MIC ≥ 256 mg/l). Daptomycin was incorporated into Ca^{2+}-supplemented Mueller-Hinton agar at subinhibitory concentrations, and synergy was screened by comparing antibiotic Etest MICs on agar, with and without daptomycin. In 11 of 15 (73.3%) strains an approximately 100-fold reduction in rifampicin MICs was observed at one eighth to one fourth the daptomycin MIC (Rand and Houck, 2004). A study using checkerboard assays with 59 *S. aureus* isolates (9 MSSA and 49 MRSA) found neither antagonism nor synergism between daptomycin and rifampicin for any of the isolates (Stein *et al.*, 2016). Synergy was also observed for 13 of 19 (68%) isolates with ampicillin (MIC ≥ 128 mg/l) (Rand and Houck, 2004). The mechanism by which daptomycin is able to reverse rifampicin resistance in some strains of VRE could not be explained by an effect of daptomycin on entry of rifampicin into or transport out of the cell, by inactivation of rifampicin, or by mutation involving the rifampicin binding site (Rand *et al.*, 2007).

The impact of administering short-course regimens of gentamicin in combination with daptomycin or vancomycin against one MSSA and one MRSA isolate using an *in vitro* pharmacodynamic model with simulated endocardial vegetations over 96 hours was evaluated. Human therapeutic dosing regimens for daptomycin (6 and 8 mg/kg of body weight) with and without gentamicin were simulated. Short-course combination regimens involving gentamicin were administered either as a single 5 mg/kg dose or as three 1 mg/kg doses for only the first 24 hours and compared with the regimens administered for the full 96-hour duration. Both regimens of daptomycin achieved 99.9% kill by 32 hours and maintained bactericidal activity against both isolates, which was significantly different from vancomycin, which displayed bacteriostatic activity ($p < 0.05$). The effects of all short-course regimens of gentamicin were equal to those of the full-duration regimens in combination with daptomycin. Adding three doses of gentamicin (1 mg/kg) to daptomycin resulted in enhancement and bactericidal activity at 24 hours against both MRSA and MSSA. The addition of a single dose of gentamicin (5 mg/kg) enhanced or improved the activity of daptomycin and resulted in early bactericidal activity at 4 hours against both isolates. These *in vitro* findings suggest that a single high dose of gentamicin in combination with daptomycin may be of utility to maximize synergistic and bactericidal activity and minimize toxicity, although clinical data to support these observations are currently lacking (Tsuji and Rybak, 2005).

In the same model, the impact of simulated standard and high-dose daptomycin in combination with gentamicin or rifampicin against daptomycin-susceptible and -nonsusceptible matched strains of *S. aureus* was evaluated. Strains were collected from the daptomycin bacteremia and endocarditis clinical trial and consisted of three susceptible strains (MIC, 0.25 mg/l) and four nonsusceptible isolates (MIC, 2–4 mg/l). Daptomycin regimens consisted of 6 and 10 mg/kg once daily alone and in combination with gentamicin, 5 mg/kg daily, or rifampicin, 300 mg every 8 hours. Rapid bactericidal activity (identified by time to 99.9% kill) was displayed in all regimens with the daptomycin-susceptible strains. Concentration-dependent activity was noted by more rapid killing with the 10 mg/kg/day dose. The addition of gentamicin improved activity in the majority of susceptible isolates. Daptomycin (6 mg/kg/day) monotherapy displayed bactericidal activity in only one of the nonsusceptible isolates and in only two isolates with increased doses of 10 mg/kg/day. Combination regimens demonstrated improvement in some but not all nonsusceptible isolates. Three isolates developed a reduction in daptomycin susceptibility with 6 mg/kg/day monotherapy, but this was suppressed with both combination and high-dose daptomycin. These *in vitro* results suggest that high-dose daptomycin therapy and combination therapy may be reasonable to consider as treatment options for difficult clinical cases with susceptible isolates (Rose *et al.*, 2008a).

More recently, daptomycin has been used in many *in vitro* studies evaluating the effect of different combinations. In these studies, for some combinations synergy or additive effect has been shown, and for some there was no difference, or even antagonism. Unfortunately, the results of these *in vitro* studies do not immediately apply to the clinical setting. Therefore the value of these studies is not clear. Some examples of drugs that have been shown to display an enhanced activity in combination with daptomycin as compared with daptomycin monotherapy are colistin (*in vitro* model, *Acinetobacter baumannii* [Córdoba *et al.*, 2015]; *Galleria mellonella* larvae model, *Acinetobacter baumannii* [Yang *et al.*, 2015]), vancomycin (biofilm-forming MRSA *in vitro* model [Luther *et al.*, 2015]), ampicillin (daptomycin-nonsusceptible enterococci, only in those with mutation in LiaSFR system, *in vitro* time-kill studies [Hindler *et al.*, 2015]; *in vitro* model, vancomycin-resistant *E. faecium* and *E. faecalis* [Smith *et al.*, 2015]), fosfomycin (MRSA osteomyelitis rat model [Lingscheid *et al.*, 2015]; foreign-body infection model, MRSA [Mihailescu *et al.*, 2014]), ceftaroline (*in vitro* model, vancomycin-resistant *E. faecium* and *E. faecalis* [Smith *et al.*, 2015]; hollow-fiber model, MRSA [Barber *et al.*, 2015]), ceftobiprole (*in vitro* model, vancomycin-resistant *E. faecium* and *E. faecalis* [Werth *et al.*, 2015]), ertapenem (*in vitro* model, vancomycin-resistant *E. faecium* and *E. faecalis* [Smith *et al.*, 2015]), rifampicin (tissue-cage MSSA infection model [El Haj *et al.*, 2015]), clarithromycin (biofilm-forming MRSA on device in broth [Fujimura *et al.*, 2015]), cloxacillin (tissue-cage MSSA infection model [El Haj *et al.*, 2014]), and gentamicin (*in vitro* model simulated endocardial vegetations, Enterococci [Luther *et al.*, 2014]).

For some combinations antagonism has been shown. Examples are linezolid (biofilm-forming MRSA *in vitro* model [Luther *et al.*, 2015]), tigecycline (two strains of nine of daptomycin-nonsusceptible enterococci *in vitro* time-kill studies [Hindler *et al.*, 2015]), and rifampicin (three strains of nine of daptomycin-nonsusceptible enterococci *in vitro* time-kill studies [Hindler *et al.*, 2015]; *in vitro* model simulated endocardial vegetations, Enterococci, delayed killing [Luther *et al.*, 2014]).