Skin and soft tissue infections caused by different genotypes of PVL-positive community-acquired methicillin-resistant *Staphylococcus aureus* strains

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Title
Skin and soft tissue infections caused by different genotypes of PVL-positive community-acquired methicillin-resistant Staphylococcus aureus strains

Authors
Tomoko Hanawa*1), Yurie Shimoda-Komatsu2), Koji Araki3), Manabu Ohyama2), Hiroaki Onishi4), Shigeru Kamiya5), Takeaki Matsuda6)

1) Department of Infectious Diseases, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka-shi, TOKYO 181-8611 JAPAN
2) Department of Dermatology, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka-shi, TOKYO 181-8611 JAPAN
3) Department of Clinical Laboratory, Kyorin University Hospital, 6-20-2 Shinkawa, Mitaka-shi, TOKYO 181-8611 JAPAN
4) Department of Laboratory Medicine, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka-shi, TOKYO 181-8611 JAPAN
5) Faculty of Health Sciences, Kyorin University, 5-4-1 Shimo-Renjaku, Mitaka-shi, TOKYO 181-8612 JAPAN
6) Department of Traumatology and Critical Care Medicine, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka-shi, TOKYO 181-8611 JAPAN

Running head: Genotypes of PVL-positive CA-MRSA

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Corresponding to
Tomoko Hanawa, PhD
Department of Infectious Diseases
Kyorin University School of Medicine,
6-20-2, Shinkawa, Mitaka, Tokyo 1818611
JAPAN
phone: +81-422-47-5511
FAX: +80-47-5511 (ext. 3492)
E-mail: thanawa@ks.kyorin-u.ac.jp
著者

花輪智子 1†, 下田（小松）由莉江 2, 荒木光二 3, 大山学 4, 大西宏明 4, 神谷茂 5, 松田剛明 6

1 杏林大学医学部感染症学教室
2 杏林大学医学部皮膚科学教室
3 杏林大学医学部付属病院臨床検査部
4 杏林大学医学部臨床検査医学教室
5 杏林大学保健学部
6 杏林大学医学部救急医学教室

†責任著者連絡先

花輪智子

〒181-8611 東京都三鷹市新川6-20-2

Tel. 0422-47-5511 内線 3492

Fax. 0422-44-7325

E-mail. thanawa@ks.kyrin-u.ac.jp
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, the isolation frequency of PVL-positive CA-MRSA has been rapidly increasing in recent years. To investigate the relevance of the *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 considered to guide the selection of 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 Panton–Valentine leukocidin (PVL) protein encoded by the lukS/F-PV, the clinical features are of cutaneous lesions with severe pain and erythema. Additionally, PVL-positive CA-MRSA can cause invasive illness 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 during 2008–2009. The USA300 clone which is CA-MRSA and PVL-positive is predominant in the USA. However, outbreaks of the USA300 and other PVL-positive clones have occurred, and a necrotizing pneumonia case involving this strain was reported more recently (2). Therefore, we investigated the effects of the lukS/F-PV and genotype on the clinical course to determine its importance for treatment and the prevention of nosocomial infections. Furthermore, we assessed the susceptibility of these strains to various antibiotics and examined the clinical courses of the patients to understand the profile of infection with PVL-positive CA-MRSA strains.
The strains isolated from patients at the Department of Dermatology, Kyorin University hospital during 2015–2016 were categorized as MRSA based on showing an MIC $\geq$ 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).

The 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 the 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). The 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 of SAarcF and 108-aR, these strains were determined to represent the USA300 clone (8).

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/F-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 from 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 has been isolated as a nosocomial infection in Zurich hospital (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 thirteen strains isolated in Bangalore exhibited the *spa* type of t852, which was identical to that of the strain from case 4 in the present study. The φ108PVL phage gene, which was integrated in the Chinese strain
was also detected in the strain from case 4 by PCR performed using the 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 of each strain using a Phoenix™ PMIC/ID-86 antibiotic susceptibility panel (BD Biosciences, Franklin Lakes, NJ) to examine the relationship between the properties of the isolated strains and the clinical course of the patients. All the isolates were resistant to erythromycin and levofloxacin in addition to β-lactams (Table 3). Furthermore, the strain of the genotype of ST217 was inducible resistant to clindamycin (Table 3). To elucidate the erythromycin resistant genes, PCR targeted to \textit{ermA}, \textit{ermB}, \textit{ermC} and \textit{msrA} genes was performed by the method described by Lina \textit{et al.} (15). The sequences of the PCR products proved the presence of the \textit{ermC} and \textit{msrA} genes.

Although CA-MRSA has been 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. 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 soft tissue infection (9). As shown in Table 1, cases 1 and 4 presented with subcutaneous abscesses respectively on the buttock and the lower abdomen, while case 2 manifested as folliculocentric pustules and on the head and as a necrotic ulcer on the lower leg, which took four months to re-epithelialize. All lesions required oral antibiotics combined with surgical incision for drainage and eventually healed after 37, 63 and 111 day outpatient follow-up, respectively. 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 of PVL-positive strains has been increasing owing to the higher frequency of bacterial isolation from clinical cases including nosocomial infections. Most of the CA-MRSA isolates reported in Japan have been PVL-negative strains causing less serious infections. In addition, infection with CA-MRSA has most frequently been reported in children and young people to date. However, 3 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 difficulties in choosing appropriate antibiotics, diagnostic detection of \textit{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 \textit{pvl}-positive MRSA strains spreading in Asian countries.

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Conflict of interest

None declared.

ETHICAL CONSIDERATIONS

Approval for this study was granted by the Faculty of Medicine Research Ethics Committee, Kyorin University (H28-122).
REFERENCES


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<table>
<thead>
<tr>
<th>Patient</th>
<th>Patient attributes</th>
<th>Symptoms</th>
<th>Presentation</th>
<th>Use of antibacterial drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54 Male None to be noted</td>
<td>Fever, right hip pain</td>
<td>Furuncle</td>
<td>Oral levofloxacin. After levofloxacin-resistant MRSA was isolated, changed to oral minocycline together with topical gentamicin ointment.</td>
</tr>
<tr>
<td>2</td>
<td>77 Male Diabetes, administration of EGFR inhibitor for lung cancer was started 3 weeks before the first medical examination</td>
<td>Folliculitis on the head, ulcer on the right lower leg</td>
<td>Skin ulcer</td>
<td>Intravenous minocycline. After diagnosis, gentamicin ointment was additionally administered.</td>
</tr>
<tr>
<td>3</td>
<td>24 Male Asthma, sinusitis</td>
<td>Swelling of the left cheek, pain, fever</td>
<td>Cellulitis</td>
<td>Intravenous ceftriaxone, oral cefcapene pivoxil, and gentamicin ointment. Intravenous sulbactam/ampicillin was added later.</td>
</tr>
<tr>
<td>4</td>
<td>54 Male None to be noted</td>
<td>Ulcer in right lower quadrant</td>
<td>Skin ulcer</td>
<td>Oral faropenem and minocycline. Silver sulfadiazine (Gohen) cream was added later.</td>
</tr>
</tbody>
</table>
Table 2 Characterization of *pvl*-positive clinical isolates

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sequence type</th>
<th>spa type (Kreiswirth IDs)</th>
<th>POT type</th>
<th>arcA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ST8</td>
<td>t008 (YHGFMBQBLO)</td>
<td>006-77-113</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>ST8</td>
<td>t008 (YHGFMBQBLO)</td>
<td>006-77-113</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>ST8</td>
<td>t008 (YHGFMBQBLO)</td>
<td>006-77-113</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>ST217</td>
<td>t852 (JNCMOMOKR)</td>
<td>104-25-49</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3 Antibiotic susceptibility of MRSA clinical isolates

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC</td>
<td>CLSI</td>
<td>MIC</td>
<td>CLSI</td>
<td>MIC</td>
</tr>
<tr>
<td>ampicillin</td>
<td>&gt;2 R</td>
<td>&gt;2 R</td>
<td>&gt;2 R</td>
<td>&gt;2 R</td>
</tr>
<tr>
<td>oxacillin</td>
<td>&gt;4 R</td>
<td>&gt;4 R</td>
<td>&gt;4 R</td>
<td>&gt;4 R</td>
</tr>
<tr>
<td>penicillin G</td>
<td>&gt;2 R</td>
<td>&gt;2 R</td>
<td>&gt;2 R</td>
<td>&gt;2 R</td>
</tr>
<tr>
<td>cefazolin</td>
<td>&gt;32 R</td>
<td>&gt;32 R</td>
<td>16 R</td>
<td>&gt;32 R</td>
</tr>
<tr>
<td>cefotaxime</td>
<td>&gt;16 R</td>
<td>&gt;32 R</td>
<td>&gt;16 R</td>
<td>&gt;32 R</td>
</tr>
<tr>
<td>imipenem/cilastatin</td>
<td>&lt;2 R</td>
<td>&lt;2 R</td>
<td>&lt;2 R</td>
<td>&lt;2 R</td>
</tr>
<tr>
<td>sulbactam/ampicillin</td>
<td>16 R</td>
<td>8 R</td>
<td>8 R</td>
<td>8 R</td>
</tr>
<tr>
<td>gentamicin</td>
<td>&lt;2 S</td>
<td>&lt;2 S</td>
<td>&gt;16 R</td>
<td>&gt;16 R</td>
</tr>
<tr>
<td>vancomycin</td>
<td>1 S</td>
<td>1 S</td>
<td>1 S</td>
<td>&lt;0.5 S</td>
</tr>
<tr>
<td>erythromycin</td>
<td>&gt;8 R</td>
<td>&gt;8 R</td>
<td>&gt;8 R</td>
<td>&gt;8 R</td>
</tr>
<tr>
<td>clindamycin</td>
<td>&lt;0.5 S</td>
<td>&lt;0.5 S</td>
<td>&lt;0.5 S</td>
<td>&lt;0.5 S</td>
</tr>
<tr>
<td>minomycin</td>
<td>&lt;1 I</td>
<td>&lt;1 I</td>
<td>&lt;1 I</td>
<td>8 I</td>
</tr>
<tr>
<td>levofloxacin</td>
<td>4 R</td>
<td>&gt;8 R</td>
<td>&gt;8 R</td>
<td>&gt;8 R</td>
</tr>
</tbody>
</table>

Minimum inhibitory concentrations (MICs) were determined using a Phoenix™ PMIC/ID-86 antibiotic susceptibility panel (BD Biosciences, Franklin Lakes, NJ) and susceptibility was decided according to CLSI M100-S22. R, resistant; I, intermediate; S, susceptible

*Inducible clindamycin resistance was determined by D-test.