Original Article
Clinical Microbiology

Ann Lab Med 2019;39:158-166
https://doi.org/10.3343/alm.2019.39.2.158
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Impact of Community-Onset Methicillin-Resistant *Staphylococcus aureus* on *Staphylococcus aureus* Bacteremia in a Central Korea Veterans Health Service Hospital

Eunsin Bae, M.D.[1], Choon Kwan Kim, M.D.[2], Jung-Hyun Jang, M.D.[3], Heungsup Sung, M.D.[3], YounMi Choi, M.D., Ph.D.[1], and Mi-Na Kim, M.D., Ph.D.[3]

Departments of [1]Laboratory Medicine and [2]Internal Medicine, Veterans Health Service Medical Center, Seoul, Korea; [3]Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea

Background: No study has examined the epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia in Korean veterans' hospitals. We investigated the microbiological and clinical epidemiology of *S. aureus* bacteremia at the central Veterans Health Services (VHS) hospital in Korea.

Methods: Patients with *S. aureus* bacteremia were consecutively enrolled from February to August 2015. Bacteremia was classified as hospital-acquired (HA), community-onset healthcare-associated (COHA), or community-acquired (CA). MRSA bacteremia risk factors were analyzed. Species identification, antimicrobial susceptibility, and presence of *luk* and *tst* were tested. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing, *spa* sequence typing *agr* polymorphism typing, and multilocus sequence typing were performed. Biofilm production and δ-hemolysin activity were measured to determine *agr* function.

Results: In total, 60 patients were enrolled (30 HA, 23 COHA, and seven CA bacteremia); 44 (73.3%) had MRSA bacteremia (26 HA, 16 COHA, and two CA). MRSA bacteremia occurred more frequently in non-CA patients and those who had received antibiotic treatment within the past month ($P<0.05$). The major MRSA strains comprised 24 ST5-*agr*2-SCC*mec*II, 11 ST72-*agr* 1-SCC*mec*IV, and five ST8-*agr*1-SCC*mec*IV strains. Of 26 *agr*2-SCC*mec*II strains, including two MSSA strains, 25 were multidrug-resistant, 18 were *tst*-positive, and 13 were *agr*-defective, whereas only five of the 18 *agr*1-SCC*mec*IV strains were multidrug-resistant, and all were *tst*-negative and *agr*-intact. *agr*1-SCC*mec*IV and ST8-*agr*1-SCC*mec*IV strains were more likely than *agr*2-SCC*mec*II strains to be COHA.

Conclusions: MRSA was highly prevalent in both COHA and HA bacteremia. The introduction of virulent CA-MRSA strains may be an important cause of increased HA-MRSA bacteremia in VHS hospitals.

Key Words: Community onset, *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, Bacteremia, Healthcare-associated, Veterans hospital, Korea

Received: January 21, 2018
Revision received: May 18, 2018
Accepted: October 4, 2018

Corresponding author: Mi-Na Kim, M.D.
https://orcid.org/0000-0002-4624-6925
Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, 88 Olympic-ro 43 gil, Songpa-gu, Seoul 05505, Korea
Tel: +82-2-3010-4511
Fax: +82-2-3010-4510
E-mail: mnkim@amc.seoul.kr

Co-corresponding author: YounMi Choi, M.D.
https://orcid.org/0000-0002-9188-4272
Department of Laboratory Medicine, Veterans Health Service Medical Center, 53 Jinhwangdo-ro 61 gil, Gangdong-gu, Seoul 05368, Korea
Tel: +82-2-2225-1459
Fax: +82-2-2225-4103
E-mail: ymchoi2000@bohun.or.kr

INTRODUCTION

Staphylococcus aureus is a major human pathogen, and methicillin-resistant *S. aureus* (MRSA) is a leading cause of the global threat of hospital-acquired (HA) multidrug-resistant (MDR) organisms [1, 2]. HA-MRSA and community-acquired (CA)-MRSA

clones are genetically distinct, and their distribution varies markedly in different regions [1, 2]. MRSA is expected to become endemic in the community, similar to penicillin-resistant *S. aureus* [3]. Over the past decade, the spread of CA-MRSA in the community and subsequently in the healthcare setting has been associated with the dissemination of specific clones such as USA300-sequence type (ST)8, a highly prominent Panton-Valentine leukocidin (PVL)-positive CA-MRSA in the USA [1, 2, 4].

In Korea, MRSA is highly endemic, constituting 60–81% of clinical *S. aureus* isolates [5, 6] and 58–64% of bacteremic isolates; ST5-SCC*mec* type II is the most prevalent HA-MRSA [7–10]. HA-MRSA strains have spread to the community [1, 2]. PVL-negative ST72 MRSA is a major CA-MRSA strain [7, 9], which has also emerged as an important healthcare-associated pathogen [11-14]. As veterans' hospitals have long-term cohorts of veterans, they are well-established models for studying HA- and CA-MRSA epidemiology and infection control in the US [15, 16]. However, no study has examined the epidemiology of *S. aureus* bacteremia in Korean veterans' hospitals. The Korea Veterans Health Service (VHS), comprising a central hospital and five regional hospitals, provides medical services for veterans nationwide. We investigated the epidemiology and microbiological characterization of *S. aureus* bacteremia in patients with bacteremia in the central VHS hospital.

METHODS

Patients and bacterial isolates

This observational case-series study was conducted at the VHS Medical Center, Seoul, Korea, which serves half of the national population of veterans, including metropolitan Seoul. This hospital contains 1,000-bed acute- and 400-bed long-term-care facilities. All patients with *S. aureus* bacteremia were consecutively enrolled from February to August 2015, and *S. aureus* isolates were collected from their initial blood culture. Patient demographic and clinical findings were retrieved from electronic medical records. The Pitt bacteremia score was used to assess bacteremia severity [17]. The Medical Review Ethics Committee of the VHS Medical Center approved this study and waived the requirement for informed consent (Institutional Review Board number: BOHUN 2015-06-016).

Phenotypic and genotypic analyses

Species identification and antimicrobial susceptibility testing of *S. aureus* isolated from blood culture were performed using MicroScan Pos Breakpoint Combo panel type 28 (PBC28; Beckman Coulter, West Sacramento, CA, USA), which included antimicrobial wells for penicillin, amoxicillin/clavulanate, azithromycin, clindamycin, daptomycin, erythromycin, fosfomycin, ciprofloxacin, levofloxacin, fusidic acid, gentamicin, imipenem, linezolid, mupirocin, oxacillin, quinupristin/dalfopristin, rifampin, teicoplanin, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin. Methicillin resistance was determined based on resistance to oxacillin or cefoxitin. For all study isolates, *agr* operon function was measured by δ-hemolysin expression assays using β-hemolysin-producing *S. aureus* strain RN4220 on blood agar plates, as previously described [18]. *agr* dysfunction was defined as no enhancement of the β-hemolytic zone. The biofilm assay was performed in polystyrene microtiter plates, as previously described [18]. PCR was conducted for *agr* polymorphism typing, staphylococcal cassette chromosome *mec* (SCC*mec*) typing, *spa* sequence typing, multilocus sequence typing (MLST), and detection of the *lukS-PV* and *lukF-PV* genes for PVL and *tst* gene for toxic shock syndrome toxin (TSST), as previously described [19-21]. *spa* types were assigned using the RidomStaphType software version 2.2.1 (Ridom GmbH, Munster, Germany) and the SpaServer (http://www.spaserver.ridom.de). MLST allele name and STs were derived from the MLST database (http://www.mlst.net).

Epidemiological investigation and definitions

Bacteremia was classified as HA if blood cultures taken ≥48 hours after admission were positive and as CA if blood cultures taken in an outpatient setting or <48 hours of hospitalization were positive [22]. It was classified as community-onset healthcare-associated (COHA) if the positive cultures were obtained in the CA time frame, but the patient satisfied one or both of the following conditions: 1) a history of hospitalization, surgery, dialysis, or residence in a long-term care facility in the previous one year and 2) the presence of a central venous catheter (CVC) within two days prior to the positive blood culture for *S. aureus*. Persistent bacteremia was defined as a culture positive for *S. aureus* ≥72 hours after the onset of appropriate antimicrobial therapy such as glycopeptide antibiotics [23]. Central line-associated bloodstream infection (CLABSI) was designated when the source of bacteremia was assumed to be a CVC, according to the criteria of the US Centers for Disease Control and Prevention's National Healthcare Safety Network [24]. MDR was defined as the resistance to three or more classes of non-β-lactam antimicrobials based on susceptibility to gentamicin, erythromycin, clindamycin, ciprofloxacin, rifampicin, tetracycline, and trimethoprim/sulfamethoxazole.

Statistical analysis

Continuous variables with normal distribution were summarized as mean±SD; variables with non-normal distribution were summarized as median and range or interquartile range (IQR). Categorical variables were expressed as N (%). Differences between MRSA and MSSA were calculated using the chi-square or Fisher's exact tests for categorical variables and Student t-test or Mann-Whitney test for continuous variables. Odds ratios and 95% confidence intervals (CIs) were calculated to measure the association between an *S. aureus* ST and HA/COHA/CA. $P<0.05$ (two-sided) was considered statistically significant. All statistical analyses were performed using MedCalc version 16.4.3 (MedCalc Software, Ostend, Belgium).

RESULTS

Patients and bacterial isolates

In total, 60 patients with *S. aureus* bacteremia were enrolled. They were categorized as having HA (30 patients), COHA (23 patients), and CA (seven patients) bacteremia (Table 1). All patients, except one, were aged >60 years, and 54 patients were males. Forty-four patients had one or more serious chronic illnesses, including diabetes, malignancy, chronic renal failure, and liver cirrhosis. The most frequent sources of bacteremia were an indwelling CVC (N=17, 28.3%) or skin and soft tissue infection (N=10, 16.6%) (Table 1). Forty-four (73.3%) patients had MRSA bacteremia. Fifteen (88.2%) of the 17 CLABSI cases and 12 persistent bacteremia cases were caused by MRSA. MRSA bacteremia was more frequent in patients who underwent antibiotic treatment within the past month ($P<0.05$).

Phenotypic and genotypic characteristics

The characteristics of all 60 *S. aureus* strains are summarized in Table 2. MRSA were grouped into three ST groups: 26 (59%) ST5-*agr*2-SCC*mec*II strains comprising 12 t2460, six t002, three t2066, two t242, and three singular types; 12 (27%) ST72-*agr*1-

Table 1. Comparison of the clinical characteristics of patients with MRSA and MSSA bacteremia

	Total (N=60)	MRSA bacteremia (N=44)	MSSA bacteremia (N=16)	*P**
Age (yr), median (range)	77 (26–89)	77 (63–88)	78 (26–89)	0.74
Males	54 (90.0)	40 (90.9)	14 (87.5)	0.93
Mode of acquisition				
Hospital-acquired	30 (50.0)	26 (59.0)	5 (31.2)	0.26
Community-onset healthcare-associated	23 (38.3)	16 (36.3)	6 (37.5)	0.96
Community-acquired	7 (11.7)	2 (4.5)	5 (31.2)	0.02
Underlying disease				
Malignant tumor	17 (24.6)	11 (22.0)	6 (31.6)	0.49
Chronic renal failure	15 (21.7)	11 (22.0)	4 (21.0)	1.00
Liver cirrhosis	4 (5.8)	4 (8.0)	0	0.24
Diabetes mellitus	33 (47.8)	24 (48.0)	9 (47.4)	0.95
Source of infection				
Central line-associated infection	17 (28.3)	15 (34.1)	2 (12.5)	0.20
Skin and soft tissue infection	10 (16.6)	7 (15.9)	3 (18.8)	0.83
Primary	33 (55.0)	21 (47.7)	11 (68.8)	0.44
Recent antibiotic treatment within one month	27 (45.0)	25 (56.8)	2 (12.5)	0.04
Pitt bacteremia score, median (IQR)	1 (0–2)	0 (0–2)	1 (0–2.5)	0.42
Outcome				
Duration of bacteremia, mean±SD	2.0±4.5	2.5±5.1	0.4±0.6	0.10
Persistent bacteremia (≥three days)	12 (20.0)	12 (32.4)	0	0.06
All-cause mortality within 14 days†	7 (11.7)	6 (13.6)	1 (6.2)	0.66

Values are given as N (%) unless otherwise indicated.
*MRSA bacteremia vs MSSA bacteremia; †Forty-four MRSA bacteremia and 14 MSSA bacteremia cases were followed up.
Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; IQR, interquartile range.

https://doi.org/10.3343/alm.2019.39.2.158

Table 2. Microbiological characteristics and epidemiological data of *Staphylococcus aureus* bacteremia

Strain type	Isolates (N)	δ-hemolysin	Biofilm	Toxin		Resistance profile	Persistent bacteremia* (day)	Source of infection (mode of acquisition)
				TSST	PVL			
ST5-*agr*2-SCC*mec*II	26							
t002	1	+	+	+	-	OXA		SSTI (CA)
	1	+	+	+	-	OXA-CIP-CC-EM-GM-TET		Primary (HA)
	1	+	+	-	-	OXA-CIP-CC-EM-GM-TET		SSTI (COHA)
	1	+	+	-	-	OXA-CIP-CC-EM-MUP-TET		CVC (HA)
	1	+	-	-	-	OXA-CIP-CC-EM-MUP		Primary (HA)
	1	+	-	+	-	OXA-CIP-CC-EM-GM-TET	3	CVC (HA)
t12703	1	-	+	+	-	OXA-CIP-CC-EM-GM-FA-MUP-TET	5	SSTI (HA)
t2066	1	+	+	+	-	OXA-CIP-CC-EM	4	CVC (HA)
	1	+	-	+	-	OXA-CIP-CC-EM-GM-TET		Primary (HA)
	1	+	-	+	-	OXA-CIP-CC-EM-GM-MUP-TET		Primary (HA)
t242	1	+	+	-	-	OXA-CIP-CC-EM		CVC (COHA)
	1	+	+	-	-	OXA-CIP-CC-EM-GM-TET		Primary (HA)
t2460	1	-	+	+	-	OXA-CIP-CC-EM-GM	6	Primary (HA)
	1	-	+	+	-	OXA-CIP-CC-EM-GM-TET	6	Primary (HA)
	1	-	+	+	-	OXA-CIP-CC-EM-FA-MUP-TET		Primary (COHA)
	2	-	+	+	-	OXA-CIP-CC-EM-GM-FA-MUP-TET		CVC (HA), CVC/SSTI (COHA)
	2	-	+	+	-	OXA-CIP-CC-EM-GM-FA-MUP-TET-RIF	4[a]	Primary (HA), CVC/SSTI (COHA)
	1	-	+	+		OXA-CIP-CC-EM-GM-FA-TET		Primary (COHA)
	2	-	+	-	-	OXA-CIP-CC-EM-GM-FA-TET		CVC (HA), Primary (COHA)
	1	-	-	+	-	OXA-CIP-CC-EM-GM-FA-MUP-TET		SSTI (HA)
	1	-	-	+	-	OXA-CIP-CC-EM-GM-FA-MUP-TET-SXT		Primary (HA)
t264	1	-	+	-	-	OXA-CIP-CC-EM-GM-TET-FA-MUP	6	CVC (HA)
t5076	1	-	+	+	-	OXA-CIP-CC-EM-GM-FA-TET	6	CVC (HA)
ST72-*agr*1-SCC*mec*IV	12							
t148	1	+	-	-	-	OXA-CIP-GM-MUP		Primary (COHA)
t324	2	+	+	-	-	OXA	5	Primary (COHA), Primary (COHA)
	1	+	+	-	-	OXA-CIP-GM		Primary (COHA)
	1	+	+	-	-	OXA-CC-EM		Primary (COHA)
	1	+	+	-	-	OXA-CC-EM-GM	31	CVC (HA)
	1	+	+	-	-	OXA-CIP-CC-EM-FA-TET	6	Primary (COHA)
	1	+	-	-	-	OXA		Primary (HA)
t5553	1	+	+	-	-	OXA-CC-EM		CVC (HA)
t664	2	+	-	-	-	OXA		Primary (COHA), CVC (HA)
t901	1	+	+	-	-	OXA	12	Primary (COHA)
ST8-*agr*1-SCC*mec*IV	5							
t008	1	+	+	-	+	OXA-CIP-EM		CVC (COHA)
	1	+	+	-	+	OXA-CIP-CC-EM-MUP-SXT		Primary (HA)
	2	+	-	-	+	OXA-CIP-EM		SSTI (CA), Primary (HA)
	1	+	-	-	+	OXA-CIP-CC-EM		Primary (COHA)

(*Continued to the next page*)

Table 2. Continued

Strain type	Isolates (N)	δ-hemolysin	Biofilm	Toxin		Resistance profile	Persistent bacteremia* (day)	Source of infection (mode of acquisition)
				TSST	PVL			
ST834-*agr*1-SCC*mec*IV	1							
t1379	1	+	+	-	-	OXA-CIP-CC-EM-GM-FA-MUP-TET		CVC (HA)
ST188-*agr*1	5							
t189	3	+	-	-	-	(–)		SSTI (COHA), Primary (HA), Primary (COHA)
	1	+	-	-	-	FA		Primary (COHA)
	1	+	-	-	-	GM-MUP		Primary (HA)
ST72-*agr*1	4							
t126	1	+	-	-	-	FA		Primary (CA)
t148	1	+	+	-	-	CIP-GM		Primary (COHA)
t324	1	+	+	-	-	(–)		SSTI (CA)
	1	+	-	-	-	(–)		SSTI (COHA)
ST5-*agr*2	3							
t002	1	+	+	+	-	CIP-CC-EM-TET		Primary (CA)
	1	+	+	-	-	CIP-CC-EM-MUP		CVC (COHA)
	1	+	-	-	-	(–)		Primary (CA)
ST30-*agr*3	2							
t012	1	+	-	+	-	CIP-CC-EM-GM-TET		Primary (HA)
t363	1	+	-	+	-	CC-EM		Primary (HA)
ST15-*agr*2	1							
t279	1	+	-	-	-	(–)		Primary (CA)
ST623-*agr*1	1							
t222	1	+	+	-	-	CIP-CC-EM-GM-TET		CVC (HA)

*Persistent bacteremia was defined as a culture-positive for *S. aureus* ≥72 hours after the onset of appropriate antimicrobial therapy such as glycopeptides.
Abbreviations: ST, sequence type; SCC*mec*, staphylococcal cassette chromosome *mec*; *agr*, accessory gene regulator; TSST, toxic shock syndrome toxin; PVL, Panton-Valentine leukocidin; OXA, oxacillin; CIP, ciprofloxacin; CC, clindamycin; EM, erythromycin; FUS, fusidic acid; GM, gentamicin; MUP, mupirocin; TET, tetracycline; RIF, rifampin; SXT, trimethoprim/sulfamethoxazole; HA, hospital-acquired; COHA, community-onset healthcare-associated; CA, community-acquired; CVC, central venous catheter; SSTI, skin and soft tissue infection.

SCC*mec*IVa strains (including seven t324, two t664, and two singular types); and five (11%) ST8-*agr*1 strains, all of which were t008 and SCC*mec*IV. Methicillin-susceptible *S. aureus* (MSSA) were heterogeneous, consisting of five ST188-t189-*agr*1 strains, four ST72-*agr*1 strains (two t324, one t126, and one t148), three ST5-t002-*agr*2 strains, two ST30-*agr*2 strains (t012 and t363), one ST15-t279-*agr*2 strain, and one ST623-t222-*agr*1 strain. ST5 MRSA was typically MDR, showing consistent resistance to erythromycin, clindamycin, and ciprofloxacin. ST5-t2460 MRSA was resistant to additional antimicrobials such as gentamicin, fusidic acid, mupirocin, and tetracycline. All strains were completely susceptible to vancomycin with a minimum inhibitory concentration of ≤1 μg/mL. *agr*1-SCC*mec*IV MRSA was unlikely to be MDR, and in particular, half of the ST72 MRSA strains were susceptible to all tested antimicrobials other than β-lactams. However, one ST834-*agr*1-SCC*mec*IV strain was highly MDR. All ST8-*agr*1 strains were resistant to ciprofloxacin, and one HA strain was resistant to high-level mupirocin and trimethoprim-sulfamethoxazole, as well as β-lactams.

A schematic summary of the genotypic and phenotypic characteristics of all 60 strains is presented in Fig. 1 based on *spa* typing. Regardless of methicillin resistance, the ST5 strains were all PVL-negative and frequently *tst*-positive (65.5%), whereas the ST72 strains were negative for both PVL and *tst*. In addition, five ST8 MRSA strains were PVL-positive, all ST30 MSSA strains were *tst*-positive, and δ-hemolysin-negative *agr* dysfunction was identified in 15 (57.7%) of the ST5 MRSA strains, mainly t2460. All MSSA strains were δ-hemolysin-positive, and one ST30 strain

https://doi.org/10.3343/alm.2019.39.2.158

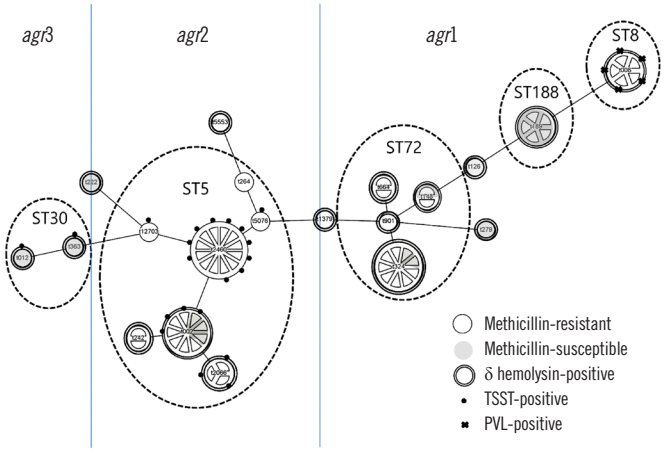

Fig. 1. Cluster analysis based on *spa* typing of 60 *Staphylococcus aureus* strains isolated from blood cultures. The minimum spanning tree was constructed using Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium). Genotypes determined by using accessory gene regulator (*agr*) polymorphism and sequence type (ST) correlated well with *spa* typing clustering. Methicillin susceptibility, δ-hemolysin production, toxic shock syndrome toxin (TSST), and Panton–Valentine leukocidin (PVL) toxin were denoted.

and two ST5 strains were MDR.

In total, 36 (60.0%) isolates were biofilm-positive, including 20 (76.9%) ST5 MRSA, eight (66.7%) ST72 MRSA, and five (31.3%) MSSA. All non-biofilm-forming strains, except two, were δ-hemolysin-positive, and all biofilm-forming strains, except two ST5 MRSA strains, were δ-hemolysin-defective. However, 22 (50.0%) of the 44 δ-hemolysin-positive strains were also biofilm-forming.

Epidemiological characteristics

The HA/COHA/CA bacteremia distribution among the *agr*II-SCC*mec*II and *agr*1-SCC*mec*IV strains was 18 (69.2%)/six (26.9%)/one (3.8%) and seven (38.9%)/11 (55.6%)/one (5.6%), respectively, while in MSSA strains, it was five (31.3%)/six (37.5%)/five (31.3%). Eighteen (69.2%) and six (26.9%) ST5 MRSA strains were isolated from HA- and COHA bacteremia, respectively. One ST5 MRSA was resistant to only oxacillin and isolated from CA bacteremia. Of the five ST8 MRSA strains, four were healthcare-associated, including two HA and two COHA. *agr*1-SCC*mec*IV strains were more likely to be COHA than *agr*2-SCC*mec*II strains (odds ratio, 3.4; 95% CI, 0.95–12.09) or MSSA (odds ratio, 2.1; 95% CI, 0.52–8.23). In particular, two thirds of the ST72 MRSA strains were isolated from COHA-bacteremia.

All δ-hemolysin-defective strains were healthcare-associated, and 10 of the 15 δ-hemolysin-defective ST5 MRSA strains were HA (Table 2). All CLABSI-causing *S. aureus* strains, except one, were biofilm-positive and 11 (64.7%) of those were HA-MRSA. Nine (75.0%) of 12 persistent bacteremia cases were HA, seven of which were caused by TSST-producing ST5 MRSA. Six of the HA-CLABSI cases resulted in persistent bacteremia, caused by five ST5 MRSA strains and one ST72 MRSA strain. No cases of persistent bacteremia were caused by PVL-producing ST8 MRSA strains.

The ST8-*agr*1-SCC*mec*IV strains were consistent with USA300, which were PVL-positive, *tst*-negative, and δ-hemolysin-positive [25-27]. The patients harboring these ST8 MRSA strains had no history of having travelled abroad in the last decade.

DISCUSSION

MRSA is highly endemic in Korea, regardless of hospital size or complexity; however, MRSA prevalence (73.3%) in this study is much higher than those reported in Korean hospitals previously (51.4–54.3%) [28, 29]. The patient population of the central VHS hospital is unique, comprising predominantly elderly males, with a high percentage of long-term repeat visitors and a high transfer rate from regional veterans' hospitals or long-term care facilities all over Korea. Therefore, more *S. aureus* bacteremia cases than usual are likely to be healthcare-associated. The proportions of MRSA in HA and COHA bacteremia were not significantly different as 83.3% and 73.9%, respectively, and were much higher than that of CA bacteremia (28.7%). HA or COHA MRSA accounted for 90% of *S. aureus* bacteremia cases, clearly contributing to the high prevalence of MRSA in this hospital. In addition, the high prevalence of COHA-MRSA bacteremia indicated the possibility of the spread of HA-MRSA into the community and subsequent re-emergence in the healthcare setting via COHA transfer.

The major HA or COHA-MRSA strains in this study were ST5 MRSA, ST72 MRSA, and ST8 MRSA. ST239 was not detected in this study, although it had been previously considered as a representative HA-MRSA strain in Korea [1, 28]. ST72, a typical CA-MRSA strain in Korea over the last decade, displayed CA-MRSA characteristics, such as non-MDR traits and the presence of SCC*mec*IV; however, all ST72 MRSA strains in this study were from healthcare-associated infections (75% COHA and 25% HA). ST72 MRSA seemed to play a major role in the spread of CA-MRSA to the hospital via COHA transfer. The prevalence of ST239 has recently decreased in Korea [6, 8, 30], and ST72 MRSA has been found to be the most common clone in both CA- and HA-MRSA infections in Korean pediatric patients [13, 31]. The absence of ST239 and the presence of only a few ma-

jor MRSA strains in this hospital provide evidence of the clonal spread of MRSA, indicating that a rapid change in MRSA clones is possible in a hospital.

The main ST5 MRSA was ST5-*agr*2-SCC*mec*II, which belongs to the New York–Japan clone and is known to be dominant in the adult Korean population [10, 13]. The ST5-t2460 strain is a well-known *tst*-positive MRSA clone in Korea [8, 9, 14], and it was a main *agr*-defective strain in the present study. *agr*-defective strains are associated with high mortality, biofilm formation, prolonged bacteremia, and reduced susceptibility to vancomycin [8, 14]. However, the minimum inhibitory concentration of vancomycin for all the isolates in this study was<2 μg/mL, in contrast to previous findings about the New York–Japan clone [32]. Therefore, to our knowledge, this is the first study to demonstrate that ST5-t2460-MRSA is a predominant and highly threatening pathogen in healthcare-associated bacteremia, with a high virulence in Korea.

ST72 MRSA in the present study was PVL-negative, harbored SCC*mec*IV, belonged to *agr*1, and was unlikely to be MDR. PVL-negative ST72-*agr*1-SCC*mec*IV, a major CA-MRSA over the last decade, has the advantage of fitness cost in the community because of the small size of SCC*mec* [33]. However, this clone has recently emerged as a healthcare-associated pathogen in Korea [11-14]. In our study, all of ST72 MRSA bacteremia cases were healthcare-associated, and the high prevalence of ST72 among MRSA bacteremia cases suggests that continuous introduction of COHA-MRSA has contributed to the high prevalence of MRSA bacteremia.

Notably, we revealed that the emergence of PVL-positive ST8-*agr*1-SCC*mec*IV strains was responsible for approximately 10% of MRSA bacteremia cases. This strain is likely to be USA300 clone, which first emerged as CA-MRSA with high virulence and then successfully spread into healthcare settings in the USA [27]. Cases of sporadic USA300 outbreaks have been reported since the first case was imported from Hawaii into Korea in 2007 [9, 25, 26, 34]. This strain was both community- and healthcare-associated in our study, consistent with a recent multicenter study [35]; however, it exhibited increased resistance to antimicrobials. The spread and evolution of this PVL-positive strain should be closely monitored because of its high virulence and ability to spread to both community and healthcare settings.

Among the MSSA strains, ST188, ST72, ST5, and ST30 occurred most frequently, consistent with previous findings in Korea [8, 36]. ST188-t198, ST72-t126, and -t324 were linked to *agr*1 and susceptible to antimicrobials, whereas ST5-t002 and ST30-t012 were linked to *agr*2 and *agr*3, respectively, and more likely to be MDR. One ST5-t002 harbored *tst* and was MDR, thus having a common trait with ST5 MRSA. These findings suggested cure of methicillin resistance by excision of the *mec*A gene from ST5-MRSA [37]. All MSSA strains had intact *agr* function. Although *agr* dysfunction in MSSA is rare, one study reported 12.5% *agr* dysfunction among MSSA blood isolates in Korea and very high *agr* dysfunction (89.4%) for ST5 MRSA [8].

This study has important limitations. Because this is the first epidemiological study on *S. aureus* in Korean VHS hospitals, more longitudinal studies are required to interpret the characteristics of high MRSA prevalence and MRSA clonality better. Furthermore, as this is an observational case-series study, clinical and laboratory evaluations were not regulated, and thus, outcome analyses were limited.

In conclusion, we identified the wide spread of ST5-*agr*2-SCC*mec*II, a typical *agr*-defective HA clone, from hospital to community and of ST72-*agr*1-SCC*mec*IV, a typical CA clone, from community to hospital in a Korean VHS hospital. We also confirmed that the PVL-positive ST8 MRSA strain was prevalent both in the community and the healthcare setting and acquired the MDR character. Clinicians and clinical microbiologists should note that COHA-MRSA is prevalent in *S. aureus* bacteremia.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

Acknowledgement

This study was supported by the VHS Medical Center Research Grant, Republic of Korea (grant number: VHSMC15006).

REFERENCES

1. Song JH, Hsueh PR, Chung DR, Ko KS, Kang CI, Peck KR, et al. Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: an ANSORP study. J Antimicrob Chemother 2011;66:1061-9.
2. Otter JA and French GL. Community-associated meticillin-resistant *Staphylococcus aureus* strains as a cause of healthcare-associated infection. J Hosp Infect 2011;79:189-93.
3. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated methicillin-resistant *Staphylococcus aureus*. Lancet 2010;375:1557-68.
4. Bal AM, Coombs GW, Holden MTG, Lindsay JA, Nimmo GR, Tattevin P, et al. Genomic insights into the emergence and spread of international clones of healthcare-, community- and livestock-associated methicillin-

https://doi.org/10.3343/alm.2019.39.2.158

resistant *Staphylococcus aureus*: blurring of the traditional definitions. J Global Antimicrob Resist 2016;6:95-101.
5. Kim D, Ahn JY, Lee CH, Jang SJ, Lee H, Yong D, et al. Increasing resistance to extended-spectrum cephalosporins, fluoroquinolone, and carbapenem in gram-negative bacilli and the emergence of carbapenem non-susceptibility in klebsiella pneumoniae: analysis of Korean Antimicrobial Resistance Monitoring System (KARMS) data from 2013 to 2015. Ann Lab Med 2017;37:231-9.
6. Kang GS, Jung YH, Kim HS, Lee YS, Park C, Lee KJ, et al. Prevalence of major methicillin-resistant *Staphylococcus aureus* clones in Korea between 2001 and 2008. Ann Lab Med 2016;36:536-41.
7. Kim ES, Song JS, Lee HJ, Choe PG, Park KH, Cho JH, et al. A survey of community-associated methicillin-resistant *Staphylococcus aureus* in Korea. J Antimicrob Chemother 2007;60:1108-14.
8. Chong YP, Kim ES, Park SJ, Park KH, Kim T, Kim MN, et al. Accessory gene regulator (*agr*) dysfunction in *Staphylococcus aureus* bloodstream isolates from South Korean patients. Antimicrob Agents Chemother 2013; 57:1509-12.
9. Park C, Lee DG, Kim SW, Choi SM, Park SH, Chun HS, et al. Predominance of community-associated methicillin-resistant *Staphylococcus aureus* strains carrying staphylococcal chromosome cassette *mec* type IVA in South Korea. J Clin Microbiol 2007;45:4021-6.
10. Park KH, Chong YP, Kim SH, Lee SO, Choi SH, Lee MS, et al. Community-associated MRSA strain ST72-SCC*mec*IV causing bloodstream infections: clinical outcomes and bacterial virulence factors. J Antimicrob Chemother 2015;70:1185-92.
11. Joo EJ, Choi JY, Chung DR, Song JH, Ko KS. Characteristics of the community-genotype sequence type 72 methicillin-resistant *Staphylococcus aureus* isolates that underlie their persistence in hospitals. J Microbiol 2016;54:445-50.
12. Park SH, Park C, Yoo JH, Choi SM, Choi JH, Shin HH, et al. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* strains as a cause of healthcare-associated bloodstream infections in Korea. Infect Control Hosp Epidemiol 2009;30:146-55.
13. Sung JY, Lee J, Choi EH, Lee HJ. Changes in molecular epidemiology of community-associated and health care-associated methicillin-resistant *Staphylococcus aureus* in Korean children. Diagn Microbiol Infect Dis 2012;74:28-33.
14. Kang CK, Cho JE, Choi YJ, Jung Y, Kim NH, Kim CJ, et al. *agr* dysfunction affects staphylococcal cassette chromosome *mec* type-dependent clinical outcomes in methicillin-resistant *Staphylococcus aureus* bacteremia. Antimicrob Agents Chemother 2015;59:3125-32.
15. Caffrey AR and LaPlante KL. Changing epidemiology of methicillin-resistant *Staphylococcus aureus* in the Veterans Affairs Healthcare System, 2002-2009. Infection 2012; 40:291-7.
16. Stenehjem E, Stafford C, Rimland D. Reduction of methicillin-resistant *Staphylococcus aureus* infection among veterans in Atlanta. Infect Control Hosp Epidemiol 2013;34:62-8.
17. Roth JA, Tschudin-Sutter S, Dangel M, Frei R, Battegay M, Widmer AF. Value of the Pitt Bacteraemia Score to predict short-term mortality in *Staphylococcus aureus* bloodstream infection: a validation study. Swiss Med Wkly 2017;147:w14482.
18. Sakoulas G, Eliopoulos GM, Moellering RC, Wennersten C, Venkataraman L, Sakoulas G, et al. Accessory gene regulator (*agr*) locus in geographically diverse *Staphylococcus aureus* isolates with reduced susceptibility to vancomycin. Antimicrob Agents Chemother 2002;46:1492-502.
19. Oliveira DC and de Lencastre H. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2002; 46:2155-61.
20. Enright MC, Day NPJ, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 2000; 38:1008-15.
21. Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, Forey F, et al. Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease. Infect Immun 2002;70:631-41.
22. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA 2007;298:1763-71.
23. Mermel LA, Allon M, Bouza E, Craven DE, Flynn P, O'Grady NP, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. Clin Infect Dis 2009;49:1-45.
24. Centers for Disease Control and Prevention and the National Healthcare Safety Network. CDC/NHSN surveillance definitions for specific types of infections. http://www.cdc.gov/nhsn/PDFs/pscManual/17pscNosInfDef_current.pdf (updated on Jan 2018).
25. Sohn KM, Chung DR, Baek JY, Kim SH, Joo EJ, Ha YE, et al. Post-influenza pneumonia caused by the USA300 community-associated methicillin-resistant *Staphylococcus aureus* in Korea. J Korean Med Sci 2012; 27:313-6.
26. Lee H, Kim ES, Choi C, Seo H, Shin M, Bok JH, et al. Outbreak among healthy newborns due to a new variant of USA300-related meticillin-resistant *Staphylococcus aureus*. J Hosp Infect 2014;87:145-51.
27. Carrel M, Perencevich EN, David MZ. USA300 methicillin-resistant *Staphylococcus aureus*, United States, 2000-2013. Emerg Infect Dis 2015; 21:1973-80.
28. Peck KR, Baek JY, Song JH, Ko KS. Comparison of genotypes and enterotoxin genes between *Staphylococcus aureus* isolates from blood and nasal colonizers in a Korean hospital. J Korean Med Sci 2009;24:585-91.
29. Oh TS, Nam YS, Kim YJ, Yang HS, Lee MY, Gu HJ, et al. Trends in bloodstream infections at a Korean university hospital between 2008 and 2013. Ann Clin Microbiol 2015;18:14-9.
30. Cha HY, Moon DC, Choi CH, Oh JY, Jeong YS, Lee YC, et al. Prevalence of the ST239 clone of methicillin-resistant *Staphylococcus aureus* and differences in antimicrobial susceptibilities of ST239 and ST5 clones identified in a Korean hospital. J Clin Microbiol 2005;43:3610-4.
31. Park JY, Jin JS, Kang HY, Jeong EH, Lee JC, Lee YC, et al. A comparison of adult and pediatric methicillin-resistant *Staphylococcus aureus* isolates collected from patients at a university hospital in Korea. J Microbiol 2007;45:447-52.
32. Mendes RE, Deshpande LM, Smyth DS, Shopsin B, Farrell DJ, Jones RN. Characterization of methicillin-resistant *Staphylococcus aureus* strains recovered from a phase IV clinical trial for linezolid versus vancomycin for treatment of nosocomial pneumonia. J Clin Microbiol 2012;50:3694-702.
33. D'Agata EM, Webb GF, Horn MA, Moellering RC Jr, Ruan S. Modeling the invasion of community-acquired methicillin-resistant *Staphylococcus aureus* into hospitals. Clin Infect Dis 2009;48:274-84.
34. Lim S, Chung DR, Baek JY, Kim SH, Peck KR, Lee NY, et al. A third case of USA300 community-associated methicillin-resistant *Staphylococcus aureus* infection in Korea. Korean J Intern Med 2013;28:258-60.
35. Jung J, Song EH, Park SY, Lee SR, Park SJ, Sung H, et al. Emergence of Panton-Valentine leucocidin-positive ST8-methicillin-resistant *Staphylococcus aureus* (USA300 clone) in Korea causing healthcare-associat-

ed and hospital-acquired bacteraemia. Eur J Clin Microbiol Infect Dis 2016;35:1323-9.
36. Park SH, Kim KJ, Kim BK, Hwang SM. Molecular characterization of community-associated methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates from children with skin infections in Busan, Korea. J Bacteriol Virol 2015;45:104-11.
37. Shore AC, Rossney AS, O'Connell B, Herra CM, Sullivan DJ, Humphreys H, et al. Detection of staphylococcal cassette chromosome *mec*-associated DNA segments in multiresistant methicillin-susceptible *Staphylococcus aureus* (MSSA) and identification of *Staphylococcus epidermidis ccrAB4* in both methicillin-resistant *S. aureus* and MSSA. Antimicrob Agents Chemother 2008;52:4407-19.

https://doi.org/10.3343/alm.2019.39.2.158