J Infect Chemother
DOI 10.1007/s10156-013-0561-5

NOTE

Virulence gene and expression analysis of community-associated methicillin-resistant *Staphylococcus aureus* causing iliopsoas abscess and discitis with thrombocytopenia

Wei-Chun Hung · Hiromitsu Mori · Sayaka Tsuji · Yasuhisa Iwao · Tomomi Takano · Akihito Nishiyama · Ivan Reva · Tatsuo Yamamoto

Received: 27 September 2012 / Accepted: 15 January 2013

Abstract Iliopsoas abscesses (IPAs) from methicillin-resistant *Staphylococcus aureus* (MRSA) are rare; however, IPAs from community-associated MRSA (CA-MRSA) may be increasing. In Japan, we previously described an adolescent athlete case of Panton–Valentine leukocidin (PVL)-positive ST30 CA-MRSA (strain NN12). In this study, we describe an IPA and discitis case from a variant of the successful PVL-negative CA-MRSA clone (ST8 CA-MRSA/J) in Japan. The patient was a 62-year-old man with intractable eczema, who had been diagnosed with IPAs and discitis (L1–L2). CA-MRSA (strain NN55) was isolated from blood, pus, and joint fluid. The invasive infections seemed to have originated in his intractable eczema, and the characteristics of this case, systemic myalgia and marked thrombocytopenia, seemed to have been caused by an exotoxin. Molecular genetic analysis revealed that NN55 possessed genotype ST8/*spa*606(t1767)/*agr*1/CoaIII and SCC*mec*IV of a novel subtype (encoding new cell-wall-anchored surface protein/J [CWASP/J]), exhibited enhanced expression of the cytolytic peptide genes, *psmα* and *hld*, and was resistant to gentamicin (caused by *aacA-aphD*), similar to ST8 CA-MRSA/J; however, NN55 lacked pathogenicity island SaPIj50 [carrying *tst*, encoding toxic shock syndrome toxin-1 (TSST-1)] of ST8 CA-MRSA/J, suggesting a variant (ST8 CA-MRSA/Jv). Strains NN12 and NN55 both caused bacteremia, IPAs, and adjacent musculoskeletal infections, preceded by intractable skin infections, and possessed high potential for adherence and enhanced expression of *psmα* and *hld*. The data suggest the role of a combination of CA-MRSA adhesin/cytolytic peptides (not PVL or TSST-1) in the pathogenesis of IPAs (and perhaps of systemic myalgia and marked thrombocytopenia).

Keywords Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) · ST8 CA-MRSA/J · Gene expression analysis · Iliopsoas abscess (IPAs) · Discitis · Thrombocytopenia

Methicillin-resistant *Staphylococcus aureus* (MRSA), which possesses staphylococcal cassette chromosome *mec* (SCC*mec*), is classified into two groups. One has been a common nosocomial pathogen since 1961 [1] and is now called healthcare-associated MRSA (HA-MRSA). HA-MRSA includes several epidemic MRSA clones with distinct multilocus sequence types (STs) [2], and in Japan, the ST5 lineage, previously referred to as the New York/Japan clone (ST5/SCC*mec*II), is dominant [2].

The other emerged in the community from 1997 to 1999 [3] and is designated community-associated MRSA (CA-MRSA). CA-MRSA is associated mainly with skin and soft tissue infections (SSTIs), but occasionally with invasive infections such as bacteremia, sepsis, and necrotizing pneumonia [2–4]. CA-MRSA includes heterogeneous ST

Electronic supplementary material The online version of this article (doi:10.1007/s10156-013-0561-5) contains supplementary material, which is available to authorized users.

W.-C. Hung · Y. Iwao · T. Takano · A. Nishiyama · I. Reva · T. Yamamoto (✉)
Division of Bacteriology, Department of Infectious Disease Control and International Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan
e-mail: tatsuoy@med.niigata-u.ac.jp

W.-C. Hung · Y. Iwao · I. Reva · T. Yamamoto
International Medical Education Research Center, Niigata, Japan

H. Mori · S. Tsuji
Division of Gastroenterology, Nagano Red Cross Hospital, Nagano, Japan

Published online: 31 January 2013

types [2, 5], but generally possesses SCC*mec*IV or V [6] and often produces Panton–Valentine leukocidin (PVL), a toxin acting against polymorphonuclear neutrophils (PMNs) and monocytes [2, 5].

PVL-positive ST8/SCC*mec*IV MRSA in the United States (USA300) is one of the most common and best characterized CA-MRSA [5, 7]. USA300 carries the arginine catabolic mobile element (ACME), which is considered to enhance the colonization and survival of USA300 [5, 7]. Moreover, CA-MRSA such as USA300 exhibits high-level expression of the cytolytic peptide (or phenol-soluble modulin, PSM) genes [*psmα* (encoding PSMα) and *hld* (encoding δ-hemolysin, Hld)] compared to HA-MRSA; the enhanced expression of *psmα* and *hld* is likely associated with bacteremia and abscess formation [5]. In Japan, successful CA-MRSA includes ST8 CA-MRSA/J [8, 9], which is negative for PVL and ACME.

Iliopsoas abscesses (IPAs) are classified as primary and secondary [10]. Primary IPA is typically caused by hematogenous spread of *S. aureus* [10, 11]. Cases from MRSA are rare [11, 12]; however, they have been increasing in the US since 2005 [10]. In this study, we describe an IPA and discitis case with thrombocytopenia caused by a variant of ST8 CA-MRSA/J in Japan.

The patient was a 62-year-old man (living in Nagano, Japan) with intractable eczema in the gluteal region for about 6 months. Fever and myalgia mainly in the gluteal region through the lower limbs had developed 10 days previously. The patient visited a hospital for shivering with chills. On admission (day 1), eczema and scratch scars were noted on the skin, and small pustules were diffusely present over the trunk and four limbs. Systemic spontaneous muscle pain was noted, and only pinching with light force caused sharp pain in the lower limbs. Body temperature was 39.6 °C; CRP, 37.99 mg/dl; WBC, 14,500/μl; and platelet count, 11,000/μl. The patient was treated with ampicillin (4 g/day, by intravenous administration) and doxycycline (200 mg/day, by oral administration) initially; this treatment was for possible leptospirosis (Weil disease), which was considered based on the patient's high fever and systemic muscle pain and also his living environment (in a garage) with rats and bats, or for possible infections from gram-positive cocci originating in the patient's scratched skin lesions. MRSA was isolated on a blood culture test on day 3. On day 4, treatment was changed to vancomycin (2 g/day, by intravenous administration) and minocycline (2 g, by intravenous administration); the latter was chosen for a drip infusion. Abscess drainage was performed on the same day (day 4). Based on magnetic resonance imaging (MRI, T_2-weighted imaging; Fig. 1) performed on day 4, the patient was diagnosed with iliopsoas muscle abscess and discitis (L1–L2). MRSA was also isolated from pus and joint fluid. Hematuria was noted initially, but urine was

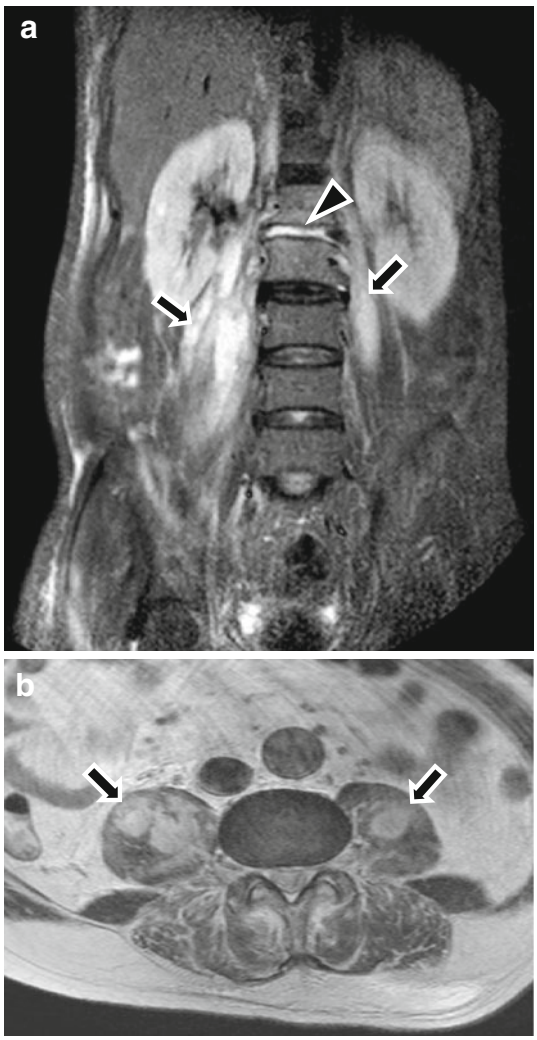

Fig. 1 Coronal view (**a**) and axial view (**b**) on T_2-weighted magnetic resonance imaging (MRI), suggesting iliopsoas abscess (IPA) and discitis. **a** A high-intensity region suggesting discitis was noted in the L1–L2 intervertebral disc (*arrowhead*). IPAs in the bilateral iliopsoas muscles are indicated by *arrows*. **b** High-intensity regions suggesting iliopsoas abscess were noted in the bilateral iliopsoas muscles (*arrow*)

MRSA negative. At the time of discharge, body temperature, CRP, WBC, and platelet count were 36.6 °C, 0.13 mg/dl, 4,100/μl, and 331,000/μl, respectively. He had no history of hospitalization, surgery, dialysis, or indwelling percutaneous medical devices and catheters in the previous year; his MRSA therefore met the Centers for Disease Control and Prevention (CDC) criteria for the CA-MRSA definition [4].

Molecular characterization of MRSA was performed, as previously described [9]. The ST type was obtained from the MLST website (http://www.mlst.net/). The *spa* type was determined using a public *spa*-type database (http://tools.egenomics.com/) or Ridom SpaServer (http://spaserver.ridom.de/). Typing of *agr* was carried out by polymerase chain reaction (PCR), as previously described