formation than negative strains, regardless of growth medium. Quantification of biofilms has been performed using crystal violet assay in carbapenemase-producing *Acinetobacter baumannii* isolates harboring the blaOXA-23 gene, and biofilm-associated protein BAP was always detected in biofilm-producing strains, suggesting that biofilm formation ability is responsible for their persistence and colonization (Sung et al. 2016). Increased bacterial persistence is also observed in biofilms and provides cross-tolerance to different clinically important antibiotics (Harms et al. 2016; Michiels et al. 2016). Several molecular mechanisms can explain this failure of antibiotics to overcome these biofilms, but many methods are currently available to cope with them.

The purpose of this review is to define the reasons for antibiotic failure in biofilms and the usual and innovative means to overcome biofilm resistance in biofilms.

9.2 Reasons for Failure of Antibiotics in Biofilms

Inside the biofilm, a high density of bacteria promotes genetic transfer, enhances the selection of resistant strains, and increases the frequency of mutation (Rodriguez-Rojas et al. 2012). A subpopulation of bacteria can enter a starved state (persisters), with modifications to physiology and metabolic rate, leading to more cells resistant to antibiotics (Mah 2012; Carvalho et al. 2018). This may be a significant contributor to recurrent infections. Kwan et al. (2013) succeeded in inducing the formation of persisters by pretreating *Escherichia coli* with rifampin to stop transcription, tetracycline to stop translation, and carbonyl cyanide m-chlorophenylhydrazone to stop ATP synthesis. There may be several explanations for the failure of antibiotics in biofilms, including a defect of penetration inside the biofilm, increased resistance of bacteria, or better adaptation of bacterial cells to their environment (Stewart 2002; Hoiby et al. 2010a, b; Jolivet-Gougeon and Bonnaure-Mallet 2014; Li et al. 2017). Kaldalu et al. (2016) also argued that mechanisms involved in persistence are more complex than supposed and a function of bacterial species, strain, growth conditions, and antibiotics is used in the experiments. Phenotypic and genetic heterogeneity within biofilms, with particular emphasis on persistence and antimicrobial tolerance, were discussed in a recent review (Sadiq et al. 2017).

9.2.1 Failure of Antibiotics to Penetrate Biofilm: Active Antibiotics on the Biofilm

Changes in biofilm architecture resulted in the accumulation of metabolites (e.g. indole for *E. coli*), which control the competition dynamics between bacterial species and influence antibiotic susceptibilities (Bhattacharjee et al. 2017). The resistance of some strains within the biofilm has been widely demonstrated (Greene et al. 2016), but antibiotic activity depends on biofilm