〈Case Report〉

A case of necrotizing pneumonia caused by co-infection with influenza B and a Panton–Valentine leucocidin negative community acquired methicillin–resistant *Staphylococcus aureus* strain

Keiji Mouri[1], Yoshihiro Kobashi[1,*], Shigeki Kato[1], Tetsuo Yamaguchi[2] and Mikio Oka[1]

[1] Department of Respiratory Medicine, Kawasaki Medical School
[2] Department of Microbiology and Infectious Diseases, Toho University School of Medicine

(Received for publication January 25, 2018)

A 63-year-old woman visited our hospital complaining of fever and dyspnea. Her inflammatory response was strongly positive, with hyperglycemia, severe hypoxia, a high level of procalcitonin, and an influenza B antigen-positive result. Chest computed tomography (CT) on admission showed multiple nodules with infiltrative shadows in the bilateral lung fields, and gram-positive coccus with phagocytosis by neutrophils was observed in a sputum sample. Although treatments using sulbactam/ampicillin (SBT/ABPC) and azithromycin (AZM) plus peramivir were initiated, the clinical effect was poor due to the delay of administration of linezolid (LZD). Because methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated from sputum, treatments were changed to LZD plus AZM. Molecular analysis of the MRSA isolate showed as follows: multilocus sequence typing (MLST) 8, staphylococcal cassette chromosome mec (SCCmec) typeIV, spa type t1767, Panton-Valentine leucocidin (PVL)-negative, arginine catabolic mobile element (ACME)-negative, and toxic shock syndrome toxin-1 (TSST)-1-positive. Influenza B and TSST-1 produced by community acquired MRSA (CA-MRSA) may have been involved in the formation of necrotizing pneumonia in this patient. However, she improved following the administration of peramivir and LZD.

*Correspondence to Yoshihiro Kobashi, Department of Respiratory Medicine, Kawasaki Medical School, 577 Matsushima, Kurashiki, 701–0192, Japan

Tel.: +81–86–462–1111, Fax: +81–86–464–1041, E-mail: yoshihiro@med.kawasaki-m.ac.jp

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA), which has the staphylococcal cassette chromosome mec (SCC*mec*), has been a known nosocomial pathogen since 1961[1]. Another class of MRSA, known as community-acquired MRSA (CA-MRSA), first emerged in the community from 1997 to 1999, and has since become a major concern worldwide[2]. CA-MRSA is mainly associated with skin and soft tissue infections, but sometimes with unusual and severe infections such as bacteremia, sepsis, and necrotizing pneumonia in healthy individuals without risk factors[2, 3].

CA-MRSA has type IV or V SCC*mec*, which exhibits low minimum inhibitory concentration (MIC) values for oxacillin or imipenem, is resistant to *β*-lactam agents only, and often produces Panton-Valentine leucocidin (PVL), a toxin acting against polymorphonuclear neutrophils and monocytes[2] in western countries. The PVL-positive sequence type (ST) 8/SCC*mec*IV MRSA in the United States (USA300) is one of the most common and best characterized forms of CA-MRSA[4]. USA300 carries the arginine catabolic mobile element (ACME), which is considered to promote the colonization and survival of USA300[4]. In Japan, most CA-MRSA consists of ST8 CA-MRSA, which is negative for PVL and ACME[5–7]. We report a rare case of necrotizing pneumonia due to co-infection of influenza B and CA-MRSA, showing negative responses for PVL and ACME, but a positive response for toxic shock syndrome toxin-1 (TSST-1).

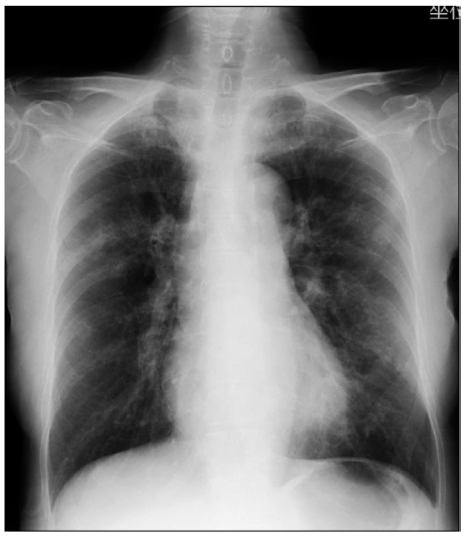
Case Report

A 63-year-old woman with diabetes mellitus (receiving no medication) was admitted to our hospital complaining of fever and dyspnea that had started three days earlier (April 1, 2014). She had a smoking history (30 cigarettes per day for 40 years), but there were no other potentially contributing factors excluding diabetes mellitus. She was not received influenza vaccine inoculation in 2013–2014. She had never been abroad. Her consciousness level was 1 on the Japan Coma Scale (14 on the Glasgow Coma Scale) on arrival and her vital signs were as follows: blood pressure 150/76 mmHg, pulse 130/min (regular), respiratory rate 30/min, oxygen saturation 95% (O_2 5 L mask), body temperature 38.6℃. On physical examinations, coarse crackles and rhonchi were auscultated in both lung fields. Laboratory examinations on admission are shown in Table 1. Inflammatory response including WBC and CRP were markedly elevated. Glucose and HbA_{1c}, related to diabetes mellitus, were also markedly elevated. Mild liver dysfunction was also recognized. Arterial blood showed respiratory and metabolic acidosis, elevation of $AaDO_2$, and degradation of PaO_2/FIO_2. Concerning serological examinations, elevation of procalcitonin and a positive response to influenza B antigens were noted. Although the blood cultures for common bacteria, urinary pneumococcal antigens, and urinary legionella antigens were negative, Gram

Table 1. Laboratory examination data on admission

Peripheral blood			Chemical screening		Blood gas($O_2$5L mask)	
WBC		13430 /μL ↑	TP	6.9 g/dL	pH	7.243↓
	Neu	87.5 %↑	Glu	401 mg/dL ↑	$PaCO_2$	47.8 mmHg ↑
	Mono	5.4 %	Bil (T)	0.9 mg/dL	PaO_2	88.3 mmHg
	Lym	7.0 %	ALP	231 IU/L	BE	-7.3 mEq/L ↓
RBC		47.9×10⁴/μL	Cho	215 mg/dL	HCO_3^-	20.2 mEq/L ↓
Hb		15.1 g/dL	γ-GTP	34 IU/L	Lactate	2.38 mg/L
Ht		44.1 %	LDH	314 IU/L ↑	$AaDO_2$	137 mmHg ↑
Plate		15.2×10⁴/μL	Alb	3.9 g/dL	PaO_2/FiO_2	220.75↑
PT		12.8 sec	Glb	3.0 g/dL	Serology	
APTT		25.1 sec	ChE	292 IU/L	Procalcitonin	44.1 ng/mL ↑
Fibrinogen		552 mg/dL ↑	AST	51 IU/L ↑	β-D-glucan	6.0 pg/mL
			ALT	46 IU/L ↑	*Mycoplasma* antibody (PA)	40 ↓
			Crn	0.54 mg/dL	*Mycoplasma* antibody (CF)	4 ↓
			BUN	17 mg/dL	*Chlamydophila pneumoniae* IgM antibody	0.19(-)
			UrA	4.1 mg/dL	*Legionella* antibody (-)	
			CRP	18.57 mg/dL ↑	Influenza antigen A(-),B(+)	
			Na	133 mg/L		
			K	4.0 mg/L		
			Cl	97 mg/L		
			HbA1c	9.5 %↑		

Fig. 1. Chest radiograph on admission showing infiltration shadows in the right middle and left middle and lower lung fields



staining of purulent sputum (Miller-Jones classification: P3) revealed many inflammatory cells phagocytizing gram-positive coccus bacilli.

Regarding the radiological findings on admission, infiltrative shadows appeared in the right middle and left middle and lower lung fields on chest radiograph (Figure 1). Infiltrative shadows and centrilobular nodular shadows were recognized in the right upper and lower lobes and left upper and lower lobes on chest computed tomography (CT) (Figure 2).

We showed the clinical course of this case in Figure 3. Although antibiotic therapy using sulbactam/ampicillin (SBT/ABPC), azithromycin (AZM) and peramivir was immediately initiated after admission, respiratory failure advanced and necessitated mechanical ventilation support on day 1. From the sputum culture on admission and aspiration sputum culture of a bronchoscopic specimen, MRSA was detected. The antimicrobial susceptibility of the isolated MRSA is shown in Table 2. Under the diagnosis of CA-MRSA pneumonia according to the definition described by Naimi *et al.*[8], the antibiotic therapy was changed to combination therapy consisting of Linezolid (LZD) and AZM on day 6. Although the respiratory condition gradually improved, several cavitary lesions appeared in both lungs with atelectasis of the left lower lobe on chest CT on day 10 (Figure 4). We considered that the pneumonia due to CA-MRSA had deteriorated to a pulmonary abscess due to the delay of administration of LZD. However, because *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were newly detected from the sputum culture, tazobactam/piperacillin (TAZ/PIPC) was added as antibiotic therapy. The clinical course of this patient gradually improved, and she was discharged on day 22.

Whole-genome sequencing of the MRSA isolate (strain TUM 14604) was carried out by

Fig. 2. Chest CT on admission showing infiltrative shadows and centrilobular shadows in the right lower lobes and left upper and lower lobes

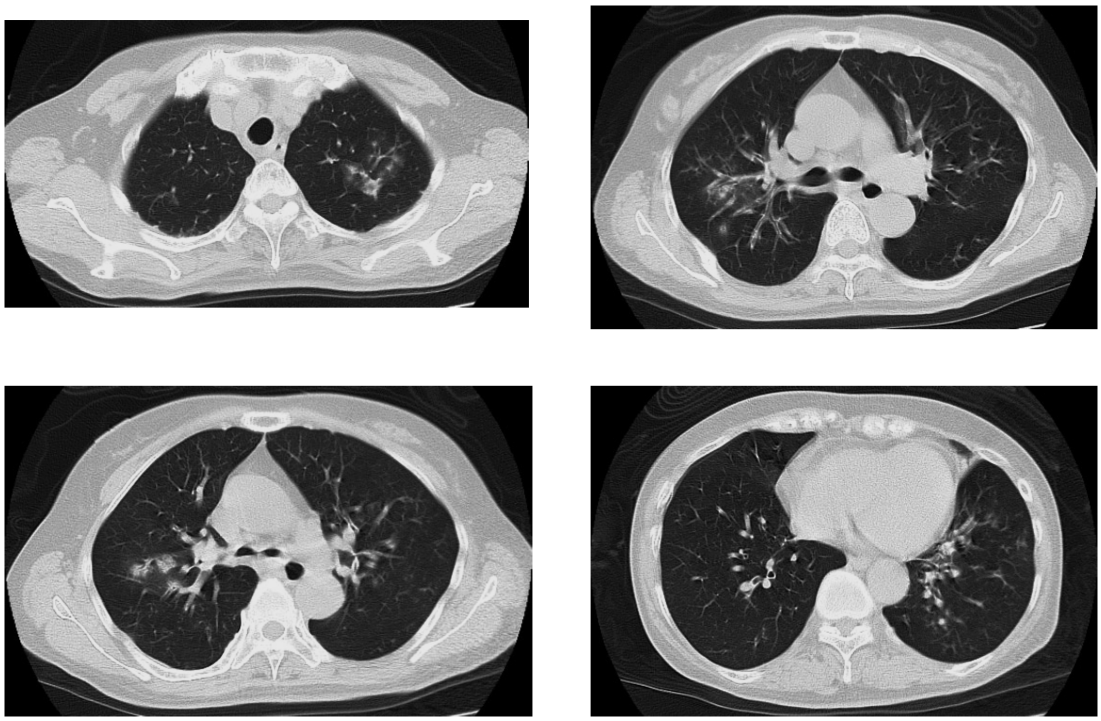


Fig. 3. The clinical course of this case showing with drugs for treatment, the transitional change of respiratory conditions and inflammatory responses

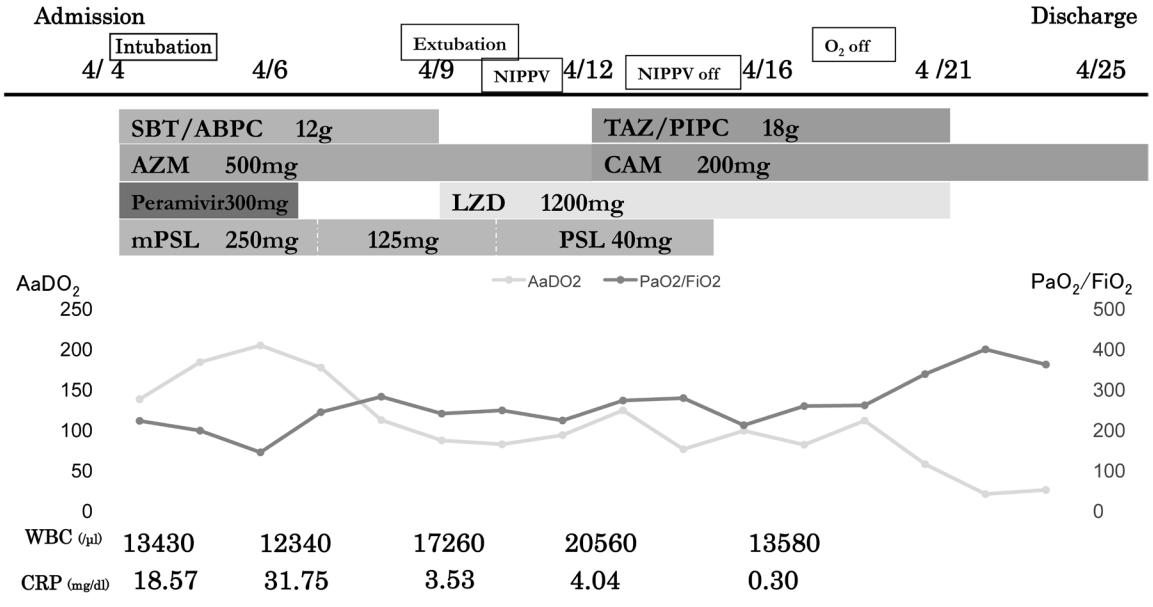


Table 2. Antimicrobial susceptibility of isolated MRSA

Agent	MIC	
ABPC	>16	R
MPIPC	>4	R
CEZ	16	R
IPM	2	R
GM	>8	R
ABK	≦1	S
EM	≦0.5	S
CLDM	≦0.5	S
MINO	≦1	S
LVFX	≦0.5	S
VCM	2	S
TEIC	≦2	S
RFP	≦1	S
ST	≦0.5	S
LZD	2	S
DAP	≦0.5	S

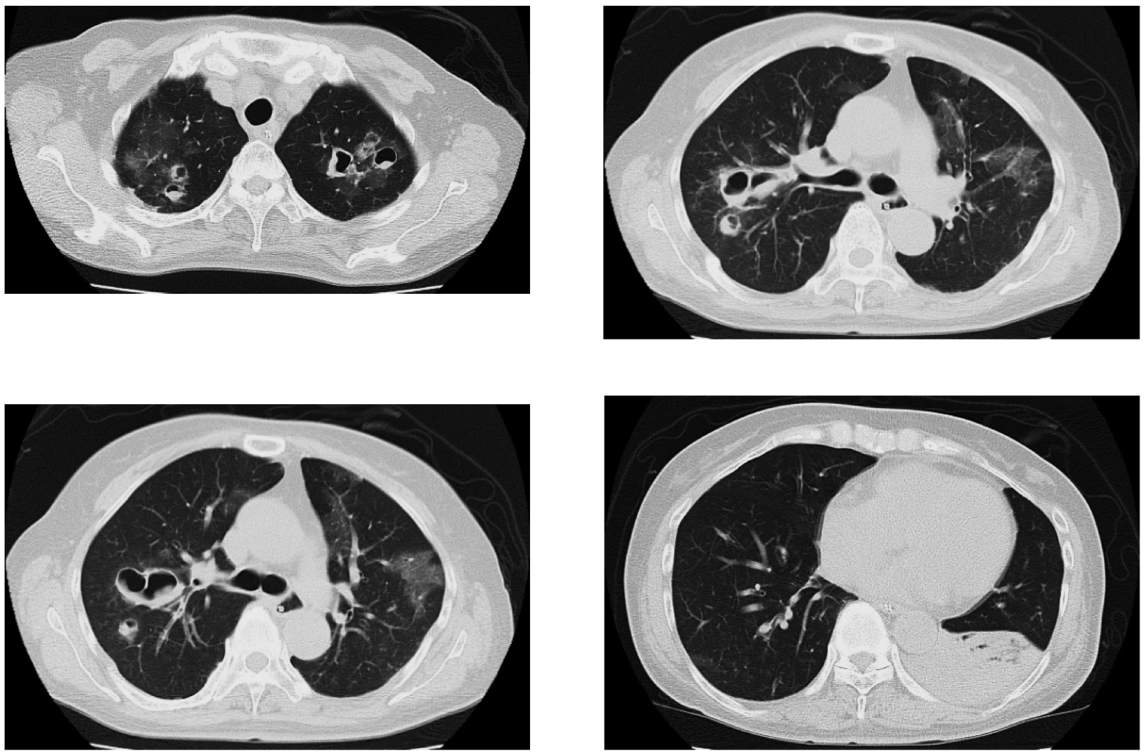
MIC: Minimum inhibitory concentration, R: Resistant, S: Sensitive, ABPC: Ampicillin, MPIPC: Oxacillin, CEZ: Cefazolin, IPM: Imipenem, GM: Gentamicin, ABK: Arbekacin, EM: Erythromycin, CLDM: Clindamycin, MINO: Minocycline, LVFX: Levofloxacin, VCM: Vancomycin, TEIC: Teicoplanin, RFP: Rifampicin, ST: Sulfamethoxazole/trimethoprim, LZD: Linezolid, DAP: Daptomycin

next-generation sequencing using MiSeq (Illumina, San Diego, CA, USA). The draft genome sequence data were assembled using CLC Genomics Workbench v 9.5.1 software (Qiagen, Aarhus, Denmark). Multilocus sequence typing (MLST) was performed using 7 housekeeping genes, and an allelic profile was obtained from the MLST website (http://www.mlst.net/). SCC*mec*type and *spa* type were determined, and the presence of virulence genes was checked. The sequence type was ST 8/SCC*mec* typeIV, and the *spa* type was t1767. The results for virulence genes were as follows: PVL (*lukSF-pv*)-negative, ACME (*arcA*)-negative, exfoliative toxin (ET)(*eta*, *etb*)-negative, and TSST-1 (*tst-1*)-positive.

Discussion

We encountered a patient with diabetes mellitus without medication who showed necrotizing pneumonia caused by co-infection with influenza B and PVL-negative CA-MRSA. Although there was a report that the frequency of MRSA increased among elderly patients with community-acquired pneumonia (CAP)[9], there are few reports concerning necrotizing pneumonia due to co-infection with influenza B and CA-MRSA in Japan.

Fig. 4. Chest CT on day 10 admission showing several newly observed cavity lesions in both lungs with atelectasis of the left lower lobe



CA-MRSA corresponds to MRSA infection occurring in patients without risk factors for MRSA infection, such as a past history of admission to a hospital or nursing home, surgery, hemodialysis, and use of an intravenous reservoir[8, 10]. Naimi *et al.* defined CA-MRSA as satisfying the following criteria: (1) the detection of MRSA in clinical specimens obtained from an outpatient or inpatient within 48 hours after admission with infectious signs, (2) no past history of MRSA detection in clinical specimen cultures, (3) no past history of admission to a hospital or nursing home within the last one year, surgical operation, or hemodialysis, and (4) no use of a reservoir such as an intravenous catheter[8]. Because our case satisfied the above four criteria and showed a positive response for the influenza B antigen, we diagnosed the patient with necrotizing pneumonia due to co-infection with influenza B and CA-MRSA.

Regarding the selection of antibiotics for this patient, we considered Gram-positive coccus bacilli as the causative microorganism because a Gram-positive coccus was isolated from the purulent sputum and aspiration sputum bronchoscopic specimens on day 1. Therefore, we initiated antibiotic therapy using SBT/ABPC and AZM combined with peramivir for influenza B infection. On day 6, the culture examination results for the gram-positive coccus identified MRSA. While CA-MRSA showed resistance to penicillin and cephem antibiotics, it showed sensitivity to anti-

MRSA drugs such as vancomycin (VCM), arbekacin (ABK), teicoplanin (TEIC), LZD, daptomycin (DAP), minocycline (MINO), levofloxacin (LVFX), and sulfamethazole-trimethoprim (ST). The MRSA of this patient showed a susceptibility pattern different from hospital-acquired MRSA (HA-MRSA). It was reported that sensitivity of CA-MRSA to erythromycin (EM) has not been preserved in Asia[11], but the CA-MRSA of this patient showed sensitivity to both EM and clindamycin (CLDM) (Table 2). Finally, we changed SBT/ABPC to LZD from day 6. The reason was that treatment was not clinically effective with VCM or TEIC and was more effective with LZD or CLDM, which suppressed the production of *Staphylococcus aureus* toxin in previous reports[8–12]. Otherwise, we administered LZD considering both the comparatively high MIC (2μg/mL) for VCM in this patient and excellent transmission to the lung tissue compared to VCM or TEIC for the treatment of CA-MRSA[13]. However, the patient's condition slightly worsened, with a newly detected infection due to the gram-negative bacilli (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). Radiologically, several cavity lesions appeared in both lung fields adding to infiltration shadows on day 10 (Figure 3). Therefore, we initiated combined therapy of TAZ/PIPC, CAM and LZD. Finally, this patient showed improvement on day 22.

As for the molecular analysis, in this case the CA-MRSA revealed the following findings: ST 8/SCC*mec*/t1767, PVL gene-negative, ACME-negative, ET-negative, and TSST-1-positive. Finally, this clone differed from the USA300 clone, and was the same genotype as CA-MRSA/J, the Japanese dominant CA-MRSA clone[6]. While most cases of CA-MRSA isolated in the USA are PVL gene-positive, CA-MRSA isolated in Japanese cases is PVL gene-negative[7,14]. Among PVL-positive CA-MRSA, it was reported that PVL was associated with the strength of pathogenesis and even healthy young adults developed fatal necrotizing pneumonia or sepsis in previous studies[15,16]. However, PVL-negative CA-MRSA caused necrotizing pneumonia (cavity formation) and was associated with a poor prognosis in Japan[2,16, 18–20]. Considering these findings, the pathogenicity and prognosis associated with CA-MRSA may be unrelated to the PVL gene and may be related to other factors. In this case, the CA-MRSA was only TSST-1 positive. TSST-1 is a superantigen that suppresses the motility of polymorphonuclear neutrophils by inhibiting the expression of MRSA exoproteins[2]. Orii *et al.* reported a fatal case of necrotizing fasciitis due to a PVL-negative MRSA strain that expressed TSST-1[19] and Otera *et al.* also reported a fetal case of necrotizing pneumonia due to a PVL-negative and TSST-1 positive MRSA strain[20]. They suggested that the pathogenesis is the result of a factor other than PVL. The difference between the patient reported by Otera *et al.*[20] and our case could have been influenza B virus infection. The synergistic effect due to TSST-1 and the products from influenza B infection may affect the formation of necrotizing pneumonia. It is necessary to perform epidemiological research involving severe cases of CA-MRSA infection and clarify pathogenetic factors other than PVL gene analysis.

Defres *et al.* reported findings suggesting CA-MRSA infection as follows: influenza-like

syndrome, severe respiratory symptoms with rapidly progressive pneumonia, fever over 39℃, hemoptysis, hypotension, leukopenia, chest radiograph showing multilobar infiltrates that may have cavitated, recent travel to an endemic area and recent contact with CA-MRSA, belonging to a group associated with increased rates of CA-MRSA colonization, and a previous family history of recurrent furuncles or skin abscess[9].

Based on this CA-MRSA case, when *Staphylococcus aureus* is detected in a case of CAP for which standard antibiotic treatment is refractory, we must consider CA-MRSA as a causative microorganism and initiate combined chemotherapy, including anti-MRSA antibiotics, at an early stage.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1) Centers for Disease Control (CDC). Community-acquired methicillin-resistant *Staphylococcus aureus* infections- Michigan. MMWR Morb Mortal Wkly Rep. 1982; 30: 185–7.
2) Yamamoto T, Nishiyama A, Takano T, *et al.*: Community-acquired methicillin-resistant *Staphylococcus aureus*: community transmission, pathogenesis, and drug resistance. J Infect Chemother. 2010; 16: 225–54.
3) Miller LG, Perdreau-Remington F, Rieg G, *et al.*: Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. N Engl J Med. 2005; 352: 1445–53.
4) Diep BA, Gill SR, Chang RF, *et al.*: Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. Lancet 2006; 367: 731–9.
5) Iwao Y, Takano T, Higuchi W, Yamamoto T: A new staphylococcal cassette chromosome *mec* IV encoding a novel cell-wall-anchored surface protein in a major ST8 community-acquired methicillin-resistant *Staphylococcus aureus* clone in Japan. J Infect Chemother. 2012; 18: 96–104.
6) Iwao Y, Ishii R, Tomita Y, *et al.*: The emerging ST8 methicillin-resistant *Staphylococcus aureus* clone in the community in Japan: associated infections, genetic diversity, and comparative genomics. J Infect Chemother. 2012; 18: 228–40.
7) Yamaguchi T, Okamura S, Miura Y, Koyama S, Yanagisawa H, Matsumoto T: Molecular characterization of community-associated methicillin-resistant *Staphylococcus aureus* isolated from skin and pus samples of outpatients in Japan. Microbial Drug Resistance 2015; 21: 441–7.
8) Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrad DJ, Etienne J, *et al.*: Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA 2003; 290: 1976–84.
9) Defres S, Marwick C, Nathwani D: MRSA as a cause of lung infection including airway infection, community-acquired pneumonia and hospital-acquired pneumonia. Eur Respir J. 2009; 34: 1470–6.
10) Vandenesch F, Naimi T, Enright MC, *et al.*: Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leucocidin gene: worldwide emergence. Emerg Infect

Dis. 2003; 9: 978–84.
11) Yamamoto T: Panton-Valentine leucocidin positive community-acquired MRSA infection in Japan. Euro Surveill Wkly 2004; 8: 27.
12) Scott T, Dunne M, Marin H: Pleuropulmonary complications of Panton-Valentine leucocidin-positive community-acquired methicillin-resistant *Staphylococcus aureus*. Chest 2005; 128: 2732–8.
13) Conte JE, Golden JA, Kipps J, Zurlinden E: Intrapulmonary pharmacokinetics of linezolid. Antimicrob Agents Chemother. 2002; 46: 1475–80.
14) Yanagihara K, Araki N, Watanabe S, *et al.*: Antimicrobial susceptibility and molecular characteristics of 857 methicillin-resistant staphylococcus aureus from 16 medical centers in Japan (2008–2009): Nationwide survey of community-acquired and nosocomial MRSA. Diagn Microbiol Infect Dis. 2012; 72: 253–7.
15) Takizawa Y, Taneike I, Nakagawa S, *et al.*: A Panton-Valentine leucocidin (PVL)-positive community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) strain, another such strain carrying a multiple-drug resistance plasmid, and other moretypical PVL-negative MRSA strains found in Japan. J Clin Microbiol. 2005; 43: 3356–63.
16) Tomita Y, Kawano O, Ichiyasu H, *et al.*: Two cases of severe necrotizing pneumonia caused by community-acquired methicillin-resistant *Staphylococcus aureus*. Nihon Kokyuuki Gakkai Zasshi 2008; 46: 395–403 (in Japanese).
17) Centers for Disease Control and Prevention. Community-acquired methicillin-resistant *Staphylococcus aureus*- Minnesota and North Dakota, 1997–1999. Murb Mortal Wkly Rep. 1999; 48: 707–10.
18) Saito M, Oikawa T, Nakagawa K, Mizuno S, Osanai K, Toga H: A case of lung abscess caused by community-acquired methicillin-resistant *Staphylococcus aureus*. Nihon Kokyuuki Gakkai Zasshi 2013; 2: 295–9 (in Japanese).
19) Orii KO, Iwao Y, Higuchi W, Takano T, Yamamoto T: Molecular characterization of Methicillin-resistant *Staphylococcus aureus* from a fatal case of necrotizing fasciitis in an extremely low-birth-weight infant. Clin Microbiol Infect. 2010; 16: 289–92.
20) Otera H, Yamamoto G, Ohkusu K, Kozuki H, Hashimoto K, Tada K: Necrotizing pneumonia in the community. Intern Med. 2012; 51: 2463–7.