Nationwide Surveillance for *Staphylococcus aureus* with Reduced Susceptibility to Vancomycin in Korea

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Methicillin-resistant *Staphylococcus aureus* (MRSA) accounts for more than 70% of *S. aureus* isolates from tertiary hospitals in Korea. Clinical isolates of *S. aureus* were collected from eight provincial, university-affiliated hospitals during the period from June 1999 to January 2001 for nationwide surveillance. All isolates were screened for reduced susceptibility to vancomycin by using brain heart infusion agar containing 4 μg of vancomycin per milliliter. Population analysis and the determination of the MIC of vancomycin were done for the isolates which grew on the screening agar plates. Of 682 total isolates, MRSA accounted for 64% (439 of 682). Of 27 (4%) isolates that grew on the screening agar plates, none showed the heteroresistance phenotype. No strains with reduced susceptibility to vancomycin were identified.

*Staphylococcus aureus* is one of the most important pathogens, causing severe morbidity and fatal infections. The rapid evolution of antibiotic resistance in *S. aureus* is of considerable concern. Since the first isolation of methicillin-resistant *S. aureus* (MRSA) in the United Kingdom in 1961 (17), the prevalence of MRSA increased rapidly worldwide, and glycopeptide antibiotics have been widely relied upon to treat MRSA infections. In 1996, the first documented infection caused by *S. aureus* with reduced susceptibility to vancomycin (vancomycin-intermediate *S. aureus* [VISA]) was reported in Japan (10). Thereafter, about 20 cases (28) of VISA infection have been reported in several countries, including Korea (20). Furthermore, 2 isolates of fully vancomycin-resistant *S. aureus* were documented in the United States during 2002 (4, 5).

In addition to VISA and vancomycin-resistant *S. aureus*, another type of vancomycin resistance called hetero-VISA (hVISA), has been described by Hiramatsu et al. (8). This strain is susceptible to vancomycin but contains a subpopulation, at a frequency of $10^{-8}$ or higher with a MIC of vancomycin of more than 4 μg per milliliter. The potential importance of hVISA is that it may be associated with treatment failure (8, 30, 31) and a precursor of VISA (15, 25). Although a number of studies have been undertaken to determine the prevalence of hVISA, reported frequencies have ranged from 0 to 20% (2, 6, 8, 14, 16, 19, 22, 24, 26, 32), depending on the definitions and methods employed for screening and confirmation.

The prevalence of methicillin resistance is known to be more than 70% among *S. aureus* isolates from tertiary hospitals in Korea (21). Considering the high prevalence of MRSA and the increased use of vancomycin, the development of vancomycin resistance in clinical strains seems likely to occur. To date, the existence of VISA or hVISA strains in countries with a high prevalence of MRSA has been rarely studied. In this study, we conducted a nationwide survey to investigate the prevalences of VISA and hVISA.

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**MATERIALS AND METHODS**

Bacterial isolates. Clinical *S. aureus* isolates were collected from eight university-affiliated hospitals, each hospital representing one of eight provinces in South Korea. From June 1999 to January 2001, each participating hospital sent consecutive isolates identified as *S. aureus*, ranging from 62 to 99 isolates per hospital. Only one isolate per patient was included in the study. Upon receipt, isolates were subcultured onto blood agar to ensure purity and identification. Initial confirmation of *S. aureus* was based on colony morphology and agglutination test with PS LATEX (Eiken Chemical, Tokyo, Japan). If necessary, further confirmatory tests were done with a Vitek system (bioMerieux, Durham, N.C.). All isolates were immediately stored at −70°C until required. Mu50 (VISA strain) and Mu3 (hVISA strain), kindly provided by Keichi Hiramatsu, were used as controls.

Screening for VISA and hVISA. To screen isolates with reduced susceptibility to vancomycin, original isolates or frozen stocks were streaked onto blood agar plates. After overnight incubation, a few colonies were inoculated into tryptic soy broth. A suspension grown overnight in tryptic soy broth equivalent to a 0.5 McFarland standard ($10^8$ CFU/ml) was prepared in 0.9% saline. Then, 10 μl ($10^4$ CFU) of the suspension was spread onto brain heart infusion agar (Becton Dickinson, Sparks, Md.) containing 4 μg of vancomycin/ml (BHI-V4). The plates were incubated for 48 h at 37°C, and the cell growth was inspected at 24 and 48 h (7). If confluent growth was apparent within 24 h, similar to that of Mu50, the strain was considered a potential VISA strain. If a countable (1 to 30) number of colonies was apparent within 48 h, similar to Mu3, the strain was designated as a possible hVISA strain. If cell growth was not apparent within 48 h, the strain was considered susceptible to vancomycin (5). Whenever screening tests were repeated, BHI-V4 plates were newly prepared and the positive controls were used. The isolates were passaged no more than twice on nonselective medium.

Population analysis. Any isolates that grew on BHI-V4 were subjected to population analysis, as previously described (27). After diluting overnight cultures to a 0.5 McFarland standard, 50 μl of the cell suspension and its serial diluents were spread onto BHI agar plates containing vancomycin concentrations in the range 1 to 10 mg/liter with 1-mg/liter increments. The plates were incubated at 37°C for 48 h, and the number of colonies was counted.
RESULTS

A total of 682 isolates were collected from eight tertiary hospitals: 199 (29%) from pus, 152 (22%) from respiratory specimens, 137 (20%) from blood, and 38 (6%) from urine. The overall prevalence of MRSA was 64% (439 of 682), which varied from 55 to 74% for hospitals and from 48 to 92% by specimen type.

In total, 27 (4%) isolates (21 MRSA and 6 methicillin-susceptible S. aureus isolates) grew on BHI-V4 after 48 h of incubation, but none possessed the heteroresistance phenotype on population analysis. Vancomycin MICs ranged from 1 to 2 μg/ml (Fig. 1).

DISCUSSION

We examined the prevalence of S. aureus with reduced susceptibility to vancomycin in hospitals with a high prevalence of MRSA. Although several hVISA or VISA isolates were expected to be found, no isolates with reduced susceptibility to vancomycin were detected.

In this study, population analysis was used as the standard method for the confirmation of hVISA instead of the method described by Hiramatsu et al. (8). Studies examining the prevalence of hVISA defined by the existence of a resistant subpopulation of cells have not generally been well standardized, which presumably is reflected by reported differences in the prevalence of hVISA. While many different methods have been evaluated (12, 14, 18, 29, 32, 33), population analysis profiling is presumed to be the only reliable way of confirming the heterogeneity of vancomycin susceptibility (13, 28). We also screened the isolates by using another method based on the antagonism of vancomycin and beta-lactam for hVISA (32; H. Hanaki, S. Ohkawa, Y. Yoko, T. Hashimoto, and K. Hiramatsu, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C-132, 1998). Briefly, after the prepared bacterial solution, adjusted to 1.0 McFarland turbidity, was swabbed on the Mu3 agar (Becton Dickinson) or BHI-V4, aztreonam (30 μg), cefoxime (5 μg), and cefminox (30 μg) disks were applied. None revealed a dense growth around beta-lactam disks after incubation at 37°C for 24 h (data not shown).

Our result differs from those of other studies; for example, hVISA strains represented 5 to 22% of the MRSA isolates in Japanese university hospitals (8) and 8.2% of the 85 MRSA strains studied in Germany by Geisel et al. (6). In contrast, hVISA strains were rarely detected in England and Wales (2), the United States (26), or a Korean university hospital (19). Even within the same countries, there have been conflicting results on the prevalence of hVISA (8, 16). These results suggest that several factors, such as the characteristics of bacterial isolates, the epidemiologic situation of antibiotic resistance, and the genetic background of clinical S. aureus isolates, might also contribute to the development of vancomycin resistance.

In this study, we collected isolates of S. aureus from the nonselective population. Studies involving a selected population, such as patients in intensive care units (18) and orthopedic patients with surgical-site MRSA infections (1), reported higher prevalence rates of hVISA. We believe that differences between the study populations may have lead to the variable results. In addition, hVISA appears to be more common in Hong Kong (30) and Germany (6) than in the United Kingdom (2, 32), the United States (14, 26), and Italy (22), which is suggestive of regional differences.

It was previously reported that some VISA isolates belonged to the same clonotype (11), which indicates that some clonotypes have a tendency to acquire vancomycin resistance compared with others. Although the isolates from Korea, Japan, and the United States were known to share clonotype II-A (9), our study indicates the low prevalence of hVISA and VISA. This could imply that the clonotype per se, though a predis-
posing factor, may not be sufficient for the development of reduced susceptibility to vancomycin.

It is possible that the resistance phenotype was not detected in some isolates with unstable vancomycin resistance because of resistance reversion during transit on nonselective medium (3). But this phenomenon has a stepwise nature of reversion during serial passage. To prevent this reversion, we plated the isolates no more than twice on routine nonselective medium. Therefore, resistance reversion is not a likely explanation for the low prevalence of hVISA observed in the present study. Despite the high prevalence of MRSA in tertiary hospitals, our investigation found no evidence of the dissemination of any hVISA or VISA strain in Korea.

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REFERENCES


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