Biofilm Tolerance Favors Rapid Emergence of Antibiotic Resistance

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Bacterial infections are a leading cause of morbidity and mortality and the increasing resistance to antibiotics among pathogenic bacteria is a major health concern. This is particularly the case for chronic infections due to the presence of biofilms developing on medical devices or mucosa, for which there is no fully efficient prevention or eradication method. In biofilms, bacteria undergo specific physiological changes and display characteristic but ill-understood high level of tolerance to both antimicrobial agents and host immune defenses. Whereas enhanced tolerance of biofilms towards antibiotics is a multifactorial process, relapse of infection is mainly explained by the presence, within biofilms, of high levels of so-called persister bacteria that can sustain extremely high concentration of antibiotics but can regrow as biofilms when treatment is stopped. Persisters are proposed to serve as a potential evolutionary reservoir from which resistance could emerge. While impact of persisters in clinical situations has been largely overlooked, recent studies demonstrated that high-levels of antibiotic tolerance, but not resistance, could be rapidly achieved by exposure of batch planktonic cultures of *E. coli* and other pathogens to cyclic treatments of lethal concentration of antibiotics.

Considering the importance of biofilms in chronic infections and the failure of their treatment there is an urgent need to characterize evolution of persister-associated tolerance and resistance within biofilms. Using laboratory evolution experiments, we exposed biofilms to intermittent exposure of lethal antibiotic concentrations, a situation mimicking clinically relevant situations. We showed that tolerance and resistance evolutionary path followed by biofilms and planktonic bacteria are different, with biofilms strongly favoring rapid emergence of genetic resistance promoted by their intrinsic high level of tolerance. These results could lead to the design of innovative strategies or clinical treatment protocols to mitigate the emergence of high tolerance and subsequent antibiotic resistance in clinically relevant situations.