Septic Arthritis Caused by Vancomycin-Intermediate *Staphylococcus aureus*

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Four methicillin-resistant *Staphylococcus aureus* isolates were consecutively isolated from synovial fluid and a knee wound of a patient with septic arthritis. Determination of the MIC of vancomycin and population analysis confirmed these isolates as vancomycin-intermediate *S. aureus* (VISA). Results of this study also revealed that cell wall thickness and reduced susceptibility to Triton X-100 were characteristic features of VISA.

We describe the first case of septic arthritis caused by vancomycin-intermediate *Staphylococcus aureus* (VISA) for a 19-year-old male who fractured his left femur in a motorcycle traffic accident. This patient had four admissions for four surgical operations at a local hospital from August 2000 to September 2001, including an open reduction and internal fixation (ORIF) and debridement on 25 August 2000, removal of dynamic compressive screws on 4 April 2001, another debridement on 19 April 2001, and another ORIF on 25 September 2001. He was also found to have osteomyelitis according to the clinical diagnosis at the time of the last surgery on 25 September 2001 and was treated with vancomycin (500 mg given intravenously every 6 h) for 3 weeks with no improvement. The patient continued to suffer from pain with erythema over his left lower leg and persistent wound discharge and swelling of the knee. He was then transferred to the Tri-Service General Hospital, Taipei, Taiwan, on 23 October 2001. Limited left knee activity with muscle atrophy of the thigh and calf was observed at the time of this admission.

An arthroscopy with debridement was performed on 30 October 2001, and the antibiotics gentamicin and cefazolin were given as empirical therapy because methicillin-resistant *Staphylococcus aureus* (MRSA) infection was suspected. Four consecutive MRSA isolates, designated MRSA1, MRSA2, MRSA3, and MRSA4, were recovered from his synovial fluid (obtained on 2 November 2001) and knee wound tissues (obtained on 4, 9, and 21 November 2001); therefore, the patient was considered to have septic arthritis with MRSA infection. These isolates were found to be susceptible to fucidic acid, teicoplanin, and vancomycin by the disk diffusion test. Therefore, sodium fucidate was used to treat the infection. After 40 days of hospitalization at the Tri-Service General Hospital, the patient showed no local heat or wound discharge and was discharged. The level of C-reactive protein had decreased from

6.71 mg/dl at admission to 1.7 mg/dl at discharge. Sodium fucidate (250 mg, three times a day) was prescribed for the following 3 months, and the patient was seen at the outpatient clinic every 2 weeks during this 3-month period.

The four MRSA isolates were characterized. The MIC of vancomycin was first determined according to the CLSI (formerly NCCLS) guidelines (12). The vancomycin MIC for the first three MRSA isolates was 8 µg/ml; therefore, they were identified as VISA. The fourth isolate, MRSA4, had a vancomycin MIC of 4 µg/ml. These MRSA isolates were then subjected to population analysis to find subpopulations of cells that were resistant to vancomycin as described previously (6). Briefly, 50 µl of the starting cell suspension of each isolate and its serial dilutions were spread on brain heart infusion (BHI) agar plates containing 1 to 10 µg/ml of vancomycin with 1-µg/ml increments. The starting cell suspension was prepared by diluting the fresh bacterial culture in BHI broth to an optical density at 580 nm (OD₅₈₀) of 0.3 by adding fresh BHI broth. The plates were incubated at 37°C for 48 h before the numbers of CFU were determined. The number of vancomycin-resistant cells in each of the 50-µl aliquots was plotted semilogarithmically. Isolates MRSA1 to -3 were homogeneously intermediately resistant to vancomycin at 4 µg/ml, whereas MRSA4 was found to have intermediate vancomycin resistance at the same concentration at a frequency of 10⁻⁵ by population analyses (Fig. 1). This type of vancomycin resistance is referred to as heterogeneous VISA (h-VISA) (6).

Pulsed-field gel electrophoresis (PFGE) was performed as described previously (11, 14). Electrophoresis was performed for 27 h, 12 min, using the CHEF MAPPER XA system (Bio-Rad, Hercules, Calif.) with a pulse time of 2.79 to 21.79 s. Isolates MRSA1 and MRSA2 were found to belong to type A, and MRSA3 and MRSA4 belonged to type A2. Since types A and A2 differ by only one band, they are considered closely related (14). Multilocus sequencing typing (MLST) was also performed and gave the same results. All isolates resulted in sequence type 239 according to the established method and the MLST database (3). These results suggest that the four MRSA isolates were derived from a single MRSA strain. To determine whether their vancomycin resistance was due to the pres-

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FIG. 1. Population analysis of control strains and MRSA isolates. Control strains *S. aureus* ATCC 29213, Mu50 (7), and Mu3 (6) were vancomycin sensitive, VISA, and h-VISA, respectively.

ence of vancomycin resistance genes, amplification of *vanA*, *vanB*, *vanC1*, and *vanC2* genes, which are commonly present in enterococci, from these MRSA isolates was performed as described previously (9). None of these four MRSA isolates were found to harbor any of the enterococcal *van* genes.

The thickness of the cell walls of these isolates was determined by transmission electron microscopy as described previously (1, 4). Photographic images of cells at a final magnification of $\times 30,000$ were examined. Thirty cells of each isolate with nearly equatorially cut surfaces were measured, and re-

sults were expressed as means $\pm$ standard deviations. The average cell wall thickness was 38.63 ± 0.52 nm for MRSA1, 30.74 ± 0.68 nm for MRSA2, and 35.92 ± 1.04 nm for MRSA3, similar to that of the vancomycin-intermediate control strain *S. aureus* Mu50 (1), which had an average thickness of 38.5 ± 2.03 nm. MRSA4 was found to have a normal cell wall thickness of 25.98 ± 0.84 nm, as does the vancomycin-sensitive control strain *S. aureus* ATCC 29213 (23.35 ± 0.47 nm). The cell wall thickness of MRSA4 was significantly different from those of MRSA1 to -3 (Mann-Whitney test, $P < 0.05$).

FIG. 2. Triton X-100 autolysis assay of control strain ATCC 29213 and MRSA isolates. Cells were grown in BHI broth to mid-logarithmic phase, washed twice in ice-cold water, and then resuspended in the Triton X-100 lysis buffer. The decrease in A_{620} was determined every 30 min for 4 h. The percentage of the remaining optical density of each isolate at each time point was plotted.

The four MRSA isolates were then assayed for susceptibility to Triton X-100 as described previously (10). The cells were grown in BHI broth to mid-logarithmic phase, pelleted, washed twice in ice-cold water, and then resuspended in the Triton X-100 lysis buffer (0.05 M Tris-HCl [pH 7.2], 0.05% Triton X-100). The decrease in A_{620} due to cell lysis was determined at 30-min intervals for 4 h, and the percentage of surviving cells, expressed as the remaining OD, was plotted. Susceptibility to Triton X-100 was found to be different between the VISA (MRSA1 to -3) and h-VISA (MRSA4) isolates. After 4 h of treatment with Triton X-100, the three VISA isolates (MRSA1 to -3) had a remaining OD of approximately 60%. In contrast, the remaining OD of the h-VISA isolate (MRSA4) was approximately 50% at 1.5 h and only 20% at the 4-h time point. The result for MRSA4 is significantly different from those for MRSA1 to -3 (Mann-Whitney test, $P < 0.05$). Similar results were observed with the vancomycin-sensitive *S. aureus* control strain ATCC 29213 (Fig. 2).

This is the first reported case of septic arthritis caused by VISA. The most characteristic feature of VISA is the thickening of cell walls, leading to increased resistance to Triton X-100. Cell wall thickening may be responsible for the vancomycin resistance, since no *van* genes were found in these MRSA isolates. This notion is supported by the observation that MRSA1, MRSA2, and MRSA3 had thicker cell walls and were more resistant to vancomycin than MRSA4, which has a cell wall of normal thickness. Reduced Triton X-100 susceptibility in h-VISA has also been reported by Hussain et al. (8). Cell wall thickening has also been shown to be a common feature of vancomycin-resistant *S. aureus* by Cui et al. (2). Reduced cross-linking in the cell wall of vancomycin-resistant *S. aureus* has been reported (13). Our results are consistent with the observations of these studies.

The PFGE types of the MRSA isolates from this study were determined to be types A and A2, which are the predominant types in Taiwan (15), suggesting that the patient acquired the MRSA through nosocomial infections. Isolate MRSA4 was previously negative by our established screening method for VISA. It was later identified as h-VISA by population analysis. Since h-VISA can be induced by antibiotics such as β -lactams and glycopeptides (5) to become true VISA, we recommend that consecutive MRSA isolates from a patient or MRSA isolates from sterile sites be subjected to population analysis in order to find subpopulations that are resistant to vancomycin.

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