

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

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23 **Abstract**

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

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

45 A murine model of hematogenous pulmonary infection was generated by the
46 inoculation of the MRSA NUMR101 strain, that was enclosed in small agar beads, into the
47 tail vein of ddY mice (6-8 weeks old, male; SLC Inc, Shizuoka, Japan) as previously
48 described (8). The Ethics Review Committee for Animal Experimentation approved all
49 experimental protocols used in this study. The MIC of vancomycin and daptomycin against
50 NUMR101 were 1 (9) and 0.25 µg/mL, respectively. The MIC of daptomycin was determined
51 by the broth microdilution method using Muller-Hinton II broth with 50mM Ca²⁺. Mice were
52 inoculated with the bacteria at 0.25-1 × 10⁸ CFU/mouse. Treatment commenced 24 h after
53 inoculation by intraperitoneal administration of the test agent. In the daptomycin-treated
54 group, daptomycin (50mg/kg) was administered every 24 h to produce similar

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

59 Survival of the mice was observed for 10 days (Fig. 1, each group; n=17). All
60 control mice died by day 8; the survival rates on day 10 in the vancomycin- and
61 daptomycin-treated groups were 52.9% ($p < 0.001$ vs. controls, Log-rank test) and 94% ($p <$
62 0.001 vs. controls and $p = 0.008$ vs. vancomycin-treated group, Log-rank test), respectively.

63 To examine the histological and bacterial findings in the early phase, animals were
64 sacrificed on day 3 (at 12 h after administration of 5 doses for vancomycin and 3 doses for
65 daptomycin) and the lungs were dissected under aseptic conditions. For histological
66 examination, lung tissue was fixed in 10% buffered formalin and stained with
67 hematoxylin-eosin. The microscopic findings revealed lung abscesses including a central
68 bacterial colony (Fig. 2). Total abscesses in a single slice were counted and lung area was
69 calculated using cross-section paper as previously described (9). The number of abscesses
70 (mean \pm SD, n=3) in a single slice of the control group was 0.297 ± 0.047 /mm², however,
71 administration of vancomycin and daptomycin resulted in a significant decrease in the
72 number of abscesses (0.107 ± 0.015 and 0.040 ± 0.010 /mm², respectively; $p < 0.01$ vs.
73 controls, Scheffe's test following the Kruskal-Wallis test) (Fig. 3). There was no significant
74 difference between these two groups ($p = 0.08$) but this may be due to small number of
75 samples.

76 For microbiological examination, the lungs were suspended in 1 mL of saline,
77 homogenized and cultured quantitatively as previously described (9) (Fig. 4). The number of
78 bacteria (mean \pm SEM, n=6) in the lungs of the control group was 7.25 ± 0.26 log₁₀CFU/mL.
79 In contrast, the numbers in the vancomycin- and daptomycin-treated groups were 4.67 ± 0.17

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

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

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

105 patients with septic pulmonary emboli.

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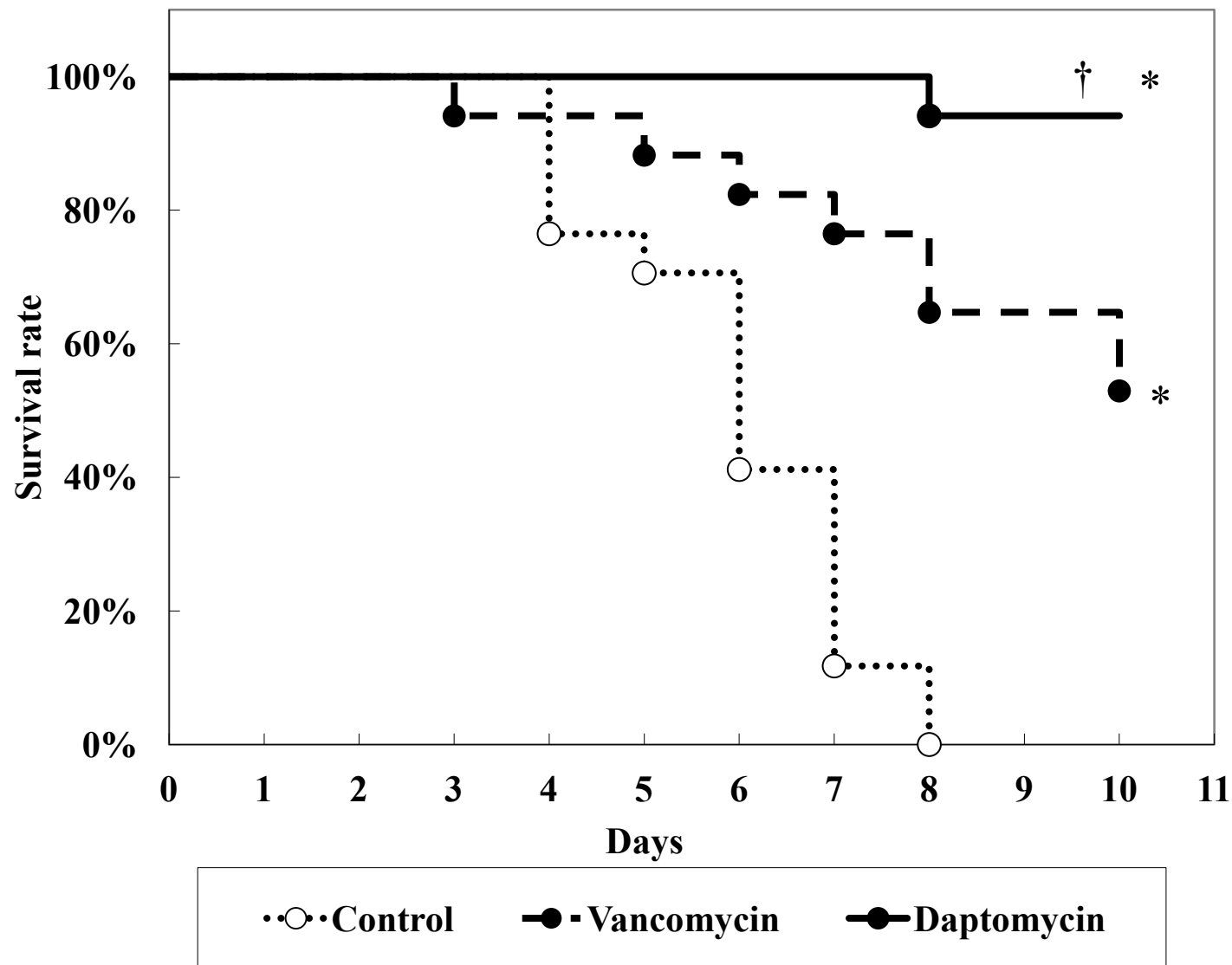
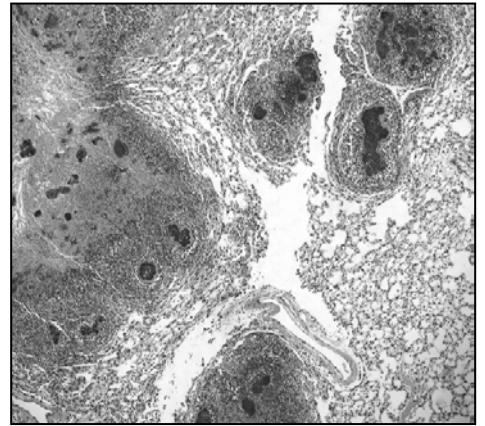
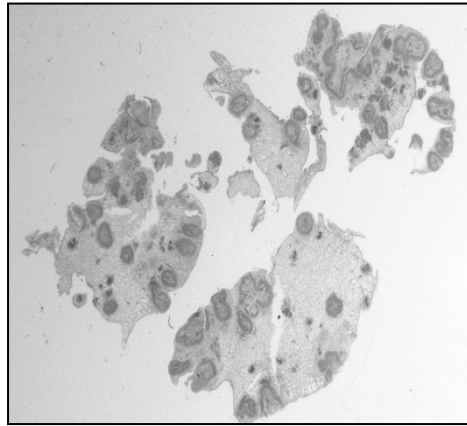


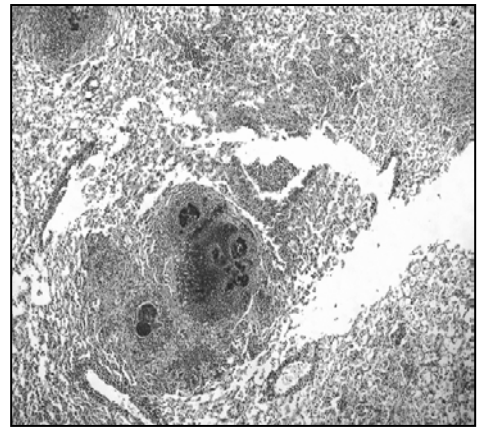
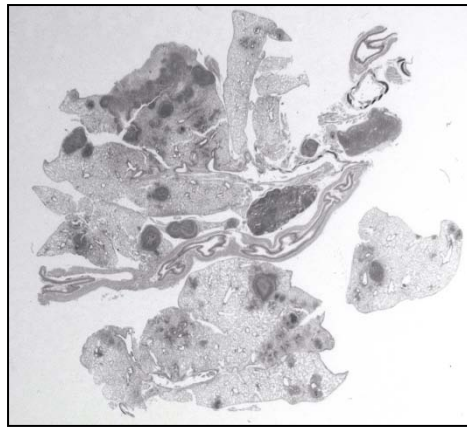
FIG 1. The survival in each treatment group during the observation period. Mice were treated for day 10. All control mice died by day 8. The survival rates in the vancomycin- and daptomycin-treated groups were significantly higher than that in the control groups. (* $p < 0.001$ vs. control, † $p = 0.008$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

× 40

(a) Control



(b) Vancomycin



(c) Daptomycin

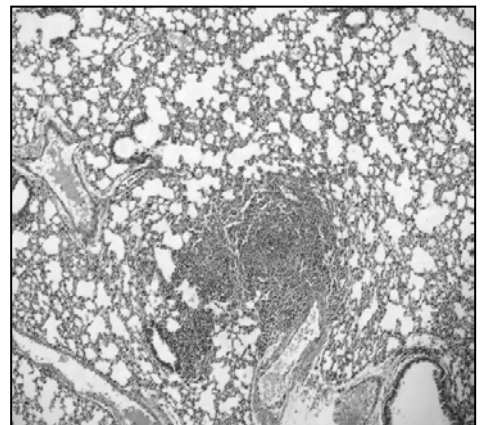
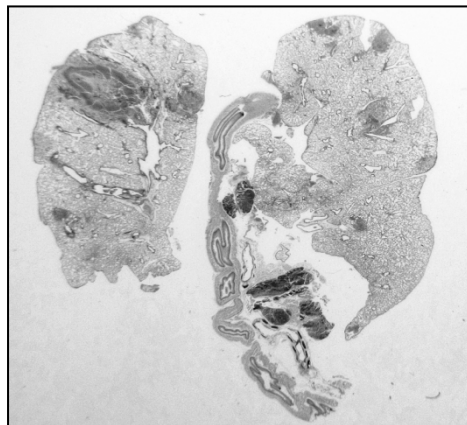


FIG 2. Histopathological examination of the lung specimens
Representative data from each group on day 3 are shown (n = 3). Many abscess lesions with central bacterial colony zones surrounded by inflammatory cells were observed in the controls (a). In the vancomycin (b) and daptomycin (c) groups, fewer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

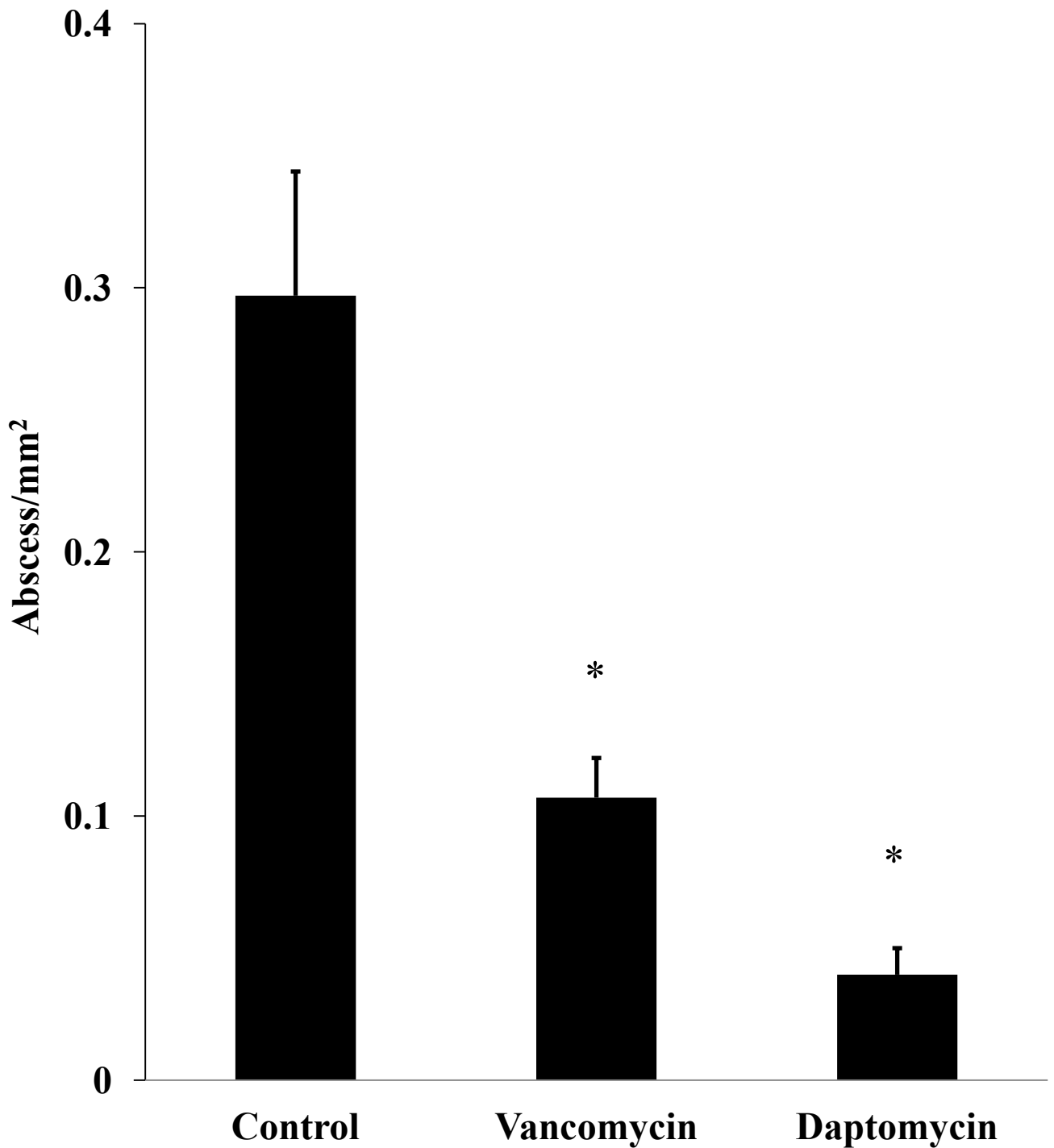


FIG 3. Histopathological examination of the lung specimens on day 3. The number of abscesses per mm² was counted. The number (mean \pm SD) of lung abscesses per mm² in the control and vancomycin- and daptomycin-treated groups was 0.297 ± 0.047 , 0.107 ± 0.015 , and 0.040 ± 0.010 /mm², respectively (n = 3 for all groups) (*p < 0.01 vs. control).

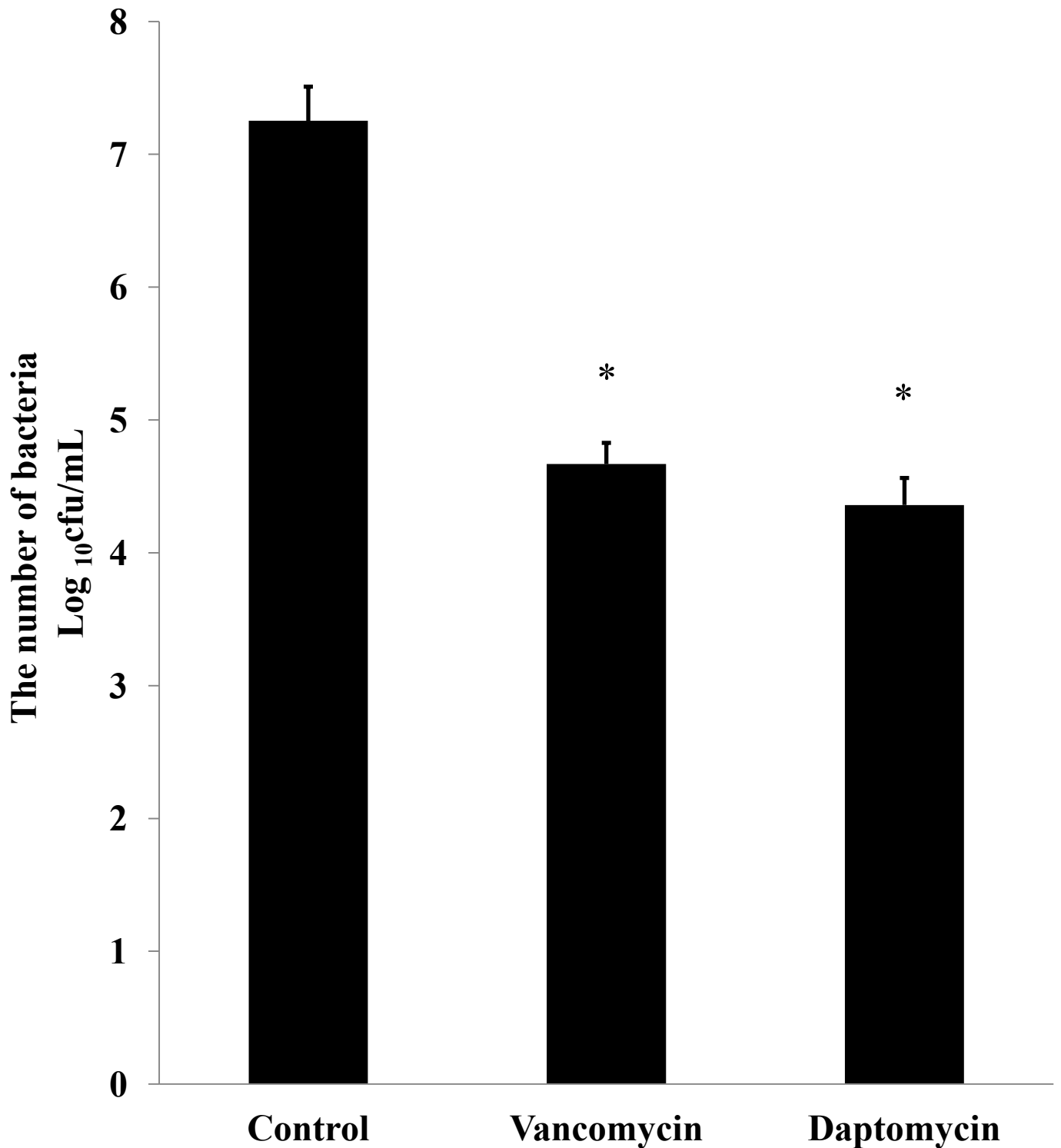


FIG 4. The number of viable bacteria in the lungs on day 3
The numbers (mean \pm SEM) of bacteria in the control and vancomycin- and daptomycin-treated groups were 7.25 ± 0.26 , 4.67 ± 0.17 , and 4.36 ± 0.20 log₁₀ cfu/mL (n = 6 for all groups), respectively (*p < 0.01 vs. control).