Short Communication

Skin and Soft Tissue Infections Caused by Different Genotypes of PVL-Positive Community-Acquired Methicillin-Resistant *Staphylococcus aureus* Strains

Tomoko Hanawa1*, Yurie Shimoda-Komatsu2, Koji Araki3, Manabu Ohyama2, Hiroaki Ohnishi4, Shigeru Kamiya5, and Takeaki Matsuda6

1Department of Infectious Diseases, 2Department of Dermatology, 3Department of Clinical Laboratory, 4Department of Laboratory Medicine, 5Faculty of Health Sciences, and 6Department of Traumatology and Critical Care Medicine, Kyorin University School of Medicine, Tokyo, Japan

**SUMMARY:** Panton-Valentine leukocidin (PVL) is a causative agent of lethal necrotizing pneumonia and is associated with epidemic strains of community-acquired (CA) methicillin-resistant *Staphylococcus aureus* (MRSA). PVL-producing strains have rarely been isolated in Japan. However, PVL-positive CA-MRSA has been isolated much more frequently in recent years. To investigate the relevance of *pvl* genes (*lukS/F-PV*) and clinical traits in epidemic *S. aureus* strains, we genotyped four PVL-positive CA-MRSA strains isolated from patients with skin and soft tissue infections and measured their susceptibility to antibiotics. Three of the isolates matched the genotype of the USA300 clone, which has predominantly been isolated in the USA. The remaining strain matched the ST217 genotype, and its *spa* type was identical to that of PVL-positive strains previously reported in India and China. Abscess drainage was necessary in all cases, and deep cutaneous ulcers were formed in three out of four cases regardless of the genotype. The ST217 genotype strain was resistant to clindamycin, in addition to quinolones, macrolides, and aminoglycosides. Thus, diagnostic determination of *lukS/F-PV* should be used as a guide for selecting the treatment regimen.

*Staphylococcus aureus* strains, especially community-acquired (CA) methicillin-resistant *S. aureus* (MRSA) clones, are often isolated from patients with skin and soft tissue infections. In cases involving strains producing the Panton-Valentine leukocidin (PVL) protein encoded by *lukS/F-PV*, the clinical features are cutaneous lesions with severe pain and erythema. Additionally, PVL-positive CA-MRSA can cause invasive illnesses such as necrotizing fasciitis or fatal necrotizing pneumonia.

According to the results of Yanagihara et al. (1), only 2.3% of MRSA isolates were positive for *lukS/F-PV* in Japan between 2008 and 2009. The USA300 clone, which is CA-MRSA and PVL-positive, is predominant in the USA. However, outbreaks of USA300 and other PVL-positive clones have occurred, and a case of necrotizing pneumonia involving this strain was reported more recently (2). Therefore, we investigated the effects of *lukS/F-PV* and genotype on the clinical course to determine its importance for the treatment and prevention of nosocomial infections. Furthermore, we assessed the susceptibility of these strains to various antibiotics and examined the patients’ clinical courses to understand the profile of infection with PVL-positive CA-MRSA strains.

The strains isolated from patients at the Kyorin University Hospital Department of Dermatology from 2015 to 2016 were categorized as MRSA, based on an MIC ≥ 4 μg/mL for oxacillin, according to the criteria of the Clinical and Laboratory Standards Institute. Ten strains were randomly selected from these MRSA strains, and *lukS/F-PV* was detected by polymerase chain reaction (PCR) using the specific primers of luk-PV-1 and luk-PV-2 (3). The DNA fragments were sequenced for confirmation. Accordingly, the presence of *pvl* was detected in four out of the ten MRSA strains (40.0%, Table 1).

**SCCmec** type was categorized according to the results of phage-open-reading frame typing (POT) using the SicaGeneous POT kit (Kanto Chemical, Tokyo, Japan) to clarify the genetic background of *lukS/F-PV* positive strains (4). Multilocus sequence typing (MLST) was performed and an allelic profile was obtained from the *S. aureus* MLST database (https://pubmlst.org/ saureus/) (5). The sequences amplified in the *spa* region were analyzed using the Ridom SpaServer database (https://www.spaserver.ridom.de/) (6). Consequently, all PVL-positive strains in the present study were classified as SCCmec type IV, according to their POT scores, and were confirmed to be negative for *tst* by PCR using the primer pair TSST-1 and TSST-2 (7). Identical POT scores of 106-77-113 in the strains isolated from cases 1–3 strongly suggested their close relationship to the USA300 clone according to the instructions provided in the test kit (Table 2). The USA300 clone is characterized by the presence of the arginine catabolic mobile element (ACME) and *lukS/F-PV* (8). Since the *arcA* associated with ACME was detected by PCR using the primer pair SAarcF and 108-aR, it was determined that these strains represent the USA300 clone (8).
Genotypes of PVL-Positive CA-MRSA

The three strains identified as representatives of the USA300 clone were classified by MLST and spa typing as ST8/t008, which matched the genotype of epidemic strains, especially in the USA (Table 2). In addition to the USA300 clone, other clones positive for lukS-PV have been found in Japan (9). Since the POT score was 104-25-49 and the arcA gene was absent, the strain isolated from Case 4 is considered to have a different genetic background than the USA300 clone. The genotype of this strain was assessed and determined as ST217/t852, which has not been isolated in Japan so far. The ST217 clone was isolated as a nosocomial infection in a hospital in Zurich (10) and is thought to be a single-locus variant of ST22 (11). Previously, a PVL-positive ST217 MRSA strain was reported in China and India (12,13) as a causative agent of healthcare-associated infections. Interestingly, the Chinese strain and nine out of 13 strains isolated in Bangalore exhibited spa type t852, which was identical to that of the strain from Case 4 in the present study. The PVL phage gene, which was integrated into the Chinese strain was also detected in the strain from Case 4 by PCR using inF-2 and 108-aR (14). Therefore, these strains probably represent the same clone distributed across Asian countries.

We then characterized the antibiotic resistance profile for each strain using a Phoenix™ PMIC/ID-86 antibiotic susceptibility panel (BD Biosciences, Franklin Lakes, NJ, USA) to examine the relationship between the properties of the isolated strains and the clinical course of the patients. All isolates were resistant to erythromycin and levofloxacin in addition to β-lactams (Table 3). Furthermore, the strain of the ST217 genotype was inducibly resistant to clindamycin (Table 3). To elucidate the erythromycin resistant genes, PCR targeted to \( \text{ermA}, \text{ermB}, \text{ermC}, \text{and msrA} \) genes was performed by the method described by Lina et al. (15). The sequences of the PCR products proved the presence of the \( \text{ermC} \) and \( \text{msrA} \) genes.

Although CA-MRSA was thought to be susceptible to a relatively large number of drugs in the past, the strain from Case 4 showed a tendency toward multidrug resistance. Furthermore, since the genes conferring drug resistance are usually contained in plasmids, they may be disseminated further in the future.

The disease courses of the four patients infected with PVL-positive MRSA strains are shown in Table 1. These cases involved skin and soft tissue infections. In general, cases of infection with PVL-positive strains tend to result in severe skin and soft tissue infections (9). As shown in Table 1, Cases 1 and 4 presented with subcutaneous abscesses on the buttocks and lower abdomen, respectively, while Case 2 manifested as a necrotic ulcer on the lower leg, which took four months to re-epithelialize. All lesions required oral antibiotics

Table 1. Case presentations of patients infected with pvl-positive MRSA

| Patient | Age (yr) | Sex | History | Symptom | Presentation | Use of antibacterial drug |
|---------|----------|-----|---------|---------|-------------|--------------------------|
| 1       | 54       | Male | None to be noted | Fever, right hip pain | Furuncle | Oral levofloxacin. After levofloxacin-resistant MRSA was isolated, changed to oral minocycline together with topical gentamicin ointment. |
| 2       | 77       | Male | Diabetes, administration of EGFR inhibitor for lung cancer was started 3 weeks before the first medical examination | Folliculitis on the head, ulcer on the right lower leg | Skin ulcer | Intravenous minocycline. After diagnosis, gentamicin ointment was additionally administered. |
| 3       | 24       | Male | Asthma, sinusitis | Swelling of the left cheek, pain, fever | Cellulitis | Intravenous ceftriaxone, oral cefcapene pivoxil, and gentamicin ointment. Intravenous sulbactam/ampicillin was added later. |
| 4       | 54       | Male | None to be noted | Ulcer in right lower quadrant | Skin ulcer | Oral faropenem and minocycline. Silver sulfadiazine (Gohen) cream was added later. |

Table 2. Characterization of pvl-positive clinical isolates

| Patient | Sequence type | spa type (Kreiswirth IDs) | POT type | arcA |
|---------|--------------|---------------------------|----------|------|
| 1       | ST8          | t008 (YHGFMBQBLO)         | 006-77-113 | +    |
| 2       | ST8          | t008 (YHGFMBQBLO)         | 006-77-113 | +    |
| 3       | ST8          | t008 (YHGFMBQBLO)         | 006-77-113 | +    |
| 4       | ST217        | t852 (JNCDMOKR)           | 104-25-49 | –    |
combined with surgical incision for drainage, and eventually healed after 37, 63, and 111-day outpatient follow-up. Case 3 initially presented as acneiform eruptions on the face which eventually progressed to cellulitis with pus discharge detected by puncture. This patient was hospitalized and treated with intravenous sulbactam/ampicillin for 12 days. Thus, all these cases exhibited furuncles, skin ulcers, and cellulitis with varying degrees of treatment resistance and required surgical interventions.

The isolation rate for PVL-positive strains has been increasing owing to the higher frequency of bacterial isolation from clinical cases, including nosocomial infections. Most CA-MRSA isolates reported in Japan are PVL-negative strains causing less serious infections. In addition, infection with CA-MRSA has most frequently been reported in children and young people. However, three out of the 4 cases included in the present study were over 50 years of age. This observation may imply that CA-MRSA infections are spreading among people of all ages. In light of the difficulties for choosing the appropriate antibiotics, diagnostic detection of lukS/F-PV is important to improve the chances of treatment success. In addition to the spread of USA300 strains in Japan, attention should be paid to the possible dissemination of other epidemic pvl-positive MRSA strains spreading across Asian countries.

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Conflict of interest None to declare.

REFERENCES

1. Yanagihara K, Araki N, Watanabe S, et al. Antimicrobial susceptibility and molecular characteristics of 857 methicillin-resistant Staphylococcus aureus isolates from 16 medical centers in Japan (2008-2009); Nationwide survey of community-acquired and nosocomial MRSA, Diagn Microbiol Infect Dis. 2012;72:253-7.

2. Takigawa Y, Fujisawa K, Saito T, et al. Rapidly progressive multiple cavity formation in necrotizing pneumonia caused by community-acquired methicillin-resistant Staphylococcus aureus positive for the Panton-Valentine leukocidin gene. Intern Med. 2019;58:685-91.

3. Lina G, Piémont Y, Godail-Gamot F, et al. Involvement of Panton-Valentine leucocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis. 1999;29:1128-32.

4. Suzuki M, Tawada Y, Kato M, et al. Development of a rapid strain differentiation method for methicillin-resistant Staphylococcus aureus isolated in Japan by detecting phage-derived open-reading frames. J Appl Microbiol. 2006;101:938-47.

5. Enright MC, Day NP, Davies CE, et al. Multilocus sequence typing for characterization of methicillin - resistant and methicillin - susceptible clones of Staphylococcus aureus. J Clin Microbiol. 2000;38:1008-15.

6. Harmsen D, Claus H, Witte W, et al. Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. J Clin Microbiol. 2003;41:5442-8.

7. Johnson WM, Tyler SD, Ewan EP, et al. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in Staphylococcus aureus by the polymerase chain reaction. J Clin Microbiol. 1991;29:426-30.

8. Diep BA, Stone GG, Basuino L, et al. The arginine catabolic mobile element and staphylococcal chromosomal cassette mec linkage: Convergence of virulence and resistance in the USA300 clone of methicillin-resistant Staphylococcus aureus. J Infect Dis. 2008;197:1523-30.

9. Nakaminami H, Ito A, Sakashita D, et al. Genetic diversity of pvl-positive community-onset methicillin-resistant Staphylococcus aureus isolated at a university hospital in Japan. J Infect Chemother. 2017;23:856-8.

10. Qi W, Ender M, O’Brien F, et al. Molecular epidemiology of methicillin-resistant Staphylococcus aureus in Zürich, Switzerland (2003): prevalence of type IV SCCmec and a new SCCmec element associated with isolates from intravenous drug users. J Clin Microbiol. 2005;43:5164-70.

11. Vignaroli C, Mancini A, Varaldo PE. Composite SCCmec element in single-locus variant (ST217) of epidemic MRSA-15 clone. Emerg Infect Dis. 2014;20:905-7.

12. Hu Q, Cheng H, Yuan W, et al. Panton-Valentine leukocidin (PVL-)

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Table 3. Antibiotic susceptibility of MRSA clinical isolates

|              | MIC1) | CLSI | MIC1) | CLSI | MIC1) | CLSI | MIC1) |
|--------------|-------|------|-------|------|-------|------|-------|
| ampicillin   | > 2   | R    | > 2   | R    | > 2   | R    | > 2   | R    |
| oxacillin    | > 4   | R    | > 4   | R    | > 4   | R    | > 4   | R    |
| penicillin G | > 2   | R    | > 2   | R    | > 2   | R    | > 2   | R    |
| cefazolin    | >32   | R    | >32   | R    | 16    | R    | >32   | R    |
| cefotaxime   | >16   | R    | >32   | R    | >16   | R    | >32   | R    |
| imipenem/cilastatin | > 2 | R   | < 2  | R   | > 2  | < 2 | < 2  | R |
| sulbactam/ampicillin | 16  | R   | 8    | R   | 8    | R   | 8    | R   |
| gentamicin   | > 2   | S    | < 2   | S    | > 16  | R    | > 16  | R    |
| vancomycin   | 1     | S    | 1     | S    | 1     | S    | < 0.5 | S    |
| erythromycin | > 8   | R    | > 8   | R    | > 8   | R    | > 5   | R    |
| clindamycin2) | < 0.5 | S   | < 0.5 | S   | < 0.5 | S   | < 0.5 | R*  |
| minomycin    | < 1   | S    | < 1   | S    | < 1   | S    | 8     | I    |
| levofloxacin | 4     | R    | > 8   | R    | > 8   | R    | > 8   | R    |

1): Minimum inhibitory concentrations (MICs) were determined using a Phoenix™ PMIC/ID-86 antibiotic susceptibility panel (BD Biosciences, Franklin Lakes, NJ, USA) and susceptibility was decided according to CLSI M100-S22. 2): Inducible clindamycin resistance was determined by D-test. R, resistant; I, intermediate; S, susceptible.
positive health care-associated methicillin-resistant *Staphylococcus aureus* isolates are associated with skin and soft tissue infections and colonized mainly by infective PVL-encoding bacteriophages. J Clin Microbiol. 2015;53:67-72.

13. Bouchiat C, El-Zeenni N, Chakrakodi B, et al. Epidemiology of *Staphylococcus aureus* in Bangalore, India: emergence of the ST217 clone and high rate of resistance to erythromycin and ciprofloxacin in the community. New Microbes New Infect. 2015;7:15-20.

14. Ma XX, Ito T, Kondo Y, et al. Two different Panton-Valentine leukocidin phage lineages predominate in Japan. J Clin Microbiol. 2008;46:3246-58.

15. Lina G, Quaglia A, Reverdy ME, et al. Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. Antimicrob Agents Chemother. 1999;43:1062-6.