

1 First outbreak of methicillin-resistant *Staphylococcus aureus* USA300 harboring the  
2 Panton-Valentine leukocidin genes among Japanese healthcare workers and hospitalized  
3 patients

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9 Abstract

10 This report describes the first outbreak of methicillin-resistant *Staphylococcus aureus*  
11 USA 300 in a general hospital ward in Japan, involving six healthcare workers and four  
12 patients. This report emphasizes the need for healthcare personnel to be alert that MRSA  
13 harboring *pvl* poses a threat for both nosocomial and occupational infection.

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16 Accumulating evidence indicates that community-associated methicillin resistant  
17 *Staphylococcus aureus* (CA-MRSA) isolates can readily produce outbreaks in hospitals,  
18 adding to the threat posed by these organisms (1-3). CA- MRSA is genetically  
19 heterogeneous, and includes a variety of clones such as the multilocus sequence (ST) 1  
20 (USA400) and ST8 (USA300) types that emerged as major clones in the United States.  
21 The USA300 clone can replace preexisting MRSA clones, and it now represents the  
22 predominant CA-MRSA clone in the United States (4,5). Until now, CA-MRSA  
23 infections reported in Japan have been sporadic, and most strains did not harbor the  
24 Panton-Valentine leukocidin (*pvl*) genes (6).

25 In September 2009, we were notified that a cluster of skin infections had broken out  
26 among healthcare workers (HCWs) and hospitalized patients in a general ward. We  
27 document herein the first outbreak of MRSA harboring *pvl* genes belonging to the  
28 USA300 clone in a healthcare setting in Japan.

29 At the time of notification, 65 patients were being cared for by 96 HCWs in a general  
30 ward at the tertiary care, 1240-bed Kyoto University Hospital (Japan), where  
31 dermatological disorders were quite prevalent. We were notified that a cluster of skin  
32 infections had developed among four healthcare workers (HCW 1-4) and it seemed  
33 compatible with *S. aureus* infection. Two weeks later, one patient (Patient 4)  
34 developed a skin abscess in the left arm from which MRSA was isolated. The isolate  
35 was susceptible to erythromycin, clindamycin and gentamicin. The antimicrobial  
36 susceptibility pattern was distinct from that of healthcare-associated MRSA  
37 (HA-MRSA) strains in Japan, which were usually multidrug-resistant and of which  
38 MIC levels of  $\beta$ -lactams were high. Subsequently, skin abscesses relapsed on the legs  
39 and chest of HCW 1 and HCW 5 and developed on the legs and buttock of HCW 6.

40 Eventually MRSA isolates were recovered from HCWs.

41 Based on information derived from these cultures, we developed a case definition in  
42 which MRSA with a specific antibiogram was recovered from a clinical specimen. The  
43 case-defined antibiogram was susceptible to erythromycin, clindamycin and gentamicin,  
44 but resistant to levofloxacin and  $\beta$ -lactams with MIC levels below those of HA-MRSA.  
45 We reviewed the antimicrobial susceptibility profiles of MRSA strains from all adult  
46 and pediatric hospitalized patients who were under care at Kyoto University Hospital  
47 during 2009 to detect any unidentified MRSA. HCWs were screened in the ward  
48 using nasal swabs to identify MRSA carriers.

49 Clinical specimens were inoculated onto mannitol salt agar plates and examined after  
50 48h. Susceptibility testing proceeded according to the Clinical and Laboratory  
51 Standards Institute. The *mecA* gene, PVL determinants and *arcA* gene on the arginine  
52 catabolite mobile element (ACME) were detected and SCC*mec* typing was performed  
53 by PCR (7-9). The typing procedure included PFGE using the restriction enzyme  
54 *SmaI* as described (5). Multilocus sequence typing proceeded as described and the  
55 nomenclature was specified as previously described. ([www.MLST.net](http://www.MLST.net))

56 Patients 1 to 3 who became infected with MRSA were newly identified based on the  
57 antimicrobial susceptibility profile described in the case definition (Table 1). A review  
58 of the medical records revealed that the first MRSA infection occurred in March 2009.  
59 Patient 1 developed catheter-related bloodstream infection followed by pneumonia and  
60 required intravenous anti-MRSA drug administration. Six of nine skin and soft tissue  
61 infections (skin abscesses, folliculitis) were treated with antibiotics, whereas three were  
62 cured by drainage alone. Patient 1 as well as HCW 1 and 5 developed recurrent  
63 infections. No case patient had a history of visiting abroad recently. All MRSA

64 isolates recovered from the case patients contained SCC *mec* type IV, the *pvl* gene and  
65 ACME-associated *arcA* gene. PFGE-based findings identified all isolates as being  
66 identical to and indistinguishable from the USA 300 clone. MLST defined all of them  
67 as ST8.

68 Excluding the isolates recovered from the case patients, four of 825 strains of MRSA  
69 isolates at our institution in 2009 had the same antimicrobial susceptibility profile as the  
70 outbreak strain. Those isolates were recovered from swab specimens and the patients  
71 did not have a symptom of infection when the specimens were taken. Screening nasal  
72 swabs of HCWs did not identify any carriers of CA-MRSA USA 300 other than the case  
73 patients.

#### 74 Discussion

75 To our knowledge, this is the first report to document an outbreak of  
76 healthcare-associated and -transmitted CA-MRSA USA300 in Japan. To date,  
77 outbreaks of PVL-positive CA-MRSA have been reported, especially in neonatal  
78 intensive care units and long term care facilities in the United states and European  
79 countries (1-3). However, no outbreaks of CA-MRSA in either community- or  
80 nosocomial settings in Japan have been described. Only one sporadic infection with  
81 the USA300 clone in Japan has been documented (10). We speculate that infected  
82 HCWs or unidentified PVL-positive MRSA carriers served as the source of infection for  
83 Patients 1 to 4, because all of them became infected while in hospital. In addition, we  
84 considered that the causative agent was community-associated because antibiograms of  
85 the outbreak strain were distinct from those of HA-MRSA strains. This was supported  
86 by a review of the antibiotic susceptibility of MRSA strains isolated at our institution  
87 during 2009; the case-defined antibiogram occurred in only 0.5% of isolates.

88 The findings of this investigation have considerable public health implications.  
89 Although HA-MRSA remains a serious threat to hospitalized patients, the introduction  
90 of CA-MRSA strains into tertiary care hospitals like our hospital represents an  
91 especially serious challenge. Many of the infections caused by these strains have been  
92 reported to cause serious infections among healthy adults and can be severer and more  
93 life-threatening to those who are highly immunocompromised. In this study, healthy  
94 HCWs suffered from skin abscesses, although most infections were mild and cured  
95 without parenteral anti-MRSA drugs. Considering that infection relapsed in some case  
96 patients, further investigations are needed to establish the management of PVL-positive  
97 MRSA carriers, especially when they are caregivers. Systematic studies involving  
98 healthcare settings are needed to reveal the transmission of such CA-MRSA isolates  
99 within the healthcare system. These would provide not only an accurate estimate of  
100 CA-MRSA prevalence, but would help monitor the emergence of more resistant and/or  
101 virulent clones and help with therapeutic infection control and patient management  
102 policies.

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- 145



146 Table 1 Characteristics of patients with CA-MRSA infections and their treatment, Kyoto  
 147 University Hospital, 2009

Case	Age, Sex	Underlying disease	Onset of infection	Type of infection	Site of infection	Antimicrobial drug treatment	Drainage
Pt 1	60,F	Inflammatory bowel disease	3/29	CRBSI	Blood- stream	TEIC	None
			8/27	Pneumonia	Lung	LZD	None
Pt 2	65,F	Decubitus ulcer	8/22	Skin abscess	Thigh	GEN	Spontaneous
Pt 3	42,M	Polyarteritis nodosa	10/19	Folliculitis	Legs	ST	Spontaneous
Pt 4	14,F	Decubitus ulcer	11/2	Skin abscess	Arm	GEN	Surgical,
HCW 1	31,F	none	9/19	Skin abscess	Arm	MINO	Surgical
			11/24	Skin abscess	Arm	CLI	None
HCW 2	27,F	none	9/21	Skin abscess	Arm	GEN	Surgical
HCW 3	27,F	none	9/21	Skin abscess	Leg	MINO	Surgical
HCW 4	25,F	none	9/21	Skin abscess	Leg	None	Surgical
HCW 5	27,F	none	11/20	Skin abscesses	Leg, Chest	CLI	Spontaneous
			12/14	Folliculitis	Arm, Finger	ST MUP	None
HCW 6	31,M	none	11/24	Skin abscess	Thigh	CLI	Spontaneous

- 148 TEIC, teicoplanin; LZD, linezolid; GEN, gentamicin(topical); ST,
- 149 Trimethoprim-sulfamethoxazole; MINO, minocyclin; CLI, clindamycin; MUP,
- 150 mupirocin (topical), CRBSI; catheter-related bloodstream infection

