P0287
Paper Poster Session
Non-culture techniques for challenging situations in diagnostics

"FISHing" in endocarditis: fluorescence in situ hybridization for the detection of microorganisms

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Background: Infective endocarditis is a microbial infection of the heart valves associated with significant morbidity and mortality. Early diagnosis of the causative microorganism is crucial for correct antibiotic therapy and, thus, the patients' outcome. Microbiological diagnosis is mainly based on conventional cultural techniques. However, bacterial growth is often prevented by previous antibiotic therapy. Furthermore, fastidious causative organisms may be missed by routine culture methods. We aimed to detect and identify microorganisms within human heart valves *in situ*.

Material/methods: Heart valve biopsies from patients with suspected endocarditis were obtained during surgery and submitted to fluorescence *in situ* hybridization (FISH) analysis. Specimens were screened for bacteria using a pan-bacterial probe along with genus- or species-specific probes for identification of streptococci, staphylococci, enterococci, and rare species like *Bartonella quintana* or *Tropheryma whipplei*. In parallel, consecutive tissue sections were analyzed by 16S rRNA gene PCR and sequencing. Results were compared with those of culture-based diagnostics and clinical data.

Results: A broad range of bacteria could be specifically detected by FISH (predominantly *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., followed by rare species such as *Tropheryma whipplei, Granulicatella adiacens, Propionibacterium acnes, or Bartonella*). Interestingly, we found FISH positive bacteria in culture negative samples and samples from patients under antibiotic therapy. The high signal intensity of FISH correlates to a high ribosomal content of the bacteria indicating metabolic activity at the time of surgery.

Further cases showed no FISH signal but bacteria could be visualized using a non-specific nucleic acid stain (DAPI). None of these DAPI positive cases resulted in growth in culture, presumably due to previous antibiotic therapy. In these cases PCR and sequencing provided species identification.

All cases were monospecies infections.

Conclusions: FISH combined with PCR is a valuable addition to conventional microbiology for successful diagnosis of infective endocarditis, in particular in culture-negative cases. In cases where otherwise broad-range antibiotics would be applied with adverse consequences for the patients' outcome, FISH in combination with PCR provides crucial information for successful targeted antibiotic therapy.