was 0, indicating that a low exposure did not select resistance. High AUC/MIC ratios (> 1970) resulted in a lower probability of decreased susceptibility (0.081) compared with the middle range (Bhavnani *et al.*, 2015).

Daptomycin displays a significant postantibiotic effect *in vitro* that is dose related and concentration dependent and lasts up to 6 hours against *S. aureus* and *E. faecalis* in the presence of free calcium at physiological concentrations (Bush *et al.*, 1989).

## THERAPEUTIC DRUG MONITORING

Pharmacokinetic studies of daptomycin have shown a marked variability among patients. Recently, Reiber *et al.* (2015) showed the pharmacokinetics to be unpredictable in 332 patients. This is in line with a study in 35 patients with severe Gram-positive infections and various creatinine clearance levels (Falcone *et al.*, 2013a). The unpredictable and highly variable pharmacokinetics and the correlation between exposure and effect warrant therapeutic drug monitoring, in particular because the need for optimal dosing and exposure has been shown in critically ill patients with MRSA bacteremia. In-hospital mortality was significantly higher (30.7% vs. 10.8%) in the subset of patients with lower exposure compared with the other patients (Falcone *et al.*, 2013b).

## IMPACT OF PROTEIN BINDING ON *IN VITRO* AND *IN VIVO* EFFICACY

The influence that the extensive binding to plasma proteins may have on daptomycin activity has been studied extensively. The effect of protein binding on the MIC values of daptomycin was studied against one MSSA and four MRSA strains using an *in vitro* pharmacodynamic model to simulate daptomycin regimens of 6 mg/kg/day. For protein-binding experiments, cation-adjusted Mueller-Hinton broth was supplemented with 4 g/dl of albumin to simulate normal human physiological levels, 50% human serum or 100% mouse serum. The activity of these agents was greater than suggested by the free fraction of drug reported previously. The kill rate of daptomycin was slowed in the presence of 4 g/dl of albumin from 0.5–8 hours; however, there was no difference in the extent of killing at 24 or 48 hours (Cha and Rybak, 2004).

The calculated protein binding based on the arithmetic means of MIC values in the presence and absence of protein for daptomycin was compared with that of telavancin, vancomycin, and teicoplanin against five *S. aureus* isolates. The calculated extent of protein binding based on MIC changes was lower than expected, 58–66%, vs. 62–70% for telavancin. These drugs may be more active than predicted based on unbound drug concentrations alone. One potential explanation for this *in vitro* finding would be that daptomycin binds weakly and reversibly to albumin (dissociation constant = 90.3 μmol/l) in contrast to its stronger irreversible binding to its site of action, the bacterial cell membrane (Tsuji *et al.*, 2008).

The bactericidal activity of daptomycin at human peak free serum concentrations ($fC_{max}$) achieved after standard doses in humans was determined against *S. aureus* (one methicillin-susceptible and two MRSA strains) in time-kill experiments and compared with vancomycin, teicoplanin, and linezolid. Daptomycin was rapidly bactericidal against all *S. aureus* strains at $fC_{max}$ of 22.0 mg/l (corresponding to 63% protein binding) and against both MRSA strains at 4.8 mg/l (corresponding to 92% protein binding). Vancomycin (18.0 mg/l) was bactericidal against only two strains, one susceptible to *S. aureus* and one to MRSA. Both teicoplanin (4.5 mg/l) and linezolid (10.4 mg/l) were consistently bacteriostatic (Brauers *et al.*, 2007).

The influence of protein binding on the bactericidal activity of daptomycin was studied by adding physiological concentrations of human albumin (4 g/dl) or human serum (90%) to kill-curve experiments against MRSA and hVISA strains. Final inocula of approximately $10^7$ CFU/ml and daptomycin concentrations similar to the $C_{max}$ obtained in serum after an i.v. dose of 4 mg/kg were used. Daptomycin was rapidly bactericidal (≥ 3 $\log_{10}$ initial inocula reduction) against *S. aureus*, regardless of the strain tested or the presence of albumin or human serum, although the latter slightly delayed bactericidal activity. Vancomycin exhibited much slower bactericidal activity against methicillin-susceptible or -resistant *S. aureus* but was never bactericidal against hVISA (Cafini *et al.*, 2007).

The influence of protein binding on the bactericidal activity of daptomycin was also studied by adding physiological concentrations of human albumin (4 g/dl) or human serum (90%) in kill-curve experiments against one vancomycin-susceptible and one vancomycin-resistant strain of *E. faecium*. Final inocula of approximately $10^7$ CFU/ml and daptomycin concentrations similar to the $C_{max}$ obtained in serum after a i.v. dose of 4 mg/kg were used. Daptomycin exhibited rapid bactericidal activity against the vancomycin-susceptible or the vancomycin-resistant *E. faecium* strain, delayed to 8 and 24 hours, respectively, by human albumin. Vancomycin was never bactericidal against the vancomycin-susceptible or -resistant strains of *E. faecium* (Cafini *et al.*, 2007).

Similarly, in another study, the bactericidal activity of daptomycin at $fC_{max}$ was determined against *E. faecalis* and *E. faecium* (one vancomycin-susceptible and one vancomycin-resistant strain of each) at 22.0 mg/l (corresponding to 63% protein binding) and at 4.8 mg/l (corresponding to 92% protein binding), respectively. All four enterococcal strains (daptomycin MIC 1–4 mg/l; AUC/MIC of approximately 100–400) demonstrated a 3-log reduction at a daptomycin concentration of 22.0 mg/l and a 2-log reduction in three strains at 4.8 mg/l. In comparison with *S. aureus* the enterococcal strains were less susceptible to daptomycin, with a lower AUC/MIC ratio, although the killing of enterococci was very effective, and regrowth did not occur (Brauers *et al.*, 2007).

The effect of 50 mg/kg of daptomycin subcutaneously on peritonitis caused by MSSA and MRSA was studied in healthy and neutropenic mice and compared with the effects of subcutaneous nafcillin at 100 mg/kg, subcutaneous vancomycin at 100 mg/kg, linezolid at 100 mg/kg via gavage (orally), or saline (10 ml/kg subcutaneously). Mice were