Intracellular growth and persistence of *Pseudomonas aeruginosa* in a lung tissue model

Luisa Lauer[1]*, A. Leoni Swart[1], Benoît-Joseph Laventie[1], Rosmarie Sütterlin[1], Tina Junne[1] and Urs Jenal[1]

[1] Biozentrum, University of Basel, Basel, Switzerland.
* Luisa.lauer@unibas.ch

The antibiotic resistance crisis is becoming an increasingly serious threat to the global health. For decades, market entry of new classes of antibiotics has stagnated and more bacterial strains develop resistances, even against last resort antibiotics. We aim to support the development of new antibiotics, by developing *in vitro* models that closely mimic human infections, enabling antibiotic efficacy assessment under physiological conditions.

We have developed an air-interface lung model from healthy human bronchial epithelial cells cultured on Transwells. Using this fully differentiated lung tissue model, we have established infection conditions and gained insight into the distinct infection kinetics of *Pseudomonas aeruginosa* using CFU assessment and confocal microscopy. Our studies have uncovered that *P. aeruginosa* is able to actively invade and rupture lung barrier tissue using T3SS and T6SS toxin delivery systems and that mutants lacking T3SS, which often emerge during chronic lung infections, switch to intracellular growth. Based on this, we hypothesized that *P. aeruginosa* is able to persist intracellularly during chronic lung infections by escaping the immune system and by tolerating specific antibiotics.

Here, we provide evidence in favor of this idea by comparing *P. aeruginosa* lung tissue infections in the presence of different concentrations of front-line fluoroquinolone (levofloxacin) and carbapenem (meropenem) antibiotics. While both drugs efficiently reduced bacterial counts and protected the host tissue, neither led to complete tissue clearance and pathogen eradication. Meropenem, although effectively eliminating extracellular pathogens, was not able to eradicate intracellular *P. aeruginosa* even at high concentrations. Intracellular pathogens not only persisted for prolonged treatment periods, but restored growth after washout of the drug, leading to tissue colonization and destruction. By identifying an intracellular pathogen reservoir and revealing its drug resilience, these studies could help elucidate fundamental mechanisms underlying recurring lung infections. Future work will address if intracellular drug tolerance is driven primarily by pharmacokinetic or pharmacodynamic properties and if intracellular reservoirs of *P. aeruginosa* also contribute to persistence in chronically infected CF or COPD patients.