Everything You Need to Know About Biofilms (But Were Afraid to Ask).

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Aim: 60% of all infections are estimated to be biofilm related and to cost more than 1 billion dollars per year in USA alone, emphasizing the importance of the field. The biofilm community, its spatial distribution and activity play an important role in the prolongation of treatment and healing of chronic infections and is clearly different from acute infections by planktonic microorganisms. The purpose of this presentation is to describe different aspects and challenges of biofilms. How to improve sampling, diagnosis and treatment of chronic infections, especially considering the biofilm issue, will be presented.

Methods: Systematic and optimized sampling of various specimen types from patients with chronic biofilm infections was performed. Extended culture and a panel of molecular biological tools were applied on the different types of specimens for improved diagnosis. For further investigation of the microbial pathogenesis, *in situ* transcriptomics and metabolomics were applied.

Results: In chronic biofilm related infections, molecular techniques detected a larger diversity of microorganisms than culture based methods in several patients. A heterogeneous distribution of bacteria in various specimens from the same patient was evident. In chronic wounds, multiple biopsies from the same ulcer showed large differences in the abundance of fx *P. aeruginosa* and *S. aureus* at different locations. Transcriptomic and metabolomic analyses resolved the important virulence genes and nutrient acquisition mechanisms of *S. aureus in vivo* and a capability to survive under severe oxygen limitation.

Conclusion: Our studies show that improved diagnosis and understanding of chronic biofilm related infections required multiple specimen types, standardized sampling, extended culture and molecular biological analysis. The use of a well-designed diagnostic algorithms, big data and personalized diagnosis and treatment has large potentials and will be discussed during the presentation.