Inconsistencies in conventional culture *vs* molecular approaches: unveiling distinct dynamics in cystic fibrosis polymicrobial communities

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The complex cystic fibrosis (CF) microbiome has been inferred from molecular approaches, since culture-based methodologies are unreliable to detect polymicrobial biofilm-mediated infections. Still, CF microbiome profiling is primarily focused on identifying disease-causative agents, disregarding how CF ecosystems are influenced by external factors, dismissing implications for the disease and providing potential basis for clinical intervention. This study aimed at examining changes in microbial composition in CF polymicrobial (dual-/three-species) communities involving the CF-traditional pathogen (*Pseudomonas aeruginosa*, PA) and two less common species (*Inquilinus limosus*, IL, and *Dolosigranulum pigrum,* DP) challenged by different oxygen environments and following antibiotic intervention. Changes were evaluated through molecular methodologies (quantitative real-time polymerase chain reaction- q-PCR- and peptide nucleic acid probe-fluorescence *in situ* hybridization - PNA-FISH) and conventional culture techniques.

Results showed no significant differences in total cells detected by conventional and molecular techniques in biofilms developed under aerobic, microaerophilic or anaerobic conditions. However, estimation of PA, IL and DP cells within dual- and three-species biofilms was notably inconsistent, with q-PCR and PNA-FISH leading to higher microbial quantification in comparison with counts obtained by specific culture media. This variability enriched for PA+IL+DP biofilms, where both molecular techniques could detect up to 4-log more than cells estimated by culture. Once exposed to tobramycin, ciprofloxacin or aztreonam (antibiotics currently used to treat CF infections), viable but non culturable bacteria within three-species biofilms was clearly detected, with cells losing ability to grow on solid media but still being detected in greater numbers by PNA-FISH and q-PCR. This was particularly observed for all species in biofilms challenged by tobramycin (>5 ΔLog cells/cm^2, culture *vs* PNA-FISH), and for IL in biofilms exposed to ciprofloxacin (>5 ΔLog cells/cm^2, culture *vs* PNA-FISH or q-PCR) and to aztreonam (up to 3 ΔLog cells/cm^2, culture *vs* PNA-FISH). Intriguingly, q-PCR presented some shortcomings by showing lower sensitivity in DP detection (values limited to below 5 Log CFU/cm^2 under all circumstances), in comparison with conventional culture and even with PNA-FISH.

This study highlights incongruities in CF polymicrobial communities` dynamics depending on the methodology used to inspect the consortia. Generally, molecular methods afforded improved sensitivity in microbial detection/quantification within polymicrobial biofilms, compared with culture-based techniques. Aware of the requirements demanded by each technique (time-consuming, cost, easy-handling), the choice for the suitable approach should also rely on the community-residing microorganisms, aiming to give accurate comprehension of how polymicrobial infections respond to external stresses and contribute for disease progression.