In Vivo Efficacy of Daptomycin Against Methicillin-resistant *Staphylococcus aureus* in a Mouse Model of Hematogenous Pulmonary Infection

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Abstract

Daptomycin is inactivated by pulmonary surfactant but its effectiveness in hematogenous pulmonary infection is poorly studied. The potential therapeutic application was evaluated in a methicillin-resistant *Staphylococcus aureus* (MRSA) hematogenous pulmonary infection mouse model. Compared with controls, daptomycin improved the survival ($p < 0.001$) and decreased the number of abscesses and bacteria in the lungs ($p < 0.01$). Daptomycin may be an effective therapeutic option for MRSA hematogenous pulmonary infection.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important bacterium that causes a variety of infections such as pneumonia, bacteremia, and skin and soft-tissue infections. In particular, bacteremia causes by MRSA is associated with a high mortality rate, even with appropriate antimicrobial treatments (1-3). Vancomycin has been a key drug for parenteral therapy for MRSA infection for many years. However, the emergence and spread of vancomycin-insensitive *S. aureus* has become of substantial concern (4). Thus, alternative drugs for the treatment of MRSA infections are required. The lipopeptide antibiotic, daptomycin, has anti-MRSA activity and possesses a novel mechanism of action that does not involve cell lysis (5). In a study of bloodstream infections due to MRSA that possessed elevated vancomycin MICs, daptomycin treatment was associated with better outcomes than vancomycin (6). For the treatment of MRSA-related lung infections including pneumonia, daptomycin is not recommended because it is inactivated by the lung surfactant (7). However, there are limited data on the effectiveness of daptomycin in MRSA hematogenous pulmonary infection. In this study, we compared the *in vivo* effectiveness of daptomycin and vancomycin in the treatment of mice with hematogenous pulmonary infection caused by MRSA.

A murine model of hematogenous pulmonary infection was generated by the inoculation of the MRSA NUMR101 strain, that was enclosed in small agar beads, into the tail vain of ddY mice (6-8 weeks old, male; SLC Inc, Shizuoka, Japan) as previously described (8). The Ethics Review Committee for Animal Experimentation approved all experimental protocols used in this study. The MIC of vancomycin and daptomycin against NUMR101 were 1 (9) and 0.25μg/mL, respectively. The MIC of daptomycin was determined by the broth microdilution method using Muller-Hinton II broth with 50mM Ca^{2+}. Mice were inoculated with the bacteria at 0.25-1 × 10^8 CFU/mouse. Treatment commenced 24 h after inoculation by intraperitoneal administration of the test agent. In the daptomycin-treated group, daptomycin (50mg/kg) was administered every 24 h to produce similar

pharmacokinetics to those in humans (10) and the saline was infected 12 h after administration of daptomycin. In the vancomycin-treated group, the same dose of vancomycin (50mg/kg) was administered every 12 h (11). For the controls, saline was injected every 12 h.

Survival of the mice was observed for 10 days (Fig. 1, each group; n=17). All control mice died by day 8; the survival rates on day 10 in the vancomycin- and daptomycin-treated groups were 52.9% ($p < 0.001$ vs. controls, Log-rank test) and 94% ($p < 0.001$ vs. controls and $p = 0.008$ vs. vancomycin-treated group, Log-rank test), respectively.

To examine the histological and bacterial findings in the early phase, animals were sacrificed on day 3 (at 12 h after administration of 5 doses for vancomycin and 3 doses for daptomycin) and the lungs were dissected under aseptic conditions. For histological examination, lung tissue was fixed in 10% buffered formalin and stained with hematoxylin-eosin. The microscopic findings revealed lung abscesses including a central bacterial colony (Fig. 2). Total abscesses in a single slice were counted and lung area was calculated using cross-section paper as previously described (9). The number of abscesses (mean ±SD, n=3) in a single slice of the control group was 0.297 ± 0.047 /mm^2, however, administration of vancomycin and daptomycin resulted in a significant decrease in the number of abscesses (0.107 ± 0.015 and 0.040 ± 0.010/mm^2, respectively; $p < 0.01$ vs. controls, Scheffe's test following the Kruskal-Wallis test) (Fig. 3). There was no significant difference between these two groups ($p = 0.08$) but this may be due to small number of samples.

For microbiological examination, the lungs were suspended in 1 mL of saline, homogenized and cultured quantitatively as previously described (9) (Fig. 4). The number of bacteria (mean ± SEM, n=6) in the lungs of the control group was 7.25 ± 0.26 $\log_{10}$CFU/mL. In contrast, the numbers in the vancomycin- and daptomycin-treated groups were 4.67 ± 0.17

and 4.36 ± 0.20 $\log_{10}$CFU/mL, respectively. Thus, administration of these agents significantly decreased the number of viable MRSA cells compared with controls ($p < 0.01$, Scheffe's test following the Kruskal-Wallis test), but there was no significance between the vancomycin- and daptomycin-treated groups. Similarly, statistical significance between these two groups was not observed on day 6 (late phase). These findings seemed not to be consistent with the result of survivals; however, our data can include the number of bacteria in the pulmonary vessels. In this model, perfusion with physiological saline through the pulmonary vessels was not performed because of concerns that it would wash out the bacteria in the abscesses.

Daptomycin is known to have a good distribution, and it penetrates well into the inflammatory sites (12); it has been confirmed to have potent antibacterial activity and long postantibiotic effects in murine thigh infection models (13-14). These advantages in pharmacodynamics and pharmacokinetics may explain the outcomes in this study. Our study suggested that daptomycin may be an effective therapeutic option for MRSA hematogenous pulmonary infection. It has been reported that daptomycin was used successfully in septic pulmonary emboli (15, 16), despite of inactivation of daptomycin by pulmonary surfactant. Unlike pneumonia, in which there is bacterial growth in the alveolar space, hematogenous infections may be little affected by the surfactant. Bacteria, probably originated from the nearby abscess, were also observed in the air space in this model, but we considered the bacteria to mainly have been in the abscess formations. However, our results do not imply that daptomycin is superior to vancomycin in the treatment of septic pulmonary emboli due to MRSA, because we did not determine the concentrations of each antibiotic in this model. In addition, the dose of vancomycin used in this study (50mg/kg, twice daily) can be lower than the estimated clinical dose (110mg/kg twice daily in mice) (17).

In conclusion, daptomycin may be effective in MRSA hematogenous pulmonary infection. However, further studies will be required to elucidate the potential benefit in

105 patients with septic pulmonary emboli.

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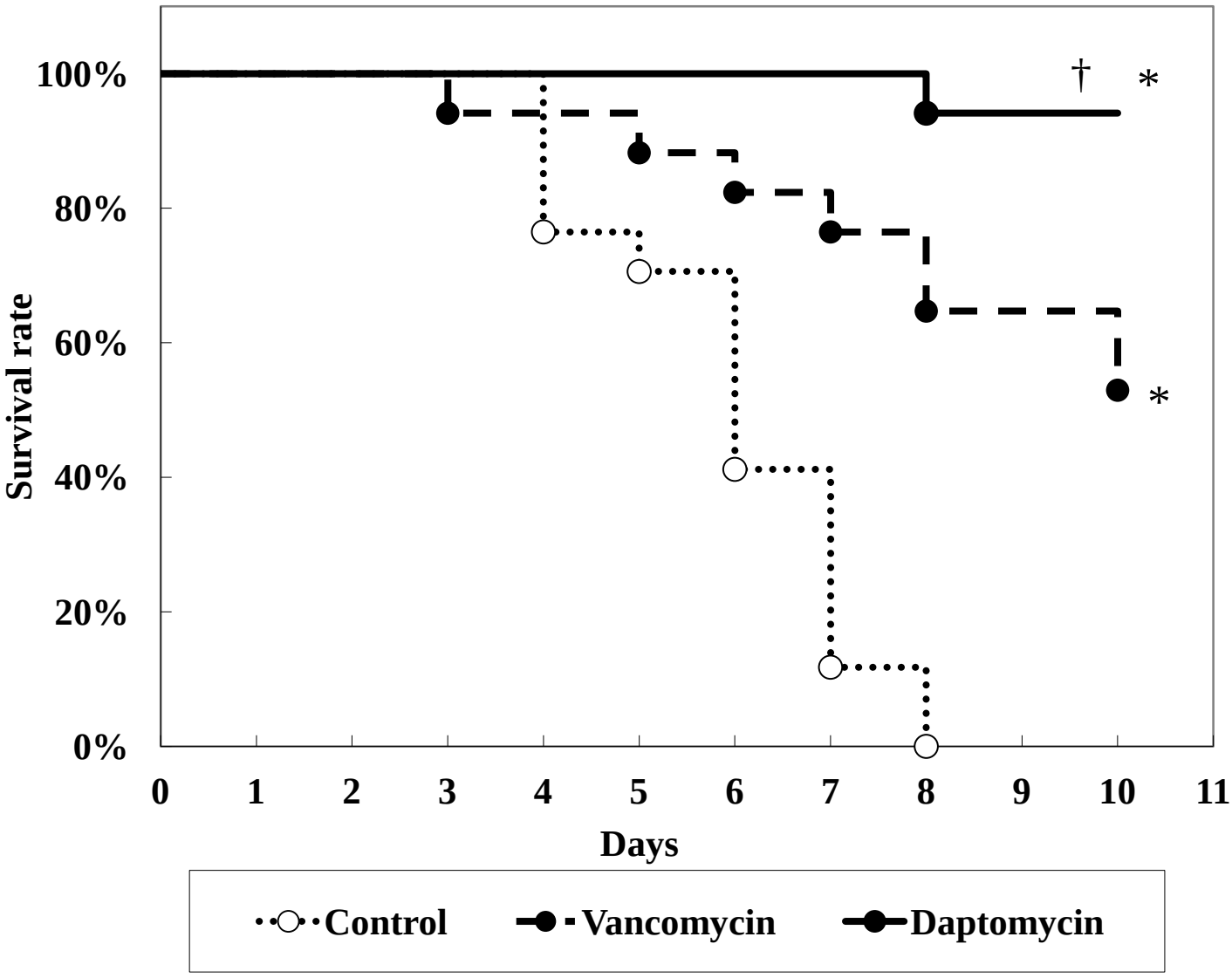

FIG 1. The survival in each treatment group during the observation period. Mice were treated for day 10. All control mice died by day 8. The survival rates in the vancomycin- and daptomycin-treated groups were significantly higher than that in the control groups. (*$p < 0.001$ vs. control, †$p = 0.008$ vs. vancomycin-treated group, $n = 17$ for all groups)

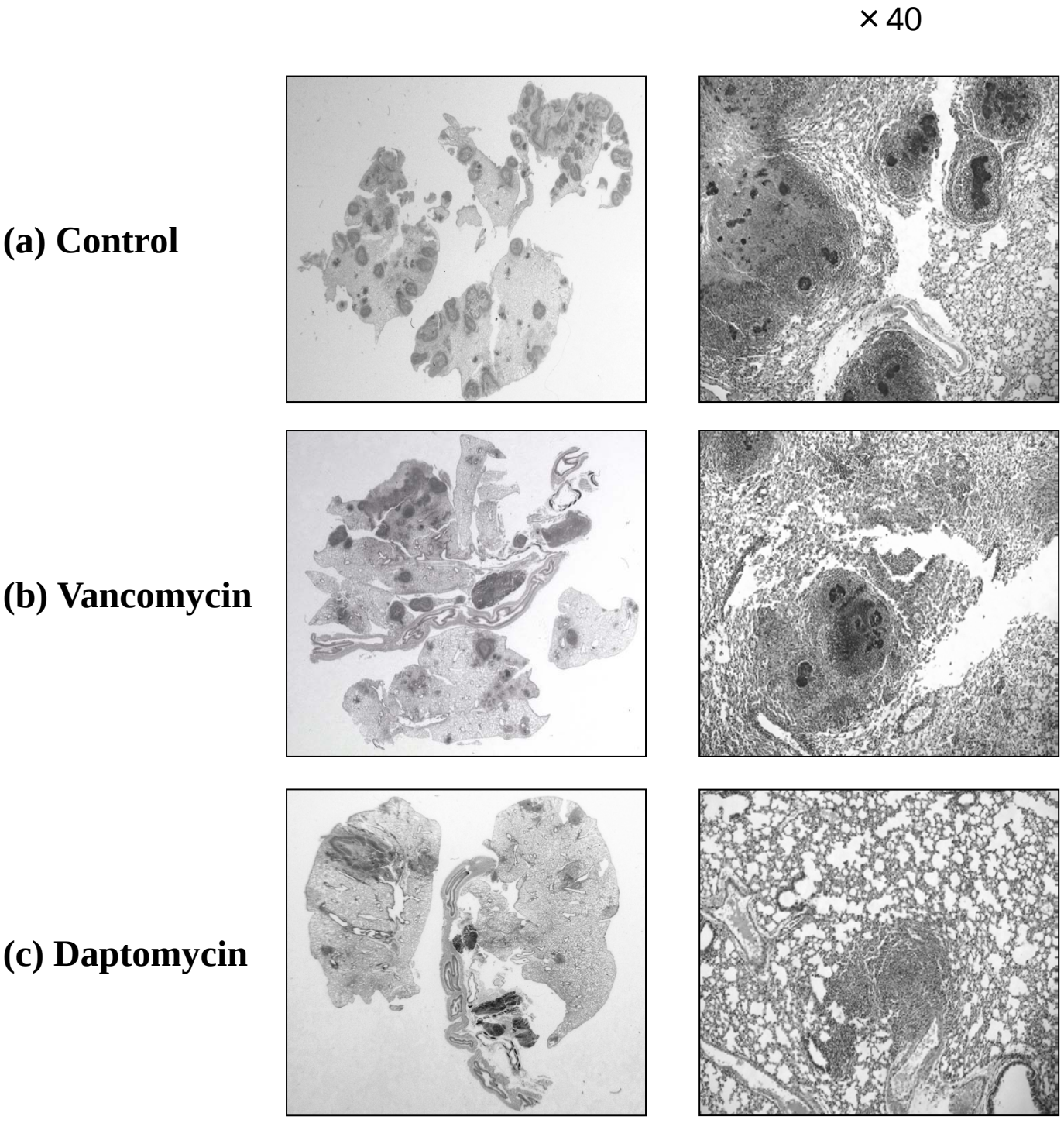

FIG 2. Histopathological examination of the lung specimens
Representative data from each group on day 3 are shown (n = 3). Many abscess lesions with central bacterial colony zones surrounded by inflammatory cells were observed in the controls (a). In the vancomycin (b) and daptomycin (c) groups, fewer abscesses were observed.

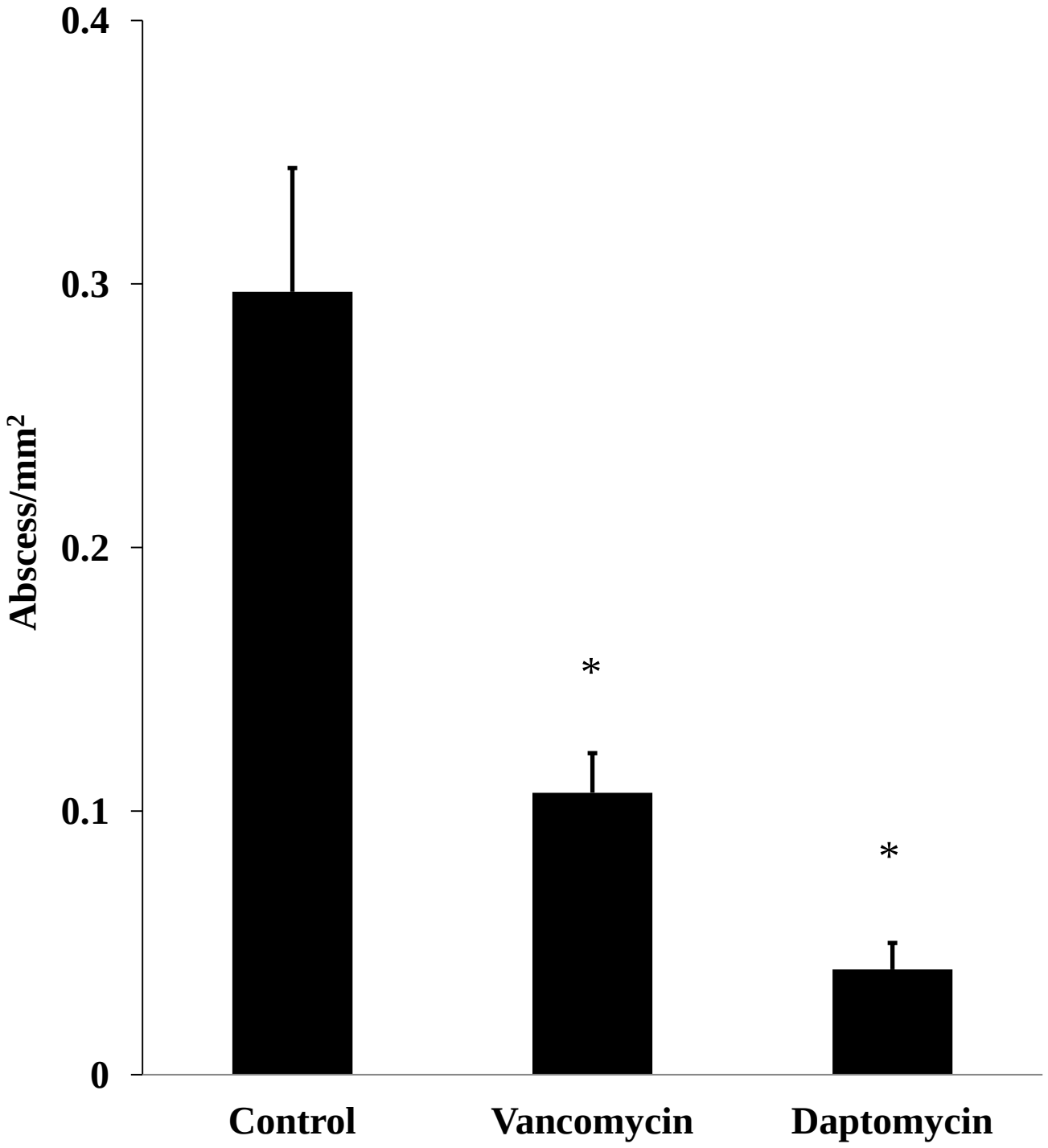

FIG 3. Histopathological examination of the lung specimens on day 3
The number of abscesses per mm^2 was counted. The number (mean ± SD) of lung abscesses per mm^2 in the control and vancomycin- and daptomycin-treated groups was 0.297 ± 0.047, 0.107 ± 0.015, and 0.040 ± 0.010/mm^2, respectively (n = 3 for all groups) (*$p < 0.01$ vs. control).

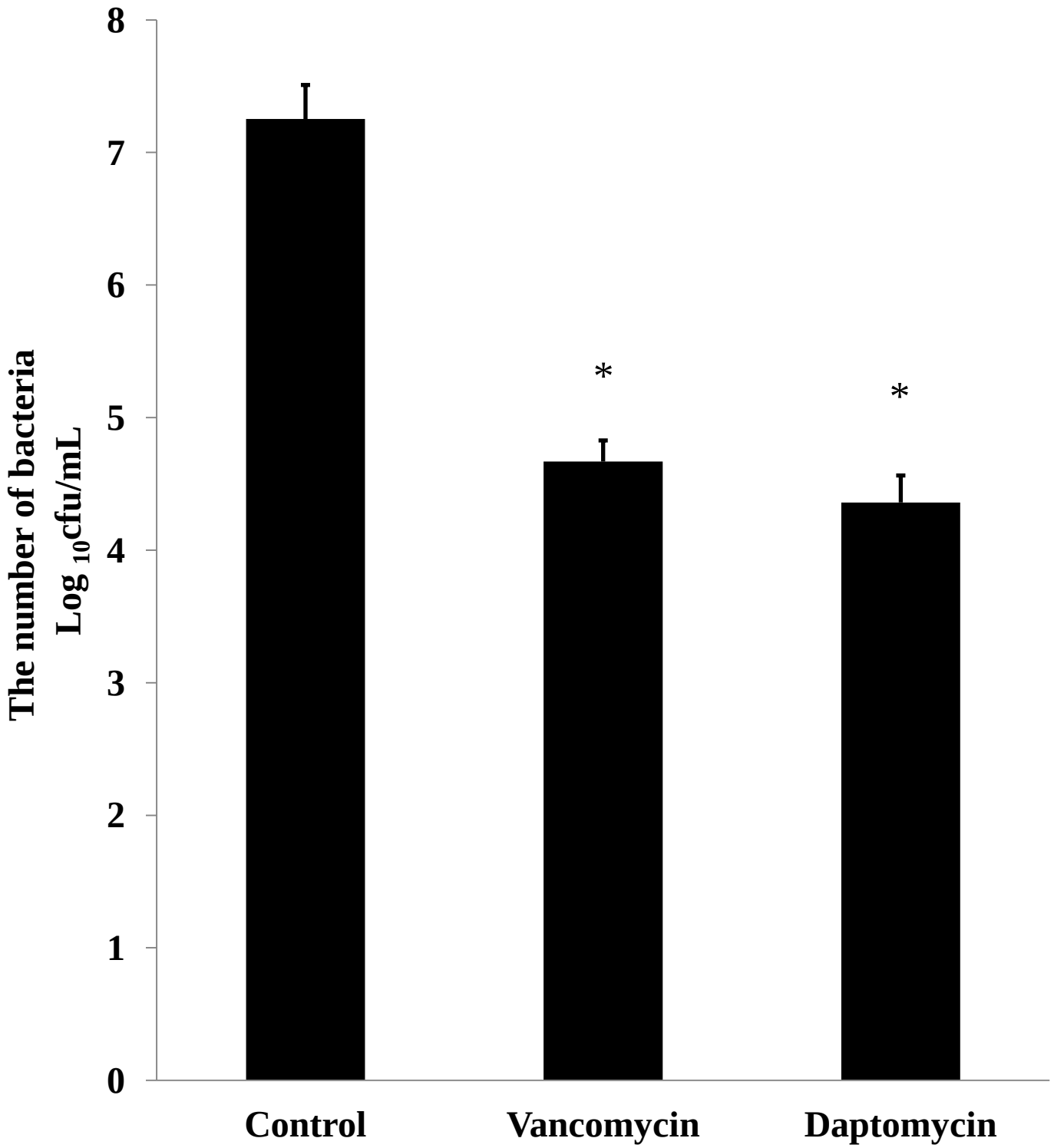

FIG 4. The number of viable bacteria in the lungs on day 3
The numbers (mean ± SEM) of bacteria in the control and vancomycin- and daptomycin-treated groups were 7.25 ± 0.26, 4.67 ± 0.17, and 4.36 ± 0.20 $\log_{10}$ cfu/mL (n = 6 for all groups), respectively (*$p < 0.01$ vs. control).