to the $MIC_{90}$ (0.5 mg/l), and $MBC_{50}$ was equal to $MBC_{90}$. No MIC creep was observed (Holmes and Jorgensen, 2008). However, in Japan, an increase of the MIC creep in MRSA isolates has been reported between 2008 and 2012. The MIC creep rate was 13% in 2012, significantly higher than the value of 3.3% in 2008 ($p < 0.01$). The $MIC_{50}$/$MIC_{80}$ values of daptomycin and vancomycin were 0.125/0.5 and 0.125/1 mg/l, respectively. The mutant prevention concentration $MPC_{50}$/$MPC_{80}$ values of daptomycin and vancomycin were both 32/64 mg/l. Of strains that were selected in the MSW, daptomycin nonsusceptible isolates accounted for 70%, whereas MRSA with a vancomycin MIC of 2 mg/l accounted for 26.7%. On the other hand, 50% of the strains that selected in the vancomycin MSW were daptomycin nonsusceptible. The detection rate of MRSA with a vancomycin MIC of 2 mg/l that selected in the daptomycin MSW was 36.7%. These results showed that MRSA with a vancomycin MIC of 2 mg/l and daptomycin nonsusceptible isolates were selected by exposure to both antibiotics (Fujimura *et al.*, 2014).

This is supported by an increase in the range of MICs for vancomycin in MRSA over the past decades. MRSA isolates were analyzed from bloodstream infections. There was a significant correlation ($p < 0.01$) between the MIC values for vancomycin and daptomycin. The daptomycin MIC values were also correlated with those for linezolid, tigecyclin, and teicoplanin. This suggests that vancomycin selective pressure may have a role in the selection of MRSA with reduced susceptibility for daptomycin and other anti-MRSA drugs (Patel *et al.*, 2009).

MRSA bloodstream isolates from patients who had received vancomycin within the preceding 30 days ($n = 38$) had higher MICs against daptomycin (not statistically significant) than non–vancomycin-exposed patients with MRSA bacteremia ($n = 43$), whereas vancomycin MICs were significantly higher. No differences in daptomycin killing of MRSA at 4 hours *in vitro* were noted, whereas vancomycin killing at 24 hours *in vitro* was significantly increased (Moise *et al.*, 2008). However, the difference in duration of the experiment for the two drugs limits the interpretation of the data.

## MECHANISMS OF RESISTANCE

Resistance to daptomycin seems to involve multiple mechanisms and steps. Many of these steps have not yet been elucidated. Some mutations and mechanisms are described here.

Daptomycin resistance in *S. aureus* appears to involve several mechanisms, including the following: (1) altered expression of two key determinants of net positive surface charge, during either exponential or stationary growth phases (i.e., dysregulation of dltA and mprF); (2) a significant increase in the D-alanylated wall teichoic acid content in daptomycin-resistant strains, reflecting DltA gain-in-function; (3) heightened elaboration of lysinylated phosphatidylglycerol in daptomycin-resistant strains, reflecting MprF gain-in-function; (4) increased cell membrane fluidity; and (5) significantly reduced susceptibility to prototypic cationic host defense peptides of platelet and leukocyte origins (Mishra *et al.*, 2014). A mutation in the *RpoB* gene was also accompanied by a thickened cell wall and a reduction of the cell surface negative charge. The *RpoB* mutation resulted in a reduced susceptibility to both daptomycin and hetero-VISA (Cui *et al.*, 2010). Point mutations resulting in dysregulation of mprF are also one of the mechanisms involved in daptomycin resistance in *S. aureus* (Bayer *et al.*, 2014).

## TOLERANCE

Tolerant strains are susceptible in laboratory testing but tolerant to antibiotic killing, and therefore they may present a challenge in treatment. Tolerance to daptomycin in *S. aureus* might occur owing to point mutation in PitA (inorganic phosphate transporter). It differs from classic resistance mechanisms in that it does not translate into an increase in MIC (Mechler *et al.*, 2015). In vancomycin-tolerant MRSA strains, the vancomycin killing was reduced, and the daptomycin activities were maintained. Exposure to daptomycin increased the expression of some genes that possess a number of regulators that are differentially expressed in strains with reduced susceptibility, such as mprF, vraSR and sltA. The most significant increase, however, was in mprF expression as compared with vancomycin-susceptible strains. Longer exposure to these antibiotics resulted in increased gene expression in vancomycin-tolerant MRSA (Rose *et al.*, 2012). Tolerance of *E. faecium* has been associated with a deletion of isoleucine in position 177 of LiaF, a member of the three-component regulatory system LiaFSR involved in the cell envelope response to antimicrobials (Munita *et al.*, 2013).

## EMERGING RESISTANCE DURING THERAPY

Several mechanisms of resistance have been described in microorganisms cultured from clinical patients during daptomycin therapy as well as in *in vitro* studies. A mutation of fabF has been described for the small-colony variant of VISA in a patient with septic arthritis during long-term treatment with daptomycin (Lin *et al.*, 2016). Daptomycin-susceptible (MIC 3 mg/l) *E. faecium* strains harbor LiaSR substitutions, causing high-dose daptomycin therapy to fail in a neutropenic patient with a bloodstream infection (Munita *et al.*, 2014). In addition to the mutations in the LiaSR system, YycFGHIJ, a system involved in the regulation of cell wall homeostasis, was also found to be important in *E. faecium* (Diaz *et al.*, 2014). In *S. aureus,* dltA overexpression and mprF mutations have been found to be important (Cafiso *et al.*, 2014). In clinical patients, resistance development has been shown for *E. faecium* (Lin *et al.*, 2016), *S. aureus* (Munita *et al.*, 2014), *Corynebacterium striatum* (McElvania TeKippe *et al.*, 2014) and viridans group streptococci (Akins *et al.*, 2015; García-de-la-Mària *et al.*, 2013). One study also described the development of resistance in vancomycin-resistant *E. faecium*–colonizing isolates and found mutations in cardiolipin synthase and in the liaFSR operon (Lellek *et al.*, 2015). Mutations do not always result in daptomycin resistance. In *E. faecium* with mutation in the liaFSR system, they may be difficult to detect in routine practice, although there is a strong association between daptomycin MICs within the upper susceptibility range and mutations in the liaFSR