

# Occurrence of MRSA on Fish in the Hillsborough River

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## ABSTRACT

MRSA is a drug-resistant strain of *Staphylococcus aureus* known to cause frequent infections in humans. Water quality assessments have identified MRSA at varying levels throughout the Hillsborough River which indicates that MRSA may be present on the organisms that reside in those waters. In this experiment, Fish were collected from multiple sites throughout the Hillsborough River and swabbed to assess the presence of MRSA on the organism. Bacterial samples were grown on Mannitol Salt Agar, isolated, and tested for drug-resistance using the Kirby Bauer Disk Diffusion Assay. MRSA was isolated from the scales and inside the mouths of fish at multiple sites on the Hillsborough River. The presence of MRSA on these fish could potentially pose a health risk to fisherman who encounter the fish harboring MRSA isolates. Fisherman with an open wound on their hand that handle these fish could acquire a MRSA infection and require subsequent medical treatment as a result.

## 1 INTRODUCTION

The Hillsborough River runs from Green Swamp through Downtown Tampa and into the Hillsborough Bay. The river is a hub for recreational activities such as boating, kayaking, and fishing but the water in the river is not monitored regularly for pathogenic bacteria. Previous samples we have collected from the Hillsborough River had a high presence of *Staphylococcus aureus* and a drug-resistant form of the bacterium known as Methicillin Resistant *Staphylococcus aureus* (MRSA). *Staphylococcus aureus* is commonly known to be an opportunistic pathogen that can cause infection in humans and is present as part of the normal microflora in one-third of the human population (Gorwitz et al., 2008). As a pathogen, *S. aureus* is the leading cause of bloodstream infections in humans (?).

The presence of *S. aureus* in the water can lead to the bacterium being present on the scales and in the mouths of fish that reside within the river. This can lead to several problems, including the spread of antibiotic resistance and possible onset of infection in humans from cuts when handling the fish. Antibiotic resistance would primarily be spread due to fish carrying the bacteria that attach to them further up- or downriver and even out into the bay. The bacteria that are carried to new locations can share their antibiotic resistance with other bacteria via a process called conjugation (Gama et al., 2017). Alternatively, the bacteria with resistance can replicate and begin a new community of drug-resistant bacteria in the location that the fish carried the bacteria to. This acclimation of drug-resistant bacteria in a new bacterial community would lead to the potential of more dangerous infections than would have previously been acquired in the area. This spread of drug-resistance in the environment makes monitoring of bacterial communities a topic of importance to researchers and public health specialists.

An infection caused by *S. aureus* originating in the river would be classified as community acquired (CA). CA-MRSA infections tend to cause abscesses and cellulitis in humans (DeLeo et al.,

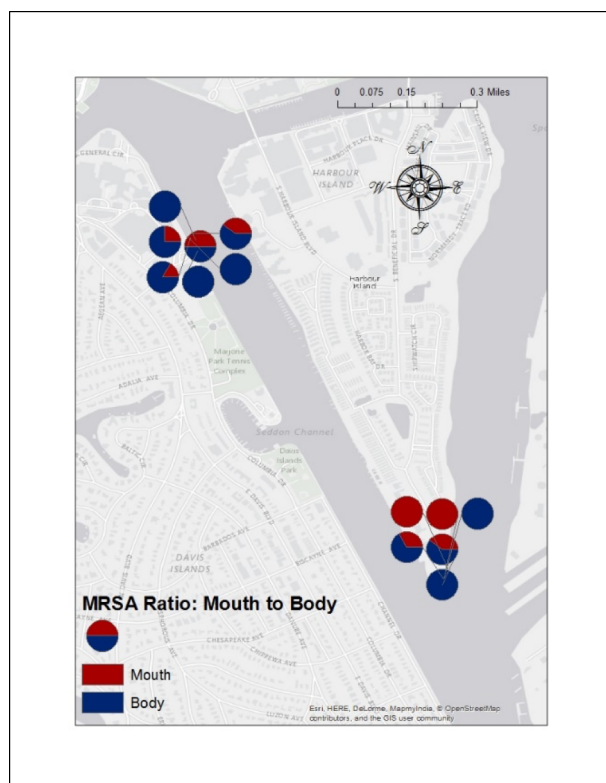
2010). Abscesses and cellulitis occur in the skin, indicating that the point of infection for the pathogen is a break in the skin. This makes contact with the water in the river potentially dangerous for people that have any breaks in the skin, however, swimming is not a popular recreational activity that occurs in the Hillsborough River. This does not eliminate the risk of infection, rather it shifts the focus to another community that is at risk. Fisherman frequently cut their hands while fishing in the river, either from handling the fish or from working with the hook or other tools. The open wounds that are exposed to the bacterium because of this provide a suitable environment for the onset of infection. As such, it is important to monitor the presence of *S. aureus* in the river and even on the fish that reside within it so that this risk of infection can be accounted for.

The purpose of this project was to determine the amount of *S. aureus* and MRSA on the scales and in the mouth of fish at a site with reportedly high levels of *S. aureus* compared to a site down-river. This information aids in our understanding of risk to fisherman who handle the fish that are caught in the Hillsborough River. Fisherman with open wounds on their hands could acquire an infection if the handled fish have *S. aureus* residing on their bodies or in their mouths. Assessing the occurrence of the bacteria on the fish could lead to a determination of the probability of fisherman encountering the bacteria while they are handling the fish. The project further assisted in our knowledge of pathogenic bacteria in the Hillsborough River. The current extent and abundance of *S. aureus* within the river is unclear; therefore, collecting data regarding the bacteria in the river would provide some insight into the bacterial communities that reside within it.

## 2 MATERIALS AND METHODS

Fish were captured using a rod and reel on the Hillsborough River at two pre-determined sites (control and treatment). The treatment site was selected because a hospital discharge pipe deposits water into the river at that location and previous work demonstrated high levels of MRSA (Froeschke et al. In Review). The control site was chosen because it has no discharge flowing into the water in that area. Captured fish were swabbed using two sterile cotton swabs and GPS coordinates for latitude and longitude were recorded using a handheld GPS each time a fish was captured. Swabs were taken from the mouth and from the body of each fish. Bacterial samples were transferred from the swabs to prepared mannitol salt agar plates. The agar plates were stored in a cooler to be transferred back to the lab.

Transferred plates were incubated at 37 °C for 24 hours. After the incubation period, colony counts were taken for *S. aureus*. Ten colonies were selected at random to undergo antibiotic susceptibility testing. Antibiotic susceptibility assays were conducted using the Kirby Bauer Disk Diffusion method. The assays were conducted on prepared Mueller-Hinton agar plates. Antibiotic susceptibility assays were conducted using oxacillin and, if a strain was resistant to oxacillin, then the bacteria were subjected to assays using vancomycin and tetracycline. Strains that were resistant to

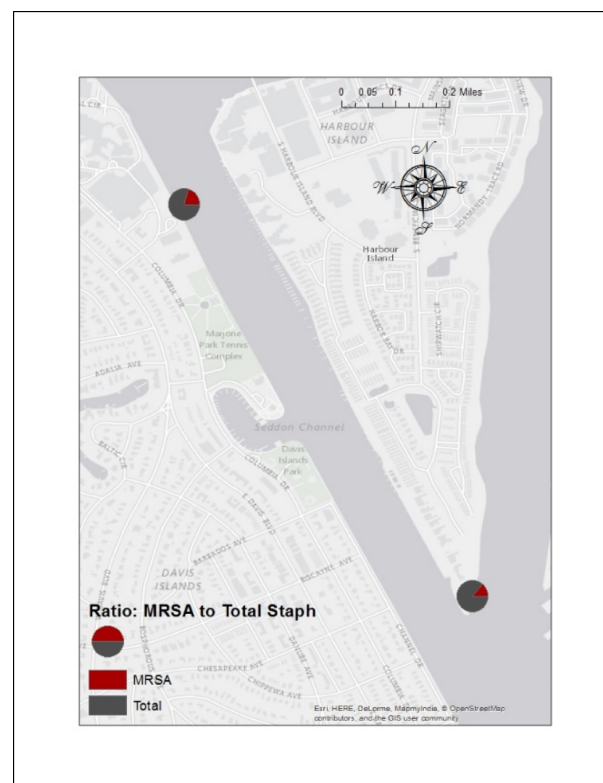


**Fig. 1.** The ratio of MRSA isolates obtained from the mouth of the fish to the amount of MRSA isolates obtained from the body of the fish. The figure displays the ratio for seven fish from the treatment site (top right) and six fish from the control site (bottom left).

oxacillin were classified as MRSA. Strains that were resistant to oxacillin and vancomycin were classified as Vancomycin Resistant *Staphylococcus aureus* (VRSA). GPS coordinates for latitude and longitude were used to create spatial maps using Arcmap 10.4.2. Spatial analysis was conducted to determine the number, location, and drug resistance of bacterial samples collected from each fish at each site.

### 3 RESULTS

A total of 13 fish were captured during the months of October and November 2016. The species caught included sheephead, red drum, catfish, pinfish, and ladyfish. *Staphylococcus aureus* colonies were isolated from all fish. MRSA was isolated from nine out of thirteen (69.2%) of the fish. The ratio of MRSA isolates recovered from the mouth of the fish compared to the body of the fish can be seen in Figure 1. Overall, 23.5% of the total MRSA isolates were found in the mouth of the fish and the rest (76.5%) were found on the bodies of the fish (Figure 1). Approximately 14.2% of the bacteria tested for antibiotic susceptibility were MRSA (Figure 2). The total number of MRSA isolates collected from the body and from the mouth of each fish can be seen in Figure 3 and Figure 4 respectively. The highest number of MRSA isolates recovered from one of the areas on the fish was ten and lowest was zero (Figures 3 and 4). 56% of the total



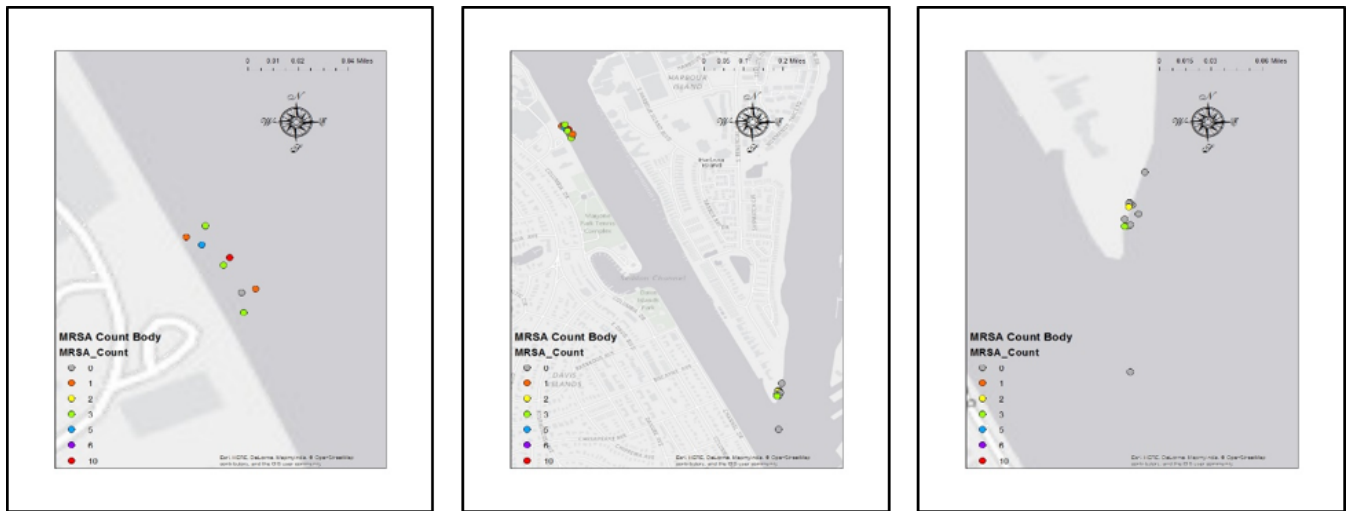
**Fig. 2.** The ratio of MRSA isolates to that of the total amount of staphylococcus isolates obtained from each location. This figure displays the ratios at both the control (bottom left) and the treatment (top right) sites.

isolates were found at the treatment site and 44% of the total isolates were found at the control site (Figure 5). 67% of the MRSA isolates were found at the treatment site and 33% of the MRSA isolates were found at the control site (Figure 6).

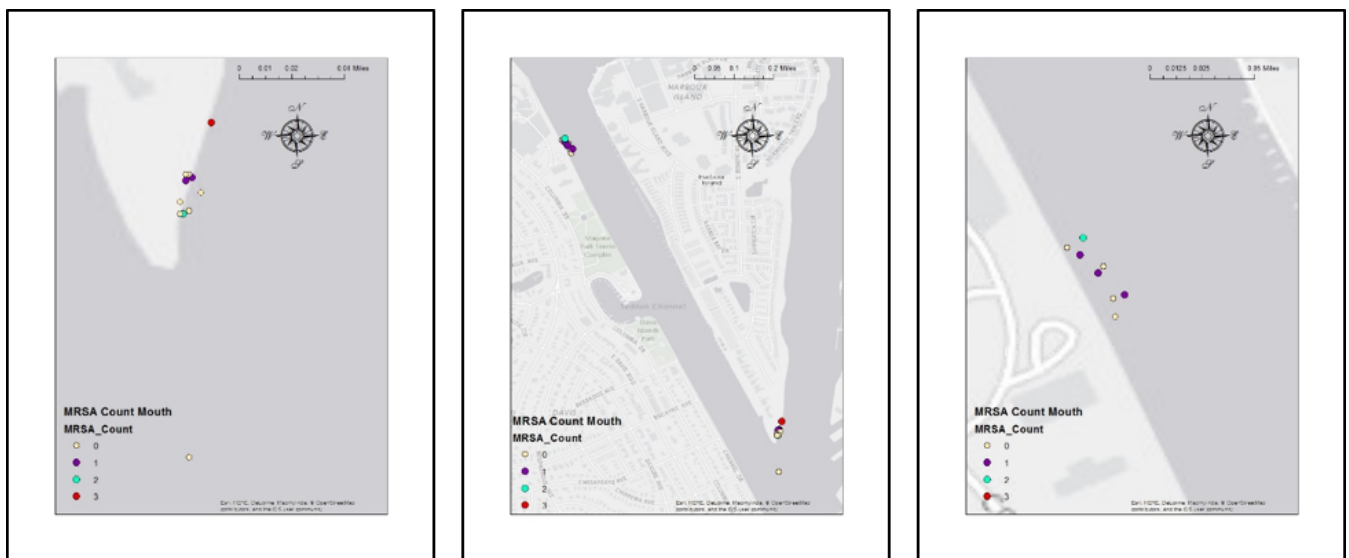
### 4 DISCUSSION

The results indicated MRSA was more likely to be collected from the body of a fish than from the mouth. This may be due to the greater amount of surface area contact between the fish's body and the surrounding water. Furthermore, most of the *S. aureus* colonies isolated from the fish are not drug-resistant, however, those that are resistant pose a considerable risk of infection. MRSA was more likely to be found on the bodies of the fish but that it is also more likely to be found on fish that are captured at the treatment site. Furthermore, the data indicated that MRSA was more likely to be found at the treatment site than at the control site. This is of interest to researchers because it indicated that the discharge pipe at the treatment site was potentially contributing to the level of antibiotic resistance that was seen in the samples.

A possible reason behind this would be that antibiotics or other pollutants are being discharged into the river at the treatment site. The selective pressure that these pollutants may induce on the bacteria in the water may lead to the death of less resistant bacteria and/or to the formation of biofilms. The death of the less



**Fig. 3.** The number of MRSA isolated collected from the body of each fish. The middle map displays both the control site (bottom right) and the treatment site (top left). Magnified versions of the control site (bottom right) and the treatment site (top left) are also provided.

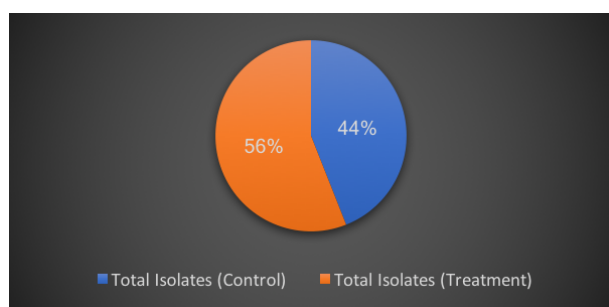


**Fig. 4.** The number of MRSA isolates collected from the mouths of the fish at each site. The map in the middle displays both sites with the control on the bottom left and the treatment site on the top right. Magnified displays of the treatment site (right map) and the control site (left map) are also provided.

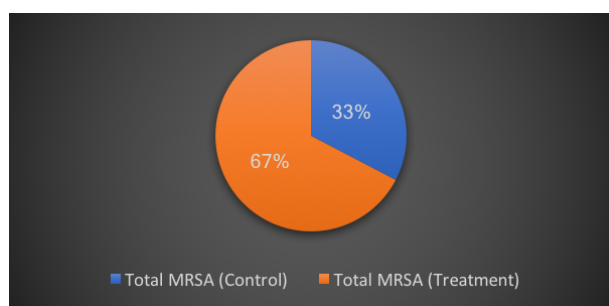
resistant bacteria due to the selective pressure of the discharge pollutants could lead to lower competition rates among the bacterial communities. This, in turn, would allow the resistant bacteria to replicate and replace the less resistant bacteria. The formation of biofilms is an even more alarming alternative mechanism of drug-resistance formation. Biofilms are extracellular matrices created by bacterial communities that require large energy expenditures; however, the creation of these biofilms bolsters the bacterial communities drug-resistance (Saville et al., 2011; Stewart & Costerton, 2001). The drug-resistance induced by the formation of biofilms in these bacterial communities make infections in humans

much more difficult to treat and, as a result, makes the infection more dangerous to the host (Stewart & Costerton, 2001). As such, it is imperative that the presence of these bacterial communities be monitored to minimize the risk of possible infections in people that use the river for recreational activities.

Fisherman would have an increased risk of infection due to exposure with the water indirectly through contact with captured fish, which carry the potentially dangerous bacteria. Fish hook related injuries were outlined in a recent study by Francesco et al. (2010). The wounds that are inflicted due to the fish hooks provide the opportunity that *S. aureus* needs to establish infection:



**Fig. 5.** The ratio of isolates found at the treatment and control sites.



**Fig. 6.** The ratio of MRSA found at the treatment and control sites.

a break in the skin. The data collected in this experiment indicated that the bacteria was present on the fish and, therefore, suggests that *S. aureus* infections are a potential risk to fisherman on the Hillsborough River. The risk would be higher at the treatment site due to the higher levels of bacteria that are present on the fish at those sites. Monitoring these bacterial levels could help prevent

potential infections in the future and prepare fisherman and medical personnel to handle them more effectively. Future studies should attempt to assess the cause behind the higher levels of antibiotic resistance at the treatment site. Discharge and river water can be collected from the treatment site and an extraction process can be conducted to determine the chemical contents of the samples. This would allow researchers to identify potential pollutants, such as antibiotics, that may be contributing to the development of antibiotic resistance in the bacterial communities that reside in the river. More information on the contents of the water could lead to the formation of policies and techniques that could prevent the pollution of the river with these stress-inducing chemicals. This would assist in curbing the development of antibiotic resistance in the bacterial communities and may reduce the risk of infection rates in fisherman. Fish captured could also be tagged and monitored via GPS to determine the extent of which they may have transferred the drug-resistant bacteria in the local environment.

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