

**Prevalence of MRSA Colonization in Surfers Following Exposure in Select Southern
California Coastal Waters**

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Abstract

Antibiotic resistant bacteria (ARB) are jeopardizing contemporary medicine's capacity to treat infections and diseases. To combat antibiotic resistance, it is critical to understand the fate and transport of both ARB and antibiotic-resistance genes (ARGs) in the environment. Recreational coastal waters may serve as an underappreciated reservoir of ARB and ARGs, ultimately increasing the likelihood of beachgoer exposure to pathogens. To identify how the human microbiome is affected by marine environmental exposure to ARB, specifically Methicillin-resistant *Staphylococcus aureus* (MRSA), and ARGs, surfers were selected due to their frequent exposure to and submergence in ocean water. Nose swab samples were obtained from surfers at two popular Santa Monica Bay surfing beaches, El Porto and Venice Beach, and culture-based methods were used to detect the presence of MRSA in each participant. A nonsurfer control group was recruited to provide nasal swab samples for comparison. Ocean water samples were also collected at each surf spot and later analyzed for the presence of MRSA and ARGs using qualitative polymerase chain reaction (qPCR). These results indicate that surfers were nearly two times more likely to be colonized by MRSA than nonsurfers. Surfers were also approximately eight times more susceptible to MRSA colonization during the first flush (precipitation above 0.75 inches). Similarly, there were higher concentrations of MRSA and ARG copies in both El Porto and Venice Beach water during the flush period, indicating that ocean water quality was significantly degraded by stormwater. These results indicate that surfers should especially avoid ocean exposure during early-season storm events.

1. Introduction

According to the Centers for Disease Control and Prevention (CDC), around two million people in the United States contract an antibiotic resistant infection per year, resulting in at least 23,000 associated mortalities (CDC, 2018). The rise of ARB jeopardizes the capacity to treat infections and diseases (Meek et al., 2015). The World Health Organization states that antibiotic resistance is one of the most significant threats to global health, food security, and development today (WHO, 2018). Thus, it is well agreed upon within the scientific community that antibiotic resistance poses a massive public health risk due to the consequential increase in scope and frequency of lethal diseases that will ultimately become untreatable (Huttner et al., 2013; Bartlett et al., 2013; Michael et al., 2014; Watkins & Bonomo, 2016). There are already several existing pathogens that are resistant to antimicrobial agents such as *Salmonella*, *Staphylococcus aureus*, *Enterococcus*, *Streptococcus pneumoniae*, and tuberculosis (CDC, 2013; Jiang et al., 2013; Huttner et al., 2013; Economou & Gousia, 2015; Kim et al., 2016). These antibiotic-resistant strains of pathogens are often associated with more severe and prolonged illnesses (Travers & Barza, 2002). The prevalence of antibiotic resistance has caused the CDC to declare that the world is now in a post-antibiotic era (CDC, 2013).

Antibiotics are chemotherapeutic agents, which diminish the growth of microorganisms through the inhibition of growth or other inhibitory mechanisms (Kümmerer, 2009). Antibiotic resistance arises from naturally-occurring genetic variations in bacteria which allow some bacteria to be resistant to antibiotics. This resistance develops while the antibiotics work to eradicate susceptible bacteria, causing selection for drug-resistant bacteria (**Figure 1**). Horizontal gene transfer (HGT), a biological mechanism by which antibiotic-susceptible bacteria inherit ARGs, further accelerates antibiotic resistance (Landers et al., 2012; Suzuki et al., 2017). Literature cites horizontal gene transfer as being variable in marine systems for a variety of reasons (Suzuki et al., 2017). Antibiotics can persist in the environment before their complete degradation, and the surrounding microbial ecosystem is selected to become resistant once exposed (Baquero et al., 2008). Dense bacterial concentration along with swift generation times provides microbes the efficiency required to successfully adapt to environmental change (Michael et al., 2014).

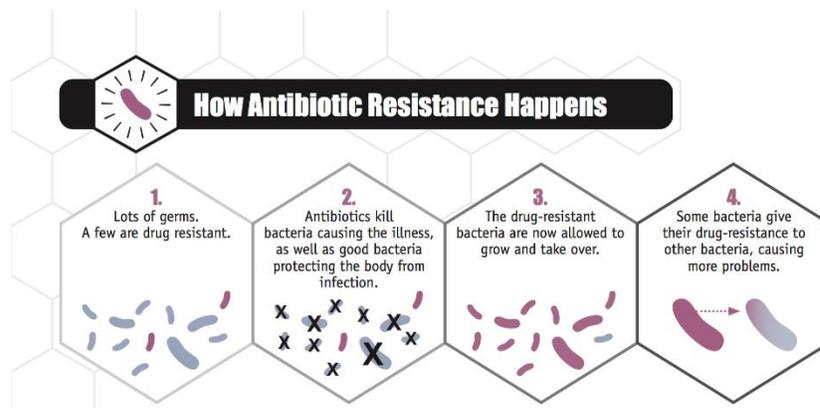


Figure 1. The Development of antibiotic-resistance. Antibiotic-resistance occurs naturally due to exposure to antibiotics (CDC, 2013).

The effects of environmental ARB on the human microbiome is unknown. The composition of the human microbiota is complex due to a variety of internal and external factors that influence individual genetic changes from HGT mechanisms, including phage transduction, transformation, and conjugation (Brinkac et al., 2017). As defined by Brinkac et al. (2017), transduction occurs when genetic material is collected from viruses due to either errors resulting in the transmittance of non-viral DNA or because the virus codes for the gene. Transformation ensues when an organism can ingest external DNA from the surrounding environment into its own genome. Lastly, conjugation takes place when genetic material is transferred from cell to cell. This transfer is facilitated by plasmids that carry both functional and replication genes. The detectability of bacterial plasmids, and their respective trends of genetic changes via conjugation, have allowed for observation of the highest rates of genetic exchange of antimicrobial resistance through HGT between microbes from farm animals, the human gut, and human foods (Brinkac et al., 2017).

The bulk of the dissemination of antibiotic resistance into the environment is caused by animal agriculture, wastewater treatment plants (WWTP), and overuse/overprescription in clinical practices (Michael et al., 2014; Rizzo et al., 2013). In the United States, livestock receive 80% of all administered antibiotics for the purpose of disease prevention, disease treatment, and growth promotion (Hollis and Ahmed, 2013; U.S. Food and Drug Administration, 2016; Cuong et al., 2018). Potential routes of ARB transmission to humans from agricultural animals or livestock are food consumption, direct contact with animals, or contaminated water (Tang et al., 2017). Agricultural runoff, effluent from WWTP and hospitals, or stormwater discharge can also

serve as potential sources of ARB exposure in urban creeks and lakes (Zhang, 2016; Rizzo et al., 2013; Kümmerer, 2009; Michael et al., 2013; Jiang et al., 2007). Therefore, these and other urban environments serve as potential sources of ARB exposure to humans via recreational use, bathing, ingestion, and aerosol inhalation in waterways.

Pathogenic bacteria can enter coastal waters through polluted stormwater discharge, which is a source of exposure for recreational beachgoers (Leonard et al., 2018). This is a major public health concern, as California beaches consistently receive approximately 550 million visitors annually (Izbicki et al., 2011). Given the magnitude of antibiotic overuse in healthcare and agriculture, humans face a potentially high risk of transmission of ARB or ARGs through exposure to ocean water. While there is no question that the rise of antibiotic resistance poses a threat to human health, there are still gaps in knowledge regarding the fate of ARGs in the environment. Thus, studies focusing on the relationship between ARB and ARGs environmental exposure and the human microbiome are essential.

1.1 Research Objective

This project aims to identify how the human microbiome is affected by marine environmental exposure to ARB, specifically Methicillin-resistant *Staphylococcus aureus* (MRSA), and ARGs. Both non-resistant *S. aureus* and MRSA has been detected in seawater and beach sand (Goodwin et al., 2012). High abundance of *S. aureus* has been associated with human activity and high bather density. However, there is a literature gap on the ocean's role in exposing humans to these and other opportunistic pathogens. In order to assess the risk of antibiotic-resistance to humans, it is necessary to understand how exposure to ARB and ARGs in seawater affects the human microbiome.

Surfers are an ideal population for evaluating the relationship between environmental exposure and ARB and ARG colonization. Surfing involves a high frequency of unanticipated head submersions as well as exposures of long duration (Harding et al., 2015). Surfers are in the ocean year-round, but particularly in the winter when storms occur. Storms often result in poor ocean water quality due to the influx of urban stormwater contaminants (Arnold et al., 2017). Surfers will often either ignore or miss warning signs regarding water quality. One survey reported that approximately 40% of surfers were not sure whether they had surfed during a health advisory, while 28% reported that they proceeded to surf despite knowing a health

advisory was posted (Harding et al., 2015). In addition, surfers also swallow up to 170 ml/day of water, which is considerably more than other beachgoers (Stone et al., 2008). Specifically, in Southern California, urban stormwater discharged into the ocean carries high concentrations of pathogens that can directly affect beach-goers - most importantly after the first flush (Steele et al., 2018). The ‘first flush’ occurs when built-up urban pollution is washed out by the first major rain event of the season (precipitation above 0.75 inches) and is subsequently discharged into the ocean (Taebi & Droste, 2004). Consequently, surfing immediately after the first flush may pose a higher risk of exposure to ARB and ARGs.

Two Los Angeles surfing beaches, Venice Beach and El Porto (City of Manhattan Beach), were identified as potential study sites due to their popularity amongst surfers and other recreational beachgoers. The surrounding water bodies in the Venice and El Porto Beach areas were found to be classified under the “impaired waterbodies” category provided by County of Los Angeles, Bureau of Land Management, Esri, HERE, Garmin, USGS, NGA, EPA, USDA, and NPS (**Figure 2**). Additionally, the Los Angeles County regional sewage pump locations were mapped utilizing arcGIS to assess the location of Venice and El Porto relative to pump locations (**Figure 3**). Based on analysis of previous literature identifying sewage runoff as sources of ARBs and ARGs, Venice and El Porto were both locations with high levels of exposure sources.

A prospective study looking at the effects of exposure to antibiotic resistant bacteria on the human microbiome was conducted between October 1, 2018 and April 24, 2019. The study’s intent was to demonstrate changes in the human microbiome following specific exposure events (in this case, impaired water as route of exposure). Both biological nasal samples and ocean water quality samples were collected, monitored, and analyzed on a weekly basis. The ocean water samples were analyzed for the following antibiotic resistant genes: *sul1*, *sul2*, *bla_{SHV}*, *intI1*, and *ermB*. These genes, such as *sul1*, may act as indicators of resistance contamination in urban runoff and are known for their propensity to persist in the environment (Roberts et al., 2009; Goodwin et al., 2012; Wang et al., 2018). Altogether, this project aims to yield an improved understanding of surfer health as well as the role of environmental exposure to MRSA and ARGs.

Venice and El Porto

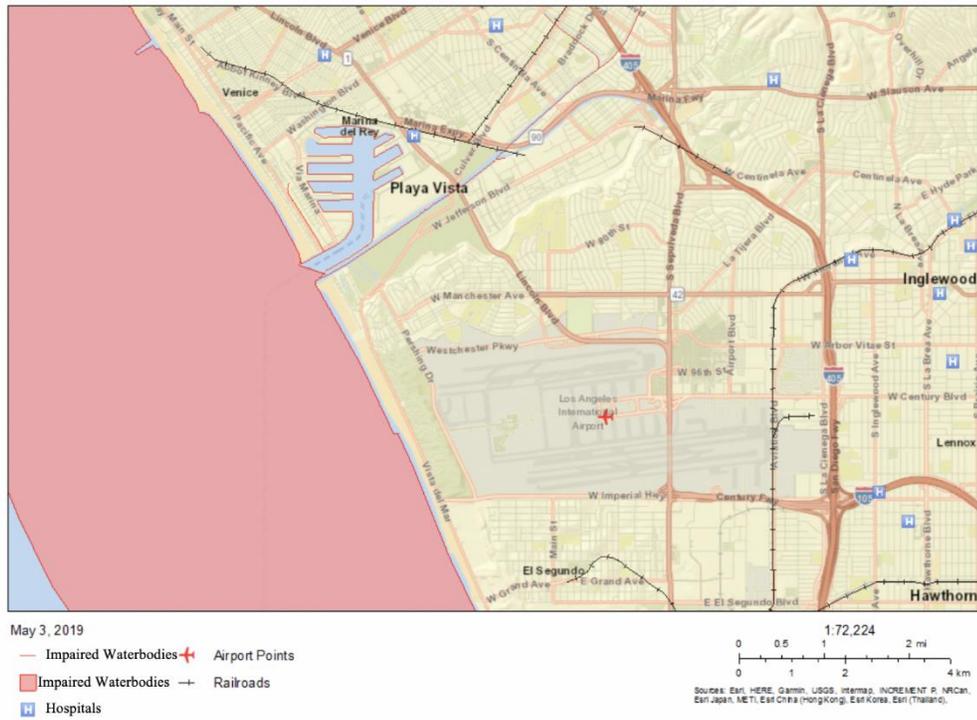


Figure 2. Locations categorized as “Impaired water bodies” near Venice and El Porto using United States EPA EnviroMapper.

Regional Sewage Pump Locations

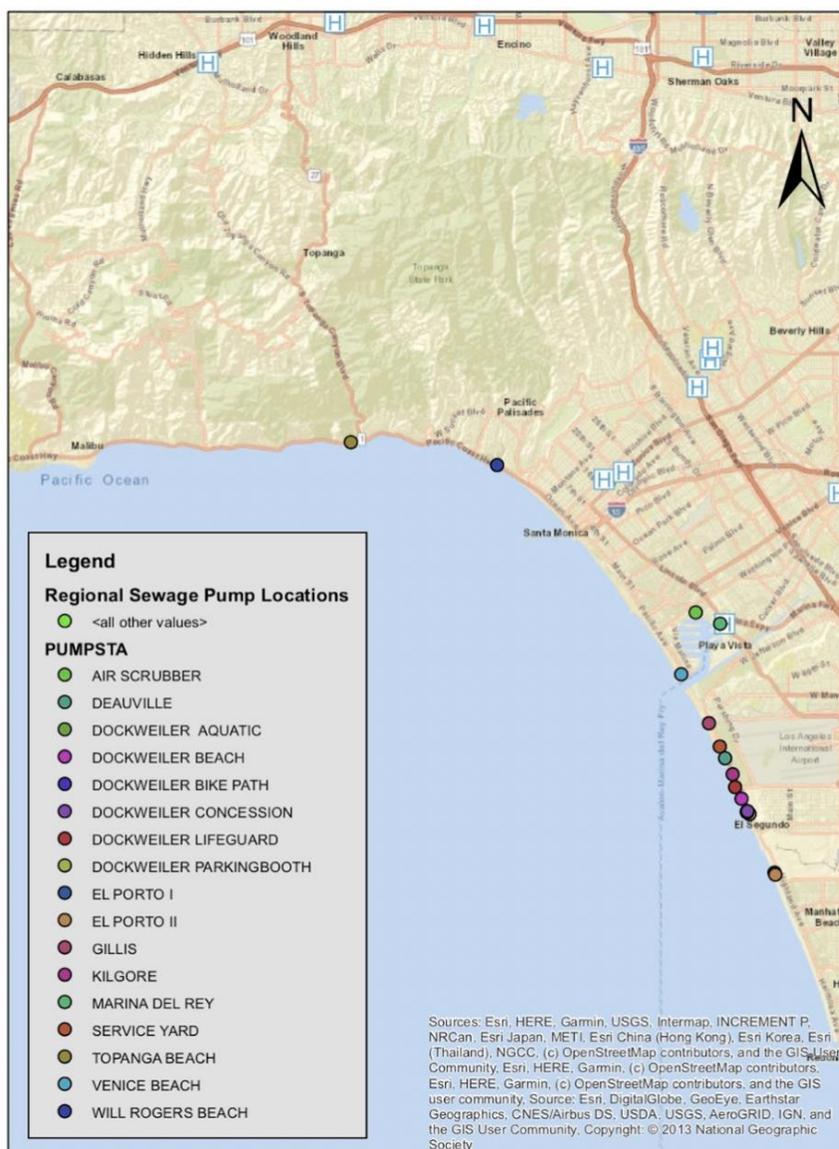


Figure 3. Regional Sewage Pump locations near Venice and El Porto plotted using Los Angeles County data and ArcGIS.

2. Materials and methods

2.1 Recruitment Method and Survey Instrument

Surfers were recruited from El Porto and Venice Beach, both directly on the beach and by posting information on the Surfrider LA Instagram account. The nonsurfer (control) population was recruited from the campuses of Heal the Bay, National Resource Defense Council, and UCLA. Prior to sample collection, all participating individuals filled out both a consent form and

an enrollment survey. Returning participants filled out a separate, shorter follow-up survey. All surveys included questions pertaining to demographic, health, and behavioral data. The survey including questions about the frequency and duration of recent infections, antibiotic prescriptions, and other lifestyle/behavioral factors influencing the microbiome, such as hospital visits. Questions pertaining to antibiotic usage included reporting dates of antibiotic administration, duration of usage, and whether the participant finished the prescription. Incompletion of antibiotic prescriptions have been seen to cause an increase in ARBs within the microbiota, representing a potential cause of the incidence of MRSA in both surfer and nonsurfer populations (WHO, 2018). Surfers were also asked to report their top three surf spots visited, to see if surfers are often exposed to other water bodies.

2.2 Human Nasal Microbiome Sampling

Human nasal samples were collected from surfers at Venice Beach and El Porto Beach between 6:30 and 8AM. Surfers were encouraged to provide two nasal swabs per month so that they could be monitored over time. However, due to the voluntary nature of the study, some surfers were swabbed just one time while other surfers chose to be swabbed multiple times. Control subjects were swabbed once a month during business hours. Similarly, some control subjects were swabbed just one time while other control subjects chose to be swabbed multiple times.

Each participant used one PurFlock™ flocked swab (Puritan, Guilford, ME) in one nostril and one BBL Stuart Media swab (BD, Franklin Lakes, NJ) in the other nostril. Both swab types were individually and sterilely packaged. The flocked swab was dipped into sterile 0.15M NaCl solution prior to nasal insertion in order to increase bacterial collection. Each swab was self-inserted while wearing gloves. Participants placed the swab approximately half an inch up their nostril, rotated the swab five times counter-clockwise and then five times clockwise. One field blank was collected for flocked nasal swabs on each sampling day as a control sample. After sample collection, the BBL swab was placed back into its transport tube and the flocked swab was aseptically cut into a 1.7 mL storage tube. Samples were immediately placed on ice and transported to the laboratory for storage and analysis. Upon arrival, the flocked swab tubes were stored at -20°C for subsequent DNA extraction and the BBL swabs were inoculated onto CHROMagar™MRSA (DRG International Inc., Springfield, NJ) media within two hours.

2.3 Water Collection

Water samples were collected in sterile 2L Nalgene containers at Venice Beach and El Porto Beach, California between 6:45-7:45 AM. Water was collected in knee-deep water during an incoming wave. Containers were rinsed once, filled completely, capped, and then placed on ice. Water samples were transported to the laboratory within two hours of collection. Upon laboratory arrival, water samples underwent filtration within three hours.

2.4 Water Filtration

Membrane filtration was utilized for ocean water samples from both Venice Beach and El Porto. In monitoring ocean water quality, filtration processing was conducted (as described in Goodwin and Pobuda (2009)) with gridded 0.45 μm filter paper (EMD Millipore, Burlington, MA). A 20 μm filter paper net NY2004700 (EMD Millipore, Burlington, Massachusetts) was stacked directly on the gridded filter to minimize difficulty counting colonies on turbid filters. The volume of seawater filtered varied according to appropriate volume necessary to yield 20 to 60 colonies. Concentrations of *S. aureus* was calculated in colony forming units (CFU) per 100 mL of water. Each volume of seawater filtered was preceded by a 20 mL phosphate buffer solution (PBS) prime and followed by a 20 mL PBS rinse. The filters were incubated on CHROMagarTMMRSA. In addition to a CHROMagarTMMRSA filter blank, a method blank filter was incubated on tryptic soy agar (TSA), which was made from Tryptic Soy Broth (BD Bacto, Fisher Scientific, Waltham, MA) and agar (Fisher Science, Hampton, NH). Filtration for DNA extraction was conducted using 0.4 μm filter paper polycarbonate (EMD Millipore, Burlington, Massachusetts) with 100 mL of PBS for the blank and 500 mL of seawater followed by a 20 mL PBS rinse for seawater samples. The filter paper was then folded into eighths and placed in a test tube for DNA extraction.

2.5 DNA Extraction

All human nasal swabs' bacterial DNA extractions were completed using QIAamp BiOstic Bacteremia DNA Kit (Qiagen, Germantown, MD) according to manufacturer's protocol. All human bacterial DNA extractions were completed within one week of receiving the sample.

All water DNA Extractions were completed using GeneRite DNA-EZ ST1 Kit (GeneRite, North Brunswick, NJ) according to manufacturer’s protocol.

2.6 Data Analysis

Quantitative data from swab sample processing and qualitative data from surfer surveys were analyzed to make conclusions about the impacts of AR on human and environmental health. Statistical methods included finding means, standard deviations, and creating Gaussian distributions across the different collected data sets. To determine if differences between two groups were statistically significant, the *z*-score and *p*-value were calculated from the website, <https://www.socscistatistics.com/tests/ztest/default2.aspx>. In a comparative analysis, the precipitation data from the National Oceanic and Atmospheric Administration (NOAA) was used to plot MRSA levels over time and observe relationships between MRSA and rain events throughout the year.

2.7 qPCR

Five genes were chosen for analysis of the prevalence of ARGs in seawater samples: *sul1*, *sul2*, *bla_{SHV}*, *int11*, and *ermB* (**Table 1**). Amplification was performed in a StepOnePlus (Applied Biosystems, Foster City, CA) in 20 µL reaction mixtures on 96-well plates containing PowerUp SYBR® Green Master Mix (Applied Biosystems, Foster City, CA), 4µL aliquots of template DNA, and forward and reverse primers at 200nM final reaction concentration. For each plate a seven-point standard curve was used along with negative control.

Table 1: Primers used in qPCR.

Gene	Forward primer/reverse primer	Amplicon Size (bp)
<i>sul1</i>	CGCACCGGAAACATCGCTGCAC/ TGAAGTTCCGCCGCAAGGCTCG	22/22
<i>sul2</i>	CTCCGATGGAGGCCGGTAT/ GGGAATGCCATCTGCCTGA	19/20
<i>bla_{SHV}</i>	TGATTTATCTGCGGGATACG/ TTAGCGTTGCCAGTGCTCG	20/19

<i>int11</i>	GGCTTCGTGATGCCTGCTT/ CATTCCTGGCCGTGGTTCT	19/19
<i>ermB</i>	AAAACCTACCCGCCATACCA/ TTTGGCGTGTTTCATTGCTT	20/20

Each sample was performed in triplicate wells, from which the standard error was calculated. A maximum cycle number of 35 was used to determine the limit of detection. Each gene had a unique limit of detection listed in **Table 2**.

Table 2: Limit of detection for each gene analyzed.

Gene	Limit of Detection (gene copies/ml of marine water)
<i>sul1</i>	2.40
<i>sul2</i>	0.47
bla _{SHV}	0.31
<i>int11</i>	1.12
<i>ermB</i>	0.16

3. Results

3.1 Demographics and Surfing Beach Preferences

3.1.1 Top Surf Spots

The 135 surfers participating in the study were asked to complete an enrollment survey that sought to determine demographic or qualitative correlations with positive MRSA colonization. One of the questions included in the enrollment survey pertained to the surfers'

favorite surf spots to understand both the potential geographic routes of exposure and general surfing behaviors. From the total surfer responses, 190 different locations were listed as ‘Top 3 Surf Spots’. The overall prevailing locations were Venice, Malibu, and El Porto (**Figure 4**). Many other locations were reported, including both local and international beaches. 95.8% of the reported locations were beaches in the Southern California Region. 4.2 % of the reported locations were in Mexico.

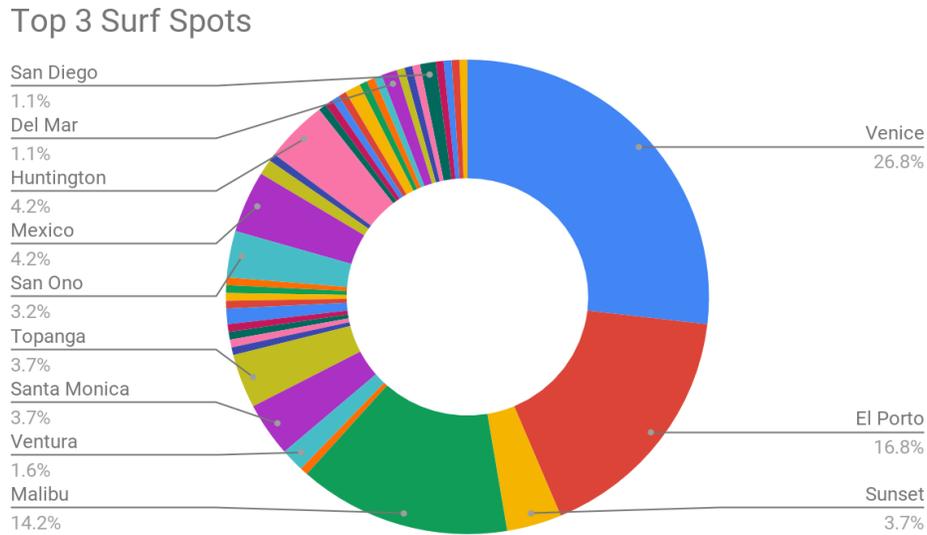


Figure 4. The top 3 reported surf spots were El Porto (16.8%), Venice (26.8%), and Malibu (14.2%). 190 different locations were reported by 135 different surfers.

3.1.2 Demographics

The total study population was comprised of 135 individuals, 26 of which left their enrollment survey completely blank and failed to provide any information. The 109 participants who completed the questionnaire had a racial background as follows: 54.1% white, 22.0% Asian, 8.3% were multiracial, and 11% were Latinx (**Figure 5a**). Only 3.7% did not report their racial background but still provided other information on their enrollment survey (did not leave it completely blank). For reported gender, 69.7% of all participants were male and 30.3% were female (**Figure 5b**).

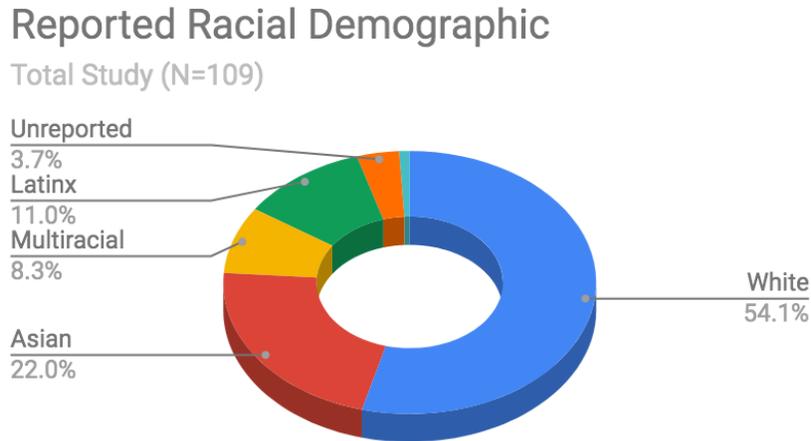


Figure 5a. represents the reported racial demographics of all participants in the study.

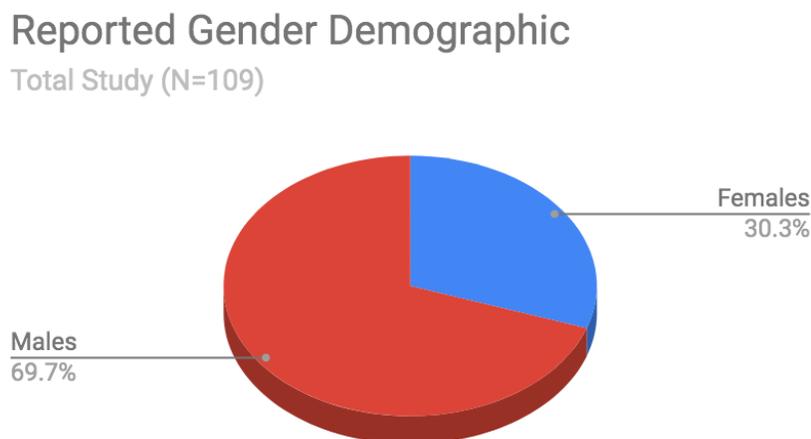


Figure 5b. represents the reported gender demographics of all participants in the study.

3.2 Prevalence of MRSA in Surfers and Nonsurfers

There were 341 total swabs collected from 105 surfers and 30 control subjects. The average surfer was swabbed 2.5 times, and the average control subject was swabbed 2.37 times throughout the duration of the study. It was found that 5.6% of swabs collected from the control population showed evidence of MRSA colonization, while 94.4% showed no evidence of MRSA colonization (**Figure 6a**). For the surfer population, 10.7% of swabs collected showed evidence of MRSA colonization, while 89.3% showed no evidence of MRSA colonization (**Figure 6b**).

While this suggests that surfers are more susceptible to MRSA colonization than nonsurfers, there was not a significant difference in MRSA prevalence between the nonsurfer and surfer groups ($P = 0.19$).

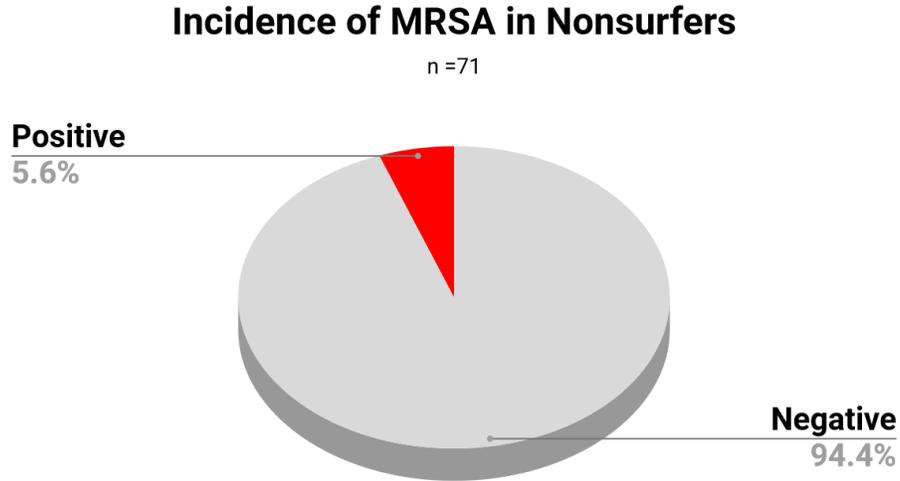


Figure 6a represents the incidence of MRSA in the control population.

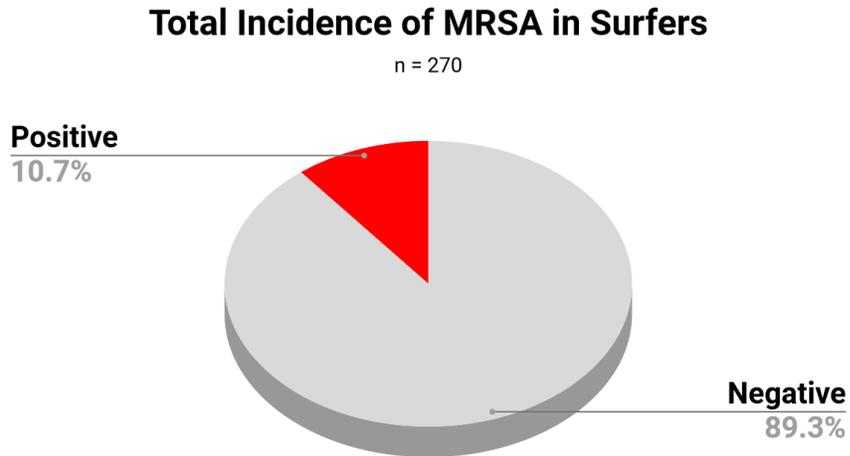


Figure 6b represents the incidence of MRSA in the surfer population.

3.3 Total Incidence of MRSA in Surfers by Location

MRSA incidence differed by beach location. There was a higher incidence of MRSA in El Porto surfers; MRSA positive detection was 15.9% over the course of the entire study, while total positive MRSA detection in Venice Beach surfers was 6.3% (**Figures 7a and 7b**). There was a significant difference in MRSA incidence between the two beaches ($P = .011$).

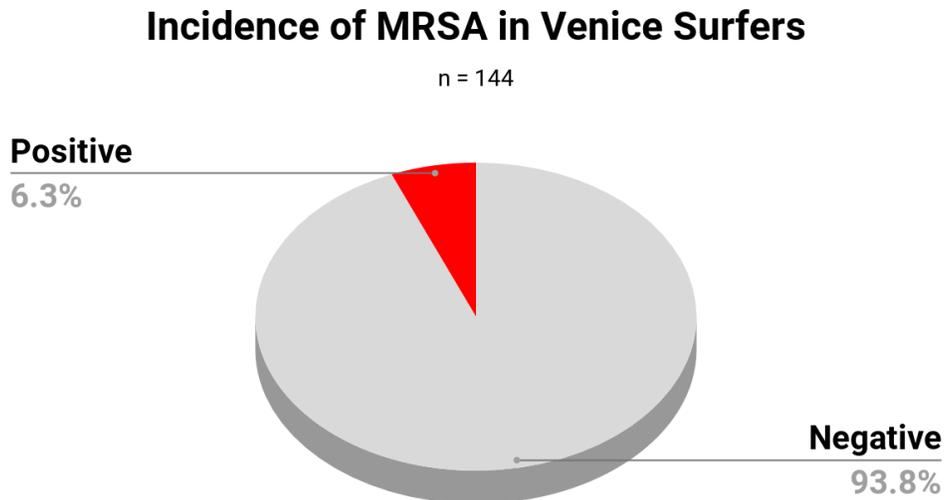


Figure 7a. Positive incidences of MRSA in Venice Beach surfers was 6.3%.

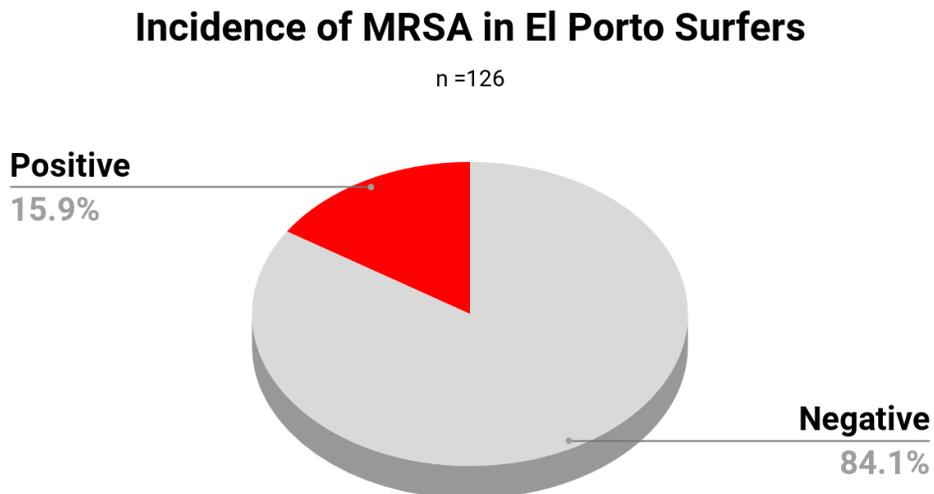


Figure 7b. Positive incidences of MRSA in El Porto surfers was 15.9%.

3.4 Incidence of MRSA in Surfers in Relation to Early Season Rain Events

The pre-flush period was defined as the time period before *major rain events* (represented as precipitation events over 0.75 inches). Positive detection of MRSA in the total (combined beaches) surfer population was found to be 3.8% during the pre-flush period, or from October 1 to November 20, 2018. (**Figure 8a**). The flush period was defined as rain events with precipitation over 0.75 inches (Water Resources Technical Report). During the flush period, from November 21 to December 21, 2018, positive detection of MRSA in the total surfer population increased to 30.6% (**Figure 8b**). There was a significant increase in MRSA colonization during the first flush compared to the pre-flush time ($P < .00001$). During the post-flush period, from December 22, 2018 to April 24, 2019, positive detection of MRSA in the surfer population decreased to 10.9% (**Figure 8c**). The difference in MRSA colonization between the flush and post-flush was statistically significant ($P = .0037$).

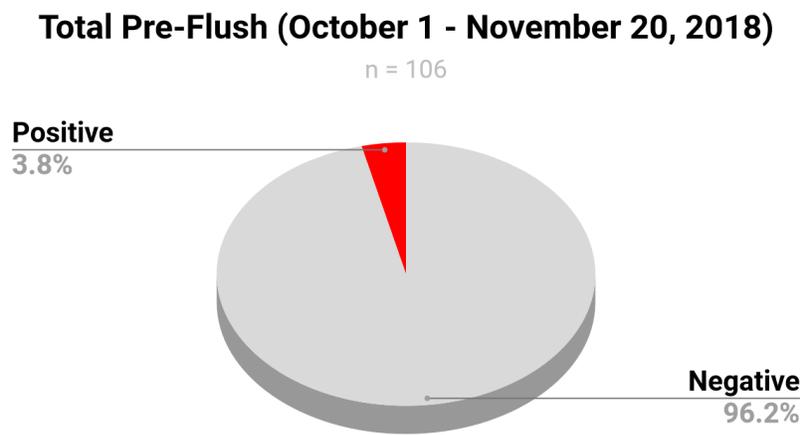


Figure 8a. There was a 3.8% positive detection of MRSA during the pre-flush period.

Total Flush (Nov. 21 - Dec. 21, 2018)

n = 36

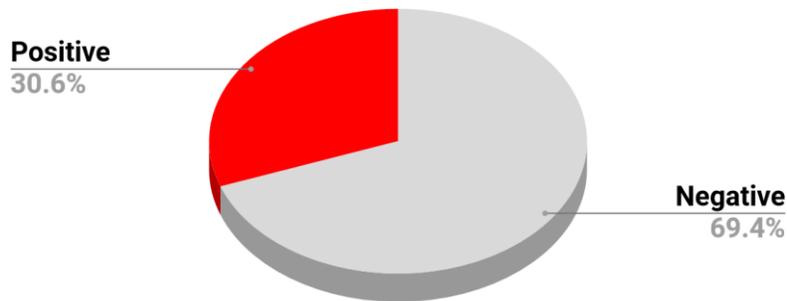


Figure 8b. There was 30.6% positive detection of MRSA during the flush period.

Total Post Flush (Dec. 22, 2018 - April 24, 2019)

n = 128

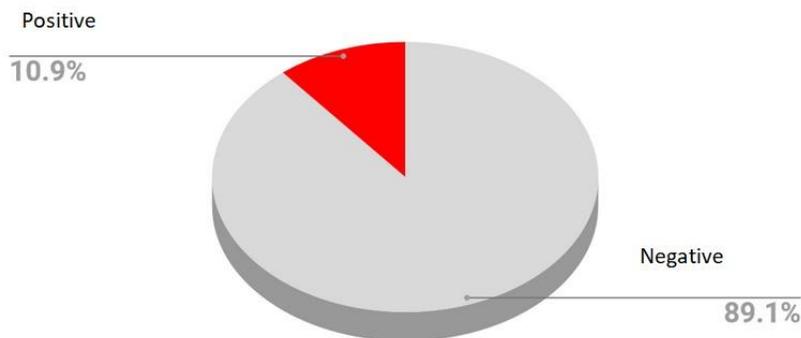


Figure 8c. There was a 10.9% positive detection of MRSA during the post flush period

The effects of the first flush followed a similar pattern at each beach. Positive detection of MRSA at Venice was 4% and at El Porto was 3% during the pre-flush period, or from October 1 to November 20, 2018. (**Figures 9a and 10a**). During the flush period, from November 21 to December 21, 2018, positive detection of MRSA in the surfer population increased to 25% at Venice and 33% at El Porto (**Figures 9b and 10b**). The increase in MRSA colonization in surfers during the first flush was significant at Venice Beach ($P = 0.0076$) and at El Porto ($P=0.0028$). During the post-flush period, from December 22, 2018 to April 24, 2019, positive detection of MRSA in the surfer population decreased to 5% at Venice and 15% at El Porto

(Figures 9c and 10c). The decrease in MRSA colonization rates during the post-flush was significant for surfers in Venice Beach ($P=0.0271$) but not significant in El Porto surfers ($P=0.059$). Additionally, the incidence of MRSA at Venice Beach and El Porto was compared for the pre-flush, flush, and post-flush time periods ($P=0.85$, $P=0.61$, $P=.066$, respectively). There were no significant differences, indicating the phenomenon of MRSA colonization in surfers occurs at each beach similarly.

Figure 9a.

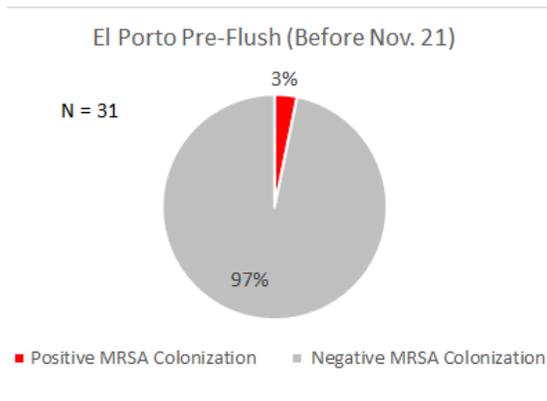


Figure 10a.

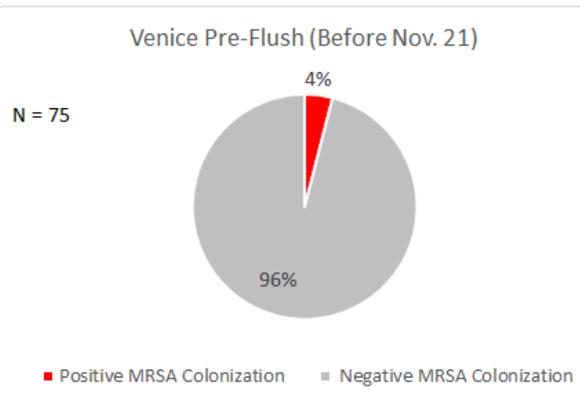


Figure 9b.

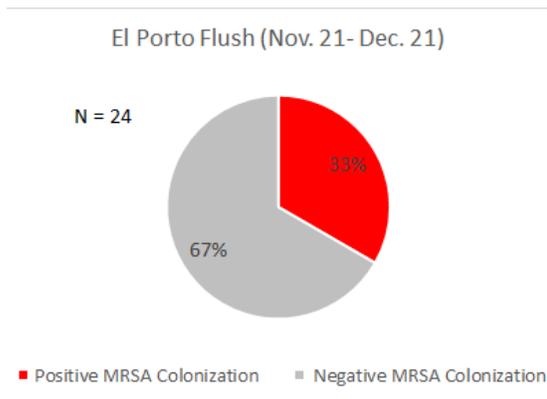


Figure 10b.

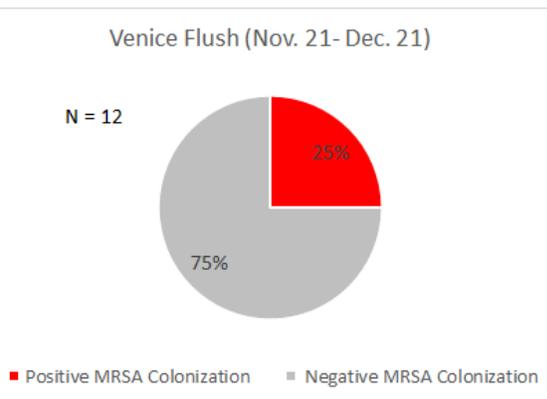
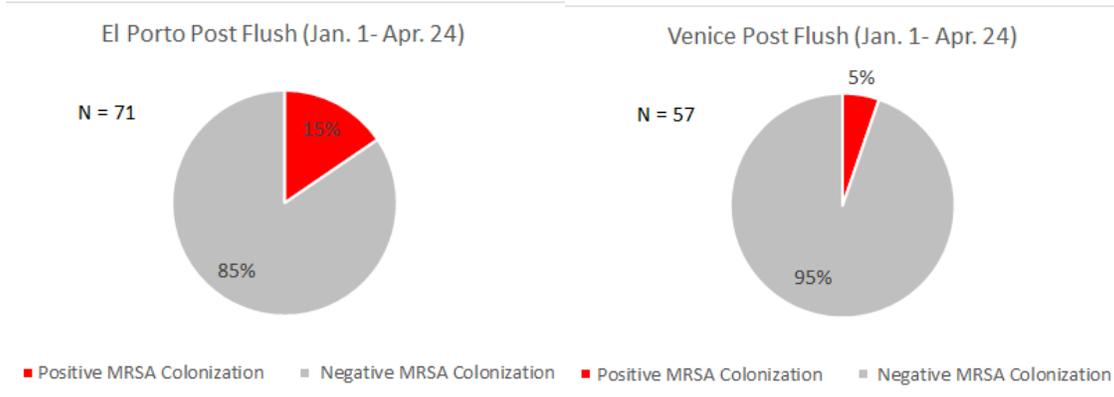


Figure 9c.



3.5 Ocean Water MRSA Concentrations

The peak concentrations of MRSA colony-forming units (CFU) per 100 mL of ocean water from El Porto and Venice Beach correspond to rain events of the wet weather season. The most elevated MRSA levels for El Porto were 800 CFU/100 mL after a rain event of 1.02 inches on November 29, 2018 and 800 CFU/100 mL after 1.91 inches on December 6, 2018. The most elevated MRSA level for Venice Beach was 1060 CFU/100 mL after a rain event of 1.91 inches of rain on December 6, 2018. Later in the season, during the period of January to April 24, 2019, the concentration of MRSA decreases to an average of 44 CFU/100 mL at El Porto and 24.22 CFU/100 mL at Venice Beach (**Figure 11**).

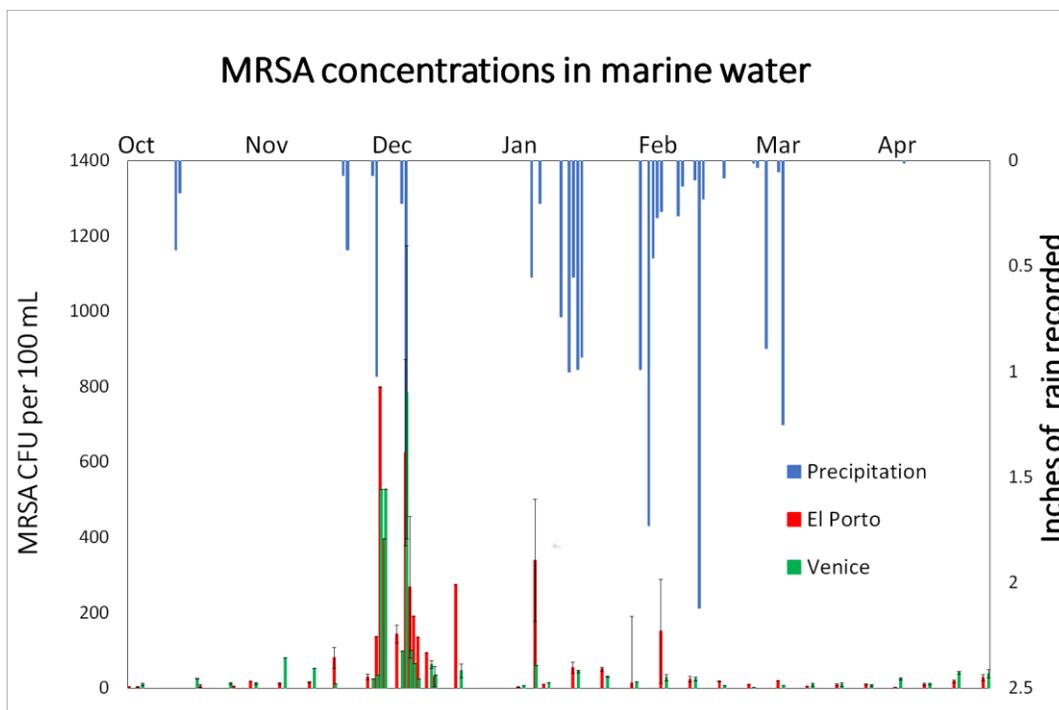


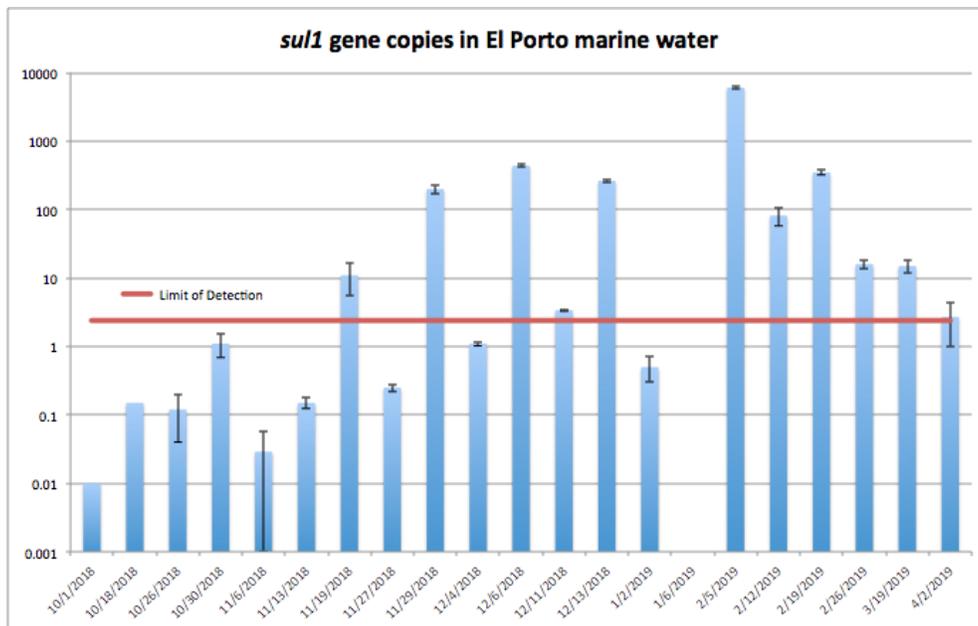
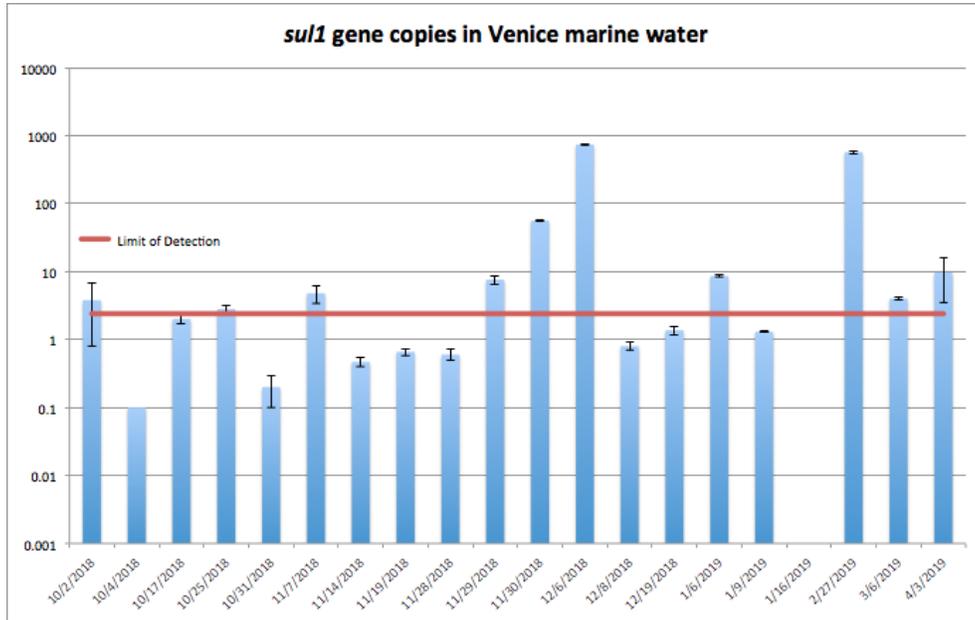
Figure 11. Highest peaks of MRSA colonies (CFU/100 mL) for El Porto (red) and Venice (green) coincide with the beginning of the wet weather season.

3.6 Ocean Water ARG Concentrations

The results of qPCR, analyzing the concentrations of *sul1*, *sul2*, *bla_{SHV}*, *intI1*, and *ermB* in marine water at Venice and El Porto, suggest a trend between increased concentrations and rain events (**Figures 12a-16b**). The red line represents the limit of detection, defined as the number of gene copies needed quantify. Anything below the line was detected but had a concentration too low to accurately calculate.

The first flush (November 21 to December 21, 2018) resulted in an observable increase in the number of gene copies of *sul1*, *sul2*, *bla_{SHV}*, *intI1*, and *ermB* at both Venice Beach and El Porto. On November 29, 2018, the Los Angeles/Oxnard area received 1.02 inches of rain. This corresponds with spikes in *sul1* at both beaches and in *ermB* at El Porto. The spikes in *sul1* at both beaches, *sul2* at El Porto, and *bla_{SHV}* and *ermB* at Venice on December 6, 2018 correspond with the 1.91 inches of rain that day. At both beaches, levels of *intI1* were elevated in the two following days after the 12/6 storm. Between February 1 and February 5, 2019, the Los

Angeles/Oxnard area received a total of 2.7 inches of rain. At El Porto, levels of *sul1* and *sul2* were elevated, and at Venice, levels of *ermB* spiked.



Figures 12a and 12b show the amount of *sul1* gene copies per milliliter of marine water from Venice Beach and El Porto respectively. The limit of detection for *sul1* is 2.40 gene copies per milliliter of marine water and is indicated by the red line.

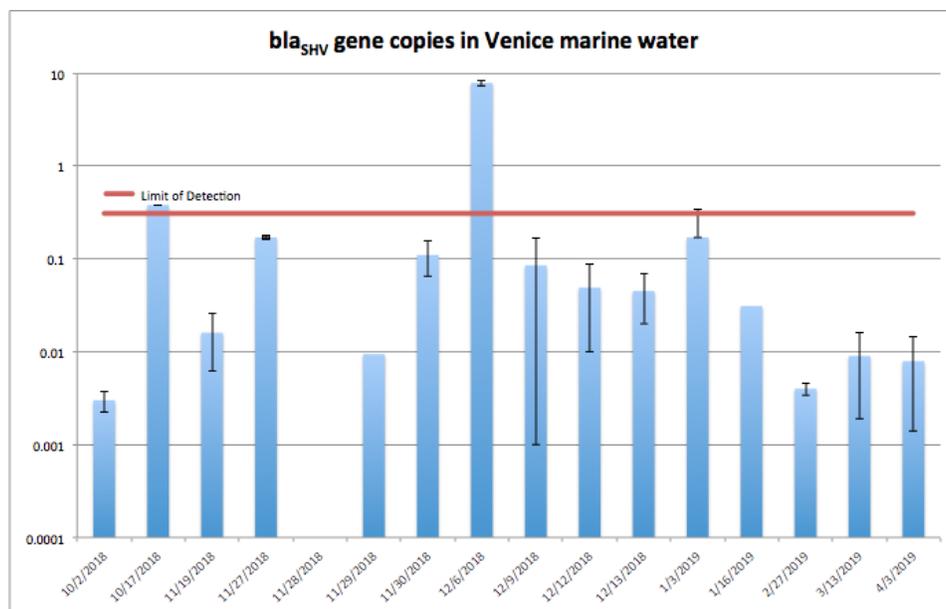
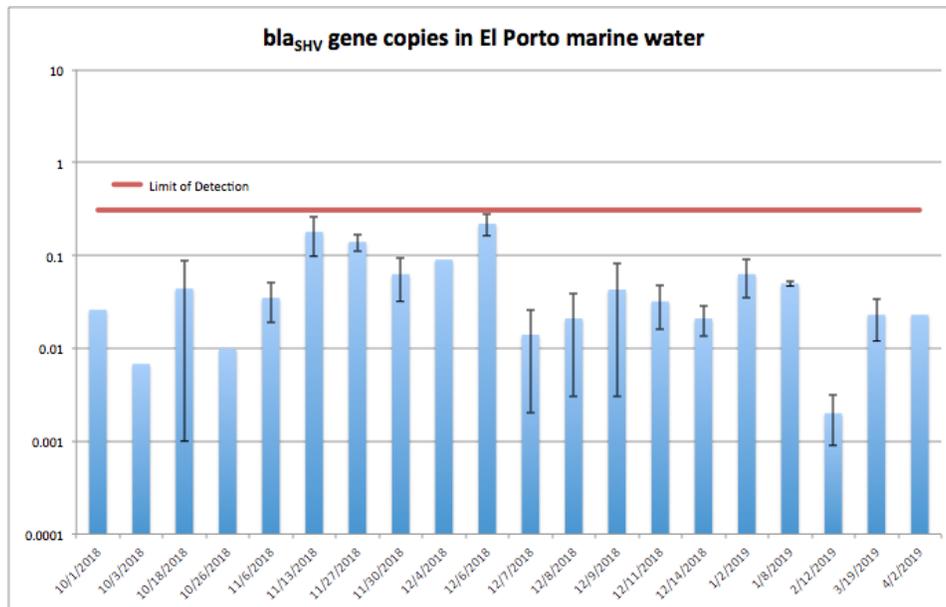
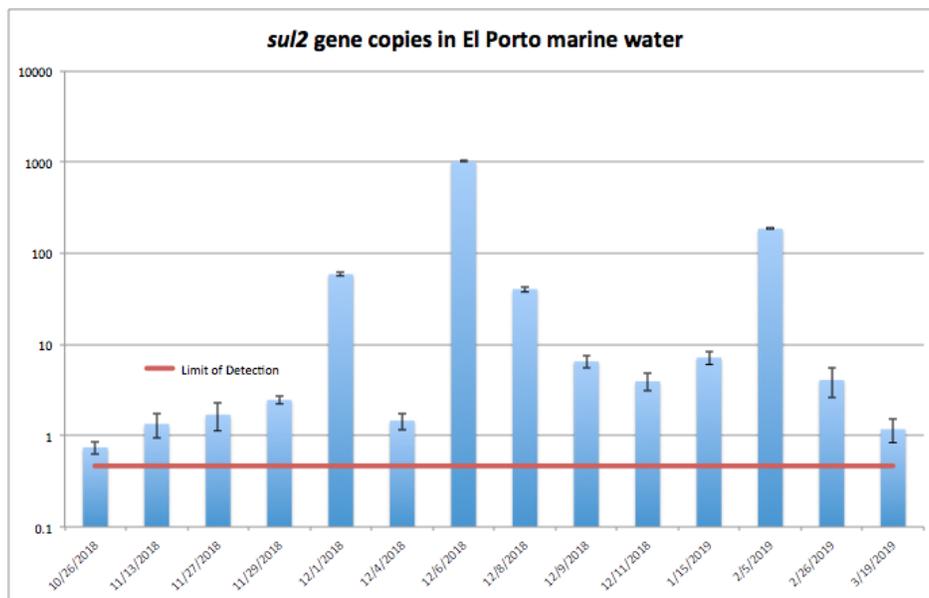
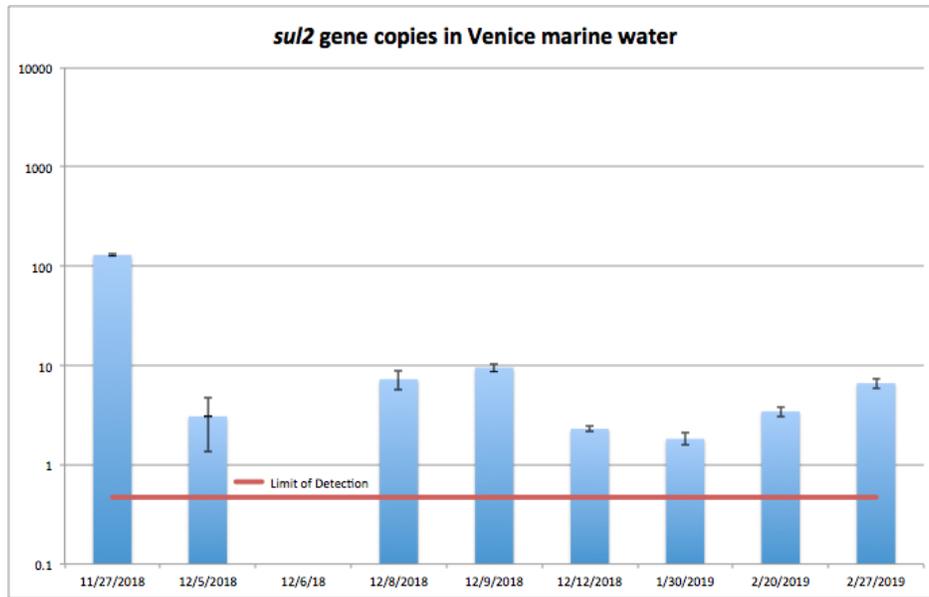
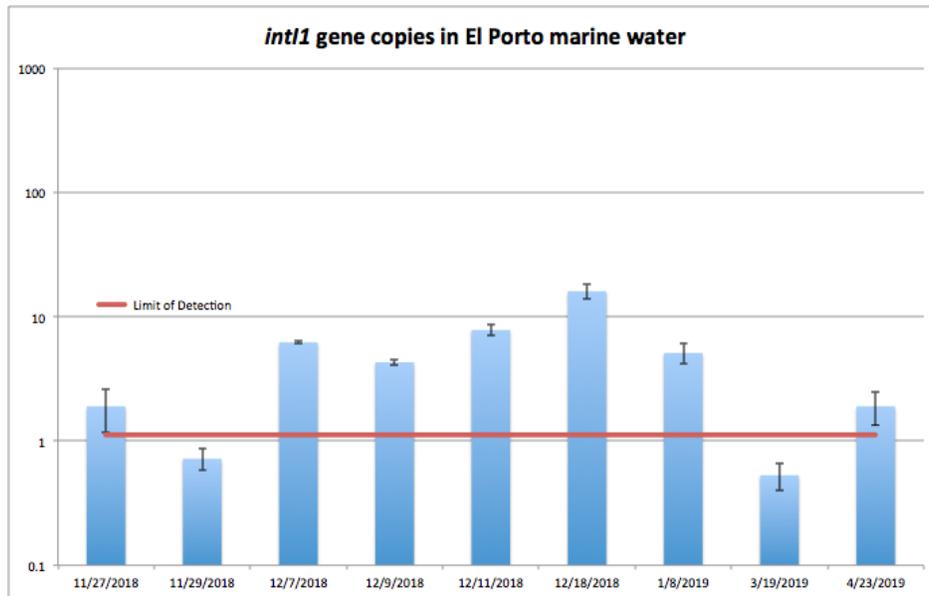
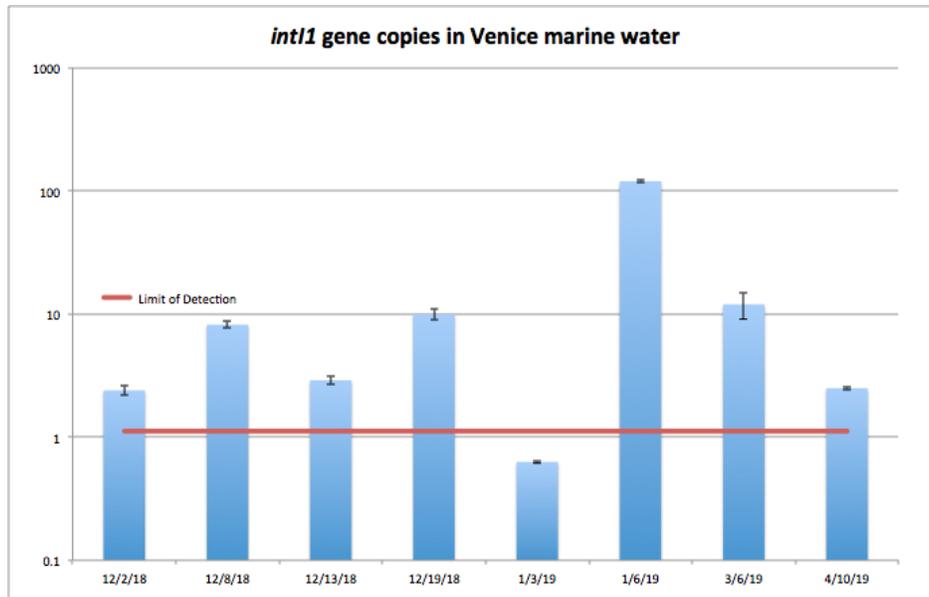


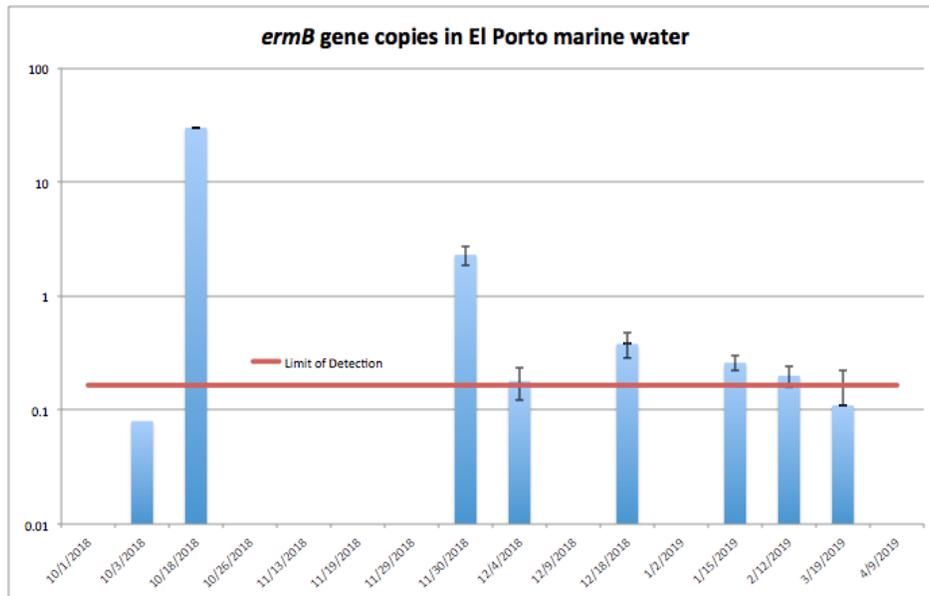
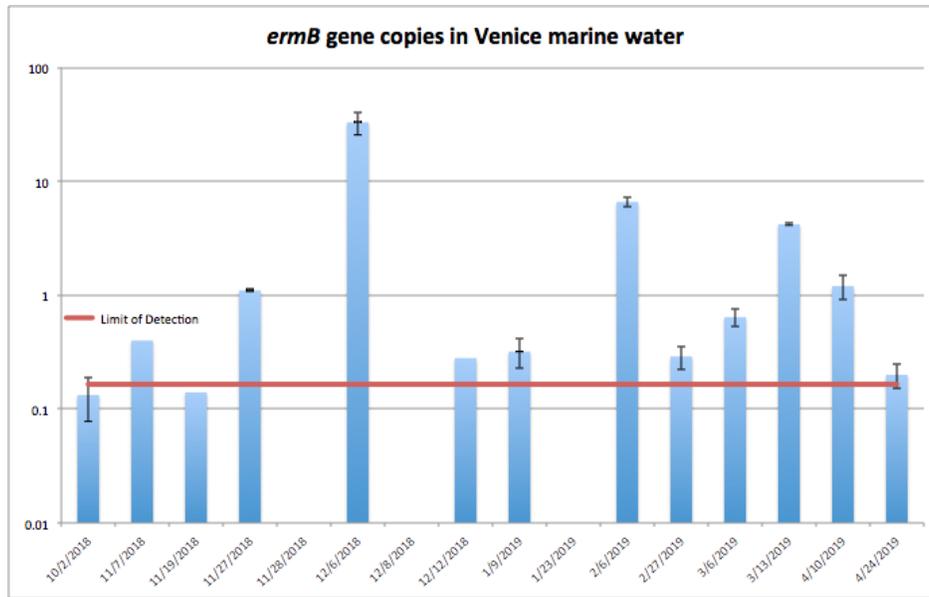
Figure 13a and **13b** show the amount of bla_{SHV} gene copies per milliliter of marine water from Venice Beach and El Porto respectively. The limit of detection for bla_{SHV} is 0.31 gene copies per milliliter of marine water and is indicated by the red line.



Figures 14a and **14b** show the amount of *sul2* gene copies per milliliter of marine water from Venice Beach and El Porto respectively. The limit of detection for *sul2* is 0.47 gene copies per milliliter of marine water and is indicated by the red line.



Figures 15a and 15b show the amount of *int11* gene copies per milliliter of marine water from Venice Beach and El Porto respectively. The limit of detection for *int11* is 1.1 gene copies per milliliter of marine water and is indicated by the red line.



Figures 16a and **16b** show the amount of *sul1* gene copies per milliliter of marine water from Venice Beach and El Porto respectively. The limit of detection for *ermB* is 0.16 gene copies per milliliter of marine water and is indicated by the red line.

3.7 Antibiotic Usage and Symptoms

The majority of participants who responded to the enrollment survey question regarding antibiotic completion indicated that they finished their respective antibiotics prescription; the question stated, “If you have been prescribed antibiotics, do you generally take your antibiotics to completion?” Based on the survey responses reported by participants throughout the study, 131 participants indicated completion of antibiotics, representing 91.6% of the total study population. The remaining 8.4% of the total study population indicated that they did not take their prescribed antibiotics to completion (**Figure 17a**).

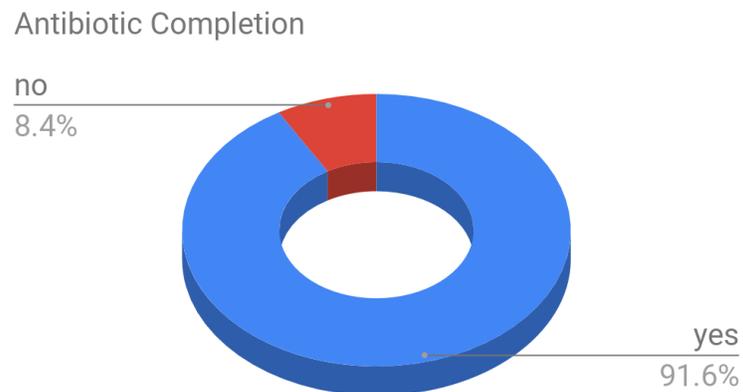


Figure 17a. Out of the total 135 participants, 131 participants indicated whether they usually take their prescribed antibiotics to completion.

A wide range of symptoms were reported by the surfers, but seemed to primarily affect the ears, head, nose, throat, eyes, and stomach. There were 194 symptoms reported throughout the entirety of the study, including all reported symptoms on all surveys (meaning if a surfer or control subject reported symptoms on two different days they were each counted). The most frequently reported symptoms were coughing and nasal congestion, 21.4 % and 20.4% respectively (**Figure 17b**). Of the overall symptoms reported, MRSA positive participants reported 92 symptoms over the course of the study. The most prevalent symptoms reported for MRSA positive participants were also coughing and nasal congestion, 21.7% and 20.6% respectively. (**Figure 17c**).

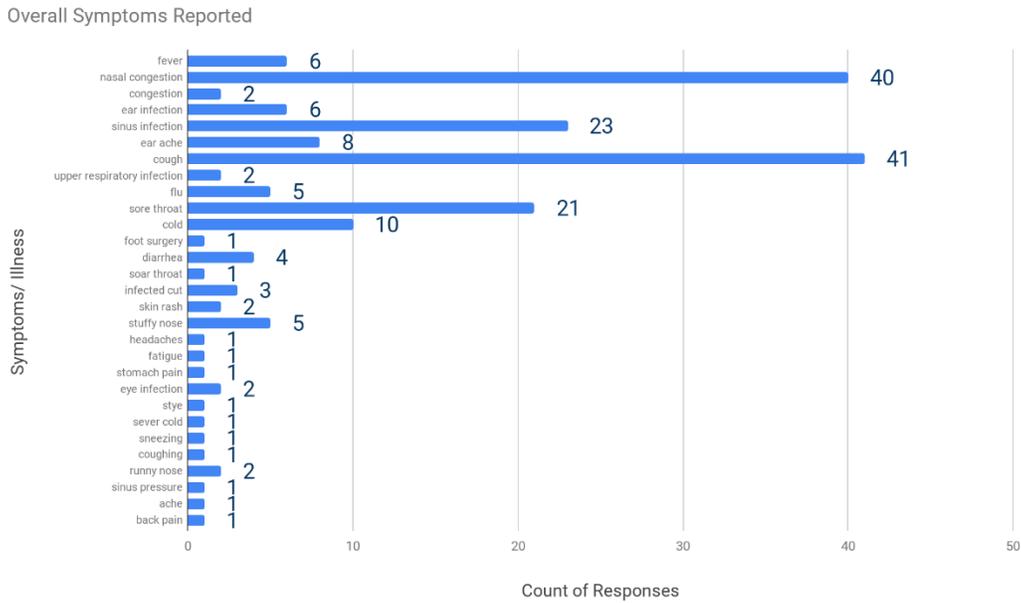


Figure 17b. Of the 135 Participants, 194 symptoms were listed over the entire course of the study. The two most prominent symptoms listed were cough and nasal congestion.

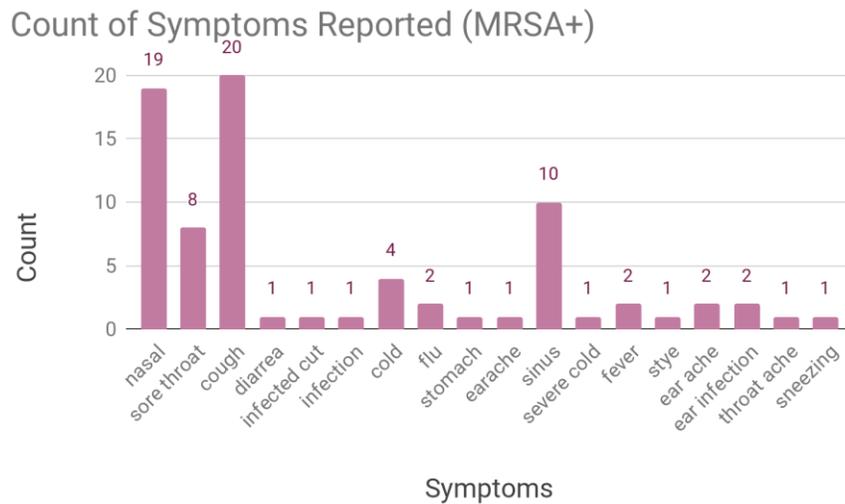


Figure 17c. Count of symptoms reported by MRSA colonized participants.

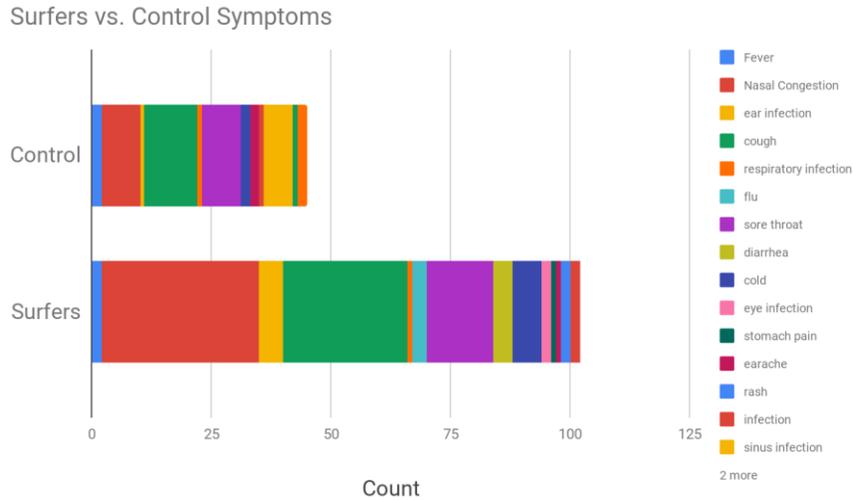


Figure 17d. Surfers reported coughing 25.5% of the time, and nasal congestion 32.35% of the time. Controls reported coughing 24.44% of the time, sore throat 17.77% of the time, and nasal congestion 17.77% of the time.

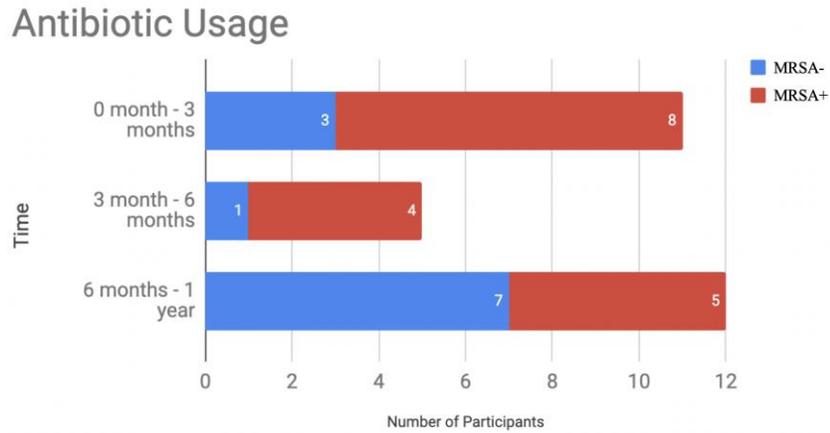


Figure 17e. Of the 135 participants, 28 reports of antibiotic (AB) usage was within last year. Each response was divided into time from AB usage, 0-3 months 39%, 3-6 months 18%, and 6-12 months 43%. Antibiotic usage within 1 year, is divided into MRSA positive and MRSA negative participants.

One of the enrollment survey questions asked the participant when their last hospital visit occurred. Out of 183 total surfer responses, accounting for more than one response per surfer in some cases (if they were swabbed more than once), 41% answered no and 59% answered yes to visiting the hospital recently. For surfers that self-reported a hospital visit within zero to three

months of the time they were swabbed, 12.5% were colonized with MRSA. For surfers that self-reported a hospital visit within three to six months of the time they were swabbed, none were colonized by MRSA. For surfers that self-reported a hospital visit within six months to one year from the time they were swabbed, 33.3% were colonized with MRSA. Lastly, both groups of surfers that self-reported a hospital visit within one to five years and over five years, none of them were colonized by MRSA (**Figure 18a**).

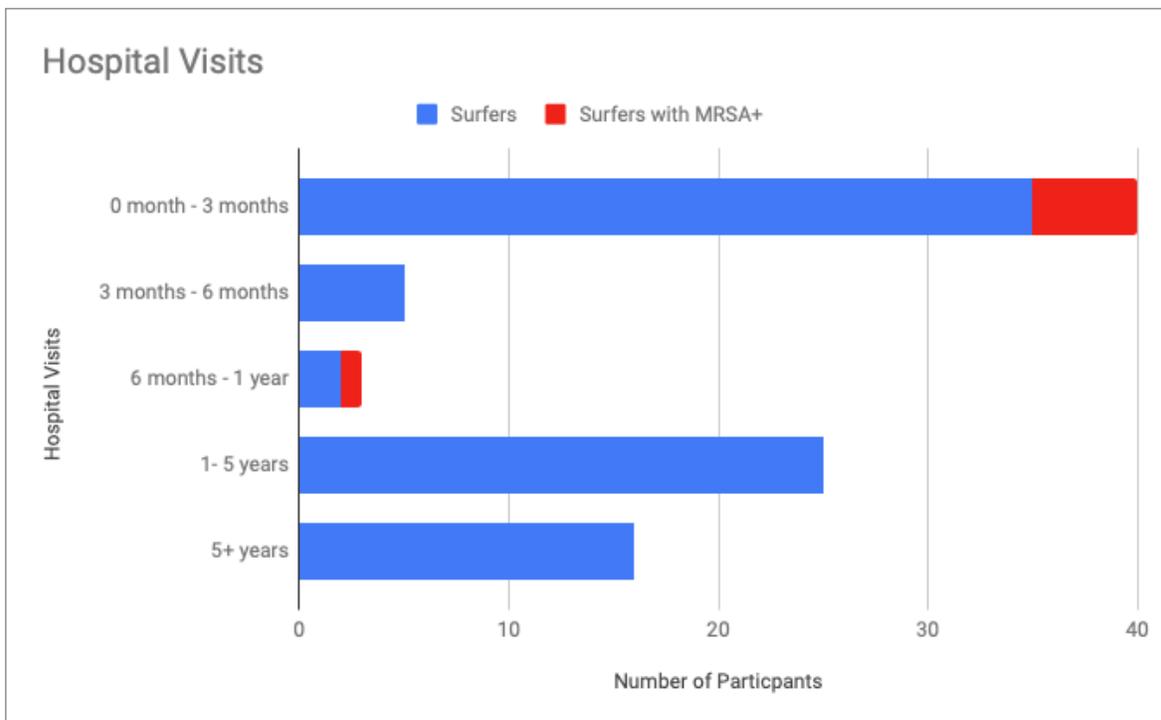


Figure 18a. While 75 surfers reported that they had not been to a hospital, 86 surfers that indicated they had been to a hospital sometime between 0 months and over 5 years ago from the time of swabbing. Within the subset of surfers who reported that they had been to a hospital in the last zero to three months, six were MRSA positive.

3.8 Risk Analysis

This data suggests that surfers exposed to El Porto beach (**Table 4**) have a greater risk (219% increase) of contracting MRSA than surfers exposed to Venice beach (**Table 3**) (42% decrease) or nonsurfers (**Table 6**) (61% increase for surfers). Additionally, data suggests antibiotics (**Table 5**) play a role in the relative risk of a subject contracting MRSA and would be an interesting area of further study.

In order to see if a sampling location was at an increased risk, the exposure/event were classified through the location subjects were sampled at (**Tables 3 and 4**). Controls were counted toward the non-exposed group. A risk analysis gives a relative risk ratio (RR) of 0.58 (and an odds ratio (OR) of 0.55) for Venice Beach (**Table 3**). For El Porto (**Table 4**), it gives a RR of 3.19 (OR=3.66). This suggests that there is less risk (i.e. contracting MRSA) associated with surfing at Venice Beach than there is with surfing at El Porto Beach. In calculating the risk of surfers versus controls (**Table 6**), the difference between surfer and control populations (RR=1.61, OR=1.68), where the RR>1 suggesting that those that surf in this study are more at risk for contracting MRSA than nonsurfers.

Lastly, the question of whether antibiotics taken within one year of survey distribution affects the risk/odds of contracting MRSA was explored. Surprisingly, it was found that the RR=1.10 and OR=1.13, which suggests that there is an increased risk for those that had taken antibiotics in the past year than those that did not or left a blank response. This is of course limited due to the vast number of blank/missense responses but does suggest an area of further study.

Table 3 shows that of the participating surfers, those that surfed at Venice during the time of sampling had 0.58 times the risk (OR=0.55, 42% reduction of risk) to be exposed to MRSA than those sampled at other locations (negative correlation).

<u>Location Sampled</u>	<u>MRSA+</u>	<u>MRSA-</u>	<u>TOTAL</u>
Venice	9	125	134
Not Venice	24	183	207
TOTAL	33	308	341
	% RELATIVE EFFECT	42%	
RELATIVE RISK	0.579291045	ODDS RATIO	0.549

Table 4 shows that of the participating surfers, those that surfed at El Porto during the time of sampling had 3.19 times the risk (OR=3.66, 219% increase of risk) to be exposed to MRSA than those sampled at other locations.

<u>Location Sampled</u>	<u>MRSA +</u>	<u>MRSA -</u>	<u>TOTAL</u>
El Porto	20	91	111
Not El Porto	13	217	230
TOTAL	33	308	341
	% RELATIVE EFFECT	219%	
RELATIVE RISK	3.187803188	ODDS RATIO	3.668639053

Table 5 shows that of the participants sampled at El Porto, individuals that took antibiotics within the last year had 1.10 times the risk (OR=1.14, 10% increase of risk) to be exposed to MRSA than those that did not take their antibiotics to completion. Discrepancies in total number and accurate responses are due to a blank or vague response but are not counted toward the total as depicted in the table.

<u>Condition</u>	<u>MRSA+</u>	<u>MRSA-</u>	<u>TOTAL</u>
AB+	4	9	13
AB-	16	41	57
TOTAL	20	50	70
	% RELATIVE EFFECT	10%	
RELATIVE RISK	1.096153846	ODDS RATIO	1.138888889

Table 6 shows that from the participants, individuals that were planning to surf during the time of sampling had 1.61 times the risk (OR=1.68, increase of 61%) to be exposed to MRSA than those that were part of the non-surfing control group.

<u>Location Sampled</u>	<u>MRSA+</u>	<u>MRSA-</u>	<u>TOTAL</u>
Surfer	29	250	279
Control	4	58	62
TOTAL	33	308	341
	% RELATIVE EFFECT	61%	
RELATIVE RISK	1.611111111	ODDS RATIO	1.682

4. Discussion

4.1 Demographic Trends

MRSA colonization did not appear to be correlated with gender or racial background. However, with the total surfer population being predominantly white and male, prospective studies should be sure to adequately represent gender and racial demographics to explore any possible links. Out of the 108 surfers who participated in the study, only 16 subjects were female. Conversely, out of the 30 control subjects, 19 were female. Survey response and non-response studies have shown that women are more likely than men to participate in voluntary surveys (Curtin et al., 2000; Moore & Tarnai, 2002; Singer et al., 2000). This issue must be addressed and accounted for in future research.

4.2 Prevalence of MRSA in Surfers and Ocean Water

The highest concentration of MRSA in El Porto and Venice Beach water coincided with the first major rain events of the winter season. This indicates that the first flush causes important degradation of water quality. This study further indicates that ocean water can potentially expose beachgoers to antibiotic resistant pathogens with the greatest risk being after the first major rain event. The lower MRSA concentrations observed from mid-January to April, despite the continued rain events, indicates that MRSA levels are at their worst during the first flush. This

reveals that southern California beaches, specifically El Porto and Venice Beach, may be a serious route of exposure to ARB during the season's first big storms.

MRSA concentrations in El Porto and Venice Beach were dynamic and did not always follow the same patterns. While both beaches contained elevated concentrations of MRSA during the onset of the wet weather season, later-season elevated concentrations occurred on different days. Thus, there was not a clear indication why the two beaches differ in ARB concentration (after first-flush events). There was a disparity in incidences of MRSA between El Porto and Venice Beach surfers; higher MRSA colonization rates in El Porto surfers compared to Venice surfers was statistically significant. Further research is needed to understand why El Porto surfers had higher incidences of MRSA than Venice Beach surfers. Investigating any differences in effluents into these spots or other potential sources of ARB may reveal why MRSA colonization differed by beach location. In addition, understanding the daily pattern of currents at these locations may also elucidate why the patterns of MRSA concentration differed.

There were more incidences of MRSA in surfers than nonsurfers, revealing that the ocean may be an important mode of transmission and exposure to ARB. The direct contact with ocean water and high frequency of long duration head immersions makes surfers vulnerable to opportunistic pathogens (Harding et al., 2015). The higher frequency of MRSA detected in surfers also coincides with wet weather events. The statistically significant increase in MRSA colonization in surfers during the flush period indicates that rain events present a high risk of exposure. While the positive detection of MRSA decreased in the post-flush period, it was still higher than the pre-flush period. The frequencies of MRSA detected in surfers seemed to follow the patterns of MRSA ocean concentration. Both the frequency of MRSA detected in humans and MRSA concentration from ocean water were highest from the first flush and decreased throughout the winter season (but are still elevated compared to the pre-first flush time period). The observed pattern is that the highest proportion of MRSA detected was when ocean water was most impaired by stormwater runoff. For public safety, Los Angeles public health agencies should consider advising surfers to avoid ocean water contact for a whole week after the first three storms of the rainy season. While it is currently recommended that beachgoers stay out of the water for 72 hours, the additional 96 hours after a first flush event may prevent incidences of MRSA colonization.

The effects of the first flush on positive detection of MRSA varied by beach. During the pre-flush period, Venice and El Porto's positive detection of MRSA percentages were within 1% of one another. During the flush period, the frequency of MRSA detection increased by 21% at Venice and 30% at El Porto. The post-flush period saw a decrease of 20% at Venice and 18% at El Porto. The positive detection during the post-flush period was 1% higher than the pre-flush period at Venice and 12% at El Porto. El Porto seemed to have had a more dynamic correspondence with the effects of the first flush. This is consistent with the aforementioned findings and further indicates a need for an in-depth examination of other potential sources of ARB at these beaches.

The first flush (November 21 to December 21, 2018) resulted in an observable increase in the number of gene copies of *sul1*, *sul2*, *bla_{SHV}*, *int11*, and *ermB* at both Venice Beach and El Porto. After the first major storm on November 29, 2018, there were spikes in *sul1* at both beaches and in *ermB* at El Porto. Additionally, the spikes in *sul1* and *int11* at both beaches, *sul2* at El Porto, and *bla_{SHV}* and *ermB* at Venice correspond with the December 6, 2018 storm. This suggests that the first flush creates elevated levels of ARGs in coastal marine water as the dry season ground contaminants are carried into the ocean.

4.3 Prevalence of ARGs in Ocean Water

Between January 12 and January 17, 2019, the Los Angeles/Oxnard area received a total of 4.21 inches of rain, yet the qPCR data shows no significant increase in ARG levels. This may be due to most of the contaminants being flushed out during the first flush. However, the storm at the start of February 2019 corresponded with elevated levels of *sul1* and *sul2* at El Porto and *ermB* at Venice. The increase in concentration was comparable to the spikes during the first flush. The lack of precipitation in the preceding two weeks may have played a role in this increase.

Among the genes analyzed, *bla_{SHV}* was the only one that consistently tested below its limit of detection. The genes that showed the largest increase in concentration after rain events were *sul1*, *sul2*, and *ermB*. *int11*'s concentration in El Porto seemed to stay relatively stable, even during rain events, whereas there were larger concentration differences in the *sul1*, *sul2*, and *ermB* gene copies. To further explore this trend, 16s can be used to standardize the number of gene copies in relation to the total amount of bacteria present; this will allow future studies to

examine whether there is a difference in the ratio of ARGs to total bacteria. Additionally, a continuation of this study should continue to run qPCR on different dates to further evaluate the levels of ARGs before and after rain events.

4.4 Hospital Visits, Antibiotic Usage, and Reported Symptoms

MRSA was found to be more prevalent in surfers that were admitted into a hospital within zero to three months. MRSA infections are common among people who have weak immune systems which are common in a hospital or other health care centers (Coello et al., 1997). Since these surfers may have been exposed to ARB while within the hospital facility, it cannot be confidently said that they were colonized by environmental MRSA. However, future work includes DNA sequencing which will indicate if the MRSA is clinically or environmentally associated.

Incidences of ARB is highest in those who do not complete a prescribed antibiotic course (WHO, 2018). Therefore, it was important to assess if participants in the study had recently taken antibiotics, and whether they had completed them. In the entirety of the study, the majority (91.6%) of participants reported that they took prescribed antibiotics to completion. Additionally, 28 subjects reported having taken antibiotics within the past 12 months. Those who had taken it most recently (within zero to three months) had the highest percentage of MRSA positive results, which could be further investigated with a larger and more diverse sample population. Limitations of the study were survey accuracy, some surfers did not respond to these specific questions, rushed through the survey, or could not remember the time frame they took antibiotics. Future studies could be more accurate if allowed to use local hospital data, however acquiring this data is difficult due to privacy concerns and HIPAA regulations.

In the investigation of the presence of ARGs and ARBs, participants reported their symptoms throughout the study. The most prevalent symptoms reported were nasal congestion and coughing (21.4 % and 20.4%, respectively). Interestingly, MRSA positive participants also reported nasal congestion and coughing as the top two symptoms listed in the survey responses. In a comparison of symptoms reported by controls versus surfers, control subjects reported similar symptoms that the surfers reported. These results are not entirely conclusive of surfers contracting illness purely from ocean water, since the controls report similar symptoms, which could indicate seasonal flu/cold. However, reported symptoms by surfers were in higher

quantity, but this could be due to the differences in sampled population sizes. Again, improvements for future research can be made with an increase in sample population size and diversity.

Conclusion

- Incidences of MRSA colonization in surfers was eight times higher during the first flush which indicates that El Porto and Venice are routes of exposure to ARB.
 - Surfers are nearly two times more at risk of contracting MRSA than nonsurfers.
 - There were higher concentrations of MRSA and *sul1* gene copies in El Porto and Venice Beach during the flush period, indicating that ocean water quality was degraded from stormwater.
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