Reduced susceptibility to daptomycin in methicillin-resistant Staphylococcus aureus isolated from catheter-related bloodstream infections

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Research

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Abstract

Background

The appearance of reduced susceptibility to daptomycin in methicillin-resistant *Staphylococcus aureus* (MRSA) has recently been reported. It is unclear how likely MRSA involved in catheter-related bloodstream infections (CRBSI) is to dampen susceptibility to daptomycin. We investigated the minimum inhibitory concentrations (MIC) of daptomycin in MRSA isolated from the blood of patients with CRBSI and examined how it was affected by previous anti-MRSA drug treatment.

Methods

A total of 115 patients whose blood culture samples were found to contain MRSA were enrolled in this study. The MIC of daptomycin and vancomycin and whether the subjects had a history of anti-MRSA drug treatment were investigated and compared between the CRBSI and non-CRBSI groups.

Results

The mean MIC of daptomycin was significantly higher for the 46 CRBSI-related MRSA isolates than for the 69 non-CRBSI-related MRSA isolates (0.78 vs. 0.33, respectively; $p<0.0001$). Among the CRBSI-related MRSA isolates, those collected from patients with a history of anti-MRSA drug treatment had significantly higher MIC (1.27 vs. 0.53, respectively; $p <0.01$). During treatment, MRSA was detected again in 10 CRBSI and 4 non-CRBSI patients, and all of the CRBSI-related MRSA isolates exhibited 1-2 log2 increases in their daptomycin MIC.

Conclusions

It is considered that when MRSA in catheter biofilms is exposed to anti-MRSA drugs, strains with reduced susceptibility to daptomycin are able to survive and disperse into the blood. Catheters should be removed if an MRSA-induced CRBSI is suspected. Further study of whether high-dose daptomycin treatment is effective when catheters cannot be immediately removed is needed.

Background

Most of the causative bacteria of catheter-related bloodstream infections (CRBSI) belong to the genus *Staphylococcus*, and they are often methicillin-resistant [1]. Therefore, vancomycin is often selected as an initial treatment when a CRBSI is suspected. However, it should be noted that there have been reports of nosocomial infections of vancomycin-resistant *Enterococcus* [2], and the frequency of heterogeneous vancomycin-intermediate *S. aureus* among methicillin-resistant *Staphylococcus aureus* (MRSA) has

increased in Japan [3]. Also, biofilms form on MRSA-colonized catheters, and vancomycin has low biofilm permeability [4].

Therefore, daptomycin was developed as an anti-MRSA drug with high biofilm permeability. Daptomycin binds to bacterial cell membranes, causes membrane depolarization, and inhibits DNA and RNA synthesis by inducing the efflux of potassium ions [5]. Ten years have passed since it was first used in Japan, and MRSA that have reduced susceptibility to daptomycin have unexpectedly been reported. Such bacteria have been detected in cases involving bloodstream infections [6], endocarditis [7], and osteomyelitis [8], and the patients in these cases were previously administered anti-MRSA drugs.

To date, the risk of the appearance of reduced susceptibility to daptomycin in CRBSI-related MRSA is unclear. In this study, we investigated the minimum inhibitory concentrations (MIC) of daptomycin in MRSA isolated from CRBSI patients and examined how it was affected by previous anti-MRSA drug treatment. Moreover, we considered the countermeasures that should be taken in the clinical setting.

Methods

Patients

A total of 115 patients who were treated at our hospital or related facilities during the four years from May 2017 to April 2021 and whose blood culture samples were found to contain MRSA were enrolled in this study. The MIC of daptomycin and vancomycin and whether the subjects had a history of anti-MRSA drug treatment were investigated and compared between the CRBSI and non-CRBSI groups. A CRBSI was defined as when a pathogen was detected in one or more blood cultures, and the detected pathogen was only associated with infection foci located in a central venous catheter [9], and the same bacterium was detected in catheter tip cultures and peripheral blood cultures.

Blood Cultures

Two sets of blood culture bottles containing BD BACTEC 23F (for aerobic cultures) or 22F (for anaerobic cultures) (Becton Dickinson and Co. Franklin Lakes, NJ, USA) in a BD BACTEC smart container GT (Becton Dickinson and Co.) were used. Bottles that had been infused with a patient's blood were immediately attached to the BD BACTEC FX system (Becton Dickinson and Co.), and culturing was performed at 35 ℃ for 5 days. Bacterial identification and drug susceptibility testing were performed using the MicroScan Walkaway 96 plus system (Beckman Coulter Inc., Brea, CA, USA) and the Pos Combo 1J panel.

Catheter Tip Cultures

The catheter tip cultures were performed according to the quantification method developed by Brun-Buisson et al [10]. Namely, a 5-cm catheter tip was placed in a 15-mL tube, soaked in 1 mL of trypticase soy broth, and vortexed. Ten μL of the resultant solution was inoculated into an agar plate, which was then cultured at 35 ℃ for 2 days. When ≥10^3 colony-forming units/mL were observed, the infection was judged to be significant, and bacterial species identification and drug susceptibility testing were performed in the same manner as for the blood cultures.

Statistical analysis

GraphPad Prism version 9.0 for Macintosh (GraphPad Software, San Diego, CA, USA) was used for the statistical analyses. Fisher's exact test was used for comparisons of the frequency of previous anti-MRSA drug treatment. The non-parametric Mann-Whitney test and multiple regression analysis were used for comparisons of MIC values. P-values of <0.05 were regarded as significant.

Results

Characteristics of the patients

Of the 115 cases in which MRSA was detected in blood cultures, 46 involved CRBSI, and 69 did not. There was no difference in mean age or sex distribution between the CRBSI and non-CRBSI groups (p=0.06 and p=0.08, respectively) (**Table 1**). Sixteen patients with CRBSI and 5 of the non-CRBSI patients had a history of anti-MRSA drug treatment (p=0.0003).

The Minimum Inhibitory Concentrations Of Daptomycin And Vancomycin

The mean MIC of daptomycin was 0.78±0.84 for the CRBSI-related MRSA isolates, which was significantly higher than that for the non-CRBSI-related MRSA isolates (0.33±0.17, p<0.0001) (Figure 1a). The mean MIC of daptomycin for the MRSA isolates collected from the patients with previous anti-MRSA drug treatment was 1.05±1.13, which was significantly higher than that for the MRSA isolates collected from the patients who had not been treated with anti-MRSA drugs (0.39±0.27, p<0.0001). In the CRBSI group, the mean MIC of daptomycin for the MRSA isolates collected from the patients with previous anti-MRSA drug treatment was 1.27±1.22, which was significantly higher than that for the MRSA isolates collected from the patients who had not been treated with anti-MRSA drugs (0.53±0.37, p<0.01). However, in the non-CRBSI group no significant difference in the mean MIC of daptomycin was observed between the MRSA isolates collected from the patients with and without a history of anti-MRSA drug treatment (0.35±0.14 vs. 0.32±0.18, p>0.05).

There was no difference in the mean MIC of vancomycin between the isolates collected from the CRBSI and non-CRBSI groups (1.18±0.41 vs. 1.13±0.4, p>0.05) (Figure 1b). The mean MIC of vancomycin for the

isolates collected from the patients who had a history of anti-MRSA drug treatment was 1.33±0.48, which was significantly higher than that for the isolates collected from the patients who had not been treated with anti-MRSA drugs (1.11±0.37, $p<0.05$). In the CRBSI group, the mean MIC of vancomycin for the isolates collected from the patients who had a history of anti-MRSA drug treatment was 1.38±0.5, which was significantly higher than that for the isolates collected from the patients who had not been treated with anti-MRSA drugs (1.08±0.32, $p<0.05$). However, in the non-CRBSI group no significant difference in the mean MIC of vancomycin was observed between the isolates collected from the patients with and without a history of anti-MRSA drug treatment (1.2±0.45 vs. 1.13±0.4, $p>0.05$).

In the multiple regression analysis, a significant CRBSI and a history of anti-MRSA drug treatment ($p=0.0036$ and $p=0.0002$, respectively) were identified as factors related to the MIC of daptomycin (**Table 2**). Only a history of anti-MRSA drug treatment was found to be related to the MIC of vancomycin ($p=0.0302$).

Fluctuations in the MIC of daptomycin due to anti-MRSA drug treatment

During the anti-MRSA drug treatment, MRSA was isolated again from the blood cultures of 10 CRBSI patients (Figure 2a) and 4 non-CRBSI patients (Figure 2b). The anti-MRSA drugs administered to the CRBSI patients were daptomycin in 5 patients, vancomycin in 3 patients, and teicoplanin and linezolid in 1 patient each, whereas those administered to the non-CRBSI patients were teicoplanin in 3 patients and vancomycin in 1 patient. In both groups, each drug was administered at the standard dose. In the CRBSI group, the infected central venous catheters were not removed in the period between the blood cultures since the CRBSI had not been diagnosed at that time.

After treatment, all of the isolates collected from the CRBSI patients exhibited 1-2 log2 increases in the MIC of daptomycin. Four of them also demonstrated increases in the MIC of vancomycin. In one non-CRBSI case, only the MIC of daptomycin was increased, and in another case only the MIC of vancomycin was increased. No change in the MIC of either drug was observed in the other two cases. Among the CRBSI patients, the mean duration of anti-MRSA drug treatment was 18.3 days (11-31 days) for the 4 patients whose MRSA isolates exhibited increases in the MIC of both daptomycin and vancomycin and 25.5 days (7-61 days) for the 6 patients whose MRSA isolates only demonstrated increases in the MIC of daptomycin. In the non-CRBSI group, the patients whose isolates exhibited increases in the MIC of daptomycin and vancomycin were administered anti-MRSA drug treatment for 39 and 36 days, respectively. The two patients whose isolates did not exhibit changes in the MIC of either drug were administered anti-MRSA drug treatment for 8 and 15 days, respectively.

The relationships between the duration of anti-MRSA drug treatment and increases in the MIC of daptomycin or vancomycin are shown in Figure 3. In the CRBSI group, increases in the MIC of daptomycin were seen in 6 cases in which anti-MRSA drug treatment was administered for ≤20 days and 3 cases in which anti-MRSA drug treatment was administered for 21-40 days, but in the non-CRBSI group an elevated MIC of daptomycin was only observed in 1 case, in which anti-MRSA drug treatment was administered for 21-40 days (Figure 3a). On the other hand, regarding the relationship between the

duration of anti-MRSA drug treatment and the MIC of vancomycin, in the CRBSI group elevated MIC were seen in 3 cases in which anti-MRSA drug treatment was administered for ≤20 days and 1 case in which anti-MRSA drug treatment was administered for 21-40 days, but in the non-CRBSI group an increased MIC was only seen in 1 case, in which anti-MRSA drug treatment was administered for 21-40 days (Figure 3b).

Discussion

In this study, we revealed that MRSA isolated from the blood of CRBSI patients had a higher MIC of daptomycin than that collected from the blood of non-CRBSI patients, especially among patients with a history of anti-MRSA drug treatment. Among the cases in which the MIC of daptomycin could be followed, the MIC of daptomycin increased in all of those involving CRBSI in which anti-MRSA drug treatment was administered for ≤20 days at the standard dose.

The factors associated with the appearance of reduced susceptibility to daptomycin have been reported to include high bacterial loads derived from refractory infections, inadequate doses of daptomycin, and the absence of necessary surgical procedures [11]. These factors may be associated with reduced susceptibility to daptomycin in endocarditis [7] and osteomyelitis [8]. CRBSI may also be affected in similar ways, and if MRSA in a catheter biofilm is exposed to an anti-MRSA drug, strains with reduced susceptibility to daptomycin may survive and disperse in the blood.

As for the mechanism responsible for reduced susceptibility to daptomycin, repulsive force associated with an increase in the positive charge of the cell membrane has been suggested [12]. Alternatively, the mechanism may involve a gene mutation or non-gene mutation-related physiological changes in bacteria. *mprF* mutations are the most common gene mutations associated with reduced susceptibility to daptomycin, and it is said that they also produce cross resistance to vancomycin [13]. In this study, since many of the CRBSI-related isolates did not show an increase in the MIC of vancomycin, such mutations were considered to be irrelevant.

As for physiological changes, it has been reported that the bacteria in biofilms become less sensitive to antibiotics by slowing their growth rate [14]. It was also reported that when bacteria were exposed to low concentrations of antibiotics, their cell walls thickened, and their susceptibility to antibiotics reduced, and when they were transferred to a drug-free medium the thickness of their cell walls normalized [15]. Furthermore, the cell walls of MRSA have been reported to thicken after the administration of daptomycin [16]. Therefore, it is considered that when MRSA biofilms form on medical devices the thickness of the cell walls of the bacteria increases, which makes it difficult for daptomycin to reach the cell membrane, resulting in an increased MIC.

Although the mechanisms responsible for reduced susceptibility to daptomycin could not be elucidated in this study, clinical measures need to be put in place to prevent the emergence of CRBSI. It is of course important to prevent the occurrence of CRBSI by employing maximal barrier precautions when inserting catheters and by using catheters made of materials that are not amenable to biofilm formation, such as polyurethane. If a CRBSI due to MRSA is suspected, it is necessary to remove the infected catheter

immediately. If the catheter cannot be removed immediately, high-dose daptomycin treatment should be considered as a way to reduce treatment tolerance and improve treatment efficacy, as it has been reported that high-dose daptomycin treatment is effective against endocarditis [17].

Conclusions

It is considered that when MRSA in catheter biofilms is exposed to anti-MRSA drugs, strains with reduced susceptibility to daptomycin are able to survive and disperse into the blood. Further study of whether high-dose daptomycin treatment is effective when catheters cannot be immediately removed is needed.

Abbreviations

CRBSI, catheter-related bloodstream infection; MIC, minimum inhibitory concentrations; MRSA, methicillin-resistant *Staphylococcus aureus*; VISA, vancomycin-intermediate *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococcus*

Declarations

Authors' contributions

SO, YM, KK designed the study. SO, TN, KM conducted the study and analyzed the data. SO, YM, KK wrote the paper. KK had primary responsibility for the final content. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the institutional review board of Teikyo University (committee number: 21-031, approval date: May 24, 2021) and was conducted in accordance with the institutional guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Characteristics of the patients

	Catheter-related bloodstream infection	Non-catheter-related bloodstream infection	P value
	n=46	n=69	
Mean age	77.7	72.2	0.06
Sex			
Female	24	24	0.08
Male	22	45	
Prior exposure to anti-MRSA drug	16	5	0.0003

Table 2. Multiple regression analysis of the minimum inhibitory concentrations

	Daptomycin			Vancomycin		
	Parameter Estimate	95%CI	P value	Parameter Estimate	95%CI	P value
Catheter-related bloodstream infection	0.3131	0.1047 to 0.5215	0.0036	-0.0076	-0.1678 to 0.1526	0.9252
Prior exposure to anti-MRSA drug	0.5207	0.2564 to 0.7849	0.0002	0.2250	0.0219 to 0.4281	0.0302

Figures

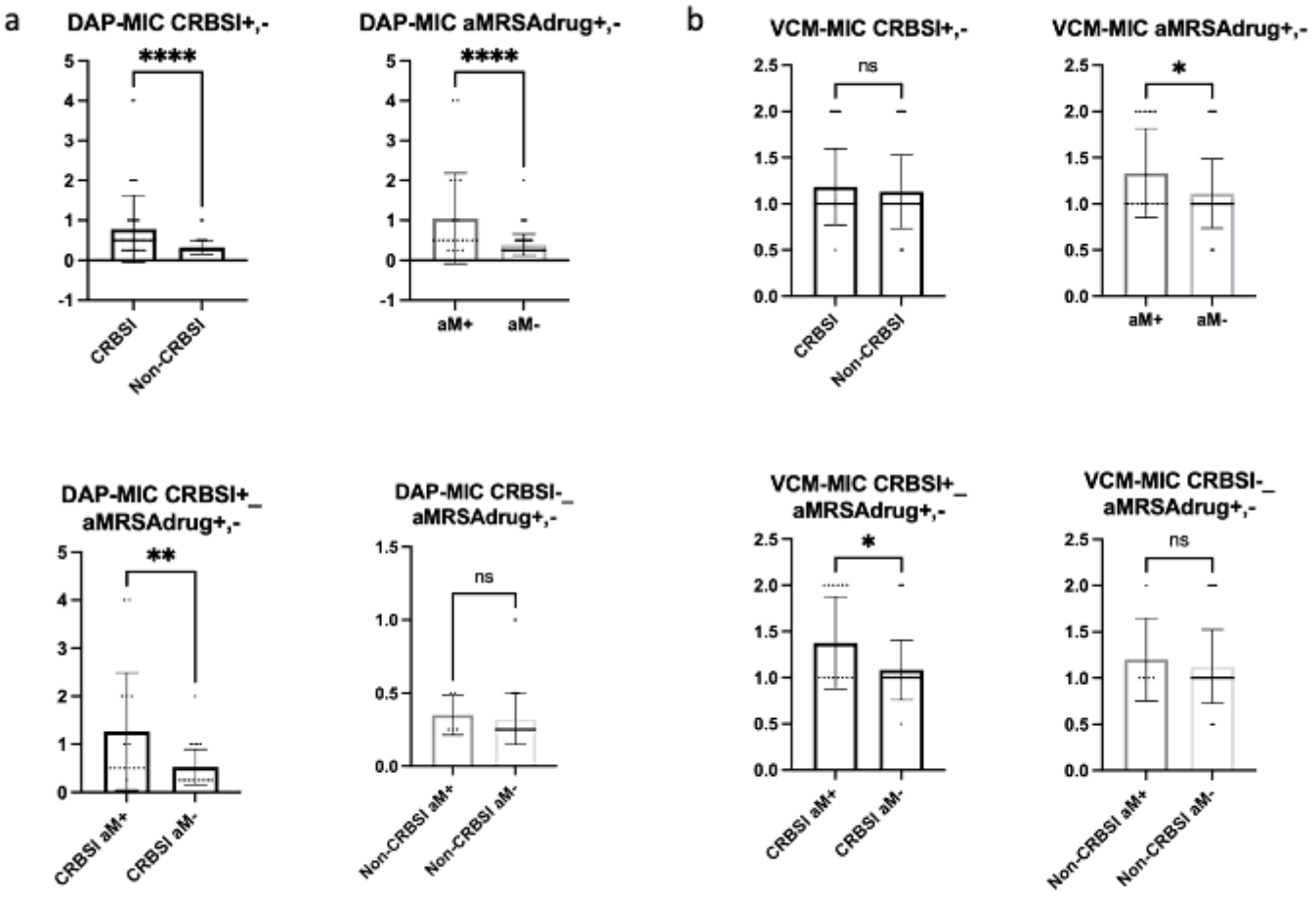

Figure 1

Figure 1

The minimum inhibitory concentrations of daptomycin (a) and vancomycin (b) for isolates collected from CRBSI or non-CRBSI patients with or without a history of anti-MRSA drug treatment. DAP, daptomycin; VCM, vancomycin; MIC, minimum inhibitory concentration; CRBSI, catheter-related bloodstream infection; aM, anti-MRSA drug treatment; *p<0.05, **p<0.01, ****p<0.0001

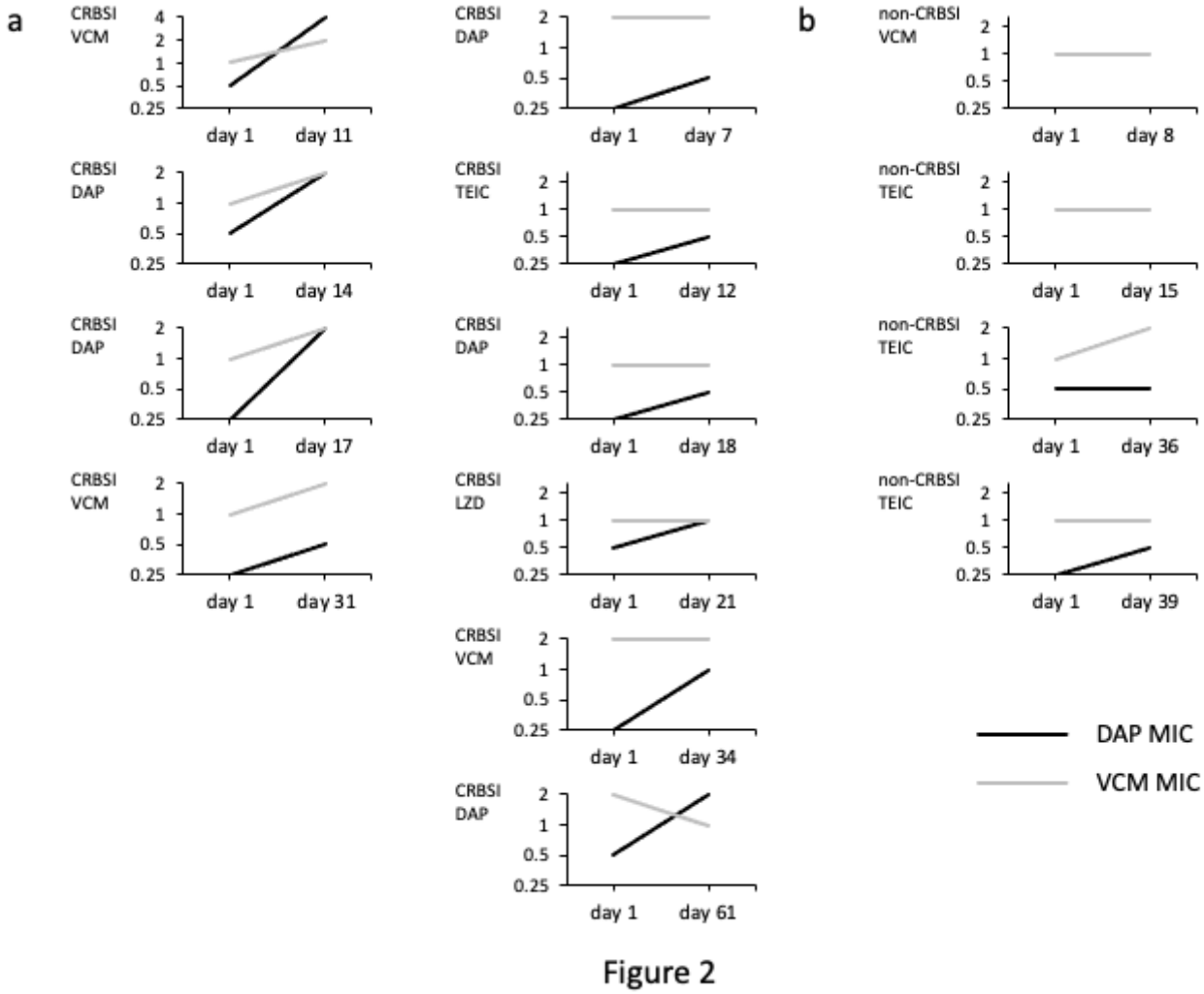

Figure 2

Figure 2

Fluctuations in the MIC of daptomycin due to anti-MRSA drug treatment. During the anti-MRSA drug treatment, MRSA was isolated again from the blood cultures of 10 CRBSI patients (a) and 4 non-CRBSI patients (b). CRBSI, catheter-related bloodstream infection; VCM, vancomycin; DAP, daptomycin; TEIC, teicoplanin; LZD, linezolid

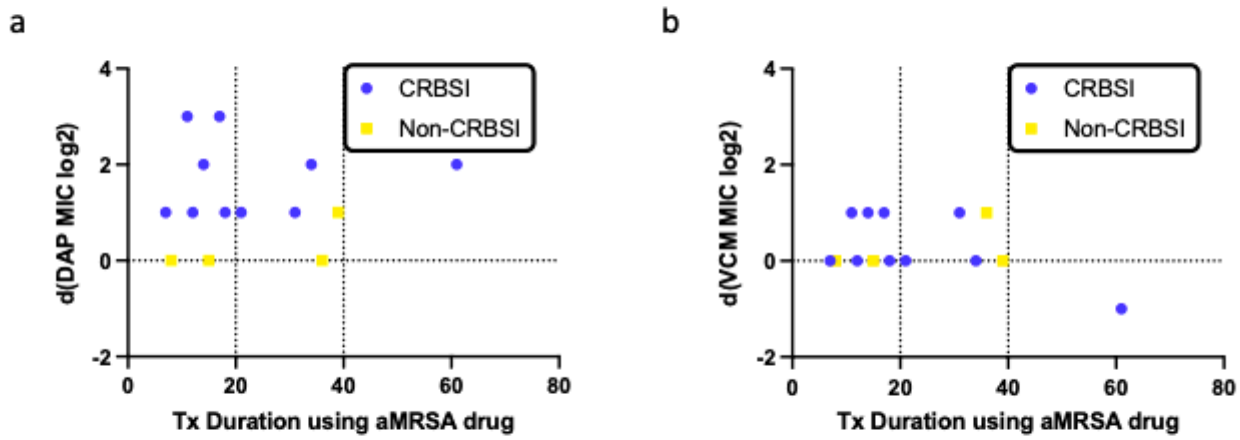

Figure 3

Figure 3

Relationships between the duration of anti-MRSA drug treatment and increases in the MIC of daptomycin (a) or vancomycin (b). Blue circles and yellow squares indicate CRBSI and non-CRBSI cases, respectively.