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Settings of Conditions Leading to Reproducible and Robust Biofilm Formation in vitro in Evaluation of Drug Activity against Staphylococcal Biofilms

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Abstract : A loss of control over antibiotic-resistant pathogens has become a global issue due to severe and often untreatable infections. This state is reflected in complicated treatment, health costs, and higher mortality. All these factors emphasize the urgent need for the discovery and development of new anti-infectives. One of the most common pathogens mentioned in the phenomenon of antibiotic resistance are bacteria of the genus Staphylococcus. These bacterial agents have developed several mechanisms against the effect of antibiotics. One of them is biofilm formation. In staphylococci, biofilms are associated with infections such as endocarditis, osteomyelitis, catheter-related bloodstream infections, etc. To author's best knowledge, no validated and standardized methodology evaluating candidate compound activity against staphylococcal biofilms exists. However, a variety of protocols for in vitro drug activity testing has been suggested, yet there are often fundamental differences. Based on our experience, a key methodological step that leads to credible results is to form a robust biofilm with appropriate attributes such as firm adherence to the substrate, a complex arrangement in layers, and the presence of extracellular polysaccharide matrix. At first, for the purpose of drug antibiofilm activity evaluation, the focus was put on various conditions (supplementation of cultivation media by human plasma/fetal bovine serum, shaking mode, the density of initial inoculum) that should lead to reproducible and robust in vitro staphylococcal biofilm formation in microtiter plate model. Three model staphylococcal reference strains were included in the study: Staphylococcus aureus (ATCC 29213), methicillin-resistant Staphylococcus aureus (ATCC 43300), and Staphylococcus epidermidis (ATCC 35983). The total biofilm biomass was quantified using the Christensen method with crystal violet, and results obtained from at least three independent experiments were statistically processed. Attention was also paid to the viability of the biofilm-forming staphylococcal cells and the presence of extracellular polysaccharide matrix. The conditions that led to robust biofilm biomass formation with attributes for biofilms mentioned above were then applied by introducing an alternative method analogous to the commercially available test system, the Calgary Biofilm Device. In this test system, biofilms are formed on pegs that are incorporated into the lid of the microtiter plate. This system provides several advantages (in situ detection and quantification of biofilm microbial cells that have retained their viability after drug exposure). Based on our preliminary studies, it was found that the attention to the peg surface and substrate on which the bacterial biofilms are formed should also be paid to. Therefore, further steps leading to the optimization were introduced. The surface of pegs was coated by human plasma, fetal bovine serum, and L-polylysine. Subsequently, the willingness of bacteria to adhere and form biofilm was monitored. In conclusion, suitable conditions were revealed, leading to the formation of reproducible, robust staphylococcal biofilms in vitro for the microtiter model and the system analogous to the Calgary biofilm device, as well. The robustness and typical slime texture could be detected visually. Likewise, an analysis by confocal laser scanning microscopy revealed a complex three-dimensional arrangement of biofilm forming organisms surrounded by an extracellular polysaccharide matrix.

Keywords : anti-biofilm drug activity screening, in vitro biofilm formation, microtiter plate model, the Calgary biofilm device, staphylococcal infections, substrate modification, surface coating
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