



## Stimulating Microbial Metabolism in Biofilm Cells Reduces Antimicrobial Tolerance

Tom Coenye, Ghent University, Ghent, Belgium

Multiple mechanisms are involved in reduced antibiotic susceptibility of bacteria in biofilms, including the micro-environmental conditions at the site of infection. While changes in microbial metabolism, (at least partly related to gradients in oxygen and nutrient levels) can have a profound effect on antimicrobial susceptibility, the exact metabolic adaptations vary between different microorganisms and settings. Nevertheless, a common pattern emerges, in which biofilm-associated bacteria typically downregulate their central metabolism (e.g. the tricarboxylic acid, TCA cycle) with a concomitant upregulation of alternative pathways (e.g. the glyoxylate shunt); by doing so they produce less reducing equivalents (NADH, FADH<sub>2</sub>) which slows down the electron transport chain and reduces the production of toxic reactive oxygen species. The observation that microbial metabolism plays a crucial role in reduced susceptibility during biofilm-associated infections raises the question whether we can counteract these metabolic changes *in vivo*. An example of such an approach is the use of carbon sources that act as potentiators of antimicrobial activity. Using *P. aeruginosa* biofilms formed in an artificial CF sputum medium, we recently showed that it is feasible to potentiate the anti-biofilm activity of ciprofloxacin (using D,L-malic acid) and ceftazidime (using sodium acetate) *in vitro*, by activating the TCA cycle. While the observed anti-biofilm effects appear to be antibiotic and strain dependent this is a proof-of-concept that direct interference with biofilm metabolism can increase antibiotic susceptibility. Ongoing work with *P. aeruginosa*-infected threedimensional epithelial cell cultures shows that such an approach could also work in more complex *in vivo*-like conditions. In addition, approaches that indirectly stimulate metabolism (e.g. baicalin hydrate stimulating gluconate metabolism) or stimulate metabolism by increasing levels of the terminal electron acceptor (e.g. hyperbaric oxygen therapy increasing microbial aerobic metabolism) also have potential to increase anti-biofilm activity of antibiotics.