maturity and bacterial strain (Bauer et al. 2013). The measures minimal biofilm inhibition concentration (MBIC) and minimal biofilm eradication concentration (MBEC) have been determined by an *in vitro* biofilm model to give an indication of the minimal inhibitory concentration (MIC) closer to real conditions in biofilms (Reiter et al. 2012). Stewart (1996) investigated antibiotic penetration into microbial biofilm and showed the theoretic inability of beta-lactams to penetrate inside the biofilm. Overproduction of chromosomally encoded AmpC cephalosporinase by gene derepression is considered the main mechanism of resistance of *Pseudomonas aeruginosa* isolates in sputum to beta-lactam antibiotics. Giwercman et al. (1992) demonstrated that strong β-lactamase inducers, such as carbapenems, allow the production of β-lactamase through all of the bacterial layers, whereas poorer inducers, such as ceftazidime, influence just the superficial layers of the biofilm, probably due to inactivation of the antibiotic by the β-lactamase. Hill et al. (2005) proposed using, in cystic fibrosis patients, colistin (tested at concentrations suitable for nebulization) either alone or in combination with tobramycin ($10\,\mu g\,ml^{-1}$), followed by meropenem combined with tobramycin or ciprofloxacin. The addition of aztreonam also improved the efficacy of ceftazidime for treatment of the biofilm, probably because aztreonam acts as a β-lactamase inhibitor (Giwercman et al. 1992).

Dynamic biofilms stained with Live/Dead probes have also been observed by confocal microscopy. Bauer et al. (2013) demonstrated that rifampin, tigecycline, and moxifloxacin are effective against mature methicillin-resistant *Staphylococcus aureus* ATCC 33591 biofilms, whereas oxacillin has demonstrated activity against methicillin-sensitive *S. aureus* ATCC 25923. Delafloxacin and daptomycin were the most potent, and fusidic acid, vancomycin, and linezolid the less potent overall. Rosales-Reyes et al. (2016) demonstrated susceptibility to polymyxin B of multidrug-resistant *A. baumannii* with a biofilm phenotype.

However, all of these results are to be considered with caution. Torres et al. (2017) used microdialysis to evaluate ciprofloxacin penetration into the lungs of healthy rats infected with *P. aeruginosa* in biofilm and showed that bacterial biofilm infection reduced the ciprofloxacin free interstitial lung concentrations and increased plasma exposure, suggesting that plasma concentrations alone are not a good surrogate for lung concentrations.

9.2.2 Outer Membrane Vesicles (OMVs)

Bacterial outer membrane vesicles are spheres of lipids released from the outer membranes (OM) of Gram-negative bacteria that are involved in cell-to-cell communication and biofilm formation (Wang et al. 2015a). Ciofu et al. (2000) have shown that the source of β-lactamase in biofilm may also be the membrane vesicles containing β-lactamase liberated by resistant *P. aeruginosa* bacteria. He et al. (2017) studied the association between biofilm formation and