The *P. aeruginosa* burden of the CF lung – Uncovering the black box

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**Background:**
- *P. aeruginosa* is the predominant pathogen in CF (Høiby 1974, Baurnfeind et al. 1987, Koch 2002)
- *P. aeruginosa* accumulates in heaps, detected in CF sputum (Høiby 1977)
- *P. aeruginosa* forms microcolonies embedded in a matrix (Lam et al. 1980)
- *P. aeruginosa* colonizes in microcolonies which are localized mainly intraluminal in the CF lung (Baltimore et al. 1989)
- The bacterial density is highest in the bronchi (Potts et al. 1995)
- The CF lung is exposed to an extensive and ongoing PMN response due to the chronic infection of *P. aeruginosa*, which is impossible to eradicate (Armstrong et al. 1995)
- Survival has significantly increased the last 30 years due to aggressive anti-*P. aeruginosa* therapy (Koch and Høiby 2000)

**Aim:** The present study was performed to investigate the significance of the aggressive therapy for the distribution of *P. aeruginosa* and how *P. aeruginosa* is organized and persists in the CF lung. The material used was: autopsies from dead short term colonized CF patients (n=12) obtained before today’s aggressive antibiotic treatment (1975-78), and explanted lungs from present long term *P. aeruginosa* infected CF patients (n=3) (2006).

**Method:** Deparaffinized sections were analyzed by fluorescence in situ hybridization (FISH) using peptide nucleic acid (PNA) probes. A mixture of Texas Red-labeled, *P. aeruginosa*-specific PNA probe and fluorescein-labeled, universal bacterium PNA probe in hybridization solution (AdvanDx, Inc., Woburn, MA) was added to each section and hybridized. Mounting media with DAPI were applied, and a cover slip was added to each slide. Slides were read using a fluorescence microscope equipped with a fluorescein isothiocyanate (FITC), a Texas Red, dual FITC/Texas Red and DAPI/Texas Red filters.

**Before aggressive antibiotic therapy**
Representative selection of autopsies from 12 CF patients dead from ~3.5 years chronic *P. aeruginosa* infection.

**Summary:** *P. aeruginosa* biofilms are observed in vast amounts, both in the conductive zone and in the respiratory zone. Most of the tissue in both zones is extensively destroyed. Upper row shows damaged bronchi, middle row shows intraluminal biofilms, and lower row shows biofilms in and destroyed respiratory tissue.

**Conclusion:** Due to the high specificity of the PNA-FISH probes employed in this study, our results provide strong evidence for that before the aggressive antibiotic therapy *P. aeruginosa* infected and destroyed the CF lung due to fast spreading into the respiratory zone. Today antibiotics suppress but can not eradicate the bacteria from the conductive zone, whereas the remaining respiratory zone is protected from massive biofilm infection for prolonged time. The conductive zone serves as a reservoir, here the bacteria are organized in microcolonies embedded in pus, a trait which is independent on the time course of the infection and amounts of antibiotics. These microcolonies consisted solely of *P. aeruginosa*. In addition, we found no bacteria adhering to the epithelial tissue. The pus consisted mainly of leukocytes, surrounding the microcolonies. A smear of DNA and dead leukocytes were detected just around the microcolonies, possible due to the Quorum sensing dependent rhamnolipid killing recently described by us (Jensen et al. 2007).

We conclude that *P. aeruginosa* persists in the CF lung due to its ability to create microcolonies i.e. biofilms. Within these biofilms the bacteria are protected against antibiotics and the host defence.