

Steven, J. and **Nelson, SM.** (2018). Characterisation of a dual species perfusion biofilm model.

Abstract

An estimated 65–80% of human infections are biofilm-related. As the biofilm mode of growth significantly contributes to bacterial tolerance of antimicrobial treatment, this has important ramifications for patient morbidity and associated healthcare costs. Biofilm infections are commonly established by more than one bacterial species which demonstrate synergistic interactions and altered behaviour, presenting yet more challenges to effective treatment. The aim of this study was to characterise a new continuous perfusion model for the culture of dual species biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa* and to compare the antibiotic sensitivities of cells eluted from the system with biofilm cells.

Biofilms of either *S. aureus* or *P. aeruginosa* were established within foam plugs, continually perfused with nutrient broth and challenged with the other species after 24 h. Biofilms were disaggregated after a further 24 h and relative species numbers determined by viable counting. Antibiotic susceptibilities of disaggregated biofilm and eluted cells were assessed by disc diffusion.

P. aeruginosa predominated in dual species biofilms, reaching 2×10^9 cfu/biofilm after 48 h. The *S. aureus* population was consistently lower, even when established before *P. aeruginosa* challenge (4.47×10^8 cfu/biofilm at 48 h and 5.5×10^8 cfu/biofilm 24 h post-challenge). Antibiotic susceptibility testing demonstrated no significant difference between eluted and disaggregated *P. aeruginosa* biofilm cells. *S. aureus* small colony variants (SCVs) were isolated from challenged biofilms, a likely result of co-culture with *P. aeruginosa*, and of importance due to their resistant phenotype.