

Comparative efficacy of telavancin and daptomycin in experimental endocarditis due to multi-clonotype MRSA strains

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Background: MRSA strains of clonal complexes (CCs) 5, 8, 30 and 45 are leading causes of complicated endovascular infections associated with suboptimal clinical outcomes. Telavancin is a novel anti-MRSA agent that both inhibits bacterial cell wall synthesis and disrupts membranes by depolarization.

Methods: In this study, we compared the *in vitro* susceptibility and *in vivo* efficacy of telavancin versus daptomycin in an experimental rabbit infective endocarditis (IE) model caused by four MRSA strains representing each of the above CC types.

Results: All study strains were susceptible to telavancin (MICs of ≤ 0.12 mg/L) and daptomycin (MICs of ≤ 0.5 mg/L). *In vitro* time–kill analyses revealed that supra-MIC levels of telavancin were effective at preventing regrowth at 24 h of incubation. In the IE animal model for all CC types, treatment with telavancin produced significantly greater reductions in MRSA counts as compared with daptomycin-treated animals in all target tissues. Moreover, telavancin-treated animals had a significantly higher percentage of sterile tissue cultures versus daptomycin-treated animals (e.g. 78%–100% versus 0% sterile vegetations and 100% versus 0%–11% sterile kidneys and spleen, in the telavancin- and daptomycin-treated animals, respectively).

Conclusions: These results suggest that telavancin exhibits significantly greater efficacies versus daptomycin in treating experimental IE caused by MRSA clinical isolates across four common CC types.

Introduction

Staphylococcus aureus is the most common cause of endovascular infections, including infective endocarditis (IE).¹ In addition, *S. aureus* IE is a life-threatening infection that is often caused by MRSA strains.¹ Despite the use of 'gold-standard' anti-MRSA antibiotics, such as vancomycin and daptomycin, morbidity and mortality associated with such infections remain unacceptably high.^{2,3} Given this potentially serious public health problem, there is a critical and urgent need to evaluate alternative anti-MRSA bactericidal agents, such as telavancin, for these invasive infections.

Telavancin is a semi-synthetic bactericidal lipoglycopeptide approved for use in Gram-positive bacterial infections including MRSA.^{4,5} It exhibits a dual mechanism of action: inhibition of bacterial cell wall synthesis and disruption of the bacterial membranes.⁴ The FDA approved telavancin in 2009 for complicated skin and skin structure infections and in 2013 for hospital-acquired and ventilator-associated bacterial pneumonia caused by *S. aureus*.⁶

In the current investigation, we evaluated the *in vitro* activity and *in vivo* efficacy of telavancin versus daptomycin in a prototypic

experimental model of IE in rabbits caused by multi-clonotypic MRSA strains. The strain-set used in these studies represents the most common clonal complex (CC) types associated with invasive *S. aureus* endovascular infections, such as IE (CC5, CC8, CC30 and CC45).^{7,8}

Materials and methods

Bacterial strains and growth conditions

The MRSA strains employed in this study were from a multinational *S. aureus* bacteraemia clinical trial collection, as well as from a collection at the Public Health Research Institute (Newark, NJ, USA) (courtesy of Dr Barry Kreiswirth).^{9–13} All *S. aureus* strains were routinely grown in tryptic soy broth (TSB; Disco Laboratories, Detroit, MI, USA) or Mueller–Hinton broth (MHB; Disco Laboratories).

Antibiotics

Telavancin was provided by Theravance Biopharma US, Inc. (South San Francisco, CA, USA). Daptomycin was purchased from Cubist Pharmaceuticals (Lexington, MA, USA). The antibiotics were reconstituted according to the manufacturers' recommendations.

MICs

The MICs of telavancin were determined by JMI Laboratories (North Liberty, IA, USA).¹⁴ The MICs of daptomycin were determined by the standard Etest method (bioMérieux, La Balme-les-Grottes, France).

In vitro time–kill curves

Time–kill experiments were performed in accordance with CLSI guidelines, with initial inocula of 10^5 or 10^7 cfu/mL in the presence of 1×, 2× and 5× MICs of telavancin or daptomycin. The two different inocula were chosen to encompass bacterial counts commonly achieved in all target tissues of animals with experimental IE.³

Experimental IE model

A well-characterized rabbit model of aortic IE was used.^{3,4} Briefly, female New Zealand White rabbits (2.2–2.5 kg body weight; Harlan Laboratories, Placentia, CA, USA) underwent indwelling transcarotid–transaortic valve catheterization and were infected intravenously (iv) with $\sim 10^5$ cfu/animal, an ID₉₅ dose established previously.^{10,11} At 24 h post-infection, animals were randomized to receive: (i) no therapy (control); (ii) telavancin at 30 mg/kg, iv, twice daily; or (iii) daptomycin at 12 mg/kg, iv, once daily. The telavancin and daptomycin doses were selected because they approximate the pharmacokinetic profiles of recommended human clinical doses (10 mg/kg, iv, once daily for telavancin, and 6 mg/kg once daily for daptomycin).^{15–17} Treatments lasted for 3 days. Control and antibiotic-treated animals were sacrificed by rapid iv injection of sodium pentobarbital (200 mg/kg; Abbott Laboratories, Lake Bluff, IL, USA) at either 24 h post-infection (untreated controls) or 24 h after the last antibiotic dose, respectively. At the time of sacrifice, cardiac vegetations, kidneys and spleen were sterilely removed and quantitatively cultured.^{10,11} The mean log₁₀ cfu/g of tissue (\pm SD) was calculated for statistical comparisons.

Ethics

Animals were maintained in accordance with the American Association for Accreditation of Laboratory Animal Care criteria and were cared for in accordance with national guidance. The Animal Research Committee (IACUC) of the LABioMed at Harbor-UCLA Medical Center approved these studies (#21294-01).

Statistical analysis

To compare tissue MRSA counts of control and antibiotic-treated animals, and between the different antibiotic treatment regimens (telavancin versus daptomycin), Student's *t*-test was employed.⁴ *P* values of <0.05 were considered statistically significant.

Results

Telavancin and daptomycin MICs

The MICs of telavancin/daptomycin for the CC5, CC8, CC30 and CC45 strains were 0.12/0.38, 0.06/0.5, 0.12/0.5 and 0.06/0.38 mg/L, respectively.

In vitro telavancin and daptomycin time–kill curves

Figure 1 shows the *in vitro* killing curves of telavancin (top panel) and daptomycin (bottom panel) on two representative MRSA strains (CC5 and CC45). All telavancin concentrations yielded an early decrease in cfu, while supra-MIC telavancin concentrations (2× and 5× MICs) prevented regrowth at 24 h of incubation in

both MRSA strains at 10^5 cfu/mL initial inocula (Figure 1, top panel, a and b). At the 10^7 cfu/mL initial inocula, only 5× MICs of telavancin prevented regrowth at 24 h of incubation for study MRSA strains (Figure 1, top panel, c and d).

A concentration-dependent killing effect of DAP was observed during the early incubation time period (Figure 1, bottom panel, a and b), but only daptomycin at 5× MICs was effective in preventing regrowth at 24 h of incubation in the CC5 strain at 5×10^5 cfu/mL initial inocula (Figure 1, bottom panel, b). However, regrowth was observed at 24 h of incubation at the 10^7 cfu/mL initial inocula, even at 5× MIC of daptomycin for both MRSA strains.

Rabbit IE model

Both telavancin and daptomycin treatments significantly reduced MRSA densities of the four common CC types in all three target tissues versus untreated controls (Figure 2). Historical data with vancomycin treatment^{10,11} are also shown for comparative purposes. As previously reported, animals with IE caused by these study MRSA strains showed one of two outcomes following vancomycin treatment: (i) no response to vancomycin treatment; or (ii) a statistically relevant, but relatively small, reduction in target tissue counts versus corresponding untreated controls.^{10,11} Importantly, telavancin treatment had significantly better efficacy as compared with both vancomycin and daptomycin therapy (Figure 2; *P*<0.001). The magnitude of these reductions in target tissue MRSA counts was dramatic, with at least 5 log₁₀ cfu/g decreases observed versus controls. Additionally, telavancin-treated animals had a significantly higher percentage of culture-negative target tissues (78%–100% in vegetations and 100% in kidneys and spleen) versus daptomycin therapy (0% in vegetations and kidneys and 0%–11% in spleen).

Discussion

Multiple factors have been implicated in the therapeutic challenge of invasive MRSA infections, including the dynamic epidemiology of genotypic lineages.^{18,19} Of note, CC5, CC8, CC30 and CC45 are among the most frequently isolated strains from complicated MRSA infections. Although vancomycin and daptomycin are standard therapeutic options, treatment failures with these agents have been increasingly reported even with 'susceptible' strains based on *in vitro* CLSI breakpoints.^{10,11} Therefore, these results have exposed the need for the development of new and improved antimicrobial agents in the treatment of persistent MRSA infections.

Given its dual mechanism of action, telavancin is a potent bactericidal agent both *in vitro* and *in vivo* in animal models, and has a low potential for resistance emergence.^{4,15,20} For instance, Madrigal *et al.*¹⁵ demonstrated that telavancin was significantly more effective than vancomycin in clearing vancomycin-intermediate *S. aureus* (VISA) isolates from cardiac vegetations in a rabbit IE model. In addition, Miro *et al.*²⁰ also reported that telavancin was efficacious in the same model caused by VISA isolates, without the emergence of telavancin-resistant clones. Recently, we demonstrated that telavancin significantly reduced MRSA densities in all target tissues versus daptomycin-treated animals in the same rabbit IE model caused by a daptomycin-non-susceptible MRSA strain.⁴ Taken together, these prior investigations suggested that telavancin may be an effective alternative to vancomycin and/or daptomycin in the

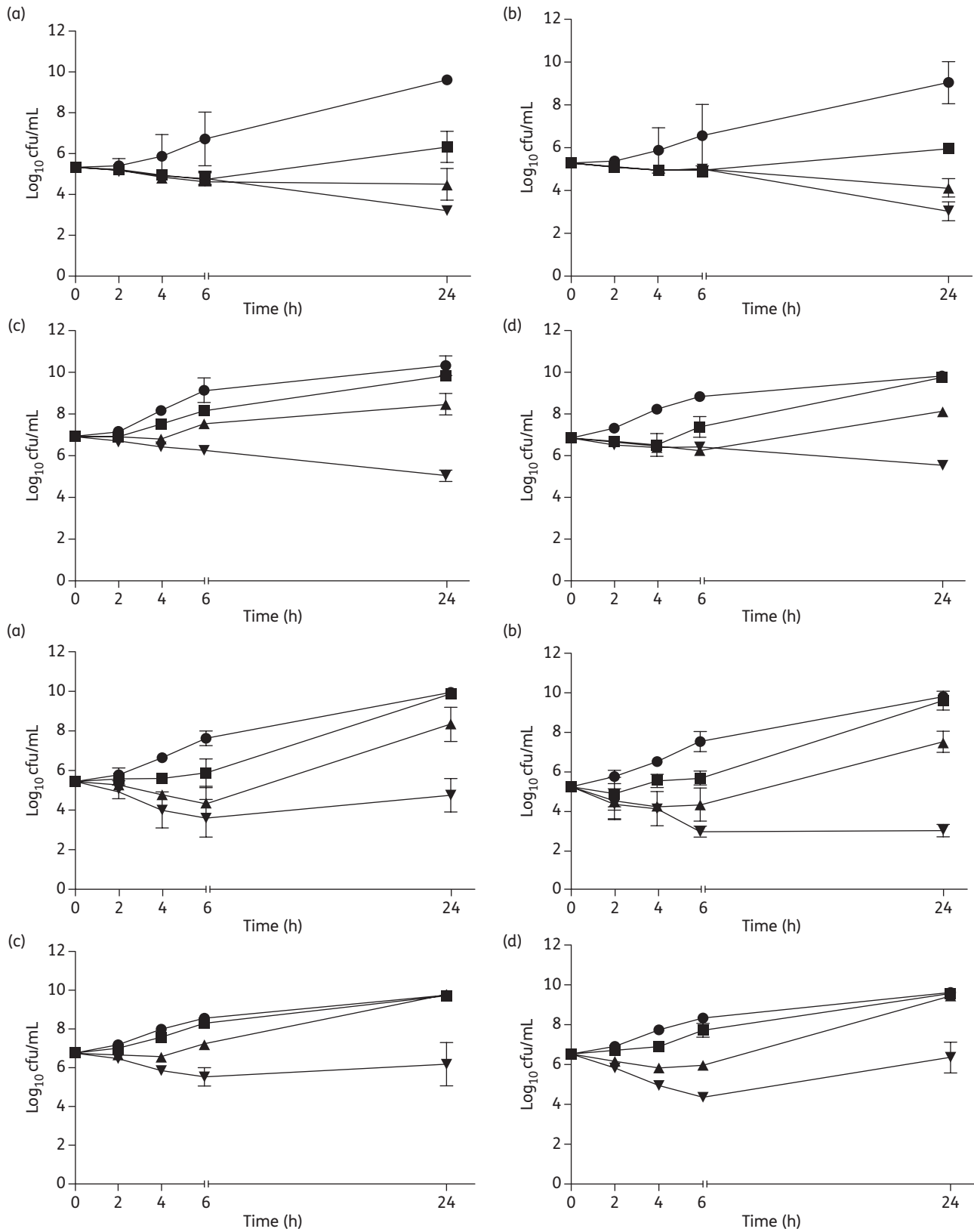


Figure 1. *In vitro* telavancin (top panel) and daptomycin (bottom panel) time–kill curves. CC45 300-169 strain (a) and CC5 300-246 strain (b) at 10^5 cfu/mL. CC45 300-169 strain (c) and CC5 300-246 strain (d) at 10^7 cfu/mL. Time–kill experiments were performed using Mueller–Hinton broth in the presence of 0 (circles), 1 \times (squares), 2 \times (triangles) and 5 \times (inverted triangles) MIC of telavancin or daptomycin.

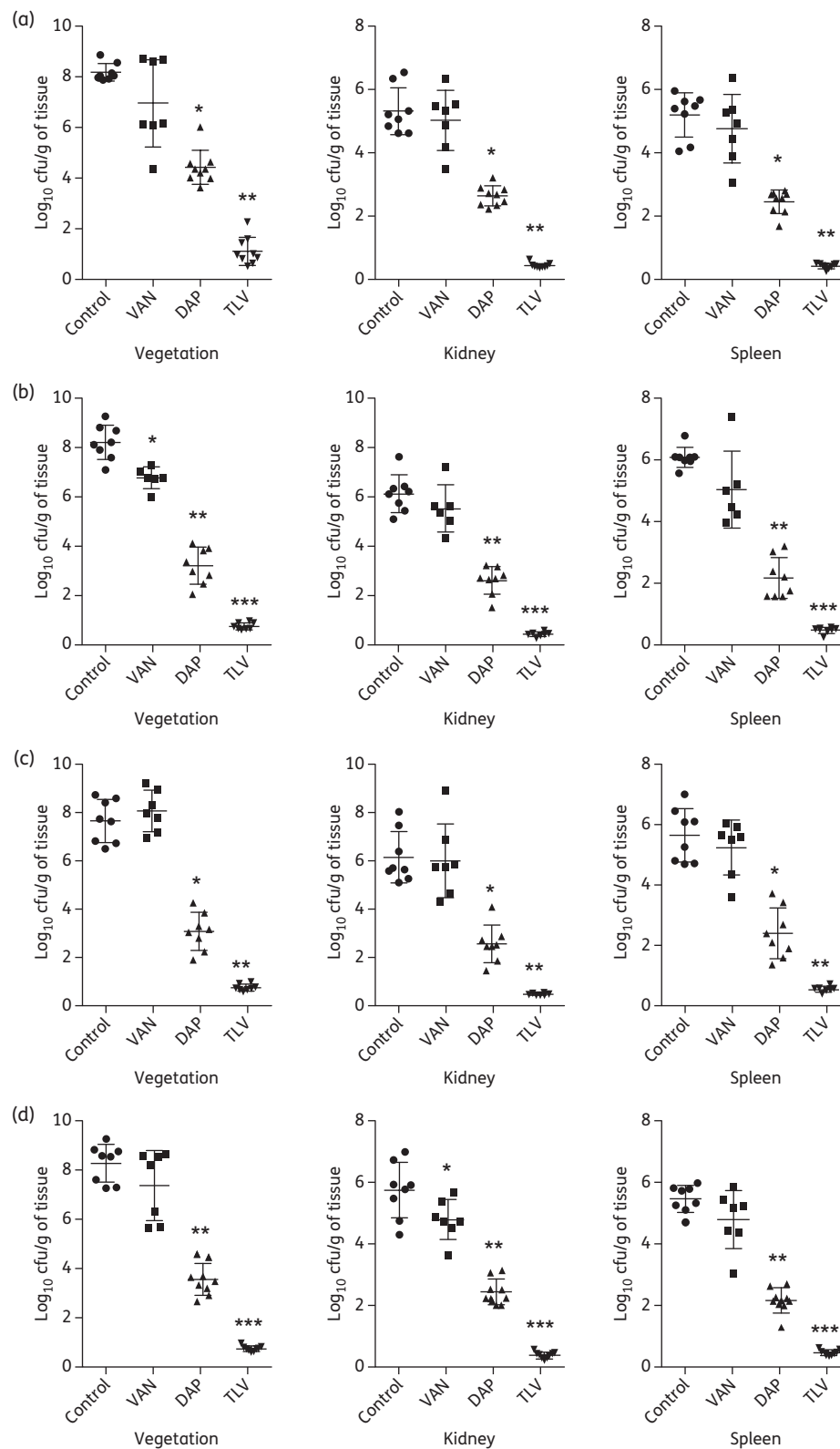


Figure 2. Densities of MRSA strains CC5 300-246 (a), CC8 BK19069 (b), CC30 BK33367 (c) and CC45 300-169 (d) (\log_{10} cfu/g) in target tissues (cardiac vegetations, kidneys and spleen) in the IE model with and without antibiotic treatment. For each group, individual data for each rabbit are represented by a dot. The means and SD are represented by horizontal and vertical bars, respectively. * $P < 0.05$, ** $P < 0.00001$ and *** $P < 0.000001$ versus untreated controls in all target tissues. $P < 0.001$ telavancin versus daptomycin in all target tissues. Historical vancomycin data are reproduced from Abdelhady *et al.*¹⁰ and Seidl *et al.*¹¹ VAN, vancomycin; DAP, daptomycin; TLV, telavancin.

treatment of serious staphylococcal infections caused by MRSA strains. However, to the best of our knowledge, there are no studies of telavancin efficacies in experimental IE across a 'relevant clonotype range' of MRSA strains representing the most common genotypes encountered in clinical practice today. Therefore, in the current studies, we focused on the four most common CC types (CC5, CC8, CC30 and CC45) predominating in complicated MRSA endovascular infections.⁷ We demonstrated that all four of these study strains were highly susceptible to telavancin and daptomycin *in vitro*. In addition, telavancin exhibited an impressive early killing effect against these MRSA strains at both 10⁵ and 10⁷ cfu/mL initial inocula. Importantly, telavancin showed significantly greater efficacies versus daptomycin and vancomycin both in terms of decreasing MRSA target tissue densities and in sterilization of such target organs, regardless of CC type.

It is important to recognize the limitations of the present study. Our data are somewhat restricted in that only a 'standard' daptomycin dose was investigated. It will be important to test higher daptomycin doses in this model to simulate doses in humans of 10 mg/kg, which may be important in clinical settings for IE treatment. In addition, we did not query whether the development of daptomycin resistance occurred after treatment in the IE model. Moreover, we did not study whether there was relapse after completion of telavancin therapy. Nonetheless, the results of the current study reveal key insights regarding the potent bactericidal activity of telavancin in the experimental IE model in terms of MRSA clearance and sterilization across four major CC types.

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Transparency declarations

None to declare.

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