Short Communication

Possible Dissemination of a Panton-Valentine Leukocidin–Positive Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* CC398 Clone in Tokyo, Japan

Hidemasa Nakaminami^{1*}, Hinako Kawasaki¹, Shunsuke Takadama¹, Hiroshi Kaneko¹, Yoshiko Suzuki², Hiroshi Maruyama³, and Norihisa Noguchi¹

¹Department of Microbiology, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Tokyo; and ²Clinical Laboratory and ³Department of Surgery, Nippon Medical School Tama Nagayama Hospital, Tokyo, Japan

SUMMARY: In the last decade, methicillin-resistant *Staphylococcus aureus* (MRSA) has been identified in livestock animals, such as swine, poultry, and veal calves, and has been termed livestock-associated MRSA (LA-MRSA). LA-MRSA sequence type (ST) 398 strains can effectively infect and colonize humans, with subsequent human-to-human transmission in both community and hospital settings. Unlike other countries, LA-MRSA had not been reported in Japanese patients until 2019. However, we recently reported a case of intractable arthritis caused by an LA-MRSA CC398 (ST1232) clone, which is a single-locus variant of ST398, in a patient in Tokyo, Japan, with no animal contact (Nakaminami H, et al. Emerg Infect Dis. 2020; 26: 795-7.). Uniquely, the strain was positive for Panton-Valentine leukocidin. Here, we report the second such case in Japan. To prevent the dissemination of LA-MRSA in the Japanese community, the prevalence of the CC398 MRSA clone should be closely monitored in the future.

Traditionally, methicillin-resistant *Staphylococcus aureus* (MRSA) is considered a nosocomial pathogen due to its prevalence mainly in hospital settings. However, the emergence of MRSA exhibiting high virulence against healthy individuals in various communities has been reported since the late 1990s. These strains are known as community-acquired MRSA (CA-MRSA) and are distinct from traditional healthcare-associated MRSA (1). In the last decade, MRSA has also been detected in livestock animals, such as swine, poultry, and veal calves, being termed livestock-associated MRSA (LA-MRSA) (2,3). LA-MRSA strains can effectively infect and colonize humans, leading to subsequent human-to-human transmission in both community and hospital settings. Human colonization with LA-MRSA sequence type (ST) 398 was first recognized among swine farmers in France and the Netherlands in the early 2000s (4). Recent reports suggest that clonal complex (CC) 398 MRSA accounts for up to 25% of all CA-MRSA in some parts of Europe (2). In contrast, ST9 LA-MRSA

has been reported as the predominant clone in Asia (3). Furthermore, unlike other countries, ST398 and ST9 MRSA isolation had not been reported in Japanese patients until 2019 (5). However, we recently reported a case of intractable arthritis of the shoulder joint caused by an LA-MRSA CC398 (ST1232) clone, which is a single-locus variant of ST398, in a patient in Japan who reported no history of animal contact (6). Hence, it is presumed that the patient with the ST1232 MRSA clone was infected by another human in the community. Extraordinarily, the strain was positive for Panton-Valentine leukocidin (PVL). Price et al. showed that ST398 strains carrying PVL genes were associated with humans but not animals (4). In relation to this, Ye et al. suggested that the absence of immune evasion cluster (IEC) genes is a useful marker of livestock association (7).

We have conducted surveillance to grasp the dissemination of MRSA in hospitals since 2009 in Tama district in Tokyo, Japan (8–10). When multilocus sequence typing (MLST) was performed on the stock strains of MRSA, we identified a PVL-positive LA-MRSA ST1232 clone. The aim of this study was to scrutinize a case associated with a PVL-positive LA-MRSA ST1232 clone and compare it with the first reported case caused by an LA-MRSA ST1232 clone.

The clinical isolate was identified as *S. aureus* through positive Gram staining, proliferation on mannitol salt agar (Oxoid, Hampshire, UK), the test for coagulase production (PS LATEX; Eiken

Received May 12, 2020. Accepted July 7, 2020.
J-STAGE Advance Publication August 1, 2020.
DOI: 10.7883/yoken.JJID.2020.345

*Corresponding author: Mailing address: Department of Microbiology, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan. Tel: +81-426-76-5642, Fax: +81-426-76-5647, E-mail: nakami@toyaku.ac.jp

Fig. 1. Pulsed-field gel electrophoresis (PFGE) of LA-MRSA CC398 (ST1232) clone. C, N315, which is a typical MRSA strain used as standard; 1 and 2, CC5 (ST5) MRSA strains used as positive control for PFGE; 3, CC398 (ST1232) strain reported previously (6); 4, CC398 (ST1232) strain isolated in this study.

Chemical, Tokyo, Japan), and *nuc* gene detection (10). MRSA was confirmed through polymerase chain reaction (PCR)-based detection of the *mecA* gene and oxacillin resistance, and *SCCmec* typing was performed as described previously (10). Pulsed-field gel electrophoresis (PFGE) was also performed as described before (10). The ST and CC of the strain were determined by MLST (10). Minimum inhibitory concentrations (MICs) were determined using the agar doubling dilution method according to the Clinical and Laboratory Standards Institute guidelines (11,12). Virulence factors were detected using PCR assays as described previously (7,10).

The study protocol was approved by the Tokyo University of Pharmacy and Life Sciences Ethics Committee (12-09). Informed consent was not required from original patient because the study did not involve clinical interactions with patients.

The patient was a Japanese man living in Tokyo, Japan, with no record of overseas travel or any animal contact. In 2017, he was admitted to the University Hospital with severe pyoderma in the groin, perineal area, and hip. He had received no antimicrobial agents prior to admission.

A MRSA strain was isolated from his open pus. Fortunately, he recovered due to treatment with multiple debridements, enucleation of abscesses, split-thickness skin grafting, and cefazolin administration.

We conducted molecular epidemiological analyses of the MRSA N1195 strain isolated in this case. *SCCmec*

Table 1. Antimicrobial susceptibility of the CC398 (ST1232)-*SCCmec* type V LA-MRSA strains isolated from patients without animal contact

Strain (reference)	MIC ($\mu\text{g/ml}$) (resistance [^R] or susceptible [^S])									
	Ampicillin	Oxacillin	Cefazolin	Cefotaxime	Gentamicin	Levofloxacin	Clarithromycin	Clindamycin	Minocycline	Vancomycin
THI2018-120 (6)	4	8 ^R	8	8	32 ^R	0.25 ^S	64 ^R	$\geq 256^R$	0.13 ^S	1 ^S
N1195 (this study)	4	8 ^R	8	8	32 ^R	0.13 ^S	16 ^R	$\geq 256^R$	$\leq 0.06^S$	1 ^S

typing and MLST analysis revealed that the strain was SCCmec type V and CC398 (ST1232). Consistent with a previous report on the CC398 strain (13), the N1195 strain could not be analyzed by PFGE due to resistance to *SmaI* digestion (Fig. 1). Furthermore, the PVL genes (*lukS/F-PV*), which are predominant virulence factors of the highly pathogenic CA-MRSA clone USA300 (10), and the *sak* and *chp* genes, which encode staphylokinase and bacterial chemokine receptor modulator (7), respectively, were detected. When determining antimicrobial susceptibilities, the N1195 strain exhibited resistance patterns similar to those of the PVL-positive LA-MRSA ST1232 strain reported previously (Table 1) (6). The PVL-positive LA-MRSA ST1232 strains exhibited high susceptibility to cefazolin.

We herein present a case of severe pyoderma caused by a PVL-positive ST1232 clone belonging to the LA-MRSA lineage CC398 in a Japanese patient without any history of animal contact. Likewise, we recently reported a case of intractable arthritis caused by an identical LA-MRSA clone in another Japanese patient without animal contact (6). Remarkably, the isolation year of the LA-MRSA strain identified in this study (2017) was very close to that of the LA-MRSA strain reported previously (2018). In addition, the areas of residence of both patients were neighboring administrative areas within Tokyo. In general, the virulence of LA-MRSA strains is considered to be lower than that of CA-MRSA strains (14). As mentioned in our previous report (6), PVL production might enhance the severity of these cases. Furthermore, the PVL-positive ST1232 MRSA strain reported herein carried the IEC genes *sak* and *chp*. Therefore, these data strongly suggest that the patient with the PVL-positive ST1232 MRSA strain originated from another human in the community. Notably, ST398 MRSA strains were recently detected in imported swine in Japan (15). Hence, we previously hypothesized that the possible transmission route of the PVL-positive ST1232 MRSA strain is not only via humans but also through imported edible meat (6). Further investigation is necessary to elucidate the transmission route of this strain.

The PVL-positive ST1232 MRSA strain exhibited high susceptibility to β -lactams, including cefazolin (Table 1). This may be one of the reasons explaining the effectiveness of multiple surgical procedures and cefazolin administration in this patient.

In conclusion, we identified a PVL-positive ST1232 clone belonging to the LA-MRSA lineage CC398 in a Japanese patient without animal contact; this is the second such case reported in Japan. To prevent the dissemination of LA-MRSA in the Japanese community, the prevalence of the CC398 MRSA clone should be closely monitored in the future.

Acknowledgments We would like to thank Editage (www.editage.jp) for English language editing. This work was supported primarily by the Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research (C) (18K06797) (H.N).

Conflict of interest None to declare.

REFERENCES

1. Takadama S, Nakaminami H, Aoki S, et al. Prevalence of skin infections caused by Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* in Japan, particularly in Ishigaki, Okinawa. *J Infect Chemother*. 2017; 23: 800-3.
2. Williamson DA, Bakker S, Coombs GW, et al. Emergence and molecular characterization of clonal complex 398 (CC398) methicillin-resistant *Staphylococcus aureus* (MRSA) in New Zealand. *J Antimicrob Chemother*. 2014; 69: 1428-30.
3. Sato T, Usui M, Motoya T, et al. Characterisation of methicillin-resistant *Staphylococcus aureus* ST97 and ST5 isolated from pigs in Japan. *J Glob Antimicrob Resist*. 2015; 3: 283-5.
4. Price LB, Stegger M, Hasman H, et al. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *mBio*. 2012; 3:e00305-11.
5. Sato T, Usui M, Konishi N, et al. Closely related methicillin-resistant *Staphylococcus aureus* isolates from retail meat, cows with mastitis, and humans in Japan. *PLoS One*. 2017; 12: e0187319.
6. Nakaminami H, Hirai Y, Nishimura H, et al. Arthritis caused by MRSA CC398 in a patient without animal contact, Japan. *Emerg Infect Dis*. 2020; 26: 795-7.
7. Ye X, Fan Y, Wang X, et al. Livestock-associated methicillin and multidrug resistant *S. aureus* in humans is associated with occupational pig contact, not pet contact. *Sci Rep*. 2016; 6: 19184.
8. Nakaminami H, Noguchi N, Ito A, et al. Characterization of methicillin-resistant *Staphylococcus aureus* isolated from tertiary care hospitals in Tokyo, Japan. *J Infect Chemother*. 2014; 20: 512-5.
9. Nakaminami H, Takadama S, Ito A, et al. Characterization of SCCmec type IV methicillin-resistant *Staphylococcus aureus* clones increased in Japanese hospitals. *J Med Microbiol*. 2018; 67: 769-74.
10. Takadama S, Nakaminami H, Sato A, et al. 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.
11. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M07-Ed11. Wayne, PA: CLSI; 2018.
12. CLSI. Performance standards for antimicrobial susceptibility testing; approved standard M100-Ed30. Wayne, PA: CLSI; 2020.
13. Welinder-Olsson C, Floren-Johansson K, Larsson L, et al. Infection with Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* t034. *Emerg Infect Dis*. 2008; 14: 1271-2.
14. Rasigade JP, Laurent F, Hubert P, et al. Lethal necrotizing pneumonia caused by an ST398 *Staphylococcus aureus* strain. *Emerg Infect Dis*. 2010; 16: 1330.
15. Furuno M, Uchiyama M, Nakahara Y, et al. A Japanese trial to monitor methicillin-resistant *Staphylococcus aureus* (MRSA) in imported swine during the quarantine period. *J Glob Antimicrob Resist*. 2018; 14: 182-4.