Beyond Resistant or Susceptible a Framework for Heteroresistance and Within-host evolution of *Pseudomonas aeruginosa* in Cystic Fibrosis

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Background and Aim

Background: Chronic *Pseudomonas aeruginosa* infection remains a major driver of morbidity in cystic fibrosis (CF) and is closely linked to antimicrobial resistance (AMR). Within the CF airway, thick mucus, biofilms and repeated antibiotic exposure create an evolutionary niche in which *P. aeruginosa* can diversify into co-existing subpopulations with different resistance and tolerance profiles. Consequently, routine susceptibility testing may not fully represent the bacterial population responsible for persistent infection.[1,2]

Aim: This poster proposes a conceptual framework to explain how within-host diversity, heteroresistance and biofilm-associated tolerance may contribute to persistent infection and recurrent antibiotic exposure in CF. It also identifies measurable indicators for future evaluation, including longitudinal culture patterns, multi-isolate susceptibility profiles, genomic diversity signals and recurrence-related outcomes.

Framework Development

An evidence-informed conceptual framework was developed by linking established mechanisms of chronic CF-associated P. aeruginosa infection with possible discordance between routine susceptibility results and clinical response.

CF Airway Microenvironment
Thick mucus and spatially structured biofilms

→

Repeated Antibiotic Exposure
Ongoing selection pressure during treatment cycles

→

Within-Host Diversity
Heteroresistant minority and antibiotic-tolerant subpopulations

→

Clinical Discordance
Apparent susceptibility despite persistence or recurrence

Framework indicators: Longitudinal cultures • Multi-isolate susceptibility profiles • Genomic diversity signals • Recurrence-related outcomes

Proposed Mechanisms

The framework identifies three interacting mechanisms that may explain discordance between routine susceptibility results and clinical response:

1. Heteroresistance

Minority resistant subpopulations may remain undetected in an apparently susceptible sample and expand during antibiotic therapy.

2. Biofilm-Associated Tolerance

Bacteria embedded within CF mucus and biofilms may survive antibiotic exposure despite susceptibility in routine planktonic testing.

3. Within-Host Evolution

Repeated antibiotic treatment cycles may select for adapted phenotypes, promoting persistent infection and progression towards difficult-to-treat disease.

Potential consequences:
Apparent susceptibility → Persistent infection or recurrence → Further antibiotic exposure and selection pressure

Clinical implications

Routine susceptibility testing may not fully capture the complexity of chronic CF-associated P. aeruginosa infection.
The framework suggests three potential clinical implications:

1. Broader Microbiological Assessment

In persistent or recurrent infection, testing multiple distinct isolates or colony morphotypes may help identify clinically relevant within-host diversity.

2. Longitudinal Surveillance

Culture and susceptibility results should be interpreted over time alongside previous treatment response and recurrence patterns.

3. Informed Antimicrobial Stewardship

Recognising possible heteroresistance and biofilm-associated tolerance may support more informed antibiotic decision-making and reduce repeated ineffective treatment cycles.

From: Single-isolate “susceptible/resistant” classification
Towards: Diversity-aware monitoring of chronic CF airway infection

Conclusions & Work in progress

- Chronic CF-associated P. aeruginosa infection is a dynamic within-host ecosystem, in which heteroresistance, biofilm-associated tolerance and bacterial evolution may contribute to persistent infection despite apparent laboratory susceptibility.
- Incorporating within-host diversity through multi-isolate testing and longitudinal surveillance may improve understanding of treatment failure and support more informed antimicrobial stewardship.

References

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2. Diaz Caballero J, Cheang M, et al. Phenotypic diversity within a *Pseudomonas aeruginosa* population infecting an adult with cystic fibrosis. *Scientific Reports*. 2015;5:10932.