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| **Identification of *Staphylococcus aureus* hypermutator strain through metagenomic sputum analysis** |
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| **Introduction/Aim:** Chronic bacterial infections in people with cystic fibrosis contribute to lung function decline and pulmonary exacerbations. Bacterial genomic traits are associated with increased pathogenicity, including hyper-mutator strains, which increase likelihood of *de novo* antimicrobial resistance development. Markers for hyper-mutator strains can be detected within airway metagenomes generated through the application of DNA sequencing technologies directly to clinical samples. Our aim was to utilise sputum metagenomes to identify pathogen traits of potential clinical importance.  **Methods:** Deep shotgunmetagenomic sequencing was performed on 321 sputum samples from 268 individuals from a national CF cohort. Reads aligning to *Staphylococcus aureus* and *Pseudomonas aeruginosa* were analysed to determine phylogenetic similarity and characterise hypermutator patterns. Suspected hypermutator phenotypes were confirmed by fluctuation analysis and whole genome sequencing (WGS) performed on pathogen isolates.  **Results:** Of 321 samples, sufficient reads aligned to *S. aureus* in120 (37.4%) and to *P. aeruginosa* in 81 (25.2%). We focused on one individual (16-year-old female, homozygous F508del), where four longitudinal samples contained the same *S. aureus* clone. The first three samples had near identical clones, with <3 single nucleotide polymorphisms (SNPs) difference. The last sample, however, had 50 SNPs difference. All SNPs were C>T or G>A transitions and included a gene involvedin DNA repair (A0A7U8F4T6), suggesting a hypermutator strain. Culture-based fluctuation analysis and isolate WGS confirmed a hypermutator phenotype. To identify potential explanators, clinical history between the third and fourth samples was investigated. While lung function was unchanged, there was a new co-infection with *Stenotrophomonas maltophilia,* which emerged subsequent to hospitalisation and IV antibiotics for a pulmonary exacerbation.  **Conclusion:** Metagenomic analysis enabled the emergence of a hypermutator *S. aureus* in a person with CF to be pin-pointed, demonstrating the potential clinical utility of culture-independent analysis.  **Grant Support:** NHMRC Project Grant (APP1102494), NHMRC Investigator Grant (APP2018745), CF Australia Innovation Grant |