Clinical and Molecular Characterization of Invasive Heteroresistant Vancomycin-Intermediate *Staphylococcus aureus* Infections in Korean Hospitals

Eu Suk Kim,^{a,b} In-Gyu Bae,^c Jeong Eun Cho,^a Yun Jung Choi,^a Il-Hwan Kim,^d Gi-Su Kang,^d Hye-yun Sin,^a Kyoung-Ho Song,^{a,b} Chulmin Park,^e Dong-Gun Lee,^{e,f} Moonsuk Kim,^{a,b} Kyoung Un Park,^g Hong Bin Kim,^{a,b} the Korea INfectious Diseases (KIND) study group

Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, South Korea^a; Department of Internal Medicine, Seoul National University College of Medicine, Seoul, South Korea^b; Department of Internal Medicine, Gyeongsang National University School of Medicine, Jinju, South Korea^c; Division of Antimicrobial Resistance, Center for Infectious Diseases, Korea National Institute of Health, KCDC, Cheongju, South Korea^d; Vaccine Bio Research Institute, The Catholic University of Korea, Seoul, South Korea^e; Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, South Korea^f; Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, South Korea^g

Invasive heteroresistant vancomycin-intermediate *Staphylococcus aureus* (h-VISA) isolates were identified and characterized in 10 Korean hospitals from July 2009 to June 2011. The prevalence of h-VISA infections was 3.3% (42/1,289). Most (41/42) were health care-associated infections caused by strains belonging to sequence type 5. Cases of persistent bacteremia were frequent (17/42), and 30-day mortality was high (16/40).

Heteroresistant vancomycin-intermediate *Staphylococcus aureus* (h-VISA) strains have a susceptible range of vancomycin MICs (MIC, ≤ 2 $\mu\text{g/ml}$) by routine methods but contain subpopulations with an intermediate range of vancomycin MICs (1). Previous nationwide surveillance studies of clinical isolates showed that h-VISA was very rare in Korea (2, 3). However, the prevalence of h-VISA among cases of methicillin-resistant *S. aureus* (MRSA) bacteremia reached 37.7% in a recent study performed in a Korean hospital (4). We conducted a multicenter study to evaluate the prevalence of h-VISA among cases of invasive *S. aureus* (ISA) infection. We also analyzed the clinical and molecular characteristics of invasive h-VISA infections and the risk factors for such infections.

Ten hospitals in four Korean regions, nine of which participated in a previous cohort study (5), took part in this study from July 2009 to June 2011. ISA infection was diagnosed when *S. aureus* was isolated from a normally sterile body fluid, as previously defined (6). We prospectively collected detailed clinical information on each ISA case. *S. aureus* isolates were collected from the enrolled cases and were sent to Seoul National University Bundang Hospital (SNUBH) where they were screened for h-VISA using a brain heart infusion agar with 4 $\mu\text{g/ml}$ of vancomycin (BHI V4) (1). If one or more colonies grew on BHI V4 within 48 h at 37°C, the isolates were screened again by macro Etest (MET) (7). h-VISA was confirmed by the modified population analysis profile-area under the curve (PAP-AUC) method (8). Multilocus sequence typing (MLST) (9) and analysis for the Pantone-Valentine leucocidin (PVL) toxin were also performed (10). Pulsed-field gel electrophoresis (PFGE) was performed in selected MRSA strains, as previously described (11). The clinical risk factors for h-VISA infection among cases in which *S. aureus* was isolated were analyzed using IBM SPSS Statistics for Windows, version 20.0 (Armonk, NY, USA). After univariate analysis to identify potential risk factors with *P* values of <0.10 using the Student *t* test, the χ^2 test, or Fisher's exact test, multivariate linear logistic regression was used to adjust covariates, and *P* values of <0.05 were consid-

ered statistically significant. The study was approved by the Institutional Review Board of SNUBH.

Clinical data for a total of 1,717 nonduplicate invasive *S. aureus* infection cases were collected during the study period. Of these, 62.1% (1,066) were MRSA infections. The *S. aureus* isolates obtained from 1,289 cases (75.1%; 825 MRSA isolates and 464 methicillin-susceptible *S. aureus* [MSSA] isolates) were sent to SNUBH. Of these isolates, 210 (16.3%) and 43 (3.3%) gave positive results for growth on BHI V4 and in the MET, respectively. Using the modified PAP-AUC method, we found 42 cases (3.3%) of h-VISA infection (Table 1). Most of the h-VISA strains (40, 95.2%) were blood isolates, and 41 (97.6%) were health care-associated infections, including 5 cases of surgical site infection. The mean value of the Charlson weighted index of comorbidities (12) was 3.17 with a standard deviation of 2.18 (range, 0 to 13). The most common primary focus of h-VISA infection was catheter-related infection (CRI). Seven cases (16.7%) were metastatic infections, and in 17 (40.5%) cases, bacteremia persisted for 7 or more days after initial culture. Thirty-day mortality for the patients whose data were available was substantially higher in h-VISA infections than that in non-h-VISA infections—40.0% (16/40) versus 25.7% (271/1,056) (*P* = 0.043), respectively. However, h-VISA itself was not a significant risk factor for mortality in multivariate analysis.

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Address correspondence to Hong Bin Kim, hbkimmd@snu.ac.kr.

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TABLE 1 Clinical characteristics of 42 cases of heteroresistant vancomycin-intermediate *Staphylococcus aureus* infection in South Korea

Characteristic	No. of cases (%)
Gender	33 male (78.6), 9 female (21.4)
Median age (range), yr	68 (16–89)
Comorbidity	37 yes (88.1), 5 no (11.9)
Oxacillin susceptibility	3 Susceptible (7.1), 39 resistant (92.9)
Type of specimen	40 blood (95.2), 1 joint fluid, 1 other sterile body fluid
Epidemiology	41 health care associated (97.6), 1 community associated
Primary site of infection	15 catheter-related infections (35.7), 6 bone and joint infections (14.3), 5 intra-abdominal infections (11.9), 4 pneumonia (9.5), 2 skin and skin structure infections (4.8), 2 central nervous system infections (4.8), 2 mediastinitis (4.8), 6 unknown (14.3)
Metastatic infection	7 yes (16.7), 35 no (83.3)
Persistent bacteremia for ≥ 7 days	17 yes (40.5), 25 no (59.5)

Most (38, 90.5%) of the strains belonged to sequence type 5 (ST5), and 3 were single-locus variants of ST72. Only one strain possessed the *pvl* gene. The prevalence of h-VISA varied according to the participating hospitals (0% to 6.1%). PFGE of 24 selected h-VISA strains showed that they were all closely related regardless of the hospital where they were isolated (Fig. 1). No outbreak related to h-VISA infection was documented in the participating hospitals during the study period. Clinical predictors of h-VISA infections were male gender, presence of indwelling percutaneous catheter, history of operation within 1 year, oxacillin resistance, and bacteremia persisting for 7 or more days (Table 2).

The data in our study cannot be extrapolated to give the nationwide prevalence of invasive h-VISA infections. However, the multicenter design and the enrollment of a relatively large number of ISA cases in a prospective manner are important factors when

considering differences between our results and those of studies performed at the level of individual centers. The prevalence of h-VISA among ISA infections was low in our study, which is in agreement with previous nationwide surveillance studies of clinical isolates (2, 3). However, the prevalence of h-VISA in a recent study by Park et al. (4) in one tertiary Korean hospital was relatively high (37.7%). Those workers performed a population analysis of all blood MRSA isolates without initial screening for h-VISA on BHI V4, and this may be one reason for the high h-VISA prevalence that they observed. The cause of this difference may also be due to a difference in the incidence of nosocomial ISA infections in the participating hospitals. Most of the h-VISA infections in this study were health care associated, and their prevalence also varied, ranging from 0% to 6.1% according to the hospital. There may be sustained ISA infections by the ST5 MRSA strain, one of the most common genotypes in Korean hospitals (13), in some endemic hospitals, and this idea is supported by our PFGE analysis. Proper infection control measures to prevent the spread of h-VISA in hospitals will be crucial.

The clinical predictors of invasive h-VISA infections are the following: hospitalization in the previous year, longer period of prior vancomycin use, initially low serum vancomycin level, persistent bacteremia for >7 days, and high inoculum MRSA infections, such as bone and joint infection, endocarditis, or prosthesis infection (4, 14–17). The most frequent kind of infection was CRI, and the presence of an indwelling percutaneous catheter, including a central venous catheter, was an independent risk factor for h-VISA among invasive MRSA infections in this study. CRI itself was not an independent risk factor for h-VISA, but all of the cases of h-VISA endocarditis were CRI in another study (14). Moreover, many cases in our study involved prolonged periods of bacteremia, and mortality was relatively high even though h-VISA itself was not a significant risk factor for mortality. Therefore, prompt detection and eradication of removable sources such as catheters and prostheses are important to improve outcomes.

This study has some limitations. First, we obtained *S. aureus* isolates from only 75.1% of the enrolled ISA cases, and this may have led to a bias in analyzing the risk factors for h-VISA infection.

FIG 1 (a) Pulsed-field gel electrophoresis (PFGE) patterns of *SmaI*-digested genomic fragments of heteroresistant vancomycin-intermediate *Staphylococcus aureus* (h-VISA) isolates from hospitals A, B, C, D, E, and F. Lane M, *XbaI*-digested DNA of *Salmonella enterica* serovar Braenderup H9812 as a reference molecular size marker. (b) Dendrogram of *SmaI*-digested PFGE profiles of h-VISA isolates.

TABLE 2 Multivariate analysis of risk factors for h-VISA among 1,289 invasive *S. aureus* (MRSA) infections in South Korea

Variable	Odds ratio (95% CI) ^a	Adjusted odds ratio (95% CI)
Male gender	2.256 (1.070–4.756)	2.475 (1.146–5.345) ^b
Charlson's weighted index of comorbidities of ≥ 3	1.758 (0.949–3.255)	1.233 (0.622–2.443)
History of hemodialysis within 1 yr	2.418 (1.215–4.812)	1.568 (0.712–3.452)
Presence of indwelling percutaneous catheter	3.702 (1.844–7.432)	2.514 (1.177–5.369) ^b
History of operation within 1 yr	3.160 (1.687–5.919)	1.981 (1.031–3.806) ^b
Catheter-related infection	2.029 (1.064–3.870)	0.845 (0.399–1.792)
Unknown primary focus	0.445 (0.186–1.065)	0.559 (0.218–1.431)
Oxacillin resistance	7.625 (2.343–24.812)	4.632 (1.383–15.510) ^b
Persistent bacteremia for 7 days or more	3.646 (1.933–6.879)	2.372 (1.219–4.618) ^b

^aCI, confidence interval.^bValue has a *P* of <0.05.

However, this effect should be minimal because we enrolled a large number of cases nevertheless. Second, we were unable to collect comprehensive data on histories of exposure to vancomycin in the cases of community-onset ISA because we were unable to obtain reliable data from patients who had previously been admitted to other hospitals and were unable to recall past vancomycin treatments. This may be the reason why we were unable to demonstrate a relationship between prior vancomycin use and h-VISA infection. Third, we may have missed some h-VISA isolates during screening because we used BHI V4 rather than BHI agar with 3 μ g/ml of vancomycin. However, the number of isolates missed is likely to be low because the h-VISA rate in the group of *S. aureus* isolates forming only one colony on BHI V4 was only 11.3% (17/150) in this study (data not shown). The h-VISA rate would have been much lower if the isolates had been screened on 3 μ g/ml of vancomycin.

In summary, the incidence of invasive h-VISA infections was low, as in previous studies in Korea, and most of them were health care associated. Cases of persistent bacteremia were frequent, and 30-day mortality was high. Rapid detection of h-VISA in hospitals and early implementation of proper antibiotic therapy will be important.

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Department of Internal Medicine, Dongguk University Ilsan Hospital, Goyang.

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