Intrinsic Fusidic Acid Resistance within *Staphylococcus aureus* Mutants

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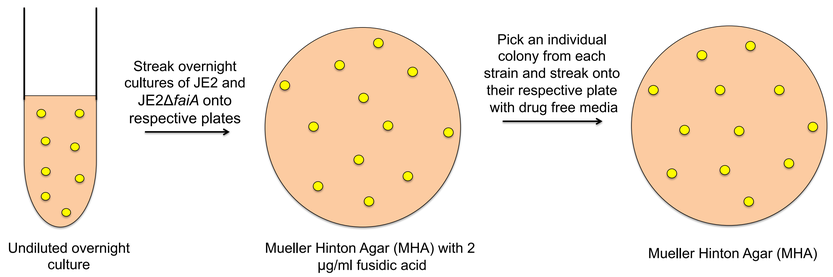
Background

Staphylococcus aureus is a Gram-positive pathogen that has the ability to both acquire genes and mutations that alleviates the affects of antimicrobials on the organism. The steroid antibiotic, fusidic acid, has been around since the 1960s and has been utilized to treat *S. aureus* infections primarily in Europe and the Commonwealth (1). Fusidic acid prevents bacterial protein synthesis by binding to elongation factor G (EF-G), guanosine diphosphate (GDP), and the ribosome; it works by stopping elongation (2). *S. aureus* resistance to fusidic acid is mediate by mutations in the gene *fusA* that encodes the target of fusidic acid, EF-G. Two genes, *faiA* and *faiB*, have been identified that code for assumed efflux pumps that are induced by fusidic acid; an *faiA* deletion mutant has also been created. This project focuses on whether these efflux pumps contribute to fusidic acid resistance within a mutated *fusA* strain. In order to do so, we isolated fusidic acid-resistant *S. aureus* mutants and determined if the JE2 fusidic acid resistance strains had a higher or lower MIC than JE2 with a *faiA* deletion.

To start the study, fusidic acid-resistant mutants for JE2 and JE2Δ*faiA* were selected for. We then used a minimum inhibitory concentration (MIC) assay to test which strain is more susceptible to fusidic acid. We also performed a Kirby-Bauer, or disk diffusion assay, to test different antibiotics and their resistance to the *S. aureus* mutant strains (3). We now present evidence that that the deletion of *faiA* affects fusidic acid resistance levels in a fusidic acid-resistant strain.

Figure 1. Isolation of fusidic acid-resistant *S. aureus* mutants



Methods

Isolation of fusidic acid-resistant *S. aureus* mutants: See Figure 1.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays: Overnight cultures for both the *S. aureus* mutants were made as above. These cultures were then diluted to an OD_{580nm} of 0.01 and added to test tubes containing fusidic acid in MHB. The test tubes were incubated for 24 hr at 37℃ after which the MIC was determined. To determine the MBC, 100 µL of test tubes containing the fusidic acid MIC and higher were then plated onto MHA plates and grown at 37℃ for 24 hrs. (Table 1). The MBC was the concentration where no growth was observed.

Kirby-Baurer disk diffusion assay: Overnight cultures were diluted to an OD_{580nm} of 0.1 and spread onto large petri plates (150 X 15 mm, Fisher Scientific, Pittsburg, PA) containing MHA with a sterile cotton swab. The following antibiotic discs: ciprofloxacin, levofloxacin, tigecycline, gentamiacin, ceftriaxone, rifampin, clyndamiacin, ceftazidime, fusidic acid, vancomycin, tetracycline, and chloramphenicol were then diffused onto the agar and the plates were incubated at 37°C for 24 hr. After incubation, zones of inhibition were measured.

Table 1. Fusidic acid MIC and MBC assays

Strain	MIC[a]	MBC[b]
JE2	2	16
JE2-FA-R-1	64	1024
JE2-FA-R-2	64	1024
JE2-FA-R-3	64	1024
JE2Δ*faiA*	1	16
JE2Δ*faiA*-FA-R-1	64	512
JE2Δ*faiA*-FA-R-2	16	512
JE2Δ*faiA*-FA-R-3	64	1024

MIC, minimum inhibitory concentration; MHB, minimum bacterial concentration
a: Concentration of fusidic acid (µg/ml)
b: Concentration of fusidic acid (µg/ml)

Table 2. Kirby Bauer disk diffusion assay for JE2 mutants

Antibiotic	JE2	JE2-FA-R-1	JE2-FA-R-2	JE2-FA-R-3
Ciprofloxacin	Resistant	Resistant	Resistant	Resistant
Levofloxacin	Intermediate	Intermediate	Intermediate	Intermediate
Tigecycline	Susceptible	Susceptible	Susceptible	Susceptible
Gentamiacin	Susceptible	Susceptible	Susceptible	Susceptible
Ceftriaxone	Susceptible	Intermediate	Susceptible	Resistant
Rifampin	Susceptible	Susceptible	Susceptible	Susceptible
Clyndamiacin	Susceptible	Susceptible	Susceptible	Susceptible
Ceftazidime	Intermediate	Susceptible	Susceptible	Resistant
Fusidic Acid	Susceptible	Resistant	Resistant	Resistant
Vancomycin	Susceptible	Susceptible	Susceptible	Susceptible
Tetracycline	Susceptible	Susceptible	Susceptible	Susceptible
Chloramphenicol	Susceptible	Susceptible	Susceptible	Susceptible

Table 3. Kirby Bauer disk diffusion assay for JE2Δ*faiA* mutants

Antibiotic	JE2Δ*faiA*	JE2Δ*faiA*-FA-R-1	JE2Δ*faiA*-FA-R-2	JE2Δ*faiA*-FA-R-3
Ciprofloxacin	Resistant	Resistant	Resistant	Resistant
Levofloxacin	Resistant	Resistant	Resistant	Intermediate
Tigecycline	Susceptible	Susceptible	Susceptible	Susceptible
Gentamiacin	Susceptible	Susceptible	Susceptible	Susceptible
Ceftriaxone	Intermediate	Resistant	Intermediate	Intermediate
Rifampin	Susceptible	Susceptible	Susceptible	Susceptible
Clyndamiacin	Susceptible	Susceptible	Susceptible	Susceptible
Ceftazidime	Resistant	Intermediate	Intermediate	Intermediate
Fusidic Acid	Susceptible	Resistant	Resistant	Resistant
Vancomycin	Susceptible	Susceptible	Susceptible	Susceptible
Tetracycline	Susceptible	Susceptible	Susceptible	Susceptible
Chloramphenicol	Susceptible	Susceptible	Susceptible	Susceptible

Results

- Using the method as shown in Figure 1, we were able to isolate fusidic acid-resistant mutants of both strains in order to test their MIC and MBC . We were able to successfully obtain colonies of each fusidic acid resistant strains on their respective plate with no mutations. To ensure none of the cultures had been contaminated with anything other then *S. aureus*, each plate was Gram stained in order to ensure all cells were Gram-positive.
- As shown in the MIC an MBC assay data, the fusidic acid-resistant strains had around the same MIC for the JE2 fusidic acid-resistant strains and the JE2 fusidic acid resistant strains containing the *faiA* deletion. However, the JE2 fusidic acid-selected *fusA* mutants had a higher minimum bacterial concentration than the JE2Δ*faiA* strains.
- The Kirby Bauer disk diffusion assay demonstrated that all fusidic acid-selected *fusA* mutants demonstrated fusidic acid resistance. Minor differences in altered susceptibility were observed for other antibiotics however these results were unexpected and need to be confirmed. We used the Kirby Bauer method because it is possible to test more than one antibiotic at a time which is advantageous when trying to determine the antibiotic susceptibility profile for a newly selected strain.

Conclusions

- The deletion of *faiA* had only a relatively small impact on the level of fusidic acid MICs/MBCs expressed by the fusidic acid selected fusA mutants.
- Selection for fusidic acid-resistance (*fusA* mutation) may have altered susceptibility to select antibiotics, but these results need to be statistically analyzed.

References

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