Susceptibility to Vancomycin of Methicillin-Resistant
*Staphylococcus aureus* Isolated in a University Hospital in Japan

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**Summary**

Intravenous vancomycin was approved in 1991 in Japan and has been widely used for treatment of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Consequently, ever since the initial discovery of vancomycin intermediate-resistant *S. aureus* in Japan, the vancomycin resistance of this organism has been a great concern in clinical settings. We investigated whether vancomycin resistance had emerged in MRSA isolated in our hospital since the approval of the use of intravenous vancomycin. Vancomycin susceptibility was evaluated on the basis of minimum inhibitory concentrations determined by the agar dilution method and a heterogeneous resistance examination. The median minimum inhibitory concentration of the 69 MRSA strains isolated in 1988 and the 74 isolated in 1998 was 0.75 µg/ml and 1.0 µg/ml, respectively (p<0.001), however, all of the strains were classified in the susceptible group. None of them was an MRSA heterogeneously resistant to vancomycin (hetero-VRSA), which has been defined as a strain having a 1/10\(^{th}\) or greater heterogeneously resistant subpopulation to vancomycin. In another set of investigations, no hetero-VRSA were found among 12 other MRSA strains isolated after intravenous administration of vancomycin for 14 or more days (range: 14 to 77 days). We conclude that while the use of intravenous vancomycin may have slightly lowered the vancomycin susceptibility of MRSA in our hospital, the decrease is so small that it may not be significant clinically. In addition, no hetero-VRSA were found in our hospital.

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**Introduction**

*Staphylococcus aureus* has been a major cause of morbidity and mortality among nosocomial infections, and nosocomial infections caused by methicillin-resistant *S. aureus* (MRSA) pose a particularly serious problem for health care facilities, because only a limited number of antibiotics are effective against them\(^{(1)}\).

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Vancomycin is one such antibiotic\textsuperscript{[13]}, and intravenous use of vancomycin for MRSA infections was approved in Japan in 1991.

Although some \textit{S. aureus} strains intermediately resistant to vancomycin have been isolated in Japan\textsuperscript{[6]}, the United States\textsuperscript{[24]}, and France\textsuperscript{[7]}, no strains definitely resistant to vancomycin according to the criteria of the National Committee for Clinical Laboratory Standards (NCCLS) have emerged yet. However, enterococci highly resistant to vancomycin have been a major problem as a cause of nosocomial infections in Europe and the United States\textsuperscript{[6]}, and high-level resistance has been transferred to \textit{S. aureus} experimentally in both \textit{in vitro} and \textit{in vivo} models\textsuperscript{[9]}. Accordingly, the emergence and prevalence of highly vancomycin resistant \textit{S. aureus} in clinical settings must be seriously considered\textsuperscript{[10]}. Another type of vancomycin resistance in MRSA, hetero-resistance, has also become a public health problem, because it can cause therapeutic failure or prolongation of vancomycin treatment of MRSA infection\textsuperscript{[11,12]}.

The importance of heterogeneously vancomycin resistant MRSA strains (hetero-VRSA) was first noted by Hiramatsu \textit{et al.} \textsuperscript{[12]}, who also demonstrated that hetero-VRSA was disseminated in hospitals in Japan. However, few investigations have been conducted on how the widespread use of intravenous vancomycin has influenced the vancomycin susceptibility of MRSA in Japan. In this report, we measured and compared susceptibility by the standard minimum inhibitory concentration (MIC) method and heterogeneous resistance examination using MRSA strains isolated in our hospital before and after the approval of the use of intravenous vancomycin. In addition, whether prolonged vancomycin use generates vancomycin resistance was also studied.

**Materials and Methods**

**Bacteria**

We randomly collected 69 MRSA strains and 74 MRSA strains isolated in 1988 and 1998, respectively, from inpatients in the University of Tokyo Hospital (877 beds; 1998 intravenous vancomycin utilization: 4kg) and stored in casitone medium (Eiken Kagaku, Tokyo, Japan). None of the strains had been isolated from the same patient. To investigate the influence of prolonged vancomycin use, we also collected other 12 pairs of MRSA strains isolated from 12 inpatients before and after intravenous vancomycin treatment for 14 or more days in 1998 and 1999. Gram-positive cocci that were catalase producing, coagulase producing, and resistant to oxacillin according to the agar screening method\textsuperscript{[13]} were identified as MRSA.

**Drug susceptibility tests**

The vancomycin susceptibility of the strains isolated was measured and classified according to the agar dilution method recommended by the NCCLS\textsuperscript{[14]}. Briefly, each isolate was suspended in normal saline to a turbidity of McFarland 0.5 (approximately $10^6$ colony forming units [CFU]/ml), and $10^6$ CFU were inoculated on Mueller-Hinton Agar Broth (Becton Dickinson, Cockeysville, USA) containing 0.25, 0.5, 0.75, 1, 1.5, and 2 μg/ml of vancomycin. The MICs were determined after incubation in air at 35°C for 16 h. The MICs of the isolates in 1988 and 1998 and those obtained from the 12 patients before and after prolonged intravenous vancomycin therapy were analyzed by the Mann-Whitney test and Wilcoxon test, respectively, and the differences between them were considered statistically significant when the $p$ values were less than 0.05.

Heteroresistance of the isolates to vancomycin was investigated according to the description by

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Hiramatsu et al., with the following modifications. Brain heart infusion broth (BHI, Difco Laboratories, Detroit, USA) agar approximately 4 mm deep and containing 4 and 8 μg/ml of vancomycin was prepared on 10 × 14 cm and 9 cm-diameter plates (by pouring 75 and 20 ml, respectively, of the broth agar). The plates were stored at 4°C and used within five days after preparation. For screening, each MRSA strain was cultured in BHI overnight, and the bacterial suspension was adjusted with BHI to an optical density of 0.15 at 540 nm. A 10 μl volume of the suspension (approximately 10⁶ CFU) was spotted on the agar plate containing 4 μg/ml of vancomycin and incubated in air for 48 h at 35°C. If one or more colonies was observed within 48 h, the strain was designated as a possible hetero-VRSA. The possible hetero-VRSA strains were re-cultured in BHI overnight, and the suspensions were adjusted with BHI to an optical density of 0.30 at 540 nm. A 50 μl volume of the adjusted suspension (approximately 10⁷ CFU) was spread on the agar plate containing 8 μg/ml of vancomycin, and after incubation in air for 48 h at 35°C, the number of colonies on the plates was counted. If more than 1/10⁶ CFU of the inoculated suspension survived, the strain was designated definitive hetero-VRSA. Each possible hetero-VRSA was confirmed to be a definitive hetero-VRSA strain three times.

Results

Vancomycin susceptibility of isolates in 1988 and 1998

The ages of the 69 patients from whom MRSA strains were isolated in 1988 and the 74 from whom they were isolated in 1998 ranged from 0 to 83 years old (mean: 54.1) and from 0 to 93 years old (mean: 45.8), respectively, and males accounted for 69.6% and 52.7%, respectively. The material from which the strains were isolated in 1988 and 1998 were wound exudates (73.9% and 24.3%, respectively), respiratory specimens (including sputum and nasal or throat swabs, 8.7% and 59.5%), and others (17.4% and 16.2%, Table 1).

The vancomycin MICs of the isolates in 1988 and 1998 ranged from 0.5 to 1.5 μg/ml (median: 0.75 μg/ml) and 0.5 to 2.0 μg/ml (median: 1.0 μg/ml), respectively (Fig. 1). All investigated strains were classified as susceptible to vancomycin according to the criteria of the NCCLS. No strains resistant to vancomycin (MIC: ≥ 32 μg/ml) or intermediate-resistant strains (MIC: 8 ~ 16 μg/ml) were found. The increase total in MIC was statistically significant (p<0.001). According to type of material, only the increase in respiratory specimens was significant (p=0.029), and the increases in the other materials were not (wound exudates: p=0.063, others: p=0.134).

Six of the isolates in 1988 (8.7%) and six in 1998 (8.1%) were positive in the screening tests for heterogeneous resistance, that is, they were possible hetero-VRSA. Seven of the 12 possible hetero-VRSA strains (two in 1988 and five in 1998) yielded one or more colonies on the BHI agar plates containing 8 μg/ml of vancomycin in at least one of the three confirmation tests. However, the maximum number of resistant subpopulations among

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<th>Table 1 Characteristics of patients and materials from which MRSA strains were isolated in 1988 and 1998</th>
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Fig. 1  Vancomycin MICs of MRSA strains isolated in 1988 (A) and 1998 (B). The open, hatched, and dotted bars indicate the numbers of strains isolated from wound exudates, respiratory specimens, and other materials, respectively. The total number of strains having each MIC is shown in parentheses.

A

B

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Discussion

In this study we found that the MRSA strains isolated in our hospital recently were slightly less susceptible to vancomycin than those isolated 10 years previously but they did not have vancomycin-resistant subpopulations that met the definition of hetero-VRSA\(^2\). No strains or subpopulations resistant to vancomycin were selected even after 14- to 77-day use of intravenous vancomycin.

The vancomycin susceptibility of MRSA isolated in our hospital has significantly decreased during the 10 years since approval of the use of intravenous vancomycin for MRSA infections. However, the median total increase MIC was only 0.25 µg/ml. Based on the NCCLS standard, all strains isolated in 1988 and 1998 were classified as susceptible, and none of them were intermediately resistant or resistant. Therefore, although widespread use of intravenous vancomycin may have influenced the susceptibility of hetero-VRSA strains in the pre- and post-treatment groups, respectively, none of them was classified as definitive hetero-VRSA.

Susceptibility of isolates from patients after prolonged vancomycin administration

The ages of the 12 patients treated with intravenous vancomycin for 14 or more days in 1998 and 1999 ranged from 26 to 75 years (mean: 58.4), and 83.3% of them were males. The materials from which MRSA strains were isolated before and after treatment were wound exudates (25.0% and 16.7%, respectively), respiratory specimens (66.7% and 83.3%), and others (8.3% and 0%). Seven patients were administered of vancomycin for a diagnosis or suspicion of hospital-acquired pneumonia, two for skin infection, two for surgical site infection, and one for meningitis. The number of days of the vancomycin administration and the total dose administered ranged from 14 to 77 days (median: 28.5) and from 9 to 74 g (27.5 g), respectively (Table 2).

Twelve MRSA strains isolated before and after the vancomycin administration were classified into pre-and post-treatment groups, respectively. The MICs of both groups were ranged from 0.5 to 1.5 µg/ml (medians: 1.0 µg/ml, Table 2), and there was no statistically significant difference between them. Although there were three and two possible
MRSA, the increase in the MIC has been very small and only statistically significant, and such a small difference may not affect the clinical outcome of intravenous vancomycin treatment. Of course, the increase in the MIC may signal the emergence of S. aureus that is highly resistant to vancomycin in the future, and we should again emphasize the prudent use of this drug.

We also examined whether heteroresistance to vancomycin could be found in the MRSA isolated in our hospital. One report showed that 9.3% of the MRSA strains isolated in seven university hospitals in Japan in 1996 were heteroresistant to vancomycin\(^\text{12}\). However, the prevalence of hetero-VRSA in university hospitals in that report was in the 0% to 26% range, and three of the seven university hospitals were free of hetero-VRSA strains. No strains that met the definition of hetero-VRSA were found in our study in spite of repeated examinations. Even intravenous vancomycin administration for 14 days or more failed to induce a vancomycin-resistant subpopulation in our hospital. In a similar study in another university hospital in Japan (where Mueller-Hinton broth agar containing 8 \(\mu\)g/ml of vancomycin was used instead of BHI agar), no hetero-VRSA that met the definition were found among the MRSA and methicillin-sensitive S. aureus strains isolated in the hospital in 1996 and 1997, which the authors did not clearly describe in their report\(^\text{13}\). Although no other studies on hetero-VRSA in Japan have been reported, we suspect that hetero-VRSA may already be prevalent in certain hospitals in Japan, and a more extensive study may be required to determine whether hetero-VRSA is now being disseminated in this country.

In this study, we used only BHI agar containing 8 \(\mu\)g/ml of vancomycin for the heteroresistance examination. Some studies have tested for resistant subpopulations by using different concentrations of vancomycin,
comycin and have reported “population curves” of the strains examined\textsuperscript{6\textsuperscript{12}\textsuperscript{15}}, however, the clinical importance of population curves is unknown, and only the percentage of resistant subpopulations at 8 µg/ml of vancomycin is included in the definition of hetero-VRSA\textsuperscript{12}. We therefore did not make population curves for any of the MRSA strains in our study.

Hetero-VRSA have been reported to be a possible risk factor for therapeutic failure of vancomycin and a precursor of a homogeneous resistant strain\textsuperscript{12}, and several studies on \textit{S. aureus} heterogeneously resistant to vancomycin have been reported since then\textsuperscript{6\textsuperscript{11}\textsuperscript{14}–\textsuperscript{18}}. However, no reports have ever clearly demonstrated that hetero-VRSA is more responsible for therapeutic failure of vancomycin treatment than non-hetero-VRSA is. Furthermore, one report has shown that the method for detecting hetero-VRSA has poor reproducibility\textsuperscript{19}. In the present study, we were unable to evaluate the reproducibility of the method and the clinical significance of hetero-VRSA ourselves, because there were no definitive hetero-VRSA in our hospital. To clarify the clinical importance of hetero-VRSA, we propose worldwide surveillance of hetero-VRSA by strictly defined methodology in terms of the kind and amount of broth used, inoculum size on broth agar, duration and temperature of incubation, appropriate ratio of resistant subpopulation compatible with clinical courses, a standard strain to calibrate the method, and, especially, reproducibility of outcome.

**Acknowledgements**

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**References**


13) Swenson JM, Hindler JA, Peterson LR: Special tests for detecting antibacterial resistance. \textit{In}: Murray PR, Baron
一大学病院で分離したメチシリン耐性黄色ブドウ球菌のバンコマイシン感受性

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静注用バンコマイシンは，1991年に保険承認されて以来，メチシリン耐性黄色ブドウ球菌（MRSA）感染症の治療に使用されている。しかし，1997年にバンコマイシン中等度耐性黄色ブドウ球菌が日本で初めて報告されてから，MRSAのバンコマイシン耐性化が問題になっている。本研究では，静注用バンコマイシンの承認後，東大病院で分離されたMRSAにバンコマイシン耐性が生じたかどうかを調べた。バンコマイシンに対する感受性は，agar dilution法による最小発育阻止濃度（MIC）と，ヘテロ耐性試験によって評価した。1988年に分離したMRSA 69株と1998年に分離した74株のMICの中央値は，それぞれ0.75 μg/ml，1.0μg/mlだった（p<0.001）が，全て感受性と判定された。ヘテロVRSA（バンコマイシン耐性の亜集団が1/10^6以上であるもの）はなかった。これとは別に，バンコマイシン静注を14日間以上（14〜77日間）行った後分離されたMRSA 12株を集め解析したが，ヘテロVRSAはなかった。以上より，静注用バンコマイシンの使用により，本院のMRSAのバンコマイシンに対する感受性はわずかに低下したものの，臨床的には問題にならないと考えた。また本院では，ヘテロVRSAは検出されなかった。