

same concentration. They also described morphologically that vacuoles were smaller when infected cells were treated with lovastatin compared with untreated cells. However, they did not use a quantitative method to prove this effect. Our method, in which we coupled imaging and software analysis, allows quantitative determination of differences in vacuole size and number.

Interestingly, pravastatin did not have any apparent effect in reducing *C. burnetii* infection. Pravastatin is hydrophilic and seems to be less effective than other statins in different models.⁴ This could be due to different pharmacokinetic properties compared with other statins⁴ and may explain the lack of activity of pravastatin in our *C. burnetii* model of infection. In our study, lovastatin was effective at a concentration achievable in the sera of patients treated with this drug. This drug does not seem to interfere with bacterial entry in cells, as internalization by cells was not different from controls, as also reported for *Salmonella* Typhimurium.⁵ We hypothesize that lovastatin indirectly reduces *C. burnetii* growth by modifying cholesterol-rich vacuoles. However, a minimal direct effect on bacteria could not eventually be eliminated, as the genome of *C. burnetii* contains some genes involved in the steroid biosynthesis pathway, especially that encoding the HMG CoA reductase, available at the KEGG web site (http://www.genome.jp/dbget-bin/get_pathway?org_name=cbu&mapno=00100). *In vitro* studies with statins in association with doxycycline could be performed in the future to look for a possible synergistic inhibitory effect on *C. burnetii*. Because the inhibitory effect of statins was seen only with pre-incubated cells, we believe that lovastatin may be effective in prophylaxis. These findings need to be confirmed using an animal model⁶ and/or epidemiological case–control study, especially in patients with chronic Q fever.

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Supplementary data

A colour version of Figure 1 is available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>).

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Effect of different Mueller–Hinton agars on tigecycline disc diffusion susceptibility for *Acinetobacter* spp.

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Sir,

We reported the *in vitro* activity of tigecycline against 148 strains of *Acinetobacter* spp. from the infectious diseases research laboratory at Siriraj Hospital, Bangkok, Thailand, which showed that only 3.4% of the *Acinetobacter* spp. strains were considered resistant to tigecycline due to their inhibition zone diameters (<13 mm) and MICs determined by broth microdilution (>2 mg/L).¹ The service microbiology laboratory of our hospital has reported tigecycline disc diffusion susceptibility results for *Acinetobacter* spp. based on the interpretative breakpoints proposed by Jones *et al.*,² which indicated that most clinical isolates of *Acinetobacter* spp. were not susceptible to tigecycline. There were several observations on the effect of the susceptibility test media on the MICs of tigecycline. Hope *et al.*³ found that the MIC of tigecycline was raised in aged media. Fernandez-Mazarasa *et al.*⁴ reported that high concentrations of manganese in Mueller–Hinton agar (MHA) increased the MIC₉₀ of tigecycline for *A. baumannii* determined by Etest from 1 to 4 mg/L. The service microbiology laboratory at Siriraj Hospital used MHA (Oxoid), whereas the infectious diseases research laboratory used MHA [Becton–Dickinson (BD)] for disc diffusion susceptibility for *Acinetobacter* spp. Therefore, a

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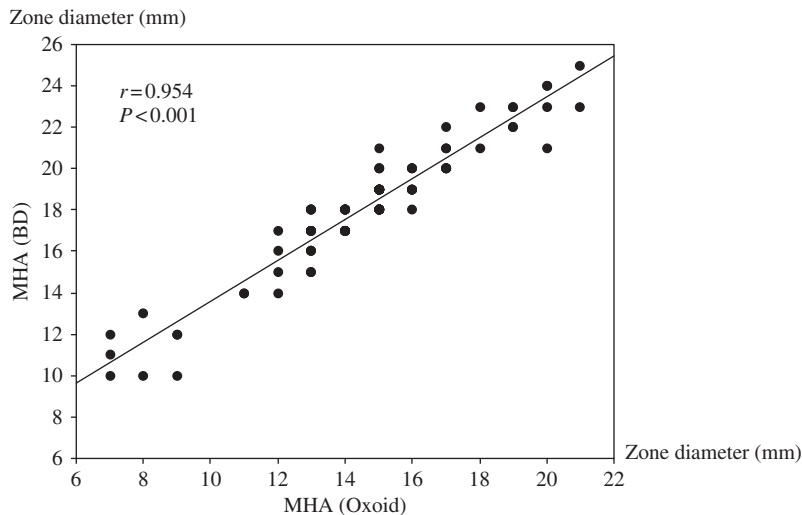


Figure 1. Comparison of tigecycline inhibition zone diameters on MHA (BD) and MHA (Oxoid) for 102 isolates of *Acinetobacter* spp.

discrepancy in tigecycline susceptibility for *Acinetobacter* spp. might be due to a difference in the type of MHA.

We performed tigecycline disc diffusion susceptibility for 102 strains of *Acinetobacter* spp. isolated from different patients. These isolates were resistant to all β -lactams, aminoglycosides and fluoroquinolones. *In vitro* susceptibility of *Acinetobacter* spp. to tigecycline was determined by the Kirby–Bauer disc diffusion method using a paper disc containing 15 μ g of tigecycline per disc (BD, USA). The MHAs were purchased from BD (Thailand) and Oxoid Company (Thailand), and they were freshly prepared. The methodology for susceptibility testing was performed by direct colony suspension according to the guidelines suggested by the CLSI.⁵ Quality control was performed by testing the susceptibility of *Escherichia coli* ATCC 25922. The susceptibility tests using both types of MHAs were performed concurrently under identical conditions. The comparison of tigecycline inhibition zone diameters on MHA (BD) and MHA (Oxoid) for 102 isolates of *Acinetobacter* spp. is shown in Figure 1. The inhibition zone diameters observed on MHA (Oxoid) were consistently smaller than those on MHA (BD), with a mean difference of 3.5 mm and a range from 1 to 6 mm. The inhibition zone diameters on MHA (Oxoid) and MHA (BD) were significantly correlated ($r = 0.95$, $P < 0.001$). The distribution of tigecycline disc diffusion susceptibility for *Acinetobacter* spp. performed on MHA (BD) and MHA (Oxoid) is shown in Table 1. The susceptibility of *Acinetobacter* spp. to tigecycline using MHA (BD) was 86.2%, whereas that using MHA (Oxoid) was only 28.5%. We also measured the content of manganese in both types of MHAs by

Table 1. Distribution of tigecycline disc diffusion susceptibility for *Acinetobacter* spp. performed on MHA (BD) and MHA (Oxoid)

Inhibition zone diameter (mm)	MHA (Oxoid)	MHA (BD)
≤ 12 (resistant)	14 (13.7%)	7 (6.9%)
13–15	59 (57.8%)	7 (6.9%)
≥ 16 (susceptible)	29 (28.5%)	88 (86.2%)

atomic absorption and found that the content of manganese in MHA (Oxoid) was three times more than that in MHA (BD). Therefore, the discrepancy in inhibition zone diameters between MHA (Oxoid) and MHA (BD) might be due to a difference in the manganese content in MHAs. However, there could be other associated factors for such a discrepancy, and more studies are required. The aforementioned observations warrant a clinical study to determine the efficacy of tigecycline for therapy of *Acinetobacter* spp. infections in order to consider which type of MHA is more appropriate for tigecycline disc diffusion susceptibility for *Acinetobacter* spp. Meanwhile, the results of tigecycline disc diffusion susceptibility for *Acinetobacter* spp. in MHA (Oxoid) should be cautiously reported and interpreted.

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