First Report of Vancomycin Intermediate Resistance in Sequence Type 72

Community-Genotype Methicillin-Resistant *Staphylococcus aureus*

Doo Ryeon Chung\textsuperscript{a,b}, Jin Yang Baek\textsuperscript{b}, Hyun Ah Kim\textsuperscript{a}, Min Hee Lim\textsuperscript{a}, So Hyun Kim\textsuperscript{b}, Kwan Soo Ko\textsuperscript{c}, Cheol-In Kang\textsuperscript{a}, Kyong Ran Peck\textsuperscript{a}, Nam Yong Lee\textsuperscript{d}, and Jae-Hoon Song\textsuperscript{a,b}

Division of Infectious Diseases, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea\textsuperscript{a}; Asia Pacific Foundation for Infectious Diseases (APFID), Seoul, Korea\textsuperscript{b}; Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon, Korea\textsuperscript{c}; Department of Laboratory Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea\textsuperscript{d}

Running head: Vancomycin intermediate resistance in CA-MRSA

Keywords: *Staphylococcus aureus*; vancomycin resistance; community-acquired infections; treatment failure
Address for correspondence:

Doo Ryeon Chung  M.D., Ph.D.
Associate Professor of Medicine
Division of Infectious Diseases, Department of Internal Medicine
Samsung Medical Center, Sungkyunkwan University School of Medicine
50 Irwon-dong, Gangnam-gu, Seoul 135-710 Korea
Telephone: +82-2-3410-0323
Fax: +82-2-3410-0064
E-mail address: drchung@skku.edu
Vancomycin intermediate resistance has not been reported among sequence type (ST) 72-
methicillin-resistant Staphylococcus aureus-SCCmec type IV, distinctive community-
genotype MRSA strains in Korea. We report the first case of vancomycin treatment failure
due to development of vancomycin intermediate resistance in infection caused by ST72-
MRSA-IV.
A 79-year-old man was admitted to the coronary care unit due to non-ST elevation myocardial infarction and sepsis. Blood culture grew methicillin-resistant Staphylococcus aureus (MRSA) which was susceptible to most non-beta-lactam agents except erythromycin (vancomycin MIC = 1 mg/l). There was no evidence of endocarditis in transesophageal echocardiogram. Vancomycin was administered for 14 days after culture became negative. Follow-up blood culture 14 days after the end of treatment grew MRSA (vancomycin MIC = 1 mg/l) again. At that time the patient complained of back pain and spine MRI revealed infectious spondylitis at T12-L1. Vancomycin was restarted and blood culture became negative nine days later. With receiving vancomycin his back pain was improved and C-reactive protein (CRP) decreased to 0.24 mg/dl until 30 days after restart of vancomycin, when back pain was aggravated and CRP suddenly elevated to 8.1 mg/dl. Blood culture grew MRSA with vancomycin MIC higher than 2 mg/l. Confirmative test using E-test method in the clinical microbiology laboratory at the time of isolation revealed that this strain had vancomycin MIC of 3 mg/l. When we re-tested the frozen isolates using broth microdilution test, vancomycin MIC was 4 mg/l. Vancomycin was switched to linezolid and continued for four weeks without any adverse effects. His back pain was improved and CRP decreased to 0.03 mg/dl. He has been free of relapse for 3 months now.

Antimicrobial susceptibility testing by MIC determination according to guidelines of the Clinical and Laboratory Standards Institute (2) showed that this vancomycin-intermediate S. aureus (VISA) strain was resistant to erythromycin and rifampicin, whereas it was susceptible to gentamicin, ciprofloxacin, clindamycin, cotrimoxazole, tetracycline, fusidic acid, linezolid, ceftobiprole, and daptomycin. A MRSA isolated at the first episode of
bacteremia and a VISA isolated at the breakthrough bacteremia were molecularly characterized and compared. Multilocus sequence typing (MLST) was carried out by PCR amplification and sequencing of seven housekeeping genes (arcC, aroE, glpF, gmk, pta, tpi and yqiL) as previously described (5). The allelic profiles and sequence types (ST) were assigned by the MLST web site (http://saureus.mlst.net/). SCCmec types were determined by the multiplex PCR method (13). Isolates were screened for the lukF-PV and lukS-PV genes encoding the components of the Panton–Valentine leukocidin (PVL) toxin as previously described (11). Pulsed-field gel electrophoresis (PFGE) was performed as described previously (17). Both isolates were determined to be PVL-negative ST72 strains and carried SCCmec type IV. The PFGE patterns were analyzed using GelCompar II software (Applied Maths, Belgium), and compared to those of the VISA strains belonging to ST5 isolated from other two patients in the same hospital during 2009-2011, Mu3 (reference heterogeneous VISA strain, kindly provided by Professor Hiramatsu, Juntendo University, Japan), and Mu50 (reference VISA strain, kindly provided by Professor Hiramatsu, Juntendo University, Japan). All the isolates from the patient displayed the identical pulsotype, whereas they were different from the types of the reference strains (FIG 1).

In order to determine if an original isolate from the patient was heterogeneous VISA (hVISA), we conducted a population analysis profile (PAP) test as previously described (15). The first isolate from the patient was determined to be vancomycin-susceptible S. aureus (VSSA) with no heterogeneous population of VISA, and two isolates of VISA were again confirmed as VISA by this method. Unfortunately, the VSSA (vancomycin MIC, 1 mg/l) isolated at the first relapse of bacteremia was not stored, and so it could not be tested for the presence of heterogeneous population.
Since the first report of *S. aureus* with reduced susceptibility to vancomycin (MIC 8 mg/l) from Japan in 1997 (9), there have been increasing reports of VISA and hVISA worldwide. In Korea, the first case of VISA (MIC, 8 mg/l) was reported in 2000 (10), and a subsequent nationwide surveillance study for VISA found 15 VISA strains through screening of 37,856 clinical isolates collected from 2001 to 2006 (1). VISA infections have been caused mostly by healthcare-associated MRSA clones (12), and all VISA strains reported in Korea had been ST5-MRSA-II or ST239-MRSA-III, which were healthcare-associated MRSA clones in this country (1, 14). Although community-associated MRSA (CA-MRSA) clones have emerged worldwide, development of VISA phenotype in these clones have been uncommon. Recently, two cases of treatment failure caused by the strains of USA300 CA-MRSA with intermediate vancomycin resistance were reported (7, 8). In particular, the VISA phenotype has never been reported among the strains of ST72-MRSA-IV, which has emerged as an important pathogen in the community and in hospitals in Korea during the past decade (16). The isolates from the patient showed susceptibility to most non-β-lactam agents and this is a typical antibiogram seen in ST72-MRSA-IV in Korea (16).

The VISA isolates in our report showed different vancomycin MICs according to the test methods. The MIC from E-test analysis was lower than that from the broth microdilution method. Such differences in vancomycin MICs of *S. aureus* according to test method have been previously reported (4).

Antecedent vancomycin use and prior MRSA infection two or three months before the current infection have been reported to be independent risk factors for VISA (6).
cooperative effect of the clogging and cell wall thickening have been reported to enable VISA to prevent vancomycin from reaching its true target in the cytoplasmic membrane (3).

This is the first reported case of vancomycin treatment failure due to development of vancomycin intermediate resistance in a patient with community-genotype ST72-MRSA-IV infection. The development of vancomycin-intermediate resistance during vancomycin treatment in patients with infections by these community-genotype MRSA strains pose difficult challenges for effective antimicrobial treatment and infection control.
References


Financial: Nothing to disclose

Conflict of Interest: Nothing to disclose
Figure legends.

FIG 1  Pulsed-field gel electrophoresis (PFGE) patterns of MRSA and VISA isolates from the patient and reference strains. Lane 1, K01-SAU-11-266 (the first strain of MRSA of ST72 and SCCmeC type IV from the patient); lane 2, K01-SAU-11-298 (a VISA strain of ST72 and SCCmeC type IV from the patient); lane 3, K01-SAU-11-300 (a VISA strain from the patient); lane 4, K01-SAU-11-075 (a VISA strain of ST5 and SCCmeC type II from other patient A); lane 5, K01-SAU-09-67 (an MRSA strain of ST5 and SCCmeC type II from other patient B); lane 6, K01-SAU-09-573 (a VISA strain of ST5 and SCCmeC type II from other patient B); lane 7, Mu3 (reference hVISA strain); lane 8, Mu50 (reference VISA strain); M, lambda marker.