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Prevalence and Clinical Impact of Heterogeneous Vancomycin-Intermediate *Staphylococcus aureus* Isolated From Hospitalized Patients

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Background: We estimated the prevalence and clinical impact of heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA). The concordance between macromethod and glycopeptide resistance detection (GRD) E tests was determined. In addition, predictors of clinical outcomes in hospitalized patients with *S. aureus* bacteremia (SAB) or pneumonia (SAP) were evaluated.

Methods: We obtained 229 consecutive *S. aureus* isolates from all hospitalized patients at two university hospitals located in Busan and Yangsan, Korea. Standard, macromethod, and GRD E tests were performed. Additionally, we reviewed the medical records of all patients. Among the 229 patients, predictors of clinical outcomes were analyzed for 107 patients with SAB and 39 with SAP.

Results: Among the 229 isolates, 34.5% of *S. aureus* isolates and 50.7% of methicillin-resistant *S. aureus* isolates exhibited the hVISA phenotype based on the macromethod E test. hVISA was nearly associated with treatment failure in patients with SAB (P=0.054) and was significantly associated with treatment failure in patients with SAP (P=0.014). However, hVISA was not associated with 30-day mortality in patients with SAB or SAP. The concordance between the macromethod and GRD E tests was 84.2%.

Conclusions: hVISA is quite common in the southeastern part of Korea. hVISA is associated with treatment failure in patients with SAP.

Key Words: Heterogeneous vancomycin-intermediate *Staphylococcus aureus*, Macromethod E test, Glycopeptide resistance detection E test, *S. aureus* bacteremia, *S. aureus* pneumonia

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has caused numerous invasive infections and deaths. Invasive MRSA infection is a major public health problem [1]. Vancomycin has been the treatment of choice for serious MRSA infections since 1958. However, its overuse has led to the emergence of vancomycin-intermediate and -resistant MRSA [2].

Vancomycin-intermediate *S. aureus* (VISA) develops via the heterogeneous vancomycin-intermediate *S. aureus* (hVISA) precursor phenotype [3]. hVISA is defined as *S. aureus* with a vancomycin minimum inhibitory concentration (MIC) within the susceptible range, but with a subpopulation of cells in the vancomycin-intermediate range [4].

hVISA was first reported in Japan in 1997 [5] and has since been identified worldwide [6-8]. In Korea, VISA was first re-

ported in 2000 [9]. hVISA prevalence was 0.5% from all clinical *S. aureus* isolates in 2002 [10], 6.1% from all clinical MRSA isolates in 2004 [11], and 37.7% from MRSA bacteremia isolates in 2012 [12]. However, these data were obtained from single-center studies conducted only in Seoul. To date, no other studies have been subsequently conducted in other regions. Therefore, it is necessary to investigate hVISA prevalence in regions other than Seoul.

Population analysis profiling (PAP) is considered the most accurate method for detecting hVISA [13]. However, PAP is time-consuming, labor-intensive, and expensive. Furthermore, results are generally not available within a clinically relevant time period, taking at least 3-5 days [14], which limits its use in most clinical microbiology laboratories.

Among hVISA detection methods, macromethod and glycopeptide resistance detection (GRD) E tests demonstrate good sensitivity and specificity, when PAP is used as a reference method [15, 16]. However, only a few studies have determined the concordance between these two E tests [15-17], and further studies are needed to address this issue.

Moreover, it is still controversial whether hVISA exacerbates clinical outcomes in patients with *S. aureus* bacteremia (SAB) [18-24]. Additionally, predictors of clinical outcomes in patients with *S. aureus* pneumonia (SAP) have not been identified.

To resolve these issues, we first performed a hVISA detection study to obtain the hVISA prevalence in the southeastern part of Korea. Second, we estimated the concordance between the macromethod and GRD E tests. Third, we evaluated predictors of clinical outcomes in hospitalized patients with SAB or SAP.

METHODS

1. Collection of *S. aureus* isolates

We obtained consecutive *S. aureus* isolates from all hospitalized patients in two university hospitals located in Busan and Yangsan, Korea. The *S. aureus* isolates were obtained by culturing blood, body fluids (pleural fluid, peritoneal fluid, and joint fluid), bronchial fluid, pus, sputum, and urine. Isolates from other specimens were excluded. *S. aureus* was identified by using the VITEK 2 system (bioMérieux, Marcy-l'Etoile, France). This study was approved by the institutional review board of the two hospitals, and the requirement for informed consent was waived.

In total, 146 *S. aureus* isolates were collected between April and November 2012 at one hospital, and 268 were collected between April 2012 and April 2013 at the other hospital. Isolates from patients younger than 18 yr were excluded. Isolates were excluded if bacterial species other than *S. aureus* were recovered from the same specimen. If *S. aureus* isolates were obtained from more than one specimen for a patient, only one isolate was selected. Isolate obtained from blood or body fluids was preferred, and the earliest cultured isolate during hospitalization was selected. Finally, 107 blood isolates and 122 non-blood isolates (body fluids, 27; bronchial fluid, 13; pus, 37; sputum, 34; urine, 11) were obtained.

2. Standard E test

Vancomycin and teicoplanin MICs were determined by standard E test according to the manufacturer's instruction. Each *S. aureus* isolate was grown for 24 hr on a blood agar plate. A single colony was inoculated into saline, and saline suspensions adjusted to match the 0.5 McFarland turbidity standard were inoculated onto a Muller-Hinton agar plate (ASAN Pharmaceutical, Hwaseong, Korea). E test strips for vancomycin and teicoplanin were applied to the same plate. All plates were incubated at 35ºC for 48 hr. Vancomycin breakpoints were defined as follows: susceptible at a vancomycin MIC of ≤2 μg/mL, intermediate at a vancomycin MIC of 4-8 μg/mL, and resistant at a vancomycin MIC of ≥16 μg/mL, according to the CLSI guideline [25].

3. Macromethod E test

Macromethod E test was performed according to the manufacturer's instruction. A McFarland suspension (200 μL) was prepared in brain heart infusion (BHI) broth, pipetted onto a 90-mm BHI agar plate (Becton, Dickinson and Company, Sparks, MD, USA), and swabbed evenly. E test strips for vancomycin and teicoplanin were applied to the same plate. All plates were incubated at 35ºC for 48 hr. The test was considered positive for hVISA, if (1) the teicoplanin MIC was ≥12 μg/mL or (2) the teicoplanin and vancomycin MICs were ≥8 μg/mL.

4. Glycopeptide resistance detection E test

Among 229 isolates, 79 were positive for hVISA based on the macromethod E test. To determine the concordance between the macromethod and GRD E tests, the GRD E test was performed on 158 isolates (79 hVISA isolates and another randomly selected 79 vancomycin-susceptible [VSSA] isolates) according to the manufacturer's instruction. A bacterial suspension corresponding to the 0.5 McFarland standard prepared in BHI broth was inoculated on Mueller-Hinton agar with 5% blood agar plate (MH-BAP; Becton, Dickinson and Company). A GRD strip consisting of a double-sided gradient with vancomycin and teicoplanin was then applied to the MH-BAP. All plates were in-

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cubated at 35ºC for 48 hr. The test was considered positive for hVISA, if the GRD E test strip result was ≥8 μg/mL for vancomycin or teicoplanin [15].

5. Predictors of clinical outcomes in patients with *S. aureus* bacteremia

We reviewed the medical records of all patients, and out of 229 patients, 107 with clinically significant SAB were analyzed for predictors of clinical outcomes. To meet the systemic inflammatory response syndrome (SIRS) criteria, two or more of the following conditions were required: (1) body temperature >38°C or <36°C; (2) respiratory rate >20 per minute or CO_2 pressure on arterial blood gas analysis (Pa_{CO2}) <32 mm Hg; (3) heart rate >90 per minute; (4) white blood cell (WBC) count >12.0 ×10^9/L or <4.0×10^9/L, or band form >10%. SAB was defined by isolation of *S. aureus* from one or more blood cultures, and patients with polymicrobial bacteremia were excluded [26]. Patients who met the SIRS criteria at SAB onset were considered to have clinically significant SAB and were included in this study [27].

Clinical outcomes were analyzed as treatment failure and 30-day mortality. Treatment failure of SAB was defined by the identification of positive blood cultures for ≥7 days [19]. 30-day mortality was defined as death within 30 days after SAB onset.

The following data from 107 patients were collected: age, gender, presence of MRSA, presence of hVISA, duration of hospital stay before SAB onset, mode of transmission, primary source of infection, presence of comorbidities, treatment prior to SAB onset (surgery within 30 days, cancer chemotherapy, immunosuppressive therapy, and vancomycin therapy within 1 yr), and appropriate empirical therapy.

The primary sources of infection were determined by the following definitions. Infective endocarditis was identified according to the Duke criteria [28]. An intravascular catheter-related infection was considered to be the source of bacteremia, if (1) the catheter had been in place for ≥72 hr; (2) the culture of the catheter tip from the insertion site showed growth of *S. aureus* with the same resistance pattern as those of culture isolates from peripheral blood, the clinical signs in the patient improved after the catheter was removed, or there was an inflammatory reaction at the catheter insertion site; and (3) no other source for bacteremia existed [29]. A diagnosis of osteomyelitis was based on radiological images showing a lytic center with a ring of sclerosis. Cultures from a bone biopsy were required to identify the specific pathogen [30]. Pneumonia was considered to be the source of SAB, if the following conditions were met: (1) the chest radiograph showed new or progressive infiltrates within 24 hr of the first *S. aureus*-positive blood collection; (2) *S. aureus* was cultured from sputum or bronchial fluid within the 3 days before the collection of culture-positive blood; (3) the pulmonary infiltrates were not attributable to other causes [31]. Skin and soft tissue infections were considered to be the source of SAB, if the following conditions were met: (1) *S. aureus* was isolated from the patient's affected tissue within the three days before the collection of culture-positive blood; (2) the patient had symptoms or signs of local infection; (3) there was no other cause of skin and soft tissue infection. Surgical wound infection was defined according to the definitions outlined by the U.S. Centers for Disease Control and Prevention [32]. If the primary focus of infection could not be determined, it was considered unknown (primary bacteremia).

The initial empirical antibiotic therapy was considered to be appropriate, if the empirical regimen provided during the first 48 hr after the onset of bacteremia included one or more antibiotics to which the isolate was susceptible (for methicillin-resistant *S. aureus*, always at least vancomycin or teicoplanin) and if the dose of the susceptible antibiotics was adequate.

6. Predictors of clinical outcomes in patients with *S. aureus* pneumonia

Of 229 patients, predictors of clinical outcomes, including treatment failure and 30-day mortality, were analyzed in 39 with clinically significant SAP. A diagnosis of SAP was made, if the following conditions were met: (1) the patient had lower respiratory tract symptoms such as cough and sputum; (2) a chest radiograph showed new pulmonary infiltrates within 24 hr of the first *S. aureus*-positive culture result; (3) only *S. aureus* isolates were cultured from sputum, bronchial fluid, or blood; (4) there were no other causes of pneumonia [33].

Treatment failure of SAP was defined as the persistence of lower respiratory tract symptoms for ≥2 weeks and radiographic abnormalities for ≥4 weeks despite treatment with antibiotics to which the *S. aureus* isolate was susceptible *in vitro* [34]. The 30-day mortality was defined as death that occurred within 30 days after the first day of sputum, bronchial fluid, or blood collection.

The following data were collected: age, gender, presence of MRSA, presence of hVISA, duration of hospital stay before SAP onset, mode of transmission, presence of comorbidities, and treatment prior to SAP onset. The initial empirical antibiotic therapy was considered appropriate, if the empirical regimen provided during the first 48 hr after the onset of pneumonia included one or more antibiotics to which the isolate was susceptible.

Table 1. Prevalence of hVISA* and VSSA phenotypes based on the macromethod E test and the vancomycin MIC of standard E test

Vancomycin MIC† (μg/mL)	MRSA (N=138)		MSSA (N=91)	
	hVISA* (%)	VSSA (%)	hVISA* (%)	VSSA (%)
0.25	0 (-)	0 (-)	0 (-)	1 (0.0)
0.5	0 (-)	7 (100.0)	0 (-)	0 (-)
0.75	1 (14.3)	6 (85.7)	1 (16.7)	5 (83.3)
1	22 (42.3)	30 (57.7)	0 (-)	45 (100.0)
1.5	38 (61.3)	24 (38.7)	7 (18.9)	30 (81.1)
2	8 (88.9)	1 (11.1)	1 (50.0)	1 (50.0)
3	1 (100.0)	0 (-)	0 (-)	0 (-)
Total	70 (50.7)	68 (49.3)	9 (9.9)	82 (90.1)

hVISA prevalences according to the vancomycin MICs were significantly different in MRSA (P=0.0007) and MSSA (P=0.0149) isolates. Categorical variables were compared using the Chi-square test by 2xN table format.
*hVISA phenotype was identified by macromethod E test; †Vancomycin MICs were determined by standard E test.
Abbreviations: VSSA, vancomycin-susceptible *S. aureus*; hVISA, heterogeneous vancomycin-intermediate *S. aureus*; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.

7. Statistical analysis

All statistical data were analyzed by using MedCalc software (version 14.12; MedCalc Software, Mariakerke, Belgium). Categorical variables were compared by Chi-square test or Fisher's exact test, and continuous variables were compared by using a t-test for sample sizes above 20 or Mann-Whitney U test for sample sizes below 20. In the multivariate analysis, all significant variables in the univariate analysis were subjected to logistic regression modeling to identify independent predictors of clinical outcomes for patients with SAB or SAP. All significance tests were two-tailed, and a P value of ≤0.05 was considered significant.

RESULTS

1. Prevalence of the hVISA phenotype based on the macromethod E test

The prevalence of hVISA and VSSA phenotypes based on the macromethod E test is shown in Table 1. Of 229 *S. aureus* isolates, 138 (60.3%) were MRSA and 91 (39.7%) were MSSA. Based on the macromethod E test, 79 (34.5%) isolates displayed the hVISA phenotype, and 150 (65.5%) had the VSSA phenotype. hVISA prevalence increased with vancomycin MICs in MRSA isolates.

2. Concordance between the macromethod and GRD E tests

Based on the hVISA and VSSA phenotypes determined by the macromethod or GRD E test, the concordance between the two analyses was 84.2% (Table 2). Of 79 isolates found to display the hVISA phenotype based on the macromethod E test, 58 (73.4%) exhibited the hVISA phenotype, and 21 (26.6%) demonstrated the VSSA phenotype as determined by the GRD E test. Of the 79 isolates found to exhibit the VSSA phenotype based on the macromethod E test, 75 (94.9%) showed the VSSA phenotype, and four (5.1%) exhibited the hVISA phenotype based on the GRD E test.

Table 2. Concordance between the macromethod E test and GRD E test

Macromethod E test	GRD E test		Total
	hVISA	VSSA	
hVISA	58	21	79
VSSA	4	75	79
Total	62	96	158

Concordance={(58+75)/158}×100=84.2%.
Abbreviations: GRD, glycopeptide resistance detection; VSSA, vancomycin-susceptible *S. aureus*; hVISA, heterogeneous vancomycin-intermediate *S. aureus*.

3. Predictors of treatment failure in patients with SAB

Results of a univariate analysis of the predictors of treatment failure in 107 patients with SAB are shown in Table 3. Of 107 patients, 34 (31.8%) patients experienced treatment failure, while 58 (54.2%) successfully responded to treatment within seven days. Data were not available for 15 (14.0%) patients. Previous surgery (P=0.0107) and prior vancomycin therapy (P=0.0214) were significantly associated with treatment failure. The hVISA phenotype was considered nearly significant (P=0.054). The multivariate analysis indicated that they were not independently associated with treatment failure in patients with SAB.

4. Predictors of 30-day mortality in patients with SAB

Results of a univariate analysis of the predictors of 30-day mortality in 107 patients with SAB are shown in Table 4. We observed that 27 (25.2%) patients died within 30 days of the onset of SAB. The duration of hospital stay before SAB onset (P=0.0008), mode of transmission (P=0.0009), infective endocarditis (P=0.014), solid cancer (P=0.0253), congestive heart failure (P=0.0338), cancer chemotherapy (P=0.0207), and prior vancomycin therapy (P=0.0019) were significantly associated with the 30-day mortality in patients with SAB. A multivariate analysis of these variables indicated that the mode of transmission (adjusted odds ratio, 7.189; 95% confidence intervals

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Table 3. Univariate analysis of predictors for treatment failure in patients* with SAB

Characteristics	Treatment failure (+) (N=34) (%)	Treatment failure (-) (N=58) (%)	*P*
Age (mean±SD, yr)	62.1±12.3	65.3±13.5	NS
Old age (≥65 yr)	14 (41.2)	36 (62.1)	NS
Male	23 (67.6)	32 (55.2)	NS
Vancomycin MIC ≥1.5 μg/mL	17 (50.5)	23 (39.7)	NS
Teicoplanin MIC ≥4 μg/mL	13 (38.2)	12 (20.7)	NS
MRSA (+)	21 (61.8)	25 (43.1)	NS
hVISA† (+)	14 (41.2)	12 (20.7)	NS (0.0540)
Duration of hospital stay before SAB onset (mean±SD, day)	14.9±27.7	7.6±14.1	NS
Mode of transmission			
Hospital-acquired	10 (29.4)	14 (24.1)	NS
Primary source of infection			
Infective endocarditis	2 (5.9)	3 (5.2)	NS
Intravascular catheter-related	1 (2.9)	1 (1.7)	NS
Osteomyelitis	1 (2.9)	3 (5.2)	NS
Pneumonia	3 (8.8)	3 (5.2)	NS
Skin and soft tissue	3 (8.8)	4 (6.9)	NS
Surgical wound	1 (2.9)	2 (3.4)	NS
Unknown (primary bacteremia)	23 (67.6)	42 (72.4)	NS
Comorbidity			
Solid cancer	5 (14.7)	14 (24.1)	NS
Hematologic malignancy	4 (11.8)	4 (6.9)	NS
Diabetes mellitus	9 (26.5)	12 (20.7)	NS
Cerebrovascular accident	9 (26.5)	12 (20.7)	NS
Congestive heart failure	7 (20.6)	7 (12.1)	NS
Chronic liver disease	3 (8.8)	12 (20.7)	NS
Chronic respiratory disease	9 (26.5)	10 (17.2)	NS
Chronic kidney disease	6 (17.6)	7 (12.1)	NS
Previous treatment			
Previous surgery	17 (50.0)	13 (22.4)	0.0107
Cancer chemotherapy	6 (17.6)	11 (19.0)	NS
Immunosuppressive therapy	2 (5.9)	4 (6.9)	NS
Prior vancomycin therapy	17 (50.0)	14 (24.1)	0.0214
Appropriate empirical therapy	25 (73.5)	42 (72.8)	NS

Categorical variables were compared using Fisher's exact test, and continuous variables were compared using t-test.
*Data from 15 patients were not available; †The hVISA phenotype was identified by macromethod E test.
Abbreviations: SAB, *S. aureus* bacteremia; MRSA, methicillin-resistant *S. aureus*; hVISA, heterogeneous vancomycin-intermediate *S. aureus*; MIC, minimum inhibitory concentration; NS, not significant.

Table 4. Univariate analysis of predictors for 30-day mortality in patients with SAB

Characteristics	30-day mortality (+) (N=27) (%)	30-day mortality (-) (N=80) (%)	*P*
Age (mean±SD, yr)	65.8±12.0	64.7±13.4	NS
Old age (≥65 yr)	16 (59.3)	43 (53.8)	NS
Male	13 (48.1)	45 (56.3)	NS
Vancomycin MIC ≥1.5 μg/mL	14 (51.9)	33 (41.3)	NS
Teicoplanin MIC ≥4 μg/mL	9 (33.3)	20 (25.0)	NS
MRSA (+)	15 (55.6)	39 (48.8)	NS
hVISA* (+)	9 (33.3)	21 (26.3)	NS
Duration of hospital stay before SAB onset (mean±SD, day)	21.6±35.6	6.4±10.1	0.0008
Mode of transmission			
Hospital-acquired	14 (51.9)	14 (17.5)	0.0009
Primary source of infection			
Infective endocarditis	4 (14.8)	1 (1.3)	0.0140
Intravascular catheter-related	1 (3.7)	2 (2.5)	NS
Osteomyelitis	1 (3.7)	3 (3.8)	NS
Pneumonia	2 (7.4)	6 (7.5)	NS
Skin and soft tissue	1 (3.7)	7 (8.8)	NS
Surgical wound	0 (0.0)	3 (3.8)	-
Unknown (primary bacteremia)	18 (66.7)	58 (72.5)	NS
Comorbidity			
Solid cancer	10 (37.0)	12 (15.0)	0.0253
Hematologic malignancy	1 (3.7)	7 (8.8)	NS
Diabetes mellitus	5 (18.5)	20 (25.0)	NS
Cerebrovascular accident	6 (22.2)	18 (22.5)	NS
Congestive heart failure	8 (29.6)	9 (11.3)	0.0338
Chronic liver disease	7 (25.9)	11 (13.8)	NS
Chronic respiratory disease	4 (14.8)	19 (23.8)	NS
Chronic kidney disease	4 (14.8)	12 (15.0)	NS
Previous treatment			
Previous surgery	13 (48.1)	21 (26.3)	NS
Cancer chemotherapy	9 (33.3)	10 (12.5)	0.0207
Immunosuppressive therapy	2 (7.4)	5 (6.3)	NS
Prior vancomycin therapy	16 (59.3)	20 (25.0)	0.0019
Appropriate empirical therapy	19 (70.4)	53 (66.3)	NS

Categorical variables were compared using Fisher's exact test, and continuous variables were compared using t-test.
*The hVISA phenotype was identified by macromethod E test.
Abbreviations: SAB, *S. aureus* bacteremia; MRSA, methicillin-resistant *S. aureus*; hVISA, heterogeneous vancomycin-intermediate *S. aureus*; MIC, minimum inhibitory concentration; NS, not significant.

Table 5. Univariate analysis of predictors for treatment failure in patients with SAP

Characteristics	Treatment failure (+) (N=12) (%)	Treatment failure (-) (N=27) (%)	*P*
Age (mean±SD, yr)	71.4±7.7	64.7±16.3	NS
Old age (≥65 yr)	10 (83.3)	15 (55.6)	NS
Male	9 (75.0)	18 (66.7)	NS
Vancomycin MIC ≥1.5 μg/mL	10 (83.3)	13 (48.1)	NS
Teicoplanin MIC ≥4 μg/mL	8 (66.6)	6 (22.2)	0.0123
MRSA (+)	9 (75.0)	18 (66.7)	NS
hVISA* (+)	9 (75.0)	8 (29.6)	0.014
Duration of hospital stay before SAP onset (mean±SD, day)	10.9±12.8	8.1±14.8	NS
Mode of transmission			
Hospital-acquired	4 (33.3)	7 (25.9)	NS
Comorbidity			
Solid cancer	2 (16.7)	5 (18.5)	NS
Hematologic malignancy	2 (16.7)	2 (7.4)	NS
Diabetes mellitus	4 (33.3)	7 (25.9)	NS
Cerebrovascular accident	2 (16.7)	7 (25.9)	NS
Congestive heart failure	1 (8.3)	3 (11.1)	NS
Chronic liver disease	1 (8.3)	1 (3.7)	NS
Chronic respiratory disease	3 (25.0)	4 (14.8)	NS
Chronic kidney disease	3 (25.0)	6 (22.2)	NS
Previous treatment			
Previous surgery	3 (25.0)	6 (22.2)	NS
Cancer chemotherapy	2 (16.7)	3 (11.1)	NS
Immunosuppressive therapy	1 (8.3)	1 (3.7)	NS
Prior vancomycin therapy	4 (33.3)	8 (29.6)	NS
Appropriate empirical therapy	7 (58.3)	17 (63.0)	NS

Categorical variables were compared using Fisher's exact test, and continuous variables were compared using Mann-Whitney U test.
*The hVISA phenotype was identified by macromethod E test.
Abbreviations: SAP, *S. aureus* pneumonia; MRSA, methicillin-resistant *S. aureus*; hVISA, heterogeneous vancomycin-intermediate *S. aureus*; MIC, minimum inhibitory concentration; NS, not significant.

Table 6. Univariate analysis of predictors for 30-day mortality in patients with SAP

Characteristics	30-day mortality (+) (N=5) (%)	30-day mortality (-) (N=34) (%)	*P*
Age (mean, yr)	58.8±16.9	67.9±13.9	NS
Old age (≥65 yr)	2 (40.0)	23 (67.6)	NS
Male	4 (80.0)	23 (67.6)	NS
Vancomycin MIC ≥1.5 μg/mL	4 (80.0)	19 (55.9)	NS
Teicoplanin MIC ≥4 μg/mL	3 (60.0)	11 (32.4)	NS
MRSA (+)	3 (60.0)	24 (70.6)	NS
hVISA* (+)	3 (60.0)	14 (41.2)	NS
Duration of hospital stay before SAP onset	21.0±24.2	7.2±11.5	0.0391
Mode of transmission			
Hospital-acquired	2 (40.0)	9 (26.5)	NS
Comorbidity			
Solid cancer	2 (40.0)	5 (14.7)	NS
Hematologic malignancy	0 (-)	4 (11.8)	NS
Diabetes mellitus	2 (40.0)	9 (26.5)	NS
Cerebrovascular accident	3 (60.0)	6 (17.6)	NS
Congestive heart failure	0 (-)	4 (11.8)	NS
Chronic liver disease	0 (-)	2 (5.9)	NS
Chronic respiratory disease	0 (-)	7 (20.6)	NS
Chronic kidney disease	2 (40.0)	7 (20.6)	NS
Previous treatment			
Previous surgery	2 (40.0)	7 (20.6)	NS
Cancer chemotherapy	1 (20.0)	4 (12.8)	NS
Immunosuppressive therapy	0 (-)	2 (5.9)	NS
Prior vancomycin therapy	2 (40.0)	10 (29.4)	NS
Appropriate empirical therapy	2 (40.0)	22 (64.7)	NS

Categorical variables were compared using Fisher's exact test, and continuous variables were compared using Mann-Whitney U test.
*The hVISA phenotype was identified by macromethod E test.
Abbreviations: SAP, *S. aureus* pneumonia; MRSA, methicillin-resistant *S. aureus*; hVISA, heterogeneous vancomycin-intermediate *S. aureus*; MIC, minimum inhibitory concentration; NS, not significant.

[CI], 2.172-23.792) was independently associated with 30-day mortality. However, hVISA was not associated with 30-day mortality in patients with SAB.

5. Predictors of treatment failure in patients with SAP

Results of a univariate analysis of predictors of treatment failure in 39 patients with SAP are shown in Table 5. Of the 39 patients, 12 (30.8%) were non-responsive to treatment, and 27 (69.2%) successfully responded to treatment. hVISA phenotype (P=0.0140) and teicoplanin MIC ≥4 μg/mL (P=0.0123) had a significant association with treatment failure in SAP patients. A multivariate analysis indicated that they were not independently associated with treatment failure in patients with SAP.

6. Predictors of 30-day mortality in patients with SAP

Results of a univariate analysis of risk factors for 30-day mortality in 39 patients with SAP are shown in Table 6. We observed that five (12.8%) patients died within 30 days from the day of SAP onset. The duration of hospital stay before SAP onset

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(P=0.0391) was significantly associated with 30-day mortality.

7. Impact of hVISA on clinical outcomes in MRSA and MSSA patients

We additionally analyzed the impact of hVISA on clinical outcomes in MRSA-SAB (Supplemental Data Tables S1 and S2), MRSA-SAP (Supplemental Data Table S3), and MSSA-SAB (Supplemental Data Tables S4 and S5) patients, respectively. hVISA was significantly associated with treatment failure in MRSA-SAP patients (P=0.0088). However, hVISA was not associated with treatment failure or 30-day mortality in MRSA- or MSSA-SAB patients. Since the cases of the treatment failure (+) group of MSSA-SAP patients and 30-day mortality (+) group of MRSA- or MSSA-SAP patients were too few (below 3), statistical analyses of these cases were inappropriate and not done.

DISCUSSION

hVISA has been reported in several countries [18-20, 35]. hVISA prevalence in the United States was 2.2% in 1986-1993, 7.6% in 1994-2002, and 8.3% in 2003-2007, among 1499 MRSA isolates by the macromethod E test and PAP at three hospitals in Detroit [35]. hVISA prevalence was found to be 14.5% based on 489 MRSA isolates from bacteremia patients by the macromethod E test at a single hospital in Detroit in 1996-2006 [19]. Approximately 9.4% of blood culture MRSA isolates obtained from a single health center in Australia between July 2001 and June 2002 were found to exhibit the hVISA phenotype by PAP [18]. Moreover, a high rate of hVISA (49.6%) was observed in 117 MRSA isolates by PAP at a single hospital in Austrailia in 2005 [20]. A meta-analysis conducted in 2011 by Sebastiaan *et al*. showed that the overall hVISA prevalence was 1.3% among all MRSA isolates [36]. The difference of hVISA prevalences may be explained by different testing methods, geographical regions, and patient populations. Additionally, higher antibiotic selection pressures at tertiary care centers may account for a higher prevalence of hVISA [37].

In Korea, hVISA prevalence was 0.5% in 2002. PAP confirmed 24 out of 4,483 *S. aureus* isolates as hVISA at a single hospital from December 1998 to August 1999 [10]. In 2004, hVISA prevalence was 6.1% (28/457 MRSA isolates) by PAP at a single health center from January 1997 to March 2000 [11]. Furthermore, among 268 MRSA bacteremia isolates, 37.7% were identified as hVISA by PAP at a single hospital from August 2008 to September 2010 [12].

In our study, PAP was not performed as a confirmatory test, since several previous studies estimated hVISA prevalence using only the macromethod E test [19, 21, 22]. Additionally, the macromethod E test has good sensitivity and specificity, with PAP used as the reference method. In 2008, Yusof *et al*. [15] reported that the macromethod E test had a sensitivity of 94% and a specificity of 96%, and GRD E test had a sensitivity of 94% and a specificity of 95%. In 2009, Leonard *et al*. [16] evaluated that the macromethod E test was 83% sensitive and 94% specific, and GRD E test was 93% sensitive and 82% specific compared with PAP. In 2011, Satola *et al*. [17] evaluated that the sensitivities of both E tests were relatively low at 57%, but the specificities were high at 96% and 97%, respectively. In our study, considering its slightly lower sensitivity, hVISA prevalence determined by the macromethod E test can be expected to be 6-17% lower than that determined by PAP. Therefore, hVISA prevalence (50.7% of MRSA isolates) in this study was greater than that (1.3-6.1% of MRSA isolates) found in previous studies in Korea [9-11]. hVISA is quite common, and its presence may be increasing in Korea.

It is controversial whether the presence of hVISA is associated with increased treatment failure. Horne *et al*. [20] reported that the rates of treatment failure were not statistically different between patients with hVISA and VSSA infection from MRSA clinical isolates. Musta *et al*. [19] reported that hVISA infection was not significantly associated with persistent MRSA bacteremia. However, Bae *et al*. [23] reported that MRSA bloodstream isolates from patients with hVISA had a higher rate of persistent bacteremia (P=0.029). Charles *et al*. [18] reported that hVISA infection from patients with MRSA bacteremia had a longer duration of fever (P<0.001), a greater number of positive blood cultures (P<0.001), a longer time until clearance of bacteremia (P=0.002), and a longer hospital stay (P=0.006). In our study, hVISA was nearly associated with treatment failure in patients with SAB, and significantly associated with treatment failure in patients with SAP. Patients with previous surgery and prior vancomycin therapy exhibit vancomycin selection pressure, which can cause VSSA to become more resistant to vancomycin [38]. Thus, VSSA isolates develop into hVISA. hVISA isolates may fail to respond to therapeutic doses of vancomycin. A significant association between the initial vancomycin trough level and vancomycin treatment failure was reported previously [39].

It is also controversial whether hVISA increases 30-day mortality for patients. Maor *et al*. [21] reported that death was related to hVISA sepsis in eight (50%) of 16 patients with MRSA bacteremia. Neoh *et al*. [24] reported that hVISA infection was significantly associated with 30-day mortality in patients with

MRSA bacteremia. However, Bae *et al*. [23] reported that in-hospital mortality did not differ between hVISA- and non-hVISA-infected patients with MRSA bacteremia. Fong *et al*. [22] reported that there was no significant difference in 30-day mortality between hVISA- and VSSA-infected patients with persistent MRSA infection. In our study, hVISA was not associated with 30-day mortality in patients with SAB or SAP. Patients with persistent bacteremia generally receive an alternative antibiotic treatment, such as linezolid, rifampicin, and fusidic acid, and these antibiotics may resolve persistent bacteremia and reduce 30-day mortality [40].

In our study, patients were hardly treated with therapeutic drug monitoring (TDM) of vancomycin (≤10% of the patients; clinicians seldom ordered vancomycin TDM in this study period). Thus, the relationship between the TDM and hVISA and/or clinical outcomes could not be analyzed. This is a limitation in our study.

In summary, 34.5% of *S. aureus* isolates and 50.7% of MRSA isolates exhibited the hVISA phenotype in our study. hVISA is quite common, and its presence may be increasing in Korea. hVISA is nearly associated with treatment failure in patients with SAB and is significantly associated with treatment failure in patients with SAP. Concordance between the macromethod and GRD E tests is 84.2%. Macromethod E test should be employed in clinical microbiology laboratories.

Authors' Disclosures of Potential Conflicts of Interest

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

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