

Cystic Fibrosis Research News

Title:

Effect of freezing sputum on *Pseudomonas aeruginosa* population heterogeneity

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What was your research question?

Many people with cystic fibrosis (CF) experience long-term (chronic) lung infection with bacteria called *Pseudomonas aeruginosa*. During potentially decades of chronic infection this bacteria is constantly 'attacked' by the body's own immune cells and exposed to frequent courses of antibiotics. As a consequence, the original infecting strain diversifies over time. The result of this diversification is a community of genetically related organisms that differ in the many traits including the ability to resist antibiotics and produce toxins. The existence of this related, but diverse population of bacteria in CF airways is fundamentally different from acute infections such as pneumonia, where all organisms behave the same. Importantly, this may explain why the prescription of antibiotics in CF based on laboratory studies that determine which antibiotics are effective against a single isolate poorly correlate with clinical outcomes. Quite simply, that isolate may not represent the diverse population of infecting organisms.

Our work looks to establish if people with CF might be able to collect and store serial sputum samples at home in their freezers and return them for later analysis in bulk, thereby decreasing the burden on them when assisting in studies assessing the role of *P. aeruginosa* communities.

Why is this important?

Other than recognizing that diversity exists in chronically infecting *P. aeruginosa* populations, we know very little about it. We do not know if changes in sub-populations

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associate with the occurrence of events such as pulmonary exacerbations or influence response to therapies. In order to understand the impact of these *P. aeruginosa* populations in CF, frequent assessments are required. However, dropping off sputum frequently poses a considerable burden on individuals. We believe that collecting serial samples at home, immediately freezing them and later transporting them to clinic may enable frequent assessments of *P. aeruginosa* population structure.

What did you do?

We enrolled ten stable people with CF with chronic *P. aeruginosa* infection and collected sputum from them. Half of the sample was processed immediately and plated on MacConkey Agar (used to isolate bacteria), from which 88 randomly chosen *P. aeruginosa* isolates were picked. The remainder of the sputum was frozen in a home freezer for 7 days and then thawed, and the same process followed.

What did you find?

As expected, freezing resulted in a 1000-fold drop in *P. aeruginosa* counts. However, across the 1760 isolates screened, no consistent difference in either antibiotic susceptibility nor virulence factor (effector molecules produced by bacteria damaging the host and releasing nutrients) production was observed between the two populations.

What does this mean and reasons for caution?

This tells us that freezing, whilst killing *P. aeruginosa*, does so indiscriminately and does not alter the distribution of traits in chronically infecting *P. aeruginosa* populations.

What's next?

Home collection and freezing of sputum may enable for the frequent and convenient assessment of *P. aeruginosa* populations in CF - enabling detailed studies assessing how *P. aeruginosa* population dynamics associate with events in CF.

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