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

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Introduction

- Patients with cystic fibrosis (CF) have unremitting airway infections with *Staphylococcus aureus*.
- Chronic *S. aureus* infections require frequent antibiotics, which increases antimicrobial resistance (AMR).
- AMR occurs by several mechanisms, including acquisition of resistance genes like SCC*mec*, 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.

Example of de novo evolution of AMR

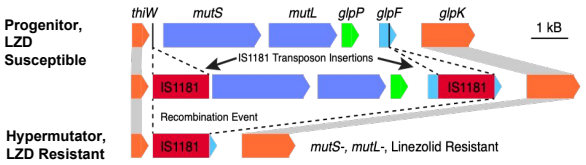

Fig 1. Deletion of DNA repair genes *mutS* and *mutL* facilitates linezolid resistance Adapted from Pitcher et al. bioRxiv 2023: 2023.2005.2002.539145.

A prevalent frameshift in *mutL:* possible hypermutation switch?

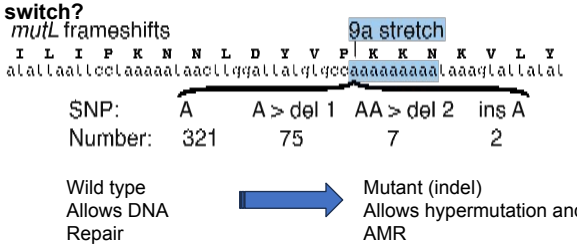

Fig 2. DNA sequence of *mutL* reveals a homopolymeric 9A stretch susceptible to frameshift mutations. Numbers represent ST5/ST105 *S. aureus* genomes collected before 2019; many of these isolates are from subjects not represented in this study.

Questions

- The new drug Elexacaftor/Tezacaftor/Ivacaftor (ETI) corrects the host defense defects in CF by increasing CFTR activity.
- Although patients remain infected by *S. aureus*, antibiotic use has decreased significantly.
- In patients who remain infected with *S. aureus*, **what happens to AMR and hypermutation following ETI?**

MRSA cultures pre and post ETI

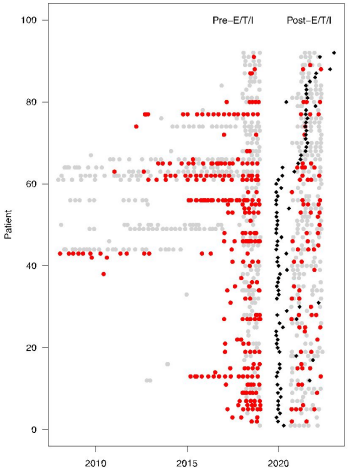

Fig 3. Culture timelines for patients with *S. aureus*. Culture: gray dots, MRSA: red dots, ETI start dates: black diamonds.

Fate of Pre-existing *S. aureus*

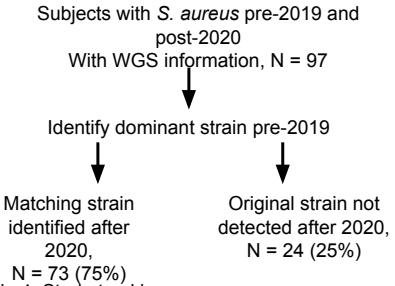

Fig 4. Strain tracking summary

Table 1. MRSA status by subject in each era

		2020-Present		
		MRSA (-)	MRSA (+)	Total
Pre-2019	MRSA (-)	34	9	43
	MRSA(+)	18	31	49
Total		52	40	92*

McNemar test p = 0.054.
*Testing of some isolates is pending

Phenotypic resistance to selected antibiotics before/after 2020

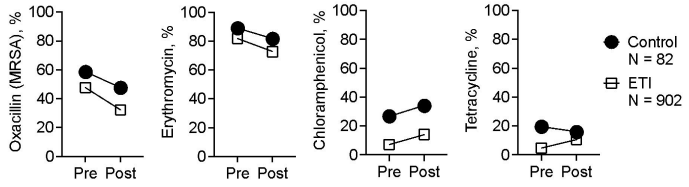

Fig 5. Declining methicillin resistance in isolates from patients ± ETI.

The loss of MRSA is mainly by strain replacement

Fig . Subjects who lost MRSA (shown with red boxes) usually had distinct strains after 2020. ST for each isolate are given inside the symbol.

Phylogenetic Tree Links:

ST5

ST8

Table 2. Prevalence of *mutL* frameshifts by Era

Era	Isolates Genotyped	*mutL-fs*	% *mutL-fs*
Pre-2019	659	10	*1.5%*
Post-2020	424	3	*0.7%*

Table 3. *mutL* frameshifts appear transiently

Subject	*mutL-fs* pre-2019	*mutL-fs* post-2020	Comment
10335	No	Yes	A new ST5 with *mutL-fs* was acquired
13594	Yes	No	An ST5 strain with *mutL-fs* was replaced by a different ST5 strain lacking the mutation
13699	No	Yes	A pre-existing ST8 evolved a new *mutL-fs* mutation
16656	Yes	No	An ST5 strain was detected before and after. A *mutL-fs* was only detected in the pre-era
19384	Yes	No	A transient infection with ST5 *mutL-fs* was detected in the pre-era
19925	No	Yes	A pre-existing ST5 evolved a new *mutL-fs* mutation
10033	Yes	No	An ST5 strain with *mutL-fs* was replaced by ST25

Conclusions

- Of subjects who remain infected with *S. aureus,* 75% have the same strain as they did before 2019.
- Methicillin resistance and hypermutation may be declining in patients with CF. This includes subject ineligible for ETI.
- The loss of MRSA by some subjects is typically associated with clonal replacement by susceptible strains.

Acknowledgements

REU was funded by the National Science Foundation. Research support through the CF Foundation and NHLBI.
Thank you to patients and families who participated in this research study.