Invasive Infection Caused by a Community-Associated Methicillin-Resistant *Staphylococcus aureus* Strain Not Carrying Panton-Valentine Leukocidin in South Korea[▽]

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Received 10 February 2009/Returned for modification 27 March 2009/Accepted 26 October 2009

Panton-Valentine leukocidin (PVL)-negative, SCCmec type IVa strains are the most common strains of methicillin-resistant *Staphylococcus aureus* (MRSA) circulating in the community in South Korea. This report describes five elderly patients presenting in 2006 to 2007 with invasive community-associated MRSA infection caused by a PVL-negative, SCCmec type IVa strain with sequence type 72 and *spa* type t324.

Panton-Valentine leukocidin (PVL) genes are prevalent among community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains and have been known as one of the key virulence determinants of CA-MRSA strains (18, 22). Recently, however, some studies have suggested that PVL genes are differentially distributed among CA-MRSA strains and that multiple factors, rather than any single determinant such as PVL, promote CA-MRSA infection (15, 19). PVL-negative, CA-MRSA strains are predominant in Asian-Pacific countries such as Australia, Japan, and China as well as South Korea (1, 3, 7, 17, 21). In particular, a new clone of CA-MRSA, sequence type 72 (ST72)-SCCmec type IVa, which lacks PVL genes, is the most predominant clonal type in South Korea (7, 14). Although there have been some epidemiologic studies of CA-MRSA, few reports are available for invasive infection caused by these PVL-negative CA-MRSA strains in South Korea. We present five Korean cases of invasive CA-MRSA infection caused by a PVL-negative, SCCmec type IVa strain with sequence type 72 and *spa* type t324.

Patient 1. An 80-year-old woman with type 2 diabetes mellitus (DM) suddenly developed painful swelling of the left proximal forearm and right knee. With a clinical diagnosis of infectious bursitis, she was initially treated with intravenous cefazolin after incision and drainage. The antibiotic regimen was changed to intravenous vancomycin since pus culture obtained at admission yielded MRSA. However, the infection progressed rapidly to necrotizing fasciitis, necessitating an emergency fasciotomy. After a 6-week course of intravenous vancomycin therapy, she was ultimately discharged without sequelae.

Patient 2. A 66-year-old woman with type 2 DM presented with painful swelling of the left buttock and was initially treated with intravenous cefazolin. On hospital day 3, the lesion spread rapidly to the left thigh and perineum, and septic shock with acute renal failure developed. Blood cultures obtained at the emergency department yielded MRSA. She was started on intravenous teicoplanin and piperacillin-tazobactam followed by an emergency fasciotomy with a diagnosis of necrotizing fasciitis. On hospital day 5, she was transferred to another tertiary-care hospital, whereupon it was learned that blood cultures taken at our hospital were positive for MRSA. Ultimately, she died of an uncontrolled infection.

Patient 3. A 68-year-old man with chronic obstructive lung disease was admitted with a 2-day history of fever and purulent sputum. Chest computed tomography revealed necrotizing pneumonia and multiple lung abscesses in the right lower lung field, the largest of which was 7.7 cm × 2.8 cm. Treatment with piperacillin-tazobactam and ciprofloxacin was initiated. His antibiotic regimen was changed to vancomycin after cultures of sputum samples obtained on hospital day 2 yielded MRSA. On hospital day 8, empyema developed and a chest tube was inserted. With a 4-week course of intravenous vancomycin, the patient was cured without sequelae.

Patient 4. A 71-year-old woman with a history of mitral valve stenosis and atrial fibrillation developed fever 1 day before admission. Chest examination revealed a grade 3 systolic murmur at apex. Janeway lesions were observed on both feet. A 1-cm vegetation in the left atrium was observed on transthoracic echocardiography. Despite administration of intravenous cefazolin and gentamicin with a diagnosis of acute infective endocarditis, septic shock developed. Blood cultures obtained at admission yielded MRSA, and cefazolin was changed to vancomycin. With the administration of intravenous vancomycin for 6 weeks, she was ultimately discharged without sequelae.

Patient 5. An 82-year-old man presented with right lower leg pain, which had gradually worsened during the previous 6

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[▽] Published ahead of print on 4 November 2009.

TABLE 1. Molecular characteristics and antimicrobial susceptibility of CA-MRSA isolates

Patient age (yr)/sex	Source(s) of MRSA	Molecular type analysis				Antimicrobial susceptibility ^a								
		PVL	SCCmec type	Sequence type (MLST)	spa type	CA	CL ^b	CIP	EM	GM	RFP	TMP-SMX	TET	VM
80/male	Pus	Negative	IVa	ST72	t324	S	R	S	R	S	S	S	S	S
66/female	Pus, blood	Negative	IVa	ST72	t324	S	R	S	R	S	S	S	S	S
68/male	Sputum	Negative	IVa	ST72	t324	S	R	S	R	S	S	S	S	S
71/female	Blood	Negative	IVa	ST72	t324	S	R	S	R	S	S	S	S	S
82/male	Pus	Negative	IVa	ST72	t324	S	R	S	R	S	S	S	S	S

^a Abbreviations: CA, chloramphenicol; CL, clindamycin; CIP, ciprofloxacin; EM, erythromycin; GM, gentamicin; RFP, rifampin; TMP-SMX, trimethoprim-sulfamethoxazole; TET, tetracycline; VM, vancomycin; S, susceptible; R, resistant.

^b These strains showed positive D-test results but were clindamycin susceptible by single-disk testing.

months. On the right lateral malleolus, puslike discharge was drained. Magnetic resonance imaging demonstrated a gadolinium-enhanced lesion consistent with osteomyelitis. Cultures of pus obtained at admission subsequently grew MRSA. The patient received a total of 6 weeks of intravenous vancomycin after incision and drainage.

All five elderly patients with invasive CA-MRSA infection were identified at Hallym University Sacred Heart Hospital from 2006 to 2007. Community acquisition of MRSA was defined as the growth of isolates either in an outpatient setting or within 48 h of hospital admission in patients who had no risk factors for MRSA acquisition (past medical history of MRSA infection or colonization; history of admission to a hospital or long-term care facility during the previous year; or history of surgery, dialysis, permanent indwelling catheters, or medical devices that pass through the skin to the body during the previous year) (5).

S. aureus strains were identified by MicroScan (Dade Behring, West Sacramento, CA) and conventional methods such as coagulase test, mannitol fermentation, and DNase test. Antimicrobial susceptibility testing was done using the MicroScan and disk diffusion methods. Resistance to methicillin was determined by the oxacillin disk susceptibility test and the presence of the *mecA* gene (10, 12). SCCmec typing was performed using multiplex PCR (11, 12). Multilocus sequence typing (MLST) was performed as previously reported (4), as was *spa* typing (16). The *spa* types were determined by comparison with the database at the Ridom SpaServer website (<http://www.ridom.de/spa-server/>). PCR amplification for staphylococcal enterotoxin genes (*sea*, *seb*, *sec*, *sed*, and *see*), the toxic shock syndrome toxin gene (*tst*), exfoliative toxin genes (*eta* and *etb*), and PVL genes was performed as previously reported (8, 10).

All five of the presently reported elderly patients had no risk factors for MRSA acquisition, and culture of all specimens was performed within 48 h of hospital admission. Antibigram patterns were identical in all CA-MRSA isolates, in which MRSA isolates were susceptible to all non- β -lactam antibiotics except erythromycin and clindamycin (Table 1). These five isolates were susceptible to clindamycin by disk diffusion testing but D test positive. All five MRSA isolates harbored the SCCmec type IVa element (12). Also, these five MRSA isolates were classified as type IV SCCmec, when they were tested by use of the updated SCCmec multiplex PCR assay described in 2007 (11). MLST and *spa* typing revealed sequence type 72 and *spa* type t324 in all CA-MRSA isolates. None of the isolates possessed the PVL gene and other staphylococcal toxin

genes tested. These ST72-SCCmec IVa-*spa* type t324 strains have been reported in children adopted to Europe from South Korea, and most cases of transmission of MRSA from these children to other family members were *spa* type t324 (6). In addition, ST72-SCCmec IV strains with *spa* type t2431, a type closely related to *spa* type t324, have been reported in Portugal (1).

CA-MRSA strains share the SCCmec type IV element and increased susceptibilities to non- β -lactam antibiotics but have multiple genetic backgrounds. PVL genes are present among the prototype CA-MRSA strain MW2 and related strains (*spa* types t131 and t194) but are differentially distributed among strains of *spa* types t1, t7, and t17 and other miscellaneous *spa* types (15). Considering the result of *spa* typing of CA-MRSA isolates found in South Korea, in which the most prevalent *spa* type was *spa* type t324 (14), it is possible that PVL genes might not be detected among CA-MRSA isolates in South Korea. Recently, a case of perianal abscess caused by PVL-positive CA-MRSA strain USA300 and *spa* type t1 in South Korea has been reported, but the organism might have been imported from Hawaii, because the patient had been to Hawaii a few weeks before the MRSA infection (13). Also, it has been suggested that PVL is not the key virulence determinant of CA-MRSA (2, 15, 19). In our study, none of the invasive CA-MRSA isolates possessed staphylococcal enterotoxin genes and the toxic shock syndrome toxin gene as well as PVL genes. Further studies of other virulence factors of CA-MRSA, such as phenol-soluble modulins (PSMs) and α -hemolysin, will be required (2, 9, 20). Although most CA-MRSA isolates were found in patients with underlying diseases and host factors might contribute to severe MRSA infection irrespective of the presence of the PVL gene, presently PVL-negative CA-MRSA isolates with ST72 and *spa* type t324 caused invasive infections including necrotizing pneumonia and fatal necrotizing fasciitis in elderly patients.

For all authors, there were no conflicts of interest.

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