staphylococcus aureus* clone in the community in Japan: associated infections, genetic diversity, and comparative genomics. J Infect Chemother 18: 228-240, 2012.
17. Vandenesch F, Naimi T, Enright MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 9: 978-984, 2003.
18. Naimi TS, LeDell KH, Como-Sabetti K, et al. Comparison of community-and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA 290: 2976-2984, 2003.
19. Diep BA, Sensabaugh GF, Somboona NS, et al. Widespread skin and soft-tissue infections due to two methicillin-resistant *Staphylococcus aureus* strains harboring the genes for Panton-Valentine leukocidin. J Clin Microbiol 42: 2080-2084, 2004.
20. Cribier B, Prévost G, Coupie P, et al. *Staphylococcus aureus* leu-kocidin: a new virulence factor in cutaneous infections? An epidemiological and experimental study. Dermatology 185: 175-180, 1992.
21. Genevier AL, Michallet MC, Prevost G, et al. *Staphylococcus aureus* Panton-Valentine leukocidin directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils. J Clin Invest 115: 3117-3127, 2005.
22. Yoong P, Torres VJ. The effects of *Staphylococcus aureus* leukotoxins on the host: cell lysis and beyond. Curr Opin Microbiol 16: 63-69, 2013.
23. Otto M. MRSA virulence and spread. Cell Microbiol 14: 1513-1521, 2012.
24. Otto M. Community-associated MRSA: what makes them special? Int J Med Microbiol 303: 324-330, 2013.
25. Lee SS, Kim YJ, Chung DR, et al. Invasive infection caused by a community-associated methicillin resistant *Staphylococcus aureus* strain not carrying Panton-Valentine leukocidin in South Korea. J Clin Microbiol 48: 311-313, 2010.

The Internal Medicine is an Open Access journal distributed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License. To view the details of this license, please visit (https://creativecommons.org/licenses/by-nc-nd/4.0/).