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SHORT COMMUNICATION

Accuracy of 0.25 µg/mL-Interval MIC Measurement of Vancomycin Among *Staphylococcus aureus*: a Pilot Study

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SUMMARY

Background: **We aimed to examine the accuracy of 0.25 µg/mL-interval minimum inhibitory concentration (MIC) measurement of vancomycin (VCM) for *Staphylococcus aureus*.**
Methods: **We collected microbiological data on *S. aureus* from June 2020 to November 2023 at Okayama University Hospital, Japan. The VCM MIC values were determined by the microdilution method using Dry Plate Eiken (Eiken Chemical Co., Ltd, Tokyo, Japan) at ≤ 0.5, 0.75, 1, 1.25, 1.5, 1.75, and 2 µg/mL. We performed day-to-day reproducibility testing for *S. aureus* standard strain (ATCC 29213) and evaluated the within-run reproducibility and inter-technician errors among clinical isolates. Finally, we investigated the possibility of VCM creeping by reviewing the clinical data.**
Results: **Fifteen day-to-day reproducibility tests revealed within one-tube differences. Within-run reproducibility testing was performed for 33 clinical isolates, and 32 isolates (97.0%) demonstrated within one-tube differences; complete agreement and one-tube differences were observed at 45.5% and 51.5%, respectively. Inter-technician reproducibility was assessed for 10 clinical isolates, and complete agreement and one-tube differences were observed at 20% and 80%, respectively. Of 19 cases receiving VCM treatment, one case showed an MIC elevation (≤ 0.5 to 1.25 µg/mL), suggesting VCM creeping.**
Conclusions: **We confirmed the accuracy of VCM MIC determination at 0.25 µg/mL increments among clinical isolates of *S. aureus,* based on the broth microdilution method.**
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INTRODUCTION

Among the various genotypic and phenotypic antimicrobial resistance (AMR) pathogens emerging worldwide, methicillin-resistant *Staphylococcus aureus* (MRSA) constitutes one of the representative pathogens of nosocomial infection. According to the Clinical & Laboratory Standards Institute (CLSI) [1], *S. aureus* isolates showing a minimum inhibitory concentration (MIC) level of vancomycin (VCM) ≤ 2 µg/mL are considered to be "clinically" susceptible. However, recent

studies have increasingly raised concerns about “MIC creep”, which is defined as an increase of VCM MIC within the susceptible range [2,3]. *S. aureus* isolates with a higher VCM MIC (≥ 1.5 μg/mL) potentially yield high mortality to patients [4,5] and clinicians should note the MIC value of *S. aureus* isolates when scheduling VCM administration [6].

The therapeutic efficacy of VCM against MRSA is correlated with the ratio of the 24-hour area under the concentration-time curve (AUC_{24}) to the MIC, denoted as AUC_{24}/MIC; the recent treatment consensus guidelines strongly recommend achieving an AUC_{24}/MIC ratio of over 400 for MRSA infection [6]. Therefore, even when the MIC of VCM is determined to be within the susceptible range (≤ 2 μg/mL), achieving the AUC_{24}/MIC ratio of ≥ 400 can be challenging when the MIC values are near the breakpoint, resulting in difficulties in the treatment of patients.

The MICs of antimicrobial agents are usually measured using a two-fold serial dilution method and measurement errors in the MIC value for a one-tube difference are generally possible. Thus, the AUC_{24}/MIC ratio easily fluctuates according to the MIC value itself; e.g., the AUC_{24}/MIC ratio doubles when the MIC value is halved. In contrast, the guidelines recommend a detailed calculation of the AUC_{24} by measuring the trough and peak concentrations of VCM [6]. The authors found a large discrepancy and inconsistency: roughly measured MIC vs. precisely measured AUC. Therefore, we consider that a detailed MIC measurement within the susceptibility range of VCM (MIC ≤ 2 μg/mL) can serve better VCM therapeutic strategy. Although the E-test can provide MIC values in small increments, it is difficult to apply in routine testing. To overcome this difficulty and complexity, we adopted a 0.25 μg/mL-interval MIC measurement of VCM at our hospital laboratory since April 2023. In this study, we aimed to examine the accuracy of 0.25 μg/mL-interval MIC measurement and its potential for clinical contribution.

MATERIALS AND METHODS

From June 2020 to November 2023, we collected microbiological data on *S. aureus* isolates from the clinical microbiology laboratory of Okayama University Hospital, Japan. The need for informed consent was waived because the data were fully anonymized without any clinical information. We excluded duplicates or more isolates from a single patient. The MICs of VCM were determined by the microdilution method using Dry Plate Eiken (Eiken Chemical Co., Ltd, Tokyo, Japan), with the following MIC points: ≤ 0.5, 0.75, 1, 1.25, 1.5, 1.75, and 2 μg/mL. We followed the manufacturer's instructions for the preparation of the bacterial suspension and MIC measurements.

Our investigations consist of three different portions to corroborate the accuracy of 0.25 μg/mL-interval MIC measurement of VCM. First, we repeatedly examined the MIC value of VCM against the standard strain of *S. aureus* ATCC 29213. Day-to-day reproducibility testing was conducted for 15 days. Second, to confirm within-run reproducibility among the clinical isolates of *S. aureus*, MICs were measured in duplicate using the same bacterial suspension of 33 clinical isolates. Third, to assess the influence of the technical process among clinical microbiologists, six technicians prepared bacterial suspensions of ten clinical isolates of *S. aureus* and independently measured the VCM MIC.

Subsequently, we retrospectively reviewed the microbiological database (from April 2023 to March 2024) of patients detected to have MRSA in their clinical specimens to confirm the MIC creeping phenomenon of VCM in real clinical settings using our 0.25 μg/mL-interval MIC measurement. VCM creeping was defined as a MIC elevation of VCM over 0.5 μg/mL (two-tube difference) after VCM treatment. Cases meeting the following criteria were excluded: i) cases without the re-identification of MRSA with more than 3 days interval and ii) no VCM administration during the clinical course.

RESULTS

The VCM MIC values for the standard strain ranged within a one-tube difference (≤ 0.5 μg/mL and 0.75 μg/mL) (Table 1A). The results of the within-run reproducibility test for 33 clinical isolates (24 methicillin-susceptible and nine methicillin-resistant) of *S. aureus* are summarized in Table 1B. Complete agreement and one-tube differences were observed for approximately half of the isolates (45.5% and 51.5%, respectively). While two-tube differences were observed in 3.0% of isolates, 32 out of 33 isolates (97.0%) showed one-tube differences. The inter-technician reproducibility results for the ten clinical isolates (three methicillin-susceptible and seven methicillin-resistant) of *S. aureus* are summarized in Table 1C. Complete agreement and one-tube differences were observed in 20% and 80% of the patients, respectively. No significant differences were observed between the two tubes.

The results of the review of the VCM MIC creeping are shown in Figure 1. Of the 48 patients who tested positive for MRSA and received VCM therapy, 18 patients were eligible for a detailed data review. Consequently, we observed one case showing a MIC elevation (≤ 0.5 to 1.25 μg/mL) after 5 days of VCM administration, meeting the VCM creeping criterion.

DISCUSSION

The present investigation demonstrated that 0.25 μg/mL -interval MIC measurement by routine broth microdilution method can provide an accurate MIC value. Notably, the MICs of 32 of 33 (97.0%) clinical isolates were tested within a one-tube difference. Additionally, there

Table 1. Reproducibility of 0.25 µg/mL-interval MIC measurement of vancomycin.

A) Day-to-day reproducibility for the standard strain of *Staphylococcus aureus* ATCC 29213 (single assay x 15 days).

	≤ 0.5 µg/mL	0.75 µg/mL	1 µg/mL	1.25 µg/mL	1.5 µg/mL	1.75 µg/mL	2 µg/mL
No. (%) of isolates	4 (26.7)	11 (73.3)	0	0	0	0	0

B) Within-run reproducibility for 33 clinical isolates of *S. aureus* (duplicate assay).

	Complete agreement	One-tube difference	Two-tube difference
No. (%) of isolates	15 (45.5)	17 (51.5)	1 (3.0)
	32 (97.0)		
	33 (100)		

C) Inter-technician reproducibility for 10 clinical isolates of *S. aureus* (single assay x 6 technicians).

	Complete agreement	One-tube difference	Two-tube difference
No. (%) of isolates	2 (20.0)	8 (80.0)	0
	10 (100)		0

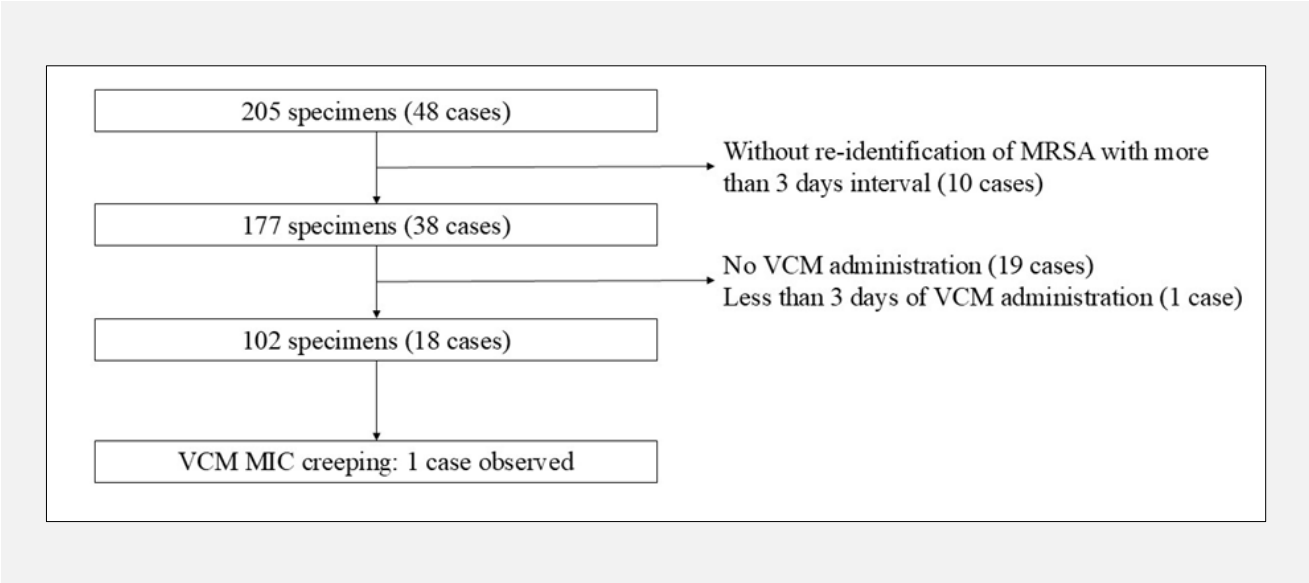

Figure 1. Retrospective review for the MIC creeping phenomenon of vancomycin in clinical MRSA isolates.

Definition of MIC creeping for VCM: an MIC elevation of more than 0.5 µg/mL (two-tube difference) after VCM treatment.

was no remarkable variation in the MIC values among technicians. Accordingly, we believe that our MIC testing method is highly reliable. In addition to its therapeutic advantages, it can contribute to the earlier detection of the VCM MIC creeping phenomenon, which was found in 1 (5.6%) of 18 *S. aureus* isolates.

For decades, VCM has been the first-line drug for MRSA infections according to the clinical guidelines [7]. VCM specifically binds to the d-alanyl-d-alanine terminus of the peptidoglycan cell wall of Gram-positive organisms, consequently inhibiting cell wall synthesis. A therapeutic drug monitoring strategy for VCM has been well established to determine the most effective and safe administration regimen [6]. In addition to its clinical effectiveness, an advantage of VCM in clinical use is the reduced likelihood of the emergence of VCM-resistant pathogens. Over 30 years have passed since its clinical use for the emergence of VCM-resistant *S. aureus* [8]. Currently, VCM-non-susceptible *S. aureus* clinical isolates are prevalent only in limited

clinical situations [9,10]. Thus, VCM has several clinical advantages over other anti-MRSA agents, implying the significance of clinical considerations in our MIC measurement.

In summary, an accuracy of 0.25 μg/mL-interval MIC measurement of VCM using the Dry Plate Eiken appeared satisfactorily high. Due to the small number of tested isolates and the single-facility nature of the study, its generalizability needs to be further examined. The clinical applicability of our methodology to both therapeutic and epidemiological aspects should be explored in future studies to provide better VCM treatment strategies based on therapeutic drug monitoring.

Data Availability:

The data in detail are available from the corresponding author upon reasonable request.

Declaration of Interest:

The authors declare that they have no conflicts of interest.

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