Supplement 2: Minimal biofilm eradication concentration (MBEC) assays.

Several assays have been used to estimate the MBEC of different antibiotics using different microorganisms [1]. The data reported from these assays were usually obtained using reference microorganisms, for example, American Type Culture Collection (ATCC) strains (**Table 2**). In contrast to ATCC strains, clinical isolates may differ in genotype and phenotype leading to different antibiotic susceptibility profiles. In regard to the biofilm growth methods for MBEC assays, typically the plates are incubated in static conditions for a period of 24 hours. After growth, the biofilms are exposed to antibiotic substances for 24 hours and then survival detection is performed after an additional 24 hour subculture. A well-tested assay is the MBEC Assay® (Innovotech, Edmonton, Canada, formerly Calgary Biofilm Device), which assays antibiotic susceptibilities of biofilms grown on pegs that are on the lid of a 96-well plate [1]. Some adaptations of this assay have been described [2]. Another limitation related to MBEC assays is that the biofilms are usually cultured under favorable conditions. The expression of biofilm-related antibiotic resistance may be driven by mechanical and biochemical stress. Providing shear forces from flow or incubation on orbital shakers may be desirable. Biofilm growth is usually in nutrient-rich media without any apparent stressors such as unfavorable pH, O_2 tension, osmolality, nutrient availability, or host defenses (antibody and cellular). Such conditions are far from the reality of the infected tissue and implant surroundings. Low availability of nutrients, pH, O_2 tension and osmolality are all important factors that drive sessile phenotype and biofilm formation. Simulated tissue fluids could be manufactured with the aim to approximate the assay to clinical infection site reality. Tissue fluids, serum and synovial fluids are examples of media that could be used or even the patient's own synovial fluid is a consideration. Further, biofilm

susceptibility may be different on the flat, nonporous, hydrophobic plastic surfaces used in research based *in vitro* assays compared with the various surfaces that may be infected by biofilm *in vivo* [3]. Rather than polystyrene, the surfaces that should be considered for the biofilm growth *in vitro* are the biological tissues that are present in the clinical infection, as for example muscle and bone [4], or epithelial tissue, which could lead to more clinically representative results. Also the biomaterials present in medical devices should be considered for implant-related infection [5]. Finally, the choice of microorganisms should focus on patient specific pathogens. As routinely done by every hospital for MIC testing, the patient's infecting microorganisms should be considered for an MBEC assay.

References

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