The objective of this study was to compare the microbial community in different biofilm-related diseases: endocarditis (n=18), chronic wounds (n=14), urinary catheter (n=24), central venous catheter (n=18), and prosthesis-related (n=9) infections. The presence of microorganisms was investigated using traditional culture-dependent methods and a range of culture-independent molecular methods including construction of clone libraries, sequencing, phylogeny, fingerprinting, FISH and quantitative PCR. In general all species detected by cultivation were also found by molecular methods. *Staphylococcus* spp were identified in 50% of the infections included in this study (n=83), and were identified in all chronic wounds, most prosthesis samples and on few urinary catheters. *Pseudomonas* spp were associated with 15% of the samples but found in all infection types, while *Stenotrophomonas* spp were abundant on catheter- and prosthesis-biofilm. *Streptococcus* spp were detected in endocarditis and prosthesis biofilms, whereas some species were primarily associated with one type of infection. Interestingly *Legionella* spp was detected in an infected heart valve by fingerprinting, specific q-PCR and in a clone library, but not by cultivation. In 75% of the investigated samples polymicrobial communities were detected and all urinary catheters, chronic wounds and prosthesis samples were polymicrobial as opposed to only 25% of endocarditis samples. FISH illustrated that microorganisms were often positioned locally in the biofilm. Some species generally appeared as microcolonies and other species as single cells in the same sample. In conclusion the significance of the findings needs further investigations, and future studies should focus on the development of optimal sampling, identification and treatment regimes.