Antimicrobial Resistance and Hypermutation in *Staphylococcus aureus* after Loss of Antibiotic Selection in Cystic Fibrosis

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Patients with cystic fibrosis (CF) have unremitting airway infections with *Staphylococcus aureus*. Chronic *S. aureus* infections require frequent antibiotics, increasing antimicrobial resistance (AMR). AMR occurs by several mechanisms, including acquisition of resistance genes like *SCCmec*, infection with a new strain that has existing AMR, or *de novo* evolution of AMR. Hypermutation facilitates evolution of AMR by allowing more mutations in the genes targeted by antibiotics. A new drug, Elexacaftor/Tezacaftor/Ivacaftor (ETI), corrects the host defense defects in CF by increasing cystic fibrosis transmembrane conductance regulator (CFTR) activity regulating salt/water on the surface of patient lungs (among other organs). Although patients remain infected by *S. aureus*, antibiotic use has decreased significantly. In patients who remain infected with *S. aureus*, we investigated what happens to AMR and hypermutation following ETI. We tested 97 patients' cryovial samples which were positive for *S. aureus* and tested each patient sample for phenotypic resistance to select antibiotics (oxacillin, erythromycin, chloramphenicol, and tetracycline) pre/post 2020 when ETI was started. Genetically, we investigated the loss of Methicillin-resistant *S. aureus* (MRSA) by collecting individual strain information for the DNA sequence of *mutL* and creating phylogenetic trees for each strain then cross checking those for strain replacement, *mutL* frameshift, and prevalence of *mutL* frameshift by era as a possible hypermutation switch for AMR. This study concluded that of subjects who remain infected with *S. aureus*, 75% have the same strain as they did before 2019 and the loss of MRSA by some subjects is typically associated with clonal replacement by susceptible strains. Additionally, methicillin resistance and hypermutation may be declining in patients with CF including subjects ineligible for ETI.