Impact of test methodology, media type and ion content on the susceptibility of Acinetobacter spp. to tigecycline

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Sir, Acinetobacter spp. are important opportunistic pathogens that mainly cause healthcare-associated infections. Although in recent years many studies have found that tigecycline exhibits antimicrobial activity against Acinetobacter,1,2 tigecycline-resistant Acinetobacter isolates have also been identified.3 Discrepancies in MICs have been reported when different methods or commercial Mueller–Hinton (MH) media have been used for in vitro susceptibility tests.4–7 Many studies have shown different tigecycline susceptibilities for Acinetobacter spp. between broth microdilution and the Etest or disc diffusion.6,7 We compared the susceptibilities of Acinetobacter spp. to tigecycline determined using agar dilution versus the broth microdilution method and then attempted to investigate the impact of the ion content of different media on tigecycline MICs for clinical Acinetobacter spp.

A total of 82 non-duplicate isolates of Acinetobacter spp. were collected, consisting of 67 Acinetobacter baumannii, 13 Acinetobacter pittii and 2 Acinetobacter nosocomialis. They were taken from patients from 13 hospitals in Sichuan, West China. Species identifications were established by partial sequencing of the recA gene.8 The MH agars (MHA) were purchased from Hopebio (Qingdao, China) and Oxoid (Hampshire, UK). MH II broth (MHB II; Becton, Dickinson (BD), USA) was used for broth microdilution. The susceptibility of each isolate was determined using both the agar dilution and broth microdilution methods, following CLSI guidelines.9 The FDA-approved tigecycline breakpoints for the Enterobacteriaceae (susceptible ≤2 mg/L; intermediate = 4 mg/L; resistant ≥ 8 mg/L) were used as provisional MIC breakpoints for the Acinetobacter isolates in this study. Each of the susceptibility tests for each isolate was performed in triplicate.

The resistance rate to tigecycline was high (47.56%) by the agar dilution method using MHA from Oxoid. However, no tigecycline-resistant isolate was found using MHA from Hopebio. The resistance rate of Acinetobacter spp. to tigecycline was found to be 19.51% by the broth microdilution method, similar to that in previous studies.5,7 A previous study had found that 86.2% of Acinetobacter isolates were susceptible to tigecycline using MHA (BD) by disc diffusion, but that only 28.5% were susceptible when using MHA (Oxoid).3 Tigecycline susceptibility results for Acinetobacter spp. should therefore be interpreted with caution.

The correlation between the agar dilution and broth microdilution methods was tested using Spearman’s rank correlation (SPSS). The tigecycline MICs determined using the agar dilution method (Oxoid/Hopebio) were significantly correlated with those obtained by broth microdilution (BD) (P < 0.001). However, the MICs determined using agar dilution (Oxoid) were higher (mean 1.56-fold dilution) than those found by broth microdilution, and the MICs determined using agar dilution (Hopebio) were consistently lower (mean 1.04-fold dilution) than those determined using broth microdilution. Thamlikitkul and Tiengrim2 also found that the inhibition zone diameters obtained using MHA (Oxoid) were consistently smaller than those obtained with MHA (BD). Different MICs were observed using different media even when using the same testing method, so the discrepancy might be related to the medium used. Discrepancies in the content of different media may have affected bacterial growth or the role of antimicrobial agents.

The concentrations of Ca, Mg, Mn and Zn ions in the three media were determined using atomic absorption spectroscopy (Varian Spectr AA-200). Spectroscopic measurements were carried out at the Analytical and Testing Center in Sichuan University. The content of manganese in Hopebio MHA
(0.0504 ppm) was higher than in Oxoid MHA (0.0196 ppm) or BD MHB II (0.0055 ppm), but the tigecycline MIC determined using the agar dilution method (Hopebio) was the lowest among the three media. The results were contrary to those of two previous studies, both of which found that the MICs of tigecycline were increased in media with a high manganese content. However, high manganese concentrations were found in MHA from Oxoid in one study and low manganese concentrations were found in MHA from Oxoid in another study.\footnote{Thamlikitkul V, Tiengrim S. Effect of different Mueller–Hinton agars on tigecycline disc diffusion susceptibility for Acinetobacter spp. \textit{J Antimicrob Chemother} 2008; \textbf{62}: 847–8.} \cite{Thamlikitkul2008} Therefore, it remains unclear whether manganese really interferes with the \textit{in vitro} activity of tigecycline or not.

To investigate the impact of the ion content of media on the MICs of tigecycline for \textit{Acinetobacter} spp., MnCl$_2$, ZnCl$_2$ or MgCl$_2$ (analytical reagent) was used to supplement MHB II to achieve Mn, Zn and Mg concentrations of 1024 mg/L. Tigecycline MICs were determined and were found to be unchanged. Hence, the discrepancy in MICs was unlikely to be due to the Mn, Zn or Mg content. Additionally, the amount of beef extract powder is different in the three media; it is still unclear whether this discrepancy is related to the different tigecycline susceptibilities. Further studies are warranted.

In conclusion, we found that MIC values varied significantly when different methods or MH media were used. The results of tigecycline susceptibility for \textit{Acinetobacter} spp. should be interpreted with caution. The content of Mn, Zn and Mg in different media may be irrelevant to the discrepancy in tigecycline MICs.

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