SUMMARY

Accurate and timely identification of pathogens and their antimicrobial susceptibility is critical for targeted treatment. However, current microbial diagnostics rely on culturing in conventional growth media that fail to mimic the infectious microenvironment, known to contribute to biofilm formation and antimicrobial tolerance. As a result, diagnostic outcomes may not fully capture the microbiological complexity of infections, particularly those involving biofilms. This thesis investigates whether incorporating *in vivo*-like conditions into the *in vitro* testing workflow yields result that better predict clinical outcome of treatment of biofilm-associated infections, focusing on cystic fibrosis (CF) lung infections and periprosthetic joint infections (PJI).

For CF lung infections, a high throughput antimicrobial susceptibility assay for *Pseudomonas aeruginosa* biofilms was developed using a synthetic CF sputum medium (SCFM2), coupled to a resazurin-based viability staining. Biofilm Prevention Concentrations (BPCs) of clinically relevant antibiotics were determined for a range of reference and clinical *P. aeruginosa* isolates. These outcomes were then compared to respective conventional susceptibility parameters (minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs)) obtained in Mueller Hinton Broth (MHB). BPCs were consistently higher than MICs and the extent of this difference appeared to be antibiotic-dependent, indicating SCFM2 better captures the antimicrobial tolerance experienced by *P. aeruginosa* biofilms *in vivo*. In addition, resazurin-derived fluorescence correlated strongly with CFU counts, validating the resazurin staining as a rapid alternative to plating.

In the context of PJI, a synthetic synovial fluid (SSF2) medium was developed, based on human diseased synovial fluid and validated across 18 PJI isolates belonging to various microorganisms. SSF2 supported growth and aggregate formation, which was confirmed by light confocal laser scanning microscopy. Antimicrobial susceptibility testing revealed that the majority of BPCs and minimal biofilm inhibitory concentrations (MBICs) of relevant antibiotics in SSF2 were higher than conventional MICs and MBCs determined in MHB. These data show that the implementation of SSF2 as a culture medium could be a

valuable addition to evaluate the antimicrobial susceptibility of biofilm aggregates in the context of PJI.

To further investigate the impact of culturing in SSF2 on bacterial behavior, RNA sequencing was performed on a PJI isolate of *S. aureus* grown in SSF2 and the conventional culture Luria Bertani (LB) medium. Transcriptomic analysis revealed that approximately half of the protein-coding genes were differentially expressed. Functional analysis showed that SSF2 induced the expression of virulence- and biofilm-associated genes. These data were in line with previously published *in vivo* gene expression data captured immediately upon collection of the same isolate during acute PJI, highlighting the potential of the SSF2 medium to approximate *in vivo* conditions experienced by *S. aureus* during PJI.

Finally, the diagnostic performance of culturing in SSF2 and isothermal microcalorimetry (IMC) were compared to the one obtained with conventional culturing (CC), using 79 synovial fluid samples collected from patients with suspected PJI. Culturing in SSF2 achieved the highest diagnostic yield and microbial diversity compared to CC, while IMC enabled rapid detection of microbial activity (<16h) in respective samples. These findings demonstrate the potential of alternative diagnostic approaches in pathogen recovery in PJI.

Taken together, this work highlights the potential of including *in vivo*-like models in microbial diagnostics. However, future work should include clinical studies (preferentially multi-center studies) with standardized protocols to validate whether antimicrobial therapy selected based on results obtained in these models will lead to better patient outcomes in biofilm-associated infections.