Telavancin and daptomycin activity against meticillin-resistant Staphylococcus aureus strains after vancomycin-resistance selection in vitro

Meticillin-resistant Staphylococcus aureus (MRSA) is a major threat worldwide. Vancomycin is still the drug of choice (Liu et al., 2011), but clinical failure in patients with serious Gram-positive infections has been increasingly reported with infections caused by MRSA strains with higher vancomycin MICs (Jones, 2006; Neoh et al., 2007; Petrosillo et al., 2010; Sakoulas et al., 2004). Daptomycin is a lipopeptide with a good activity against MRSA, vancomycin-intermediate Staphylococcus aureus (VISA) and vancomycin-resistant Staphylococcus aureus (VRSA) (Jung et al., 2004; Moubareck et al., 2009). The emergence of S. aureus strains with a daptomycin MIC at the upper range of susceptibility has been reported (van Hal et al., 2011). An association between reduced susceptibility to daptomycin and to vancomycin in S. aureus has been found (van Hal et al., 2011). In these cases, no definitive daptomycin resistance mechanism has been identified (Hobbs et al., 2008; van Hal et al., 2011). It has been shown that a thickened cell wall is a common characteristic for VISA strains: the thickened cell wall serves as a physical barrier against the penetration of vancomycin molecules (Cui et al., 2005; Gander et al., 2005; Peleg et al., 2012). Daptomycin and vancomycin molecule sizes are comparable, and it has been suggested that a thickened cell wall acts as a common obstacle to both drugs’ penetration. This would be a possible explanation of the correlation between vancomycin and daptomycin resistance.

Telavancin is active against MRSA, VISA and VRSA (Cui et al., 2003; Draglić et al., 2008; Kanafani, 2006; Pace et al., 2003). We evaluated the in vitro activity of telavancin and daptomycin against MRSA strains with a vancomycin MIC \( \leq 0.5 \mu g \text{ ml}^{-1} \), and again after the induction of the same strains to a vancomycin MIC of 2 \( \mu g \text{ ml}^{-1} \).

A total of 19 MRSA strains with a vancomycin MIC \( \leq 0.5 \mu g \text{ ml}^{-1} \), isolated from patients with bloodstream (nine patients), respiratory tract (four patients), and skin and soft skin infections (six patients), were considered. All the isolated strains showed a vancomycin MIC \( \leq 0.5 \mu g \text{ ml}^{-1} \) when tested with the VITEK 2 system (bioMérieux). All the strains were retested utilizing 96 Sensititre plates (Dri Panel CMP2STA; Theravance/ Astellas) for susceptibility to oxacillin, vancomycin, daptomycin and telavancin. The inoculum was adjusted to \( \approx 5 \times 10^5 \) c.f.u. \text{ ml}^{-1} \) in a 100 \( \mu l \) final volume of freshly prepared Mueller–Hinton (MH) broth (Oxoid), and microtitre plates were read after incubation for 24 and 48 h at 37 °C. S. aureus ATCC 29213 was used as an internal quality control strain. MIC results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) breakpoint criteria. Multistep resistance selection was performed using the broth macrodilution method. Vancomycin was obtained as a laboratory-grade powder from Sigma. Exposure was performed with fresh MH broth prepared 24 h before inoculation. Serial passages were performed daily in MH broth for each strain in sub-inhibitory concentrations of vancomycin until strains were generated with a vancomycin MIC of 2 \( \mu g \text{ ml}^{-1} \). Each passage was performed three consecutive times for each vancomycin concentration. All adapted strains were subcultured for 10 passages on MH agar supplemented with vancomycin at a concentration of 2 \( \mu g \text{ ml}^{-1} \), followed by 10 additional passages on MH agar. At this time, all the MRSA strains were tested again for susceptibility to oxacillin, telavancin and daptomycin using Sensititre plates, and read after 24 and 48 h of incubation at 37 °C. Susceptibilities of the MRSA isolates were assessed according to the CLSI breakpoints for oxacillin, vancomycin and daptomycin, and according to Food and Drug Administration (FDA) breakpoints for telavancin. After induction of an increased vancomycin MIC of 2 \( \mu g \text{ ml}^{-1} \), the cell walls of all MRSA strains were measured by electron microscopy. The Sensititre plate method confirmed that all the strains tested were resistant to oxacillin, only two strains (10.5 %) were confirmed with a vancomycin MIC of 0.5 \( \mu g \text{ ml}^{-1} \), 11 strains (57.9 %) showed a vancomycin MIC of 1 \( \mu g \text{ ml}^{-1} \) and six strains (31.6 %) showed a vancomycin MIC of 2 \( \mu g \text{ ml}^{-1} \). For telavancin, two strains (10.5 %) showed a MIC of 0.25 \( \mu g \text{ ml}^{-1} \), 16 strains showed a MIC of 0.5 \( \mu g \text{ ml}^{-1} \) (84.2 %) and only one (5.3 %) showed a telavancin MIC of 1 \( \mu g \text{ ml}^{-1} \); this MRSA isolate had an initial vancomycin MIC of 2 \( \mu g \text{ ml}^{-1} \). For daptomycin, three strains had a MIC of 0.25 \( \mu g \text{ ml}^{-1} \) (15.8 %), seven (36.8 %) had a MIC of 0.5 \( \mu g \text{ ml}^{-1} \) and nine (47.4 %) had a MIC of 1 \( \mu g \text{ ml}^{-1} \); all of these nine strains had an initial vancomycin MIC range between 1 and 2 \( \mu g \text{ ml}^{-1} \). Multistep resistance selection was therefore performed, increasing the vancomycin MIC of all the MRSA to 2 \( \mu g \text{ ml}^{-1} \). Four (21 %) of the initial MRSA strains were found to be oxacillin susceptible with a MIC of 0.5 \( \mu g \text{ ml}^{-1} \) at 24 and 48 h. This result was confirmed with a Kirby–Bauer test. At 24 h, 13 (68.4 %) and six (31.6 %) of the strains showed a telavancin MIC of 0.5 and 1 \( \mu g \text{ ml}^{-1} \), respectively. At 48 h, four more strains had an increased telavancin MIC of 1 \( \mu g \text{ ml}^{-1} \). None exceeded this value. At 24 h for daptomycin, only one strain (5.3 %) was confirmed with a MIC of 0.25 \( \mu g \text{ ml}^{-1} \); three (15.8 %) had a MIC of 0.5 \( \mu g \text{ ml}^{-1} \) and the remaining 15 strains (78.9 %) showed a MIC of 1 \( \mu g \text{ ml}^{-1} \). The MIC of daptomycin for the strains after 48 h of incubation showed the same results as at 24 h. Electron microscopy was performed before and after resistance selection, and showed a cell wall thickening in all the MRSA strains with a vancomycin MIC of 2 \( \mu g \text{ ml}^{-1} \) (Fig. 1).

We have demonstrated that an in vitro increase of vancomycin MIC in MRSA strains does not affect telavancin and...
significant cell wall thickening among our with electron microscopy, we found a between 0.25 and 1

daptomycin maintained a MIC range in vitro

Fig. 1. Electron micrographs of different S. aureus strains showing differences in cell wall thickness. The values under each panel are the mean thickness ±sb.

daptomycin MICs. Some reports demonstrated that prior vancomycin therapy may cause an increase of cell wall thickening, with a subsequent increase of vancomycin and daptomycin MICs (Leutner et al., 2006). Reports of cross-resistance between vancomycin and telavancin after vancomycin exposure are not reported in the literature. Our data showed that among our MRSA strains with an induced vancomycin MIC of 2 μg ml⁻¹, the in vitro activity of both telavancin and daptomycin maintained a MIC range between 0.25 and 1 μg ml⁻¹. Moreover, with electron microscopy, we found a significant cell wall thickening among our MRSA strains after inducing the vancomycin MIC of 2 μg ml⁻¹. This cell wall thickening does not appear to be associated with a significant MIC increase for daptomycin and telavancin. In conclusion, daptomycin and telavancin seem to represent, at least in vitro, a good alternative treatment for MRSA strains with a vancomycin MIC of 2 μg ml⁻¹, even in the presence of cell wall thickening.

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