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**Title:** Is primary ciliary dyskinesia a “biofilm” disease?

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**Body:** Introduction Bacterial biofilms are structured communities of adherent bacteria enveloped in self-produced matrix which are refractory to antibiotics and the host immune response. They play a key role in the chronicity of respiratory infection in cystic fibrosis. Haemophilus influenzae is the most common pathogen isolated in primary ciliary dyskinesia (PCD). We hypothesise that impaired mucociliary clearance in PCD will predispose these patients to biofilm infections. Aims To investigate the biofilm-forming capacity of clinical H. influenzae isolates from PCD patients. Methods The biofilm-forming ability of 4 H. influenzae clinical isolates from different PCD patients were compared using a crystal violet (CV) assay, colony forming unit counts (CFUs), fluorescence in situ hybridisation (FISH) and scanning electron microscopy (SEM). Results CV staining demonstrated bacterial biomass adherent to plastic for all isolates (OD<sub>600</sub> 0.04 for isolate 1 to 0.12 for isolate 4, p<0.05). SEM permitted visualisation of a characteristic matrix surrounding the bacteria. Biofilm thickness varied for each isolate, qualified by FISH (13µm for isolate 1; 82µm for isolate 4). CFUs quantified the number of viable bacteria within each day 4 biofilm, ranging from 9x10<sup>6</sup> CFU/cm<sup>2</sup> in isolate 1 to 2x10<sup>8</sup> CFU/cm<sup>2</sup> in isolate 4. Conclusion We have characterised biofilm-forming capacity in all 4 H. influenzae isolates from PCD patients. 2 isolates from chronically colonised patients (over 4 years) consistently formed thicker, cell dense and structurally more complex biofilms than the other, more recently isolated, strains. These data suggest that H. influenzae is capable of biofilm formation and that PCD patients, like cystic fibrosis, might harbour bacteria in biofilms.