Emergence in Asian Countries of *Staphylococcus aureus* with Reduced Susceptibility to Vancomycin

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Received 10 June 2004/Returned for modification 12 August 2004/Accepted 19 August 2004

To investigate the prevalence of *Staphylococcus aureus* with reduced susceptibility to vancomycin among methicillin-resistant *S. aureus* (MRSA) strains in Asian countries, a total of 1,357 clinical isolates of MRSA collected from 12 Asian countries were screened by using brain heart infusion agar plates containing 4 mg of vancomycin per liter. The presence of strains that were heterointermediately resistant to vancomycin (hVISA) was confirmed by population analysis. Of 347 (25.6%) MRSA isolates that grew on the screening agar plates, 58 isolates (4.3%) were hVISA. hVISA strains were found in India, South Korea, Japan, the Philippines, Singapore, Thailand, and Vietnam. However, neither vancomycin-intermediate *S. aureus* nor vancomycin-resistant *S. aureus* isolates were found among MRSA isolates from Asian countries in this survey.

Treatment of *Staphylococcus aureus* infections is becoming increasingly more complicated due to the emergence of various types of antimicrobial resistance. As a consequence of selective pressure of glycopeptide use, the first strain of *S. aureus* with reduced susceptibility to vancomycin (MIC, 8 mg/liter) (strain Mu50) was reported from Japan in 1997 (10). Since then, vancomycin-intermediate *S. aureus* (VISA) isolates have been reported from the United States, Europe, and the Far East (12, 19). Moreover, three reports of isolates of vancomycin-resistant *S. aureus* (VRSA) (MIC, ≥32 mg/liter) in 2002 and 2004 from the United States have added more serious concern (4, 5, 6).

Although the incidence of VISA or VRSA remains low, there is an increasing number of reports from many countries of strains of *S. aureus* showing heterointermediate resistance to vancomycin (hVISA) (1, 8, 13, 16, 20) since the first report of the prototype strain (Mu3) by Hiramatsu et al. (11). Generally, hVISA strains are defined as strains of *S. aureus* that contain subpopulations of vancomycin-intermediate daughter cells but for which the MICs of vancomycin for the parent strain are only 1 to 4 mg/liter (9, 11). Although the clinical significance of hVISA is controversial, there have been reports that suggested vancomycin treatment failure associated with hVISA (2, 11, 15, 20).

In this study, a total of 1,357 methicillin-resistant *S. aureus* (MRSA) clinical isolates were collected from 12 centers in 12 Asian countries that were part of the Asian Network for Surveillance of Resistant Pathogens during the period from January 1997 to March 2000. The number of MRSA isolates from each country ranged from 28 (the Philippines) to 457 (South Korea) (Table 1). Each strain was isolated from a specimen from a different patient. Isolates of *S. aureus* were confirmed as hVISA strains by a population analysis in which all isolates representing one or more colonies on brain heart infusion agar plates containing 4 mg of vancomycin per liter were tested within 48 h of specimen collection as described previously (11). A strain was considered to be an hVISA strain if it contained subpopulations of cells that grew on the 4-mg/liter vancomycin plate at a frequency of 10⁻⁶ or higher (11). Mu50 (VISA, Japan) and Mu3 (hVISA, Japan) strains were used as controls (10, 11). Population analyses were conducted at the Asian-Pacific Research Foundation for Infectious Diseases (ARFID), Seoul, South Korea, and at Juntendo University, Tokyo, Japan.

Of 1,357 MRSA isolates, 347 (25.6%) showed growth of cells on the screening plates (brain heart infusion agar plates containing 4 mg of vancomycin per liter). The rate of screen-positive strains was the highest for strains from South Korea (42%) and Japan (29.9%), while it was less than 10% for strains from Singapore, Sri Lanka, and Saudi Arabia (Table 1). hVISA strains from Asian countries showed features typical of the population analysis profile of the Mu3 strain (Fig. 1). All of these isolates were susceptible to vancomycin, with the MICs for them being ≤2 μg/ml. Of 347 screen-positive strains, 58 strains (4.3% of total MRSA strains) were finally confirmed as hVISA by population analysis (Table 1). Heterointermediate resistance to vancomycin was found among MRSA isolates from Japan (8.2%), India (6.3%), South Korea (6.1%), the Philippines (3.6%), Vietnam (2.4%), Singapore (2.3%), and Thailand (2.1%), but it was not found among strains from China, Indonesia, Saudi Arabia, Sri Lanka, or Taiwan.

Given the high prevalence of MRSA strains and the popular
TABLE 1. Prevalence rates of hVISA among MRSA isolates from Asian countries by screening method and population analysis

<table>
<thead>
<tr>
<th>Country</th>
<th>No. of MRSA isolates</th>
<th>No. of screen-positive strains (%)</th>
<th>No. of hVISA isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>84</td>
<td>12 (14.3)</td>
<td>0</td>
</tr>
<tr>
<td>India</td>
<td>80</td>
<td>16 (20.0)</td>
<td>5 (6.3)</td>
</tr>
<tr>
<td>Indonesia</td>
<td>114</td>
<td>14 (12.3)</td>
<td>0</td>
</tr>
<tr>
<td>Japan</td>
<td>231</td>
<td>69 (29.9)</td>
<td>19 (8.2)</td>
</tr>
<tr>
<td>South Korea</td>
<td>457</td>
<td>192 (42.0)</td>
<td>28 (6.1)</td>
</tr>
<tr>
<td>The Philippines</td>
<td>28</td>
<td>6 (21.4)</td>
<td>1 (3.6)</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>34</td>
<td>3 (8.8)</td>
<td>0</td>
</tr>
<tr>
<td>Singapore</td>
<td>87</td>
<td>2 (2.3)</td>
<td>2 (2.3)</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>55</td>
<td>4 (7.3)</td>
<td>0</td>
</tr>
<tr>
<td>Taiwan</td>
<td>50</td>
<td>7 (14.0)</td>
<td>0</td>
</tr>
<tr>
<td>Thailand</td>
<td>96</td>
<td>17 (17.7)</td>
<td>2 (2.1)</td>
</tr>
<tr>
<td>Vietnam</td>
<td>41</td>
<td>5 (12.2)</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>Total</td>
<td>1,349</td>
<td>347 (25.6)</td>
<td>58 (4.3)</td>
</tr>
</tbody>
</table>

*a Strains were tested on brain heart infusion agar plates containing 4 mg of vancomycin per liter.

*b Determined by population analysis.

FIG. 1. Population analysis profile of hVISA strains from Asian countries. Two hVISA strains, Korea 116 and India 93, contained subpopulations that grew in the presence of a vancomycin concentration of 4 mg/liter at frequencies of ≥10⁻⁶.

The emergence of VISA or VRSA strains in this region might be anticipated. The emergence of hVISA strains is another cause for concern with regard to the epidemiology of infections and implications for clinical practice. Since the first report of hVISA from Japan (11), hVISA strains have been found in many parts of the world (1, 13, 17, 20). Based on the published reports, the prevalence rate of hVISA varied widely from 0% (16) to 73.7% (1). Among South Korean isolates of MRSA, hVISA has been found with very low frequencies (0 to 0.5%) (18). However, our data showed that 6.1% of MRSA isolates were heterointermediately resistant to vancomycin. A previous report from Germany, which included 91 MRSA isolates from South Korea for screening for VISA, showed that 2.1% of South Korean MRSA isolates were hVISA (3). The frequency of hVISA strains may vary according to the characteristics of bacterial strains tested, specimen sources or types of infections (1), screening methods, media and test conditions (18), the local epidemiology or situation with regard to antibiotic selective pressure (14), and the unstable nature of the vancomycin resistance phenotype (9, 14). Our study confirmed the presence of hVISA strains in India, the Philippines, Singapore, and Vietnam for the first time. India showed a relatively high rate of hVISA strains (6.3%). However, the relationship between vancomycin usage and the prevalence of hetero-VRSA isolates in Asian countries could not be evaluated in this study, because data on vancomycin usage were not available.

The clinical significance of hVISA strains is still controversial. The presence of hVISA may be a significant indicator of the potential decline of the therapeutic effectiveness of vancomycin against MRSA infections (9). However, the clinical significance of hVISA is difficult to evaluate because there are very few cases and because conventional susceptibility tests in a microbiology laboratory cannot detect hVISA strains. Therefore, prospective controlled clinical studies to ascertain the clinical relevance of hVISA are hard to perform in clinical practice. At present, sporadic case reports show contradictory data on the clinical outcomes of hVISA infections. Some reports suggest that hVISA was not associated with the failure of vancomycin treatment (13), while other reports document the failure of vancomycin to treat hetero-VRSA infections, sometimes with fatal outcomes (1, 2, 11, 15, 20). Although there have been no data from controlled clinical studies which document the increased mortality from hVISA infections, clinicians should bear in mind that an inadequate response to vancomycin in the treatment of MRSA infections could be due to heteroresistance to vancomycin (9, 17). However, it is difficult to confirm the presence of heteroresistance in clinical practice because conventional microbiological methods, including MIC determination, cannot detect hVISA. Therefore, if hVISA infection is strongly suspected based on clinical features, population analysis has to be performed at a reference laboratory.

In summary, data from an international survey of VISA and hVISA strains among MRSA strains from Asian countries showed that hVISA strains were found in some Asian countries. Given the prevalence of hVISA strains (6.1, 8.2, and 6.3% in South Korea, Japan, and India, respectively) and the high prevalence of MRSA strains in the Asian region, continued regional surveillance of S. aureus with reduced susceptibility to vancomycin and clinical studies to evaluate the relevance of hVISA should be performed.

This study was partly supported by the SBRI (Samsung Biomedical Research Institute) (grant C-A4-216) and by the National Fund of the Ministry of Education, Tokyo, Japan.

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