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More accurate measurement of vancomycin minimum inhibitory concentration indicates poor outcomes in meticillin-resistant *Staphylococcus aureus* bacteraemia

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ABSTRACT

Meticillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen associated with community-acquired and nosocomial infections. The aim of this study was to validate the vancomycin (VAN) minimum inhibitory concentration (MIC) and administration of VAN that may affect the prognosis of patients with MRSA bacteraemia. In total, 140 clinical MRSA strains from blood cultures were collected from January 2009 to December 2013 at a university hospital in Tokyo (Japan). Patient background, their clinical situation and the susceptibility of isolates to anti-MRSA agents in all cases were reviewed, and factors contributing to 30-day mortality were analysed. Susceptibility to anti-MRSA agents was measured by a microdilution susceptibility testing method. The VAN MIC was further evaluated at 0.25 μg/mL intervals from 0.5 μg/mL to 2.0 μg/mL. Multiple logistic regression analysis revealed a 4-fold increase in mortality of patients with a VAN MIC ≥1.5 μg/mL [odds ratio (OR) = 3.952, 95% confidence interval (CI) 1.471–10.614; $P = 0.006$]. A one-score increase in the Charlson co-morbidity index resulted in a 1.2-fold increase in the risk of death (OR = 1.199, 95% CI 1.054–1.364; $P = 0.006$). However, no significant difference was found in the ratio of the VAN 24-h area under the concentration–time curve to MIC between VAN MIC ≥1.5 μg/mL and <1.5 μg/mL. A significant increase in the MICs of teicoplanin and daptomycin was observed in strains with high VAN MICs. For patients with high VAN MICs, administration of these anti-MRSA antibiotics may have a poor outcome owing to cross-resistance.

1. Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA) causes important community-acquired and nosocomial infections and is the most common bacterial pathogen worldwide. Managing these infections is costly [1]. When MRSA is isolated from blood cultures, it carries a high mortality rate despite appropriate initial antimicrobial therapy. A mortality rate between 20% and 30% is observed in most studies [2].

For more than four decades, vancomycin (VAN) has been the primary treatment for infections caused by MRSA. The area under the concentration–time curve (AUC) to minimum inhibitory concentration (MIC) ratio is a representative pharmacokinetic/pharmacodynamic parameter for VAN [3]. A consensus review in the USA recommends a target AUC/MIC ratio of >400 for the treatment of MRSA-associated lower respiratory tract infections [4]. It is reported that an AUC/MIC ratio of >398 is needed for successful treatment of MRSA bacteraemia [5].

Recently there have been reports about an increase in the VAN MIC of MRSA isolates over time, termed 'MIC creep' [6,7]. According to current standards established by the Clinical and Laboratory Standards Institute (CLSI), isolates with a VAN MIC of 2 μg/mL are considered to be VAN-susceptible [8]. However, for MRSA strains

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with high VAN MICs, it is difficult to attain a VAN AUC/MIC of 400, predicting failure to achieve a sufficient therapeutic response. High mortality with MRSA bacteraemia has been reported among patients treated with VAN for infections caused by strains with high VAN MICs [2,9].

AUC/MIC values vary greatly between patients with MRSA bacteraemia when the VAN MIC is 1 μg/mL or when it is 2 μg/mL, and this can affect the clinical outcome. Recent reports indicate poor clinical outcomes for VAN MICs of ≥1.5 μg/mL as determined by Etest [10,11]. Controversial results, however, were obtained in large-scale meta-analyses: VAN MICs of ≥1.5 μg/mL affected the prognosis of patients in one study, but not in another [2,9]. This is mainly because the treatment and background of individual patients were not considered in the meta-analyses [12].

Here we report detailed results of MIC creep and the relationship between VAN MIC and patient prognosis and therapeutic response. These were obtained through determination of VAN MICs of MRSA strains isolated from blood culture using the broth microdilution (BMD) method.

2. Materials and methods

2.1. Patients and bacterial strains

In total, 140 clinical MRSA strains from blood cultures were collected from inpatients at Showa University Hospital, a 1000-bed tertiary referral centre and teaching institution in Tokyo (Japan), from January 2009 to December 2013. Blood cultures were analysed using a BD BACTEC FX System (BD Diagnostics, Sparks, MD). All strains were screened using a MicroScan® WalkAway® system (Beckman Coulter K.K., Tokyo, Japan) and those identified as MRSA were subjected to oxacillin or cefoxitin testing. These strains were stored in a Microbank™ System (IWAKI, Tokyo, Japan) at −80 °C. Patients who had a fever of ≥38 °C and who tested positive for only one strain from at least one blood culture bottle were included in this study. In patients with more than one strain or with recurrent episodes, only the first strain was analysed. Patient background [i.e. age, sex, body weight, underlying diseases, colonisation of MRSA, duration of central venous catheter, onset day after admission and Charlson co-morbidity index (CCI)] [13,14]), laboratory data (white blood cell count, serum albumin and creatinine clearance), source of infection (soft tissue and bone, central nervous system, pneumonia, intra-abdominal, urinary tract, intravascular or medical devices) were reviewed and the factors contributing to 30-day mortality were analysed.

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of MRSA strains was performed using a Sensititre® microbroth dilution system (Trek Diagnostic Systems Inc., Cleveland, OH) in accordance with the manufacturer's instructions. Overnight cultures were transferred to sterile demineralised water (Trek Diagnostic Systems) to achieve a 0.5 McFarland standard. Then, 30 μL of each suspension was transferred to sterile cation-adjusted Mueller–Hinton broth (Trek Diagnostic Systems) and 100 μL of the broth solution was then dispensed into the original MIC plates (Trek Diagnostic Systems Inc.) with the following antibiotics: VAN; teicoplanin (TEC); arbekacin (ABK); daptomycin (DAP); and linezolid (LZD). Only the VAN MIC was strictly evaluated as 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0 μg/mL. *Staphylococcus aureus* ATCC 29213 was used for quality control. MICs were recorded as the lowest concentration of an antimicrobial that completely inhibited bacterial growth. Resistance breakpoints by the CLSI were used [8].

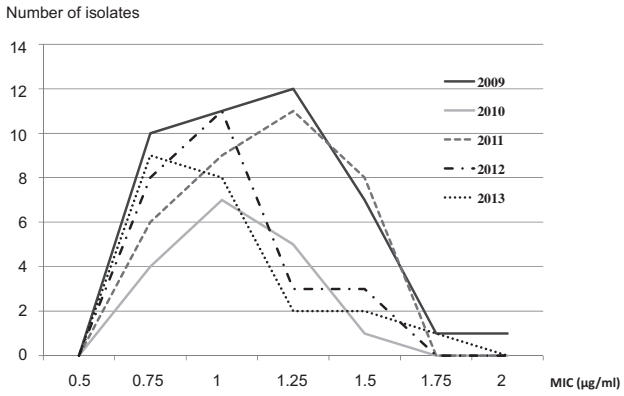

Fig. 1. Vancomycin (VAN) minimum inhibitory concentration (MIC) distribution against meticillin-resistant *Staphylococcus aureus* (MRSA) over a 5-year period.

2.3. Pharmacokinetic/pharmacodynamic analyses

VAN area under the 24-h concentration–time curve (AUC_{0-24}) calculations were performed if the patient received >48 h of VAN therapy and had one or more VAN level measured at the initial steady-state. Individual pharmacokinetic parameters were estimated for each patient using SHIONOGI-VCM-TDM S-edition software v.2009 (Shionogi & Co., Ltd., Osaka, Japan). VAN serum concentrations were fitted to a two-compartment volume–clearance model using the maximum posterior probability Bayesian approach to each individual patient's pharmacokinetic profile.

2.4. Statistical analysis

Continuous variables are presented as the mean ± standard deviation or median and interquartile range and were compared between groups using the independent *t*-test or Mann–Whitney *U*-test. Categorical variables are presented as the number and percentage of patients within each group and were compared using the χ^2 or Fisher's exact test. Survival was analysed using the Kaplan–Meier method. Plots were compared using the log-rank test. Correlations were calculated using Spearman's rho (ρ) test. Backward stepwise logistic analysis was used to identify independent variables associated with 30-day mortality and those variables with P <0.10 were included in the univariate analysis.

All statistical tests were two-sided and a P-value of <0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics for Windows v.21.0 (IBM Japan Ltd., Tokyo, Japan).

2.5. Ethics

This study was approved by the Ethical Committee of Showa University (Tokyo, Japan). Informed consent was not needed according to the ethical principles of the Declaration of Helsinki.

3. Results

3.1. Evaluation of vancomycin MIC creep

Fig. 1 shows the distribution of VAN MICs against MRSA within a 5-year period from 2009 to 2013. The number of MRSA strains collected in each year was 42, 17, 34, 25, and 22 in 2009, 2010, 2011, 2012, and 2013, respectively.

Only one strain collected in 2009 showed an MIC of 2 μg/mL. The MIC_{90} (MIC that inhibits 90% of the isolates) for strains collected in