system. Identifying these mutations might be useful in detecting these *E. faecium* first-step mutants (Munita *et al.*, 2012). However, in patients treated with daptomycin for MRSA bacteremia, significant increases in MIC in MRSA strains isolated from blood were observed in 7 of 18 (39%) episodes of persistent bacteremia. Daptomycin MIC increase was significantly associated with microbiological failure (odds ratio [OR]: 27; confidence interval [CI]: 1.9–368.4) and therapeutic failure of any cause (OR: 16; CI:1.3–194.6) (Gasch *et al.*, 2014). In another study in patients with *S. aureus* bacteremia with or without right-sided endocarditis, the emergence of daptomycin resistance appeared to occur relatively commonly (Fowler *et al.*, 2006; Grayson, 2006). About one third (6 of 19) of daptomycin-treated patients who suffered microbiological failure were found to have isolates that had developed resistance to daptomycin (MIC ≥ 2 mg/l) during treatment (for further discussion see section 7, Clinical uses of the drug).

One of the factors involved in the development of resistance during therapy is suboptimal dosing. This is demonstrated in VRE strains in a model simulating a 6 mg/kg/day and a 10 mg/kg/day dosing regimen. The daptomycin-resistant mutants displayed a 43–58% increase in cell wall thickness ($p < 0.0001$), whereas daptomycin membrane depolarization decreased from 53–65% compared with that of the susceptible strains. The response and regrowth were dose dependent against all three strains studied (Steed *et al.*, 2011). However, the regrowth during daptomycin therapy is not (only) related to suboptimal concentrations. Regrowth during daptomycin therapy in an *in vitro* endocarditis model for viridans group streptococci (MICs 1 or 2 mg/l) has been shown with doses equal to 6 and 8 mg/kg/day. Regrowth was seen even at concentration eight times the MIC. The underlying mechanism is unknown (Akins *et al.*, 2015). The expression of virulence factors in MRSA (Panton–Valentine leukocidin, alpha-hemolysin, and protein A) has shown not to be increased in the presence of subinhibitory concentrations of daptomycin and vancomycin (Otto *et al.*, 2013).

EMERGING RESISTANCE AND COMBINATION THERAPY

Restored susceptibility has been demonstrated with several antibiotics when they are administered in combinations with daptomycin. Daptomycin's bactericidal activity was abolished in the presence of liaFSR or yycFGHIJ mutations in *E. faecium* regardless of the MIC and was restored in the presence of ampicillin, but only in representatives of the LiaFSR pathway. Reduced binding of daptomycin to the cell surface was the predominant finding correlating with resistance in isolates with daptomycin MICs above the susceptibility breakpoint (Diaz *et al.*, 2014). The increased daptomycin cell membrane binding in the presence of a beta-lactam was also seen by Dhand *et al.* (2011), who reported enhanced daptomycin bactericidal activity, increased membrane-daptomycin binding, and decrease in positive surface charge induced by antistaphylococcal beta-lactams against daptomycin-nonsusceptible MRSA. In persistent or refractory MRSA infections the daptomycin–beta-lactam combination may significantly enhance both the *in vitro* and the *in vivo* efficacy of anti-MRSA therapeutic options against DAP-resistant MRSA infections and represent an option in preventing daptomycin resistance selection (Mehta *et al.*, 2012).

The combination of daptomycin with other antibiotics has also identified some beneficial combinations. High-level daptomycin resistance (MIC ≥ 256 mg/l) develops rapidly and frequently *in vitro* and *in vivo* among mitis group streptococci. Combining daptomycin with gentamicin might enhance its activity and prevent the development of high-level daptomycin resistance (García-de-la-Mària *et al.*, 2013). Combining daptomycin with oxacillin or clarithromycin in MRSA may delay the development of daptomycin resistance in cases requiring prolonged antibiotic therapy (Berti *et al.*, 2012). Combinations with rifampin or fosfomycin were effective in delaying the emergence of daptomycin, but the development of resistance was delayed 1 week only (Berti *et al.*, 2012).

3. MECHANISM OF DRUG ACTION

Daptomycin exerts bactericidal activity by altering the bacterial cell envelope homeostasis by interacting with the phospholipids of the cell membrane. The process of these interactions has not been fully elucidated, and the mechanism leading to daptomycin-induced bacterial cell death has not been fully established (Straus and Hancock, 2006; Tran *et al.*, 2015). It is clear that the activity of daptomycin is dependent on the presence of ionized calcium (Jung *et al.*, 2004). The daptomycin–calcium complex facilitates the insertion of the antibiotic into the bacterial cell membrane. The presence of calcium also appears to stimulate the formation of daptomycin micellar structures, which have been postulated to serve as vehicles for delivery of daptomycin to the bacterial cell membrane (Ho *et al.*, 2008; Scott *et al.*, 2007).

Interactions between the daptomycin–calcium complex and the cell membrane have not fully been elucidated, but there are some important steps (Tran *et al.*, 2015). The daptomycin–calcium complex inserted into the cell membrane oligomerizes in the outer leaflet of the cell membrane, and this process is dependent on the present of phospholipid phosphatidylglycerol (Muraih *et al.*, 2011; Muraih *et al.*, 2012). There is also a translocation of the oligomers into the inner leaflet of the cell membrane in a model in which two opposing structures (on the inner and outer leaflet of the cell membrane) form a multifunctional porelike structure (Zhang *et al.*, 2014a). These porelike structures allow potassium efflux from the bacterial cytoplasm, thus destroying the ion concentration gradient. The translocation of daptomycin oligomers is influenced by the presence of cardiolipin, a phospholipid that plays important roles in the cell membrane homeostasis of bacteria. Increased concentrations of cardiolipins might prevent, at least in part, the antibacterial activity of daptomycin (Zhang *et al.*, 2014b).

Recently an alternative model for the mechanism of action of daptomycin has been proposed (Chen *et al.*, 2014). The activity of daptomycin was studied using microscopic imaging