


**RESEARCH ARTICLE**

# Detection and modeling of *Staphylococcus aureus* and fecal bacteria in Hawaiian coastal waters and sands

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## Funding information

This project was supported by grants from the National Institute of Health (NIH) COBRE (Grant No. P20GM125508), National Institute of General Medical Sciences (NIGMS), IDeA Networks of Biomedical Research Excellence (INBRE: Award no: P20GM103466), Hau'oli Mau Loa Foundation, and the United States Geological Survey (USGS) via the Pacific Islands Climate Adaptation Science Center (PI-CASC, Cooperative Agreement No. G12AC00003). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Undergraduate research support was provided in part by the National Oceanic and Atmospheric Administration, Educational Partnership Program with Minority Serving Institutions through an undergraduate scholarship awarded from academic years 2015 – 2017, UH Hilo's Pacific Internships Program for Exploring

## Abstract

Microbial pollution of recreational waters leads to millions of skin, respiratory, and gastrointestinal illnesses globally. Fecal indicator bacteria (FIB) are monitored to assess recreational waters but may not reflect the presence of *Staphylococcus aureus*, a global leader in bacterial fatalities. Since many community-acquired *S. aureus* skin infections are associated with high recreational water usage, this study measured and modeled *S. aureus*, methicillin-resistant *S. aureus* (MRSA), and FIB (*Enterococcus* spp., *Clostridium perfringens*) concentrations in seawater and sand at six beaches in Hilo, Hawai'i, USA, over 37 sample dates from July 2016 to February 2019 using culturing techniques. Generalized linear models predicted bacterial concentrations with physico-chemical and environmental data. Beach visitors were also surveyed on their preferred activities. *S. aureus* and FIB concentrations were roughly 6–78 times higher at beaches with freshwater discharge than at those without. Seawater concentrations of *Enterococcus* spp. were positively associated with MRSA but not *S. aureus*. Elevated *S. aureus* was associated with lower tidal heights, higher freshwater discharge, onsite sewage disposal system density, and turbidity. Regular monitoring of beaches with freshwater input, utilizing real-time water quality measurements with robust modeling techniques, and raising awareness among recreational water users may mitigate exposure to *S. aureus*, MRSA, and FIB.

Maria Steadmon is a member of the Water Environment Federation (WEF).

Science (PIPES, National Science Foundation Grant No. 1005186, 1461301), UH Hilo's Students of Hawai'i Advanced Research Program (SHARP, National Institutes of Health Research Initiative for Scientific Enhancement Award No. R25GM11347), and UH Mānoa's Center for Microbial Oceanography Research and Education (C-MORE, National Science Foundation Grant No. 0424599).

### Practitioner Points

- *Staphylococcus aureus* and fecal bacteria concentrations were higher in seawater and sand at beaches with freshwater discharge.
- In seawater, *Enterococcus* spp. positively correlated with MRSA, but not *S. aureus*.
- Freshwater discharge, OSDS density, water turbidity, and tides significantly predicted bacterial concentrations in seawater and sand.
- Predictive bacterial models based upon physicochemical and environmental data developed in this study are readily available for user-friendly application.

### KEYWORDS

groundwater, on-site sewage disposal systems, pathogens, recreational water quality, rivers, turbidity

## INTRODUCTION

Microbial pollution of surface waters leads to millions of skin, respiratory, and gastrointestinal illnesses annually worldwide (Shuval, 2003; Yau et al., 2009). *S. aureus* is the most common cause of skin infections, and community-acquired infections are rising where young, healthy individuals are becoming infected (Akanbi et al., 2017; Elmir et al., 2007; Zetola et al., 2005). In 2019, *S. aureus* was the leading cause of death from bacterial infections across 135 countries, with methicillin-resistant *S. aureus* (MRSA), an antibiotic-resistant strain of *S. aureus*, accounting for more than 100,000 deaths globally (Antimicrobial Resistance Collaborators, ARC, 2022a, 2022b). One environmental route to *S. aureus* exposure is recreational water use at beaches. Recreational water users report skin infections, including those from *S. aureus*, four to six times more than non-water users, with staphylococcal colonies isolated from seawater having similar antibiotic sensitivity as those recovered from clinical infections (Charoencra & Fujioka, 1995; Fleisher et al., 2010). *S. aureus* and MRSA have been documented in seawater and sands at beaches worldwide (Esiobu et al., 2013; Goodwin et al., 2012; Papadakis et al., 1997; Thapaliya et al., 2017). This poses potential health risks for beachgoers, as MRSA is classified as a high-priority antibiotic-resistant pathogen by the World Health Organization (WHO, 2017).

Presence of *S. aureus* and MRSA in seawater has been largely attributed to shedding from human skin and mucous membranes during bathing; less is known about their sources in sands (Elmir et al., 2007; Enns et al., 2012; Goodwin et al., 2012; Halliday & Gast, 2011; Plano et al., 2011). As 20–40% of the worldwide population carries *S. aureus*, it is not surprising that seawater *S. aureus* concentrations are positively correlated with beachgoer density (Enns et al., 2012; Friedman et al., 2015). Even at beaches

with relatively low human use, beachgoers risk exposure since *S. aureus* and MRSA are detected throughout watersheds (wildlife, streams, and soils), urban runoff, sewage, and beach sands, which all may function as potential sources of these bacteria in recreational waters (Economy et al., 2019; Gerken et al., 2021; Gerken et al., 2022; Halliday & Gast, 2011; Porrero et al., 2013). Once in recreational waters, physicochemical parameters such as salinity, water temperature, dissolved oxygen, nutrients, and turbidity may impact concentrations of *S. aureus* (Burton et al., 1987; Economy et al., 2019). Environmental factors like rainfall, surface runoff, river discharge, and high wave action can elevate turbidity levels from erosion and sediment resuspension (Christensen et al., 2001). These sediments can transport adsorbed bacteria (including FIB and pathogens) from land to water, contributing to high turbidity conditions, with physicochemical parameters impacting their exchange between sediments and water (Hassard et al., 2017). Temporal drivers, including seasons and tides, may also impact microbiota abundance through altering physicochemical and environmental conditions, as demonstrated in estuarine environments (Kaestli et al., 2017).

While there are currently no recreational water quality standards for *S. aureus*, universal standards exist for *Enterococcus* spp., a fecal indicator bacteria (FIB) used to assess sewage pollution levels (Byappanahalli et al., 2012). However, *Enterococcus* spp. are widespread in tropical soils and streams, with exceedances occurring in the absence of sewage pollution (Byappanahalli et al., 2012; Fujioka & Shizumura, 1985; Gerken et al., 2022). In tropical locations, like Hawai'i, the Department of Health (HDOH) has adopted *C. perfringens* as a secondary sewage indicator (HDOH, 2017). *C. perfringens* abundance in Hawaiian recreational waters is low during ambient conditions but increases after sewage pollution spills and storms (Economy et al., 2019; Fujioka et al., 2015). Given that

*S. aureus* has been detected in raw sewage (Börjesson et al., 2010; Economy et al., 2019), it remains unclear whether FIB are reliable predictors for *S. aureus* in recreational waters.

The U.S. Environmental Protection Agency (U.S. EPA) employs multiple linear regression models (facilitated by the software Virtual Beach 2.0), to predict log-transformed FIB seawater concentrations like *Enterococcus* spp. (U.S. EPA, 2010). These models consider real-time and forecasted physicochemical and environmental data as predictors, with a ranking process that automatically excludes models with highly correlated predictors, which increases the risk of type II errors (false negatives). This process may overlook significant variables crucial for accurate predictions of fecal and pathogenic bacteria for assessing safe swimming conditions (Zuur et al., 2010).

The goal of this study was to measure the abundance of *S. aureus*, MRSA, and FIB in seawater and sand while identifying the physicochemical and environmental parameters associated with elevated concentrations of these bacteria. Additionally, this research aimed to define the predictive relationships among these bacteria using robust modeling techniques. This research was conducted at beaches in Hilo, Hawai'i, which is situated in the state with some of the highest annual rates of community-acquired MRSA infections in the United States (Estivariz et al., 2007; Jarvis et al., 2007; Li et al., 2005). Notably, Hilo, the second-most populous city in Hawai'i, also exhibits high rates of inpatients with community-acquired MRSA infections, with roughly 350 cases a year (HSL, 2021). This study is the first to investigate this combination of bacteria in beach waters and sands using advanced mixed modeling techniques. To contextualize our findings and gain insights into at-risk populations and the recreational activities occurring at beaches with elevated concentrations of *S. aureus*, MRSA, and FIB, we also conducted surveys among beachgoers at our study sites. These findings will allow for targeted awareness among high-risk groups based on popular activities across beaches.

## METHODS

### Study Site

Hilo is located on the eastern side of Hawai'i Island, USA (Figure 1). It has one of the highest annual precipitation rates in the U.S., receiving ~305 cm of rainfall by the coast and ~700 cm at higher elevations, with wet winters and dry summers (Giambelluca et al., 2013). Hilo Bay has a 2.9-km breakwater that reduces water circulation and retains watershed materials (M & E Pacific, 1980). For this study, six beaches in Hilo were sampled: Honoli'i, Bayfront, Reed's Bay, Puhi Bay, Onekahakaha, and

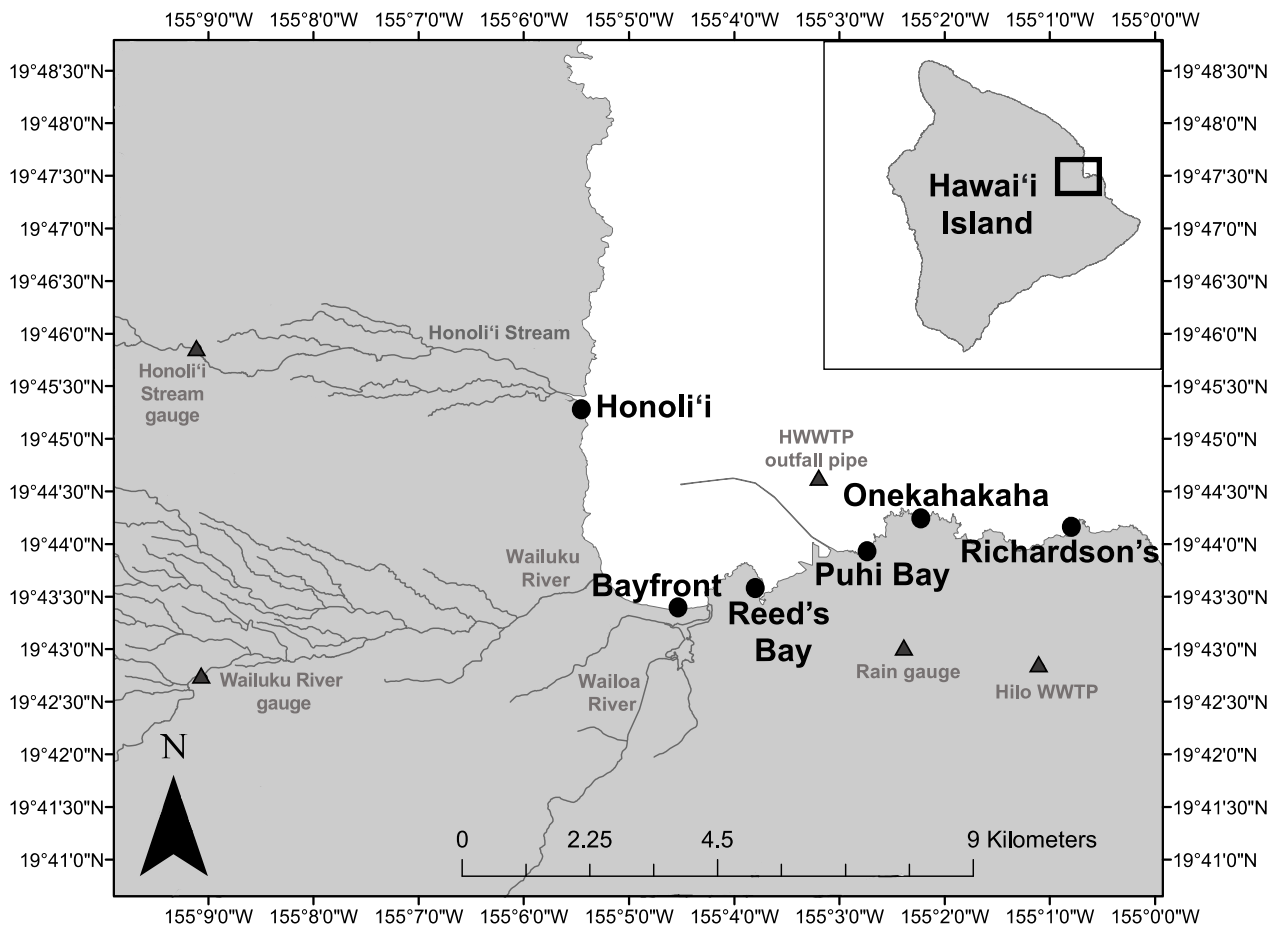
Richardson's Ocean Park (Figure 1). Honoli'i, Bayfront, and Richardson's Ocean Park are wave-dominated, while Reed's Bay, Puhi Bay, and Onekahakaha have barriers like seawalls sheltering these sites from high wave action. Honoli'i Stream discharges at Honoli'i Beach, and the Wailuku River and Wailoa River discharge into Bayfront Beach. There is submarine groundwater discharge (SGD) at each beach, with the highest SGD at Reed's Bay and Puhi Bay (Fischer et al., 1966). Honoli'i, Hilo Bayfront, and Onekahakaha are designated Tier 1 "Core" beaches for Hawai'i Island by the HDOH Hawai'i Beach Monitoring Program based upon high-usage or high-risk, necessitating sampling three times weekly (U.S. EPA, 2014; HDOH, 2017).

### Water and Sand Collection

Water and sand collection took place from July 2016 to February 2019 over 37 sampling dates. The selection of sampling dates was primarily guided by practical considerations, including safe weather conditions for sample collection and logistical constraints such as availability of resources and personnel. Triplicate seawater samples were collected at beaches roughly 1 m from shore (often in the surf zone at beach sites with high wave-action) where bather contact is most likely to occur (Alm et al., 2003; Boehm et al., 2002; Boehm et al., 2005; HDOH, 2012). At wave-driven beaches, the surf zone is defined as the location between where waves break and the shoreline that can facilitate the rapid transport of pathogens that pose a potential health risk to beachgoers (Alm et al., 2003; Boehm et al., 2005). Samples were consistently collected before or shortly after sunrise to reduce photo-inactivation of cells that reduce bacteria concentrations (Whitman et al., 2004). Seawater was collected in an 18-L plastic bucket, pre-rinsed with sample water, and stored in 1-L sterile polypropylene bottles on ice for 4–6 h until laboratory analysis. On-site, physicochemical parameters including salinity (in practical salinity units PSS78), water temperature, and dissolved oxygen (DO) concentrations were measured with a calibrated YSI Pro 2030 multi-parameter probe. Roughly 100 g of wet sand was collected at beaches at a depth of 5 cm perpendicular to the shoreline (within the foreshore and the intertidal swash zone) located ~1 m from the high tide line. Sand samples were placed in sterile 100-mL polypropylene bottles and stored on ice for 4–6 h until laboratory analysis.

### Bacterial enumeration

For enumeration of *S. aureus* and MRSA, 10 ml of seawater was filtered through 47-mm 0.45- $\mu$ m pore size



**FIGURE 1** Map of beaches sampled in Hilo, Hawai'i, USA. Water and sand samples were collected for analysis of *Staphylococcus aureus*, Methicillin-Resistant *S. aureus* (MRSA), and Fecal Indicator Bacteria (FIB) concentrations from July 2016 – February 2019. Beaches (marked by black circles) include (from left to right): Honoli'i, Bayfront, Reed's Bay, Puhi Bay, Onekahakaha Beach Park, and Richardson's Ocean Park (a.k.a., Richardson's). Also shown on the map are locations of Honoli'i Stream, Honoli'i Stream gauge, Wailuku River, Wailuku River gauge, Wailoa River, Hilo International Airport rainfall gauge, Hilo Wastewater Treatment Plant (HWWTP) and the HWWTP outfall pipe.

cellulose nitrate filters consistent with previous publications (Charoencra & Fujioka, 1993; Goodwin & Pobuda, 2009; Tice et al., 2010; Viau et al., 2011). Ten grams of wet sand from each sample was shaken with 200 ml of 0.15 M NaCl at 100 rpm for 45 min, and 10 ml, 25 ml, and 50 ml of sand slurry supernatant were filtered through 0.45- $\mu$ m pore size cellulose nitrate filters. Past studies revealed that *S. aureus* colonies have limited passage through 0.45- $\mu$ m pore size filters despite an average cell diameter of 0.5–1.5  $\mu$ m (Harris et al., 2002; Onyango et al., 2010). *S. aureus* and MRSA were cultured on BBL CHROMagar™ *S. aureus* (Becton, Dickinson Co. MD) and BBL CHROMagar™ MRSA II Select media (Becton, Dickinson Co., MD), respectively. *S. aureus* and MRSA cultures were incubated at 37°C for 24 h, and mauve morphologies were counted (Goodwin & Pobuda, 2009; Viau et al., 2011). To confirm morphologies, putative *S. aureus*, and MRSA colonies were re-streaked on BBL

CHROMagar™ *S. aureus* and BBL CHROMagar™ MRSA II Select media, respectively, and incubated again for 24–48 h. Economy et al. (2019) confirmed that 21 of 87 mauve colonies displayed gram-positive cocci cluster morphology, and 14 of these tested positive for *S. aureus* and MRSA using 24-h tube coagulase tests (BD BBL Coagulase Plasma, BD Diagnostics). CHROMagar has a positive predictive accuracy of 70–99% for *S. aureus* and 92% for MRSA (Goodwin & Pobuda, 2009; Tice et al., 2010). Thus, our reported *S. aureus* and MRSA concentrations are presumptive. *Enterococcus* spp. were quantified using the Enterolert MPN method and QuantiTray®/2000 from IDEXX Laboratories Incorporated, following the manufacturer's recommendations (10 ml seawater and 90 ml of sterile water) that have been found to have a 99.8% sensitivity (Budnick et al., 1996; Palmer et al., 1993). Quanti-Tray wells that exhibited no fluorescence were considered below the

detection limit (<10 MPN/100 ml for 1:10 dilution of the sample) and reported as 5 MPN/100 ml (Budnick et al., 1996). *C. perfringens* in seawater and sand were cultured on mCP medium following identical water filtering volumes, filter sizes, and sand processing as described above for *S. aureus* and MRSA in seawater and sand (Acumedia, Baltimore, MD), incubated anaerobically at 45°C for 24 h, and then exposed to ammonium hydroxide. Bright-pink colonies were counted (Bisson & Cabelli, 1979). Fujioka and Shizumura (1985) had a 91% recovery of *C. perfringens* using mCP medium, with 89/91 colonies being true positives. All reported *S. aureus*, MRSA, *Enterococcus* spp., and *C. perfringens* concentrations were corrected for sample dilution in seawater.

## Physicochemical Analysis

Samples were analyzed for nutrient concentrations of nitrate plus nitrite ( $\text{NO}_3^- + \text{NO}_2^-$ ; U.S. EPA 353.4, Detection Limit [DL] = 0.07  $\mu\text{mol/l}$ ), ammonium ( $\text{NH}_4^+$ ; United States Geological Survey, USGS I-2525, DL = 0.36  $\mu\text{mol/l}$ ), phosphate ( $\text{PO}_4^{3-}$ ; U.S. EPA 365.5, DL = 0.03  $\mu\text{mol/l}$ ), total dissolved phosphorus (TDP; USGS I-4650-03, DL = 0.25  $\mu\text{mol/l}$ ), and silicic acid ( $\text{H}_4\text{SiO}_4$ ; U.S. EPA 366, DL = 1.00  $\mu\text{mol/l}$ ) with a Lachat Quikchem 8500, flow-injection autoanalyzer using standard methods and reference materials. Dissolved organic carbon (DOC; U.S. EPA 415.1, DL = 10.0  $\mu\text{mol/l}$ ) and total dissolved nitrogen (TDN; ASTM D5176, DL = 5.0  $\mu\text{mol/l}$ ) were analyzed on a Shimadzu TOC-V CSH, TNM-1 analyzer (Sharp et al., 2002). Dissolved organic nitrogen (DON) values were calculated by subtracting  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NH}_4^+$  concentrations from those of TDN. Dissolved organic phosphorus (DOP) values were calculated from the difference between TDP and  $\text{PO}_4^{3-}$ . Select samples were analyzed for inorganic and organic nutrients ( $n = 23$  across all beaches). Turbidity was measured using a calibrated Turbidimeter (HACH 2100P). Analyses were conducted at the University of Hawai'i at Hilo Analytical Laboratory.

The proportion of moisture in the sand was calculated by taking the difference between wet and dry weights (10 g of wet sand dried at 60°C), divided by the initial weight. Sand moisture (proportional dry weight) and the specific density of freshwater (1 g/ml) were used to convert concentrations to MPN or colony forming units (CFU) per g dry weight of sand. Sand organic matter content was determined by combusting samples at 500°C for 5.5 h. Sand grain size was determined using modified methods from Poppe et al. (2000). Approximately 10 g of dried sand from the samples were individually distributed into stacked wire-mesh sieves (-2, -1, 0, 1, 2, 3,

and 4 phi) and agitated for 10 min. After sieving, the weight of each phi size-fraction was weighed and the relative percentages of gravel, sand, and silt/clay fractions were calculated.

## Environmental data

Tidal stage (ebb or flood) and tidal height (m) data were obtained through the National Oceanic and Atmospheric Administration (NOAA) Buoy for Hilo, Hilo Bay, Kuhio Bay, HI (<https://tidesandcurrents.noaa.gov/waterlevels.html?id=1617760>) (NOAA, 2023). Maximum river discharge ( $\text{m}^3/\text{s}$ ) within 24 h of sampling ( $Q_{\text{max}}$ ) for USGS gauges for Honoli'i stream (16717000) and the Wailuku River (16704000) were used for Honoli'i and Bayfront beaches (<https://waterdata.usgs.gov/hi/nwis/current/?type=flow>) (USGS, 2023). Note that the Wailoa River, which also discharges into Hilo Bay (Figure 1), does not have a stream gauge. Locations of on-site sewage disposal systems (OSDS) on Hawai'i Island were accessed from the State of Hawai'i, Office of Planning and Sustainable Development (OPSD) (<https://planning.hawaii.gov/gis/download-gis-data-expanded/>) (State of Hawai'i, OPSD, 2023). The total OSDS within a 1-km circumference of each beach (referred to as OSDS density), and the straight-line beach distance (m) to a discharging stream (further referred to as DS) were quantified using ArcGIS Desktop v.10.4.1 (Environmental Systems Research Institute, Redlands, CA). To account for variation in rainfall and consistent with other studies, cumulative 24-h rainfall (cm) prior to sampling was accessed from the NOAA National Weather Service (NWS) Hilo International Airport rainfall gauge (4 m above sea level) (<https://www.weather.gov/wrh/Climate?wfo=hfo>) (Economy et al., 2019; NWS, 2023; Strauch et al., 2014).

## In-person Beach Survey Design

An in-person survey was developed to obtain information on adult (18 + years) beach goers and water activities across six beach sites. Surveys were conducted for six weeks (January 20th to February 10th, and September 4th to 17th, 2017) over 1-h periods at each beach at one of the three following times (6:30–7:30 am, 12:30–1:30 pm, 5:00–6:00 pm) to capture varying activities. Participants had the opportunity to share relevant comments to the survey. Every week, each beach was surveyed daily, with its survey time systematically varying to reduce bias. Each beach was surveyed 2–3 times for each survey time. Informed consent was obtained from all participants involved in the study. The study was conducted

according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the University of Hawai'i, (Protocol # 2016–31,151, approval 26 January 2017).

## Statistical analyses

A Principal Component Analysis (PCA) was run to collapse correlated variance along orthogonal axes and reduce collinearity for multivariate analysis among physicochemical and environmental parameters. Principal component scores for each variable in the PCA are demonstrated in Equation (1):

$$PC_{score} = X_1L_1 + X_2L_2 + \dots + X_nL_n \quad (1)$$

where  $PC_{score}$  stands for the principal component (PC) score of variable  $x$ , where  $X_n$  denotes the observed raw data variable value, and  $L_n$  signifies the corresponding principal component loading derived from the PCA. The PC scores for each observation in the dataset based on the PCA components were used as predictor variables in subsequent model analyses (Quinn & Keough, 2002). PCA ordinated quantitative data including temperature, salinity, turbidity, DO, tidal height, rainfall, beach distance to a stream (DS), and OSDS density. The analysis excluded  $Q_{max}$  (river discharge), nutrients, and sand physicochemical parameters due to the limited sample size for each.

The normality of *S. aureus*, MRSA, *Enterococcus* spp., and *C. perfringens* concentrations in seawater and sand were assessed using Shapiro-Wilks tests, with all concentrations following non-parametric distributions ( $p > 0.05$ ). Mood's Median Tests (MMT) followed by paired MMT were used to assess differences among median non-parametric concentrations of bacteria sampled in water and sand across beaches. Medians rather than means are reported for all bacteria concentrations to account for outliers. Negative binomial generalized linear mixed models (GLMM) were run to determine significant correlations between *S. aureus*, MRSA, and FIB in seawater and sand. A negative binomial GLMM was employed as a robust modeling approach for non-parametric count data, avoiding the need for data transformations and effectively addressing concerns related to overdispersion (Fávero & Belfiore, 2019). All bacteria concentrations in seawater and sand were fixed effects and the sampled beach was included as a random effect in all models. Log-likelihood ratio tests (LRT), which assume differences in deviance, were approximated with a Chi-square distribution and used to determine the significance of each predictor on the response variable and were used

since only one variable was a predictor in each model (Bates et al., 2015). A Pseudo- $R^2$ ,  $\Omega_0^2$ , was included for significant associations in bacteria-only models and was used to measure goodness of fit by indicating the proportion of variance explained by the predictors of each GLMM (Nakagawa et al., 2017).

Negative binomial models, including both generalized linear models (GLM) and GLMM, were utilized to evaluate the effectiveness of physicochemical and environmental parameters for predicting concentrations of *S. aureus*, MRSA, and FIB in seawater and sand. The negative binomial GLM is represented by Equation (2):

$$\mu = E(Y) = \exp(\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n) \quad (2)$$

Here,  $E(Y)$  signifies the expected value of the response variable, while  $\beta_0$  denotes the model intercept, and  $\beta_n$  represents the coefficient for the predictor  $X_n$ . Similarly, negative binomial GLMM introduces a random effect  $u$  into the model, as demonstrated by Equation (3):

$$\mu = E(Y) = \exp(\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n + u) \quad (3)$$

Whereas the random effect follows a normal distribution, described by Equation (4):

$$u \sim N(0, \sigma_u^2) \quad (4)$$

Here,  $N$  indicates a normal distribution and  $\sigma_u^2$  represents the variance parameter of the random effect. In the case of zero-inflated negative binomial models, the zero-inflation probability  $p$  is incorporated, as expressed in Equation (5):

$$\begin{aligned} p &= P(Y = 0) \\ &= \text{logit}^{-1}(\beta_{zi,0} + \beta_{zi,1} X_1 + \beta_{zi,2} X_2 + \dots + \beta_{zi,n} X_n) \end{aligned} \quad (5)$$

Here  $P(Y = 0)$  refers to the probability of the response being 0 (also known as the probability of non-structural zeros). The intercept of the zero-inflation model is denoted by  $\beta_{zi,0}$  and the zero-inflation coefficient for the predictor  $X_n$  is represented by  $\beta_{zi,n}$  (if applicable). Further description, derivation, and application of equations for each model are included in the [supplemental material](#). Principal components were used as predictors instead of individual raw physicochemical and environmental parameters (with the exclusion of tidal height) to reduce collinearity in models. The principal components and tidal height were included as fixed effects and sampled beach as a random effect in all GLMM. Select bacteria

were modeled with GLM rather than GLMM, where principal components and tidal height were fixed effects, but did not include a random effect to reduce complexity and improve model fit. Diagnostic tests run on models were run to reduce type I and type II errors and assess model fit. These tests included evaluating predictor collinearity, examining the necessity of zero-inflation (to address the potential disproportionate occurrence of zero counts in the data), convergence issues, and singularity (Allison, 2008; Matuschek et al., 2017; Zuur et al., 2010). Zero-inflation terms were included in negative binomial GLMM models if deemed necessary. For nested model comparisons, models were quantitatively ranked based on “Corrected” Akaike Information Criterion (AICc),  $\Delta$  AICc, and Akaike weights (weight) (Portet, 2020). Best-fitting models are determined by the lowest AICc and  $\Delta$  AICc, and highest Akaike weights (Portet, 2020). To conduct nested model comparisons, an intercept-only model, also known as the null model, was incorporated (Long & Freese, 2001). For comparisons to previously published work, the  $\Omega_0^2$  was calculated for the highest-ranked models. Visualizations of each predictor's effect size, estimated from the model's coefficients, are included in the [supplementary material](#).

Survey data were compared among beaches using chi-square tests. Due to the limited availability of data on nutrients, river discharge, and sand physicochemical parameters collected in this study, these data were exclusively used to enhance the understanding of conditions across each beach and are excluded from statistical analyses. RStudio Statistical Software (Affero General Public License, RStudio, Inc.) was used for statistical analysis with the following packages: “glmmTMB,” “MuMIn,” “plyr,” “dplyr,” “effects,” “car,” and “performance” ( $\alpha = 0.05$ ).

## RESULTS

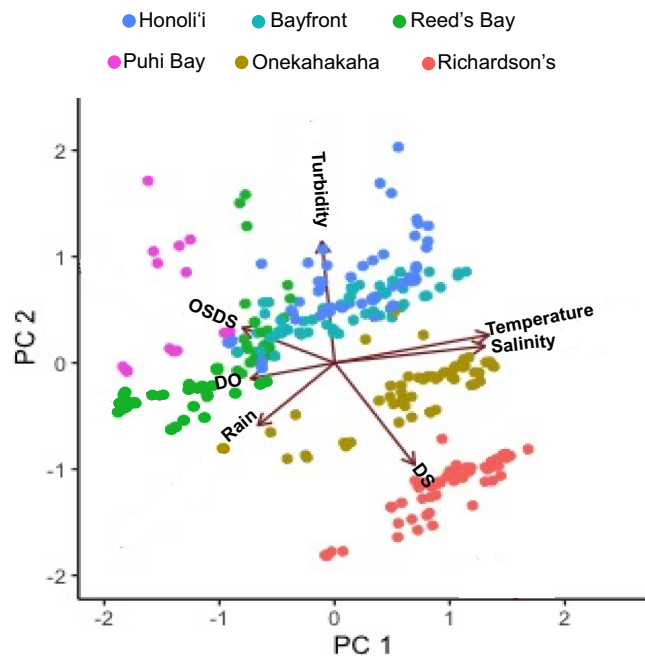
### Physicochemical and Environmental Parameters

Physicochemical water and sand parameters varied across beaches (Table 1, Table S1). Puhi Bay and Reed's Bay had the lowest mean salinities (range: 13.22–14.90) and water temperatures (20.51–21.09°C) that were roughly half as salty with temperatures cooler than other beaches. Bayfront had the highest average turbidity (mean±SD, 13.09 ± 28.95 NTU) that was three times greater than at other beaches (0.93–5.44 NTU), with  $Q_{\max}$  rates three times higher at Wailuku River (7.2 ± 12.0 m<sup>3</sup>/s) than Honoli'i Stream (2.4 ± 3.9 m<sup>3</sup>/s). Puhi Bay has the highest OSDS densities of all beaches

TABLE 1 Average ± SD and (n) values of physicochemical parameters of seawater and environmental parameters for all six beaches sampled in Hilo, Hawai'i, USA, collected from July 2016 – February 2019.

	Honoli'i	Bayfront	Reed's Bay	Puhi Bay	Onekahakaha	Richardson's
Salinity*	24.97 ± 6.26 (75)	23.78 ± 4.53 (66)	13.22 ± 4.88 (75)	14.90 ± 3.74 (36)	27.04 ± 5.37 (69)	27.25 ± 3.10 (69)
Water Temp. (°C)	24.28 ± 1.19 (63)	24.26 ± 1.02 (54)	21.09 ± 1.15 (63)	20.51 ± 1.05 (24)	24.20 ± 0.95 (57)	24.02 ± 0.90 (57)
DO (mg/L)	6.82 ± 1.03 (75)	6.45 ± 0.85 (66)	6.35 ± 0.70 (75)	6.84 ± 0.93 (36)	5.59 ± 1.09 (69)	6.32 ± 0.97 (69)
Turbidity (NTU)	5.44 ± 6.18 (75)	13.09 ± 28.95 (66)	4.88 ± 7.72 (75)	4.86 ± 9.63 (36)	2.18 ± 3.36 (69)	0.93 ± 1.33 (69)
Tidal height (m)	0.43 ± 0.16 (63)	0.39 ± 0.16 (54)	0.37 ± 0.17 (63)	0.32 ± 0.17 (24)	0.38 ± 0.15 (57)	0.38 ± 0.15 (57)
DS (m)	160	500	860	2760	3830	6180
OSDS density	43	29	30	100	40	13
$Q_{\max}$ (m <sup>3</sup> /s)	2.4 ± 3.9 (75)	7.2 ± 12.0 (54)	-	-	-	-

\*Salinity is in practical salinity units (PSS78) (United Nations Educational, Scientific, and Cultural Organization, UNESCO, 1985); Temp. = temperature; DO = dissolved oxygen concentrations; DS = Beach distance to a stream (approximate linear beach distance to a discharging stream); OSDS density = approximate amount of On-Site Sewage Disposal Systems within 1-km diameter of each beach;  $Q_{\max}$  = Maximum river discharge (m<sup>3</sup>/s) within 24 h of sampling; - = data not available or applicable.



**FIGURE 2** PCA biplot of physicochemical and environmental parameter data collected from six beaches sampled in Hilo, Hawai'i, USA, from July 2016 – February 2019. Each point represents a PCA score that is colored by the beach where the data was observed alongside data collected for bacteria ( $n = 99$ ). PCA loadings (correlations) are represented by arrows where the direction demonstrates the relationship between the parameter and the PC, while the length demonstrates the strength of the loading (a longer arrow indicates a stronger correlation). Variation explained by Principal Component (PC) 1 is 34% and PC 2 is 17% for a cumulative variation of 51%. PC 1 is strongly correlated with salinity and temperature and PC2 is associated with turbidity.

( $\sim 100$  OSDS  $\text{km}^{-1}$ ), and it is 13 times greater than the density at Richardson's (13 OSDS  $\text{km}^{-1}$ ), which has the lowest density of all beaches. Onekahakaha sand had the highest moisture ( $21.95 \pm 5.92\%$ ) and organic matter content ( $0.75 \pm 0.57$  g), which was 7 and 3 times greater than Richardson's. Sand grain size composition across beaches was similar and consisted mostly of sand (72.01–97.28%) and gravel (0.81–27.30%).

PCA identified correlated axes of variation among the quantitative physicochemical and environmental parameters (Figure 2). Principal component 1 (PC1, x-axis) had positive loadings on salinity, temperature, and DS and negative loadings on DO, rainfall, and OSDS density, consistent with a low to high freshwater discharge gradient. Functionally, PC1 scores distinguished beaches like Richardson's and Onekahakaha, with positive values associated with warm, saltier waters further from stream mouths. Negative PC1 scores were associated with closer proximity to stream mouths and greater DO, rainfall, and OSDS densities. PC2 (y-axis) contained positive loadings on turbidity, temperature, and OSDS density, and negative loadings on rainfall, and DS. Positive scores in PC2 distinguish beaches with higher turbidity conditions that are frequently closer to streams, like Honoli'i and Bayfront beaches, and negative scores group beaches further from streams that receive high rainfall. PC1 and PC2 cumulatively explained 51% of the variation in the data of quantitative physicochemical and environmental parameters included in the analysis (Table 2).

**TABLE 2** Loadings (correlations) for principal components (PC) from PCA on physicochemical and environmental parameter data collected alongside bacteria concentrations across six beaches sampled in Hilo, Hawai'i, USA, from July 2016 – February 2019. The further the loadings are from zero (closer to  $-1$  or  $1$ ), the greater the influence of the variables on the principal component. Values bolded are the variables that are strongly correlated to each PC. Variables strongly correlated with each component include PC1 with salinity and temperature, PC2 with turbidity, PC3 with DO, PC4 with rainfall, PC5 with OSDS density, PC6 with beach site distance to a stream (DS), and PC7 with temperature. Cumulative variance is the cumulative percentage that is explained for each component. PC1 and PC2 were included in subsequent model analyses.

Parameter	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Salinity <sup>a</sup>	<b>0.551</b>	-	0.245	0.221	0.328	0.178	0.663
Temperature ( $^{\circ}\text{C}$ )	<b>0.566</b>	0.157	0.179	-	0.336	-0.173	<b>-0.692</b>
DO <sup>b</sup> (mg/L)	-0.307	-	<b>0.752</b>	-0.303	0.182	0.44	-0.117
Turbidity (NTU)	-	<b>0.683</b>	0.216	0.432	-0.495	0.212	-
Rain (cm)	-0.281	-0.352	0.343	<b>0.694</b>	0.116	-0.429	-
DS <sup>c</sup> (m)	0.293	-0.574	-0.12	0.293	-0.329	<b>0.577</b>	-0.207
OSDS density <sup>d</sup>	-0.339	0.198	-0.405	0.322	<b>0.617</b>	0.428	-0.124
Cumulative Variance	34%	51%	65%	77%	89%	98%	100%

<sup>a</sup>Salinity is in practical salinity units (PSS78) (UNESCO, 1985), <sup>b</sup> Dissolved oxygen concentrations, <sup>c</sup> DS = each distance to a stream, <sup>d</sup> Amount of onsite sewage disposal systems (OSDS) within 1-km of beach, - = data not applicable.

## Bacterial Concentrations

*Seawater* — *S. aureus*, MRSA, and FIB were detected in seawater at all beaches (Figure 3). *S. aureus* was detected in 67% (255/378) of seawater samples, with concentrations ranging from 0 to 4000 CFU/100 ml and differed across beaches. Honoli‘i had the greatest median seawater *S. aureus* concentrations (median±SD, 30 ± 689 CFU/100 ml), while Onekahakaha had the lowest (0 ± 21 CFU/100 ml). Methicillin-resistant *S. aureus* was detected in 61% (109/180) of the seawater samples, with concentrations ranging from 0 to 1146 CFU/100 ml and differed among beaches. Hilo Bayfront had the highest MRSA concentrations (62 ± 180 CFU/100 ml) and Onekahakaha had the lowest (0 ± 4 CFU/100 ml). *Enterococcus* spp. were present in all seawater samples, with concentrations ranging from <10–6131 MPN/100 ml and differed among beaches. In contrast to *S. aureus* and MRSA, Reed’s Bay had the highest median *Enterococcus* spp. concentrations (359 ± 878 MPN/100 ml), and Richardson’s had the lowest (30 ± 31 MPN/100 ml). In contrast to *S. aureus*, MRSA, and *Enterococcus* spp., *C. perfringens* was only detected in 21% (30/144) of samples. Concentrations ranged from 0 to 33 CFU/100 ml and differed among beach sites. Like with *Enterococcus* spp., Reed’s Bay had the highest *C. perfringens* concentrations (0 ± 4 CFU/100 ml). *C. perfringens* also had the lowest concentrations at Onekahakaha (0 ± 0 CFU/100 ml) similar to *S. aureus* and MRSA.

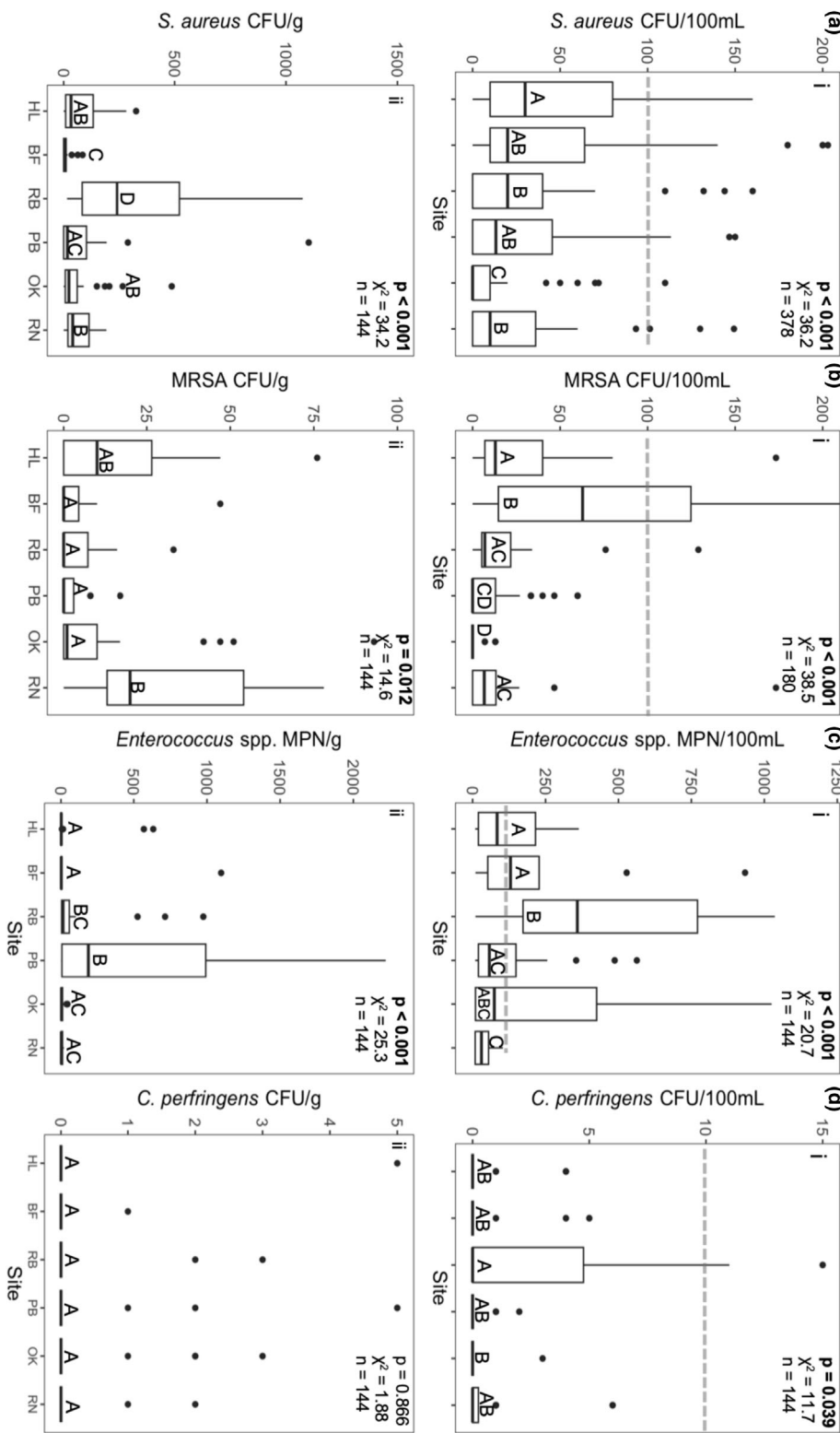
*Sand* — Similar to seawater, concentrations of *S. aureus*, MRSA, *Enterococcus* spp., and *C. perfringens* across beaches differed in sand (Figure 3). *S. aureus* concentrations in sands ranged from 0 to 8710 CFU/g and were detected in 84% of samples (121/144). The highest *S. aureus* concentrations were measured at Reed’s Bay (241 ± 350 CFU/g) and the lowest concentrations at Hilo Bayfront (5 ± 4 CFU/g). MRSA was detected in 50% (67/132) of the sand samples and concentrations ranged from 0 to 605 CFU/g. Richardson Ocean Park had the highest concentrations of MRSA in the sand (20 ± 11 CFU/g) and Puhi Bay had the lowest (0 ± 1 CFU/100 ml). *Enterococcus* spp. was detected in 90% (131/144) of sand samples, with its concentrations ranging from 0 to 5553 MPN/g and differing among beaches. Puhi Bay had the highest *Enterococcus* spp. concentrations (187 ± 252 MPN/g) and Honoli‘i, had the lowest (2 ± 30 MPN/g). *C. perfringens* was detected in 11% (16/144) of sand samples, with its concentrations ranging from 0 to 147 CFU/g, and was similar across beaches. The highest observation of *C. perfringens* in sand occurred at Reed’s Bay (147 CFU/g), while the lowest observations were frequently at Bayfront (0 ± 0 CFU/g).

## Associations among bacteria in seawater and sand

There were several significant associations between *S. aureus*, MRSA, and FIB across beaches in seawater and sand (Figure 4). Models of *S. aureus* in seawater indicated a significant positive correlation with MRSA concentrations in seawater (LRT,  $p = 0.002$ ,  $\Omega_0^2 = 0.54$ ), but not with seawater concentrations of *Enterococcus* spp. and *C. perfringens* ( $p > 0.05$ ). *Enterococcus* spp. concentrations were a significant predictor of increasing seawater MRSA concentrations ( $p = 0.04$ ,  $\Omega_0^2 = 0.41$ ), while *C. perfringens* concentrations were not ( $p > 0.05$ ). *Enterococcus* spp. concentrations in seawater were found to be significantly positively predictive of *C. perfringens* seawater concentrations ( $p < 0.001$ ,  $\Omega_0^2 = 0.48$ ). Models of *S. aureus* and *Enterococcus* spp. concentrations in sand exhibited a significant negative association ( $p = 0.03$ ,  $\Omega_0^2 = 0.59$ ), whereas other bacteria in sand were not significantly correlated with one another ( $p > 0.05$ ). *S. aureus* concentrations in seawater were significantly positively correlated with sand concentrations of *S. aureus* ( $p < 0.001$ ,  $\Omega_0^2 = 0.52$ ) and inversely with *Enterococcus* spp. in sand ( $p < 0.001$ ,  $\Omega_0^2 = 0.69$ ), but not with MRSA and *C. perfringens* sand concentrations ( $p > 0.05$ ). Similarly, MRSA concentrations in seawater exhibited a significant negative association with both *S. aureus* ( $p < 0.001$ ,  $\Omega_0^2 = 0.75$ ) and *Enterococcus* spp. in sand ( $p = 0.04$ ,  $\Omega_0^2 = 0.63$ ), while MRSA and *C. perfringens* sand concentrations did not exhibit significant associations ( $p > 0.05$ ). *Enterococcus* spp. in seawater models demonstrated significant inverse correlations with *S. aureus* in the sand ( $p = 0.01$ ,  $\Omega_0^2 = 0.63$ ) and positive with *Enterococcus* spp. in the sand ( $p = 0.009$ ;  $\Omega_0^2 = 0.73$ ), while MRSA and *C. perfringens* in the sand were not significantly correlated ( $p > 0.05$ ). For modeling *C. perfringens* in seawater, only *Enterococcus* spp. in sand exhibited a significant positive association ( $p = 0.03$ ,  $\Omega_0^2 = 0.65$ ), while other bacteria in sand were not significantly associated ( $p > 0.05$ ).

## Modeling bacterial concentrations using physicochemical and environmental parameters

Models of *S. aureus*, MRSA, and FIB demonstrated that physicochemical and environmental parameters were strong predictors of bacterial concentrations in seawater and sand. Best-fitted models uniformly included PC2 scores for all bacteria tested in this study (Table 3). The highest ranked for seawater concentrations of *S. aureus*



**FIGURE 3** Concentrations of (a) *Staphylococcus aureus*, (b) Methicillin-Resistant *S. aureus* (MRSA) (c) *Enterococcus* spp., and (d) *Clostridium perfringens* in both seawater (i) and sand (ii) across six beaches in Hilo, Hawai'i, USA, collected from July 2016 – February 2019. Beaches include Honoli'i (HL), Bayfront (BF), Reed's Bay (RB), Puhi Bay (PB), Onekahakaha (OK), Richardson's (RN). Boxes on the figure are interquartile ranges, horizontal lines are the median, whiskers are the range, solid points are outliers, and dashed lines are approximate representations of proposed and established water quality standards for an individual sample. Proposed: staphylococci = 100 CFU/100 ml (Favero et al., 1964), *Enterococcus* spp. = 130 MPN/100 ml Beach Action Value (BAV) (HDOH, 2017), recommended: *C. perfringens* < 10 CFU/100 ml for single sample exceedances (Fung et al., 2007). P-values, letter groupings, and  $\chi^2$  from Mood's Median Tests (values bolded are less than  $\alpha = 0.05$ ).

was the full model containing PC1, PC2, and tidal height (TH) (weight = 0.916,  $\Omega_0^2 = 0.22$ ; Figure S1). A model with only PC2 was best fitted to MRSA concentrations in seawater (weight = 0.526,  $\Omega_0^2 = 0.23$ ) and was a similar fit with the model containing PC1 and PC2 ( $\Delta$

AICc = 1.86,  $\Omega_0^2 = 0.25$ ) (Figure S2). A model including PC1, PC2, and TH outperformed other models for *Enterococcus* spp. concentrations in seawater (weight = 0.863,  $\Omega_0^2 = 0.53$ ) (Figure S3). *C. perfringens* in seawater highest ranked model was the full model containing PC1,

	<i>S. aureus</i> CFU/100 mL	MRSA CFU/100 mL	<i>Enterococcus</i> spp. MPN/100 mL	<i>C. perfringens</i> CFU/100 mL	<i>S. aureus</i> CFU/10 g	MRSA CFU/10 g	<i>Enterococcus</i> spp. MPN/10 g	<i>C. perfringens</i> CFU/10 g
<i>S. aureus</i> CFU/100 mL	<b>0.002 (+)</b> $\Omega_0^2 = 0.54$	<b>0.27</b>	<b>0.06</b>	<b>&lt;0.001 (+)</b> $\Omega_0^2 = 0.52$	<b>0.07</b>	<b>&lt;0.001 (-)</b> $\Omega_0^2 = 0.69$	<b>0.93</b>	
MRSA CFU/100 mL	<b>0.002 (+)</b> $\Omega_0^2 = 0.54$		<b>0.04 (+)</b> $\Omega_0^2 = 0.41$	<b>0.08</b>	<b>&lt;0.001 (-)</b> $\Omega_0^2 = 0.75$	<b>0.97</b>	<b>0.04 (-)</b> $\Omega_0^2 = 0.63$	<b>0.05</b>
<i>Enterococcus</i> spp. MPN/100 mL	<b>0.27</b>	<b>0.04 (+)</b> $\Omega_0^2 = 0.41$		<b>&lt;0.001 (+)</b> $\Omega_0^2 = 0.48$	<b>0.01 (-)</b> $\Omega_0^2 = 0.63$	<b>0.05</b>	<b>0.009 (+)</b> $\Omega_0^2 = 0.73$	<b>0.95</b>
<i>C. perfringens</i> CFU/100 mL	<b>0.06</b>	<b>0.08</b>	<b>&lt;0.001 (+)</b> $\Omega_0^2 = 0.48$		<b>0.79</b>	<b>0.59</b>	<b>0.03 (+)</b> $\Omega_0^2 = 0.65$	<b>0.30</b>
<i>S. aureus</i> CFU/10 g	<b>&lt;0.001 (+)</b> $\Omega_0^2 = 0.52$	<b>&lt;0.001 (-)</b> $\Omega_0^2 = 0.75$	<b>0.01 (-)</b> $\Omega_0^2 = 0.63$	<b>0.79</b>		<b>0.14</b>	<b>0.03 (-)</b> $\Omega_0^2 = 0.59$	<b>0.79</b>
MRSA CFU/10 g	<b>0.07</b>	<b>0.97</b>	<b>0.05</b>	<b>0.59</b>	<b>0.14</b>		<b>0.67</b>	<b>0.40</b>
<i>Enterococcus</i> spp. MPN/10 g	<b>&lt;0.001 (-)</b> $\Omega_0^2 = 0.69$	<b>0.04 (-)</b> $\Omega_0^2 = 0.63$	<b>0.009 (+)</b> $\Omega_0^2 = 0.73$	<b>0.03 (+)</b> $\Omega_0^2 = 0.65$	<b>0.03 (-)</b> $\Omega_0^2 = 0.59$	<b>0.67</b>		<b>0.62</b>
<i>C. perfringens</i> CFU/10 g	<b>0.93</b>	<b>0.05</b>	<b>0.95</b>	<b>0.30</b>	<b>0.79</b>	<b>0.40</b>	<b>0.62</b>	

p-value
<b>&lt;0.001</b>
<b>0.001 - 0.009</b>
<b>0.01 - 0.04</b>
<b>0.05 - 0.19</b>
<b>0.20 - 0.39</b>
<b>0.40 - 0.69</b>
<b>0.70 - 1.00</b>

**FIGURE 4** Negative binomial generalized linear mixed models (GLMM) of *Staphylococcus aureus*, Methicillin-Resistant *S. aureus*, and fecal indicator bacteria concentrations in seawater and sand across six beaches in Hilo, Hawai'i, USA, from samples collected from July 2016 – February 2019. Bacteria were included as fixed effects and beach was included as a random effect in all models. For mixed model application with sand, bacterial concentrations were scaled from CFU/g or MPN/g up to CFU/10 g or MPN/10 g. Pseudo- $R^2$ ,  $\Omega_0^2$ , is a proportion that indicates the variance explained by each predictor in GLMM with values that range between 0 and 1. Direction of modeled relationship is indicated by (+) for positive and (-) for negative. P-values are from log-likelihood ratio tests (LRT) to test the significance of each predictor ( $\alpha = 0.05$ ).

PC2, and TH (weight = 0.411,  $\Omega_0^2 = 0.30$ ), but was indistinguishable from the second and third highest ranked models, which were the full model containing PC1 and TH ( $\Delta AICc = 1.29$ ,  $\Omega_0^2 = 0.28$ ) and a model with only TH ( $\Delta AICc = 1.62$ ,  $\Omega_0^2 = 0.21$ ) (Figure S4).

The top two best-fitting models of *S. aureus* in sand were virtually identical in terms of their performance. These models included the full model (PC1, PC2, and TH) (weight = 0.417,  $\Omega_0^2 = 0.26$ ), followed by a model with PC2 and TH ( $\Delta AICc = 0.01$ ,  $\Omega_0^2 = 0.31$ ) (Figure S5). Similar to *S. aureus* in sand, top models of MRSA concentrations in sand performed similarly. The top models were a model containing PC1 and TH (weight = 0.689,  $\Omega_0^2 = 0.37$ ) and the full model (PC1, PC2, and TH) ( $\Delta AICc = 1.67$ ,  $\Omega_0^2 = 0.37$ ) (Figure S6). The four highest-ranked best-fit models for *Enterococcus* spp. in sand exhibited little discernible differences in how they fit the data. These included the full model (weight = 0.41,  $\Omega_0^2 = 0.86$ ), a model with only TH ( $\Delta AICc = 1.11$ ,  $\Omega_0^2 = 0.76$ ), a model with PC2 and TH ( $\Delta AICc = 1.37$ ,  $\Omega_0^2 = 0.75$ ), and a model with PC1 and TH ( $\Delta AICc = 1.89$ ,  $\Omega_0^2 = 0.82$ )

(Figure S7). Lastly, model comparisons demonstrated that a model with PC2 and TH was the best fit for *C. perfringens* in sand (weight = 0.699,  $\Omega_0^2 = 0.82$ ) (Figure S8).

### Beach surveys

A total of 427 surveys were completed, but only 422 were included in this analysis due to response discrepancies. Ninety-eight percent of beachgoers asked to participate in the survey agreed and completed it. There was an equal gender ratio in beachgoers who completed the survey ( $n$  male = 237, 56%,  $n$  female = 185, 44%;  $\chi_1 = 3.60$ ,  $p = 0.058$ ), and their ages ranged from 18 to 66+ years, with the majority ranging in age from 25 to 44 years ( $n = 207$ , 49%), followed closely by 18–24 ( $n = 38\%$ ) (Table S2). Different water activities dominated at different beaches. At Bayfront, beachgoers preferred paddling (64%), with surfing at Honoli'i (66%), and swimming at Onekahakaha (40%), Reed's Bay (38%), Puhi Bay (37%), and Richardson's (76%).

**TABLE 3** Negative binomial generalized linear model (GLM) and GLMM comparisons predicting concentrations of *S. aureus*, MRSA, and FIB in seawater and sand including the null (intercept only) model. Physicochemical and environmental parameters were summarized by principal components (PC). These components were included as fixed effects along with tidal height (TH) in every model. Zero-inflated models are denoted by †, and GLMM are denoted by \*. Rankings were based on AICc,  $\Delta$  AICc, and Akaike weights.  $\Delta$  AICc of less than 2 means both models are indistinguishable in their fit to the data from one another. Models are in descending order starting with the best-fit model. For mixed model application with sand, bacterial concentrations were scaled from CFU/g or MPN/g up to CFU/10 g or MPN/10 g.

Bacteria	Model	Intercept	Log-Lik	AICc	$\Delta$ AICc	Weight
<i>S. aureus</i> †* (CFU/100 ml)	PC1 + PC2 + TH	3.201	−1301.229	2616.8	0	0.916
	PC1 + PC2	4.278	−1304.670	2621.6	4.79	0.084
	PC2 + TH	3.400	−1310.436	2633.1	16.32	0
	PC2	4.564	−1313.650	2637.5	20.67	0
	PC1	4.380	−1321.472	2653.1	36.32	0
	PC1 + TH	3.944	−1321.096	2654.5	37.64	0
	Intercept	4.544	−1326.868	2659.8	42.99	0
	TH	4.164	−1326.274	2662.7	45.92	0
MRSA†* (CFU/100 ml)	PC2	3.188	384.427	779.3	0	0.526
	PC1 + PC2	3.123	−384.257	781.2	1.86	0.208
	PC2 + TH	3.040	−384.353	781.4	2.05	0.189
	PC1 + PC2 + TH	2.927	−384.138	783.2	3.85	0.077
	PC1	3.431	−394.307	799.1	19.76	0
	TH	3.863	−394.616	799.7	20.38	0
	PC1 + TH	4.009	−393.558	799.8	20.46	0
	Intercept	2.675	−400.523	807.2	27.90	0
<i>Enterococcus</i> spp.* (MPN/100 ml)	PC1 + PC2 + TH	6.245	−815.234	1643.1	0	0.863
	PC2 + TH	6.352	−818.197	1646.9	3.73	0.134
	PC1 + PC2	5.154	−822.739	1656.0	12.81	0.001
	TH	6.861	−824.306	1656.9	13.79	0.001
	PC2	5.287	−824.525	1657.4	14.23	0.001
	PC1 + TH	6.854	−823.682	1657.8	14.70	0.001
	Intercept	5.417	−835.778	1677.7	34.60	0
	PC1	5.422	−835.775	1679.9	36.73	0
<i>Clostridium perfringens</i> † (CFU/100 ml)	PC1 + PC2 + TH	1.418	−138.547	289.8	0.00	0.411
	PC1 + TH	2.030	−140.290	291.1	1.29	0.216
	TH	2.027	−141.538	291.4	1.62	0.182
	PC2 + TH	1.653	−140.813	292.1	2.34	0.128
	PC1 + PC2	−0.229	−142.237	295.0	5.18	0.031
	Intercept	0.321	−145.999	296.1	6.32	0.017
	PC2	0.190	−144.302	296.9	7.15	0.012
	PC1	0.144	−145.526	299.4	9.60	0.003
<i>S. aureus</i> †* (CFU/10 g)	PC1 + PC2 + TH	6.631	−1008.109	2031.1	0	0.417
	PC2 + TH	6.718	−1009.232	2031.1	0.01	0.414
	PC1 + PC2	7.516	−1010.743	2034.2	3.04	0.091
	PC2	7.691	−1012.147	2034.8	3.65	0.067
	TH	6.635	−1014.467	2039.4	8.29	0.007
	PC1 + TH	6.646	−1014.440	2041.6	10.43	0.002
	PC1	7.601	−1016.889	2044.3	13.13	0.001
	Intercept	7.524	−1019.350	2044.9	13.76	0

TABLE 3 (Continued)

Bacteria	Model	Intercept	Log-Lik	AICc	Δ AICc	Weight
MRSA† (CFU/10 g)	PC1 + TH	8.049	−557.092	1124.7	0	0.689
	PC1 + PC2 + TH	8.251	−556.828	1126.3	1.67	0.300
	TH	7.697	−562.722	1133.8	9.10	0.007
	PC2 + TH	7.957	−562.297	1135.1	10.41	0.004
	PC1 + PC2	6.353	−566.436	1143.3	18.69	0
	PC2	6.341	−569.366	1147.0	22.39	0
	PC1	6.357	−572.436	1153.2	28.53	0
	Intercept	5.69	−585.247	1174.6	49.93	0
<i>Enterococcus</i> spp.* (MPN/10 g)	PC1 + PC2 + TH	8.481	−867.735	1748.1	0	0.405
	TH	8.253	−870.468	1749.3	1.11	0.233
	PC2 + TH	8.284	−869.516	1749.5	1.37	0.205
	PC1 + TH	8.363	−869.779	1750.0	1.89	0.157
	PC1 + PC2	6.591	−883.833	1778.1	30.00	0
	PC1	6.512	−885.395	1779.1	30.96	0
	Intercept	6.275	−887.343	1780.9	32.73	0
	PC2	6.268	−887.313	1782.9	34.80	0
<i>C. perfringens</i> † (CFU/10 g)	PC2 + TH	5.010	−100.427	211.3	0	0.699
	PC1 + PC2 + TH	5.055	−100.401	213.5	2.14	0.239
	PC2	3.752	−104.235	216.8	5.45	0.046
	PC1 + PC2	3.723	−104.218	218.9	7.58	0.016
	TH	6.285	−109.878	228.1	16.74	0
	PC1 + TH	7.040	−109.616	229.7	18.38	0
	PC1	3.760	−111.317	230.9	19.62	0
	Intercept	2.662	−144.000	292.1	80.75	0

## DISCUSSION

### Abundance of *S. aureus*, MRSA, and FIB in seawater

*S. aureus* and MRSA have been detected in recreational waters worldwide and frequently documented throughout the Hilo Bay watershed—in rivers and nearshore waters, wastewater, urban runoff, soils, and beach sands (Economy et al., 2019; Gerken et al., 2021; Gerken et al., 2022) (Table 4). Observed *S. aureus* and MRSA concentrations in Hilo coastal waters align with findings from other beach studies in California, Florida, Mexico, Central Israel, and Egypt (Table 4). In this study, *S. aureus* and MRSA were detected in seawater and sand at all beaches. Approximately 12% (47/378) and 9.4% (17/180) of samples in this study had concentrations of *S. aureus* and MRSA that exceeded the Favero et al. (1964) 100 CFU staphylococci/100 ml recommendation

for pool water. Despite repeated observations of elevated concentrations of *S. aureus* and MRSA in coastal waters, these bacteria are not monitored by any federal or state agency throughout the world.

Worldwide microbial recreational water quality standards utilize measurements of FIB such as *Enterococcus* spp., and less frequently *C. perfringens*, which may not be indicative of pathogens like *S. aureus* (Hardina & Fujioka, 2011; WHO, 2021). *Enterococcus* spp. concentrations exceeded the HDOH BAV of 130 MPN *Enterococcus* spp./100 ml in ~40% (57/144) of observations, yet concentrations were similar to previous studies (Table 4). High concentrations of *Enterococcus* spp. in our samples may be partially due to the prevalence of this bacteria in tropical soil microbiota, but may also still indicate non-point source sewage pollution (Economy et al., 2019; Fujioka & Byappanahalli, 1996; Fujioka & Shizumura, 1985; Gerken et al., 2022). *C. perfringens* has also been detected in tropical soils and streams, but

**TABLE 4** *Staphylococcus aureus*, Methicillin-resistant *S. aureus* concentrations (MRSA), total staphylococci, *Enterococcus* spp., and *Clostridium perfringens* quantified in seawater (CFU/100 ml or MPN/100 ml) and sand (CFU/g or MPN/g) at beaches worldwide using membrane filtration methods or enrichment broth and quantified using IDEXX trays. Concentrations yielded using enrichment broth methods are denoted by \* since these values may not be directly comparable to studies not utilizing these techniques. Ranges listed under means are a range of reported means in papers (occasionally geometric means). Medians are medians of reported means. Methods used for the studies in the table are provided in the footnotes with letter superscripts. Note, that some concentrations from studies are approximated (based upon tables/figures or reported in the text) and may not represent exact values. Concentrations that were previously log-transformed, were reconverted to their original, non-transformed values.

Bacteria	Location	Mean	Median	Range	Reference	
<i>S. aureus</i>	Seawater	8–234	39	0–938	This study	
	Hilo, Hawaii, US (6 beaches)					
	Hilo Bay, Hawaii, US (6 stations)	30–256	30	0–687	Economy et al., 2019 <sup>a</sup>	
	O'ahu, Hawaii, US (19 'low staph' beaches)	< 2–22	2	< 2–234	Charoena & Fujioka, 1993 <sup>b,n</sup>	
	O'ahu, Hawaii, US (8 'high staph' beaches)	< 2–93	57	< 2–2600	Charoena & Fujioka, 1993 <sup>b,n</sup>	
	Southern California, US (3 beaches)	2–23	5	1–33	Goodwin et al., 2012 <sup>c</sup>	
	South Florida, US (2 beaches)	-	-	< 2–780	Plano et al., 2013 <sup>d*</sup>	
	Virginia Key, Florida, US	9	-	< 2–32	Abdelzaher et al., 2010 <sup>e</sup>	
	Central Israel (34 beaches)	-	-	< 2–600	Yoshpe-purer & Golderman, 1987 <sup>f</sup>	
	Red Sea, Egypt (3 water bodies)	14–2260	-	0–5000	El-Shenawy, 2005 <sup>i</sup>	
Sand	Hilo, Hawaii, US (6 beaches)	368.1	33.5	0–8710	This study	
	Southern California, US (3 beaches)	1.87	0.07	0.98–3.90	Goodwin et al., 2012 <sup>c</sup>	
	Virginia Key, Florida, US	463	-	20–1421	Abdelzaher et al., 2010 <sup>e</sup>	
	Hilo, Hawaii, US (6 beaches)	12–163	33	0–1147	This study	
	Hilo Bay, Hawaii, US (6 stations)	0–5	0	0–13	Economy et al., 2019 <sup>a</sup>	
	South Florida, US (2 beaches)	-	-	< 2–68	Plano et al., 2013 <sup>d,m</sup>	
Total staphylococci	Seawater	Hilo, Hawaii, US (6 beaches)	29.58	1	0–605	This study
		Southern California, US (3 beaches)	~ 0.03	-	0.05–0.78	Goodwin et al., 2012 <sup>h</sup>
		Hilo, USA (12 stations)	~ 50–150	-	-	Gerken et al., 2021 <sup>g*</sup>
		Kona, Hawaii, USA (9 stations)	~ 30–340	-	-	Gerken et al., 2021 <sup>g*</sup>
		Puna, Hawaii, USA (2 stations)	~ 80–90	-	-	Gerken et al., 2021 <sup>g*</sup>
		O'ahu, Hawaii, US (19 'low staph' beaches)	~ 2–90	-	1–2304	Charoena & Fujioka, 1993 <sup>b</sup>
	Sand	O'ahu, Hawaii, US (8 'high staph' beaches)	~ 122–690	-	< 2–2880	Charoena & Fujioka, 1993 <sup>b</sup>
		Hilo, Hawaii, USA (5 stations)	~ 9–100	-	-	Gerken et al., 2021 <sup>g*</sup>
		Kona, Hawaii, USA (4 stations)	~ 9–4500	-	-	Gerken et al., 2021 <sup>g*</sup>
		Puna, Ha, USA (2 stations)	~ 9–45	-	-	Gerken et al., 2021 <sup>g*</sup>

TABLE 4 (Continued)

Bacteria	Location	Mean	Median	Range	Reference
<i>Enterococcus</i> spp.	Seawater	<10–6131	75	372	This study
	Hilo, Hawaii, US (6 beaches)	234–1137	-	10–10,670	Economy et al., 2019 <sup>a</sup>
	Hilo Bay, Hawaii, US (6 stations)	42	6	28–59	Goodwin et al., 2012 <sup>z</sup>
	Southern California, US (3 beaches)	21; 40	-	< 2–110; < 10–100	Abdelzaher et al., 2010 <sup>h,i</sup>
	Virginia Key, Florida, US	149–157	-	4–7115	Curriel-Ayala et al., 2012 <sup>j</sup>
	Acapulco, Mexico (3 beaches)	0–196,600	-	0–460,000	El-Shenawy, 2005 <sup>i</sup>
	Red Sea, Egypt (3 water bodies)	155.72	3.5	< 10–5553	This study
	Hilo, Hawaii, US (6 beaches)	50.86	0.13	0.04–10.88	Goodwin et al., 2012 <sup>z</sup>
	Southern California, US (3 beaches)	337; 271	-	4–1088; 6–1006	Abdelzaher et al., 2010 <sup>h,i</sup>
	Virginia Key, Florida, US	~ 3.74	-	-	Alim et al., 2003 <sup>i</sup>
	Michigan, US (6 stations)	6.4	-	0.1–4924	Heaney et al., 2014 <sup>a</sup>
	Fairhope Municipal Park Beach, Alabama, US	1.5	-	0.1–1596	Heaney et al., 2014 <sup>a</sup>
	Goddard Memorial State Park Beach, Rhode Island, US	-	-	4–40.0	de Oliveira & Pinhata, 2008 <sup>i</sup>
Sao Vicente, Brazil (2 stations)	-	-	-	-	
<i>C. perfringens</i>	Seawater	1	0	0–33	This study
	Hilo, Hawaii, US (6 beaches)	3–12	-	0–45	Economy et al., 2019 <sup>a</sup>
	Hilo Bay, Hawaii, US (6 stations)	35–89	-	1–1082	Curriel-Ayala et al., 2012 <sup>k</sup>
	Acapulco, Mexico (3 beaches)	1.4	-	0–147	This study
	Hilo, Hawaii, US (6 beaches)	-	-	0–15,000	Buchholtz ten Brink et al., 2000 <sup>a</sup>
	Long Island Sound, US (219 stations)	39	-	11–64	Abdelzaher et al., 2010 <sup>l</sup>
	Virginia Key, Florida, US	-	-	-	-

<sup>a</sup>Same as this study; <sup>b</sup>Tellurite Glycine Agar and Vogel-Johnson Agar with sodium azide; <sup>c</sup>CHROMagar™ Staph aureus with select colonies confirmed through a combination of morphology and PCR; <sup>d</sup>Baird Parker agar with egg yolk tellurite enrichment, followed by mannitol salt agar, and then trypticase soy agar with 5% sheep blood, with final confirmation using a latex agglutination test, protein A using BACTISTAPH™ Latex Agglutination Test, and PCR confirmation of isolates; <sup>e</sup>CHROMagar™, subculture to mannitol salt agar, further test for catalase, coagulase, and protein A using BACTISTAPH™ Latex Agglutination Test. Isolates then gram-stained and subcultured on sheep blood agar and Oxacillin Resistance Screening Agar Base media; <sup>f</sup>Modification of 4-S agar containing NaCl, egg yolk, and tellurite developed by Mintzer-Morgenstern & Katzenelson (1982); <sup>g</sup>Culturing using broth enrichment 1.5 X m Staphylococcus broth, 75 µg/ml polymyxin B, and 0.01% potassium tellurite in Quanti-Tray 2000® (MPN/g); <sup>h</sup>CHROMagar™ MRSA with select colonies confirmed through PCR; <sup>i</sup>m-Enterococcus agar (mEI) culture media for enterococci (CFU/g); <sup>j</sup>Nutrient agar, mannitol salt agar, and confirmation tests included microscopic examination, catalase, aerobic and anaerobic fermentation of mannitol, DN<sub>ase</sub>, and coagulase; <sup>k</sup>Tryptose Sulfite Cyloserine Agar supplemented for *C. perfringens*; <sup>l</sup>U.S. EPA method 600-R-95/030; <sup>m</sup>PCR confirmation for presence of methicillin resistant gene (*mecA*); <sup>n</sup>*S. aureus* confirmation based upon coagulase tube test and latex agglutination test and speciated with STAPHTrac Test; <sup>z</sup> Not described; \* = enrichment; – = data not applicable.

evidence suggests this organism is a more conservative indicator of direct sewage pollution compared to *Enterococcus* spp. (Fujioka & Shizumura, 1985; Gerken et al., 2022; Medema et al., 1997; Stelma, 2018; Zhang et al., 2015). No samples collected in this study exceeded the single sample standard for *C. perfringens* in marine waters of >50 CFU/100 ml (HDOH, 2012). However, Reed's Bay and Bayfront exceeded the recommended geometric means for marine water in Hawai'i (<5 CFU/100 ml), with values of 5 CFU/100 ml (Fujioka et al., 2015).

### Associations between *S. aureus*, MRSA, and FIB in seawater and sand

Sand is known to be a reservoir for bacteria such as *S. aureus*, MRSA, and FIB, yet no universal microbial public health criteria exist for sand despite the possibility of sand being a vector of exposure for CA – infections (Fujioka et al., 2015). Studies have suggested that correlations between seawater and sand concentrations of bacteria, such as *Enterococcus* spp., indicate that sand can be a potential source or sink of these bacteria into overlying waters, especially since sand can provide nutrients for survival and protection from UV light (Abdelzaher et al., 2010; Goodwin et al., 2012). Our study found few significant associations between bacterial concentrations in seawater and sand. *S. aureus* and MRSA concentrations in seawater were correlated, which is expected since MRSA is a strain of *S. aureus*. Significant positive relationships between FIB, *Enterococcus* spp., and *C. perfringens*, in both seawater and sand is not surprising since both are abundant in sewage. Seawater *S. aureus* and *Enterococcus* spp. were correlated to their respective sand concentrations since both bacteria are capable of replicating in sand, which allows sand to function as a potential sink that is influenced by physical forces, like tides (Boehm et al., 2004; Kaestli et al., 2017). A positive association between seawater MRSA and *Enterococcus* spp. was present; future studies are recommended to further investigate this relationship and to assess if *Enterococcus* spp. concentrations, which are regularly measured, can be used as a proxy for MRSA concentrations. Numerous inverse associations were detected between concentrations of *S. aureus* or MRSA in seawater and sand with *Enterococcus* spp. in sand. These inverse relationships are likely driven by how these bacteria interact with sand. For example, flood tides and higher tidal heights can detach bacteria from sand in the intertidal zone into the water column, a process called over-beach transport (Yamahara et al., 2007). This trend was likely not observed for *C. perfringens* since this

bacterium was frequently not detected in sand. Further surveillance is required to differentiate whether sand is a potential source or sink to overlying waters, as well as understanding the potential risks of these bacteria in sands to human health.

### Physicochemical factors associated with elevated pathogens and fecal indicators

Freshwater discharge via streams into coastal waters is a documented source of *S. aureus* and fecal contamination on beaches (Boehm et al., 2004; Economy et al., 2019; Futch et al., 2011; Gerken et al., 2022). Our study revealed that beaches receiving stream inputs, such as Honoli'i and Bayfront, exhibited higher concentrations of *S. aureus* and MRSA in seawater, while those with SGD like Reed's Bay and Puhi Bay demonstrated elevated levels of FIB in seawater and *S. aureus* in sand. Notably, increased freshwater discharge linked to low salinity, low water temperature, and heavy rainfall emerged as significant predictors in models of seawater *S. aureus*, MRSA, and FIB concentrations. This indicates the crucial role of storms, river inputs, and groundwater discharge in the transport of these bacteria to coastal waters (Economy et al., 2019). In our study, the only beaches that exceeded the proposed Favero et al. (1964) standard of 100 CFU staphylococci/100 ml were Honoli'i, Bayfront, and Reed's Bay, which all receive a freshwater discharge through streams or groundwater. Similarly, at a Mexican beach, *S. aureus* concentrations exceeded the proposed Favero et al. (1964) threshold twice as often in the rainy season than in the dry season (Curiel-Ayala et al., 2012).

During heavy rainfall events, the impact of OSDS in watersheds, such as those in Hilo, might be amplified, potentially leading to increased contaminated groundwater flux to the coast (Engott, 2011; Whittier & El-Kadi, 2014). The presence of OSDS was significantly associated with higher *S. aureus*, MRSA, *Enterococcus* spp., and *C. perfringens* seawater concentrations, hinting at the potential of non-point source pollution from OSDS into coastal waters (Börjesson et al., 2010; Economy et al., 2019; Gerken et al., 2021). When applying the Fung & Fujioka *C. perfringens* scale to observations in this study, ~4.2% (6/144) of samples fell between 10 and 100 CFU/100 ml, suggesting non-point source sewage pollution when these samples were collected (Fung et al., 2007; Wiegner et al., 2017). Groundwater in Hawai'i County faces significant microbial pollution risk due to the presence of ~59,000 OSDS countywide (Whittier & El-Kadi, 2014). In Hilo, where only 30% of the area is sewered, approximately 8700 OSDS (i.e., cesspools, septic tanks) release 21 million liters of

untreated sewage into Hilo Bay daily, with dye-tracer studies identifying OSDS pollution into popular Hilo beaches within 20 hours (HDOH, 2017; Waiki, 2022). The risk of OSDS-polluted SGD discharging into the coast is not uncommon in the tropics since more than 50% of individuals on tropical islands (and Sub-Saharan Africa) lack proper forms of sanitation and sewage infrastructure, and often rely on OSDS (Wear et al., 2021). Thus, tropical regions are at high risk of OSDS pollution to SGD, especially since they account for a 56% share of the world's SGD, while mid-latitude arid regions contribute only 10% (Zhou et al., 2019).

*S. aureus* and FIB in beach sands, especially at beaches with high SGD rates, may signal long-term persistence of *Enterococcus* spp. from historical spills, in addition to OSDS pollution (Roszakt & Colwell, 1987). Not only were sand concentrations of *S. aureus* positively associated with densities of OSDS, but the sand concentrations of *S. aureus* and *Enterococcus* spp. also peaked at beaches with high SGD, such as Reed's Bay and Puhi Bay. Puhi Bay has a high prevalence of OSDS (~100) within a kilometer of this beach, but also historically suffers from sewage spills due to Hilo's Wastewater Treatment Plant outfall pipe located ~1.3 km offshore (Tetra Tech in support of USEPA Region 9, 2010). The highest observation of *C. perfringens* in the sand was also at Reed's Bay, which often experiences direct wastewater pollution and has a storm drain that empties directly into the coastal waters (HDOH, 2021). Thus, groundwater discharge may provide a continuous, low-level input of not only *Enterococcus* spp. and *C. perfringens*, but also a source of *S. aureus* and MRSA into beaches especially in regions with deficient sanitation infrastructure (Nakoa, 2022).

Impacts of freshwater input into the coast through groundwater or streams not only lead to increases in coastal water turbidity from washing of fine-grained terrigenous particulates but also can resuspend sand particulates, which may impact concentrations of pathogens in coastal waters and sands (Yamahara et al., 2007). Models demonstrated that water turbidity was positively correlated with seawater concentrations of *S. aureus*, MRSA, and FIB across all beaches, and this finding is consistent with other studies in Hilo and elsewhere (Cira et al., 2022; Steadmon et al., 2023; Wiegner et al., 2017). Notably, models also demonstrated that tidal height was significantly associated with all bacteria in seawater and sand in which elevated *S. aureus* and MRSA frequently occurred at higher tidal heights, while FIB were greater at lower tidal heights. Particle transport such as resuspension from sands from tidal forces and stream discharge can deliver *S. aureus* from upstream sources and beach sands into coastal waters. Tidal forces and river discharge

also increase water turbidity, which reduces sunlight penetration allowing enhanced *S. aureus* persistence (Fujioka & Unutoa, 2006). In contrast, FIB concentrations in seawater and sand decreased with higher tidal heights. Previous studies conducted in Florida and in Southern California also observed greater concentrations of *Enterococcus* spp. at outgoing tides leading to low tides (Boehm et al., 2002; Enns et al., 2012). Additionally, groundwater discharge is the greatest at low tides, hence the combination of low tides and high SGD may be an effective indicator of elevated FIB in coastal waters particularly where OSDS are used (McKenzie et al., 2021; Peterson et al., 2009).

## Public health concerns and community risks

Visitors to beaches may significantly contribute to bacterial levels by shedding *S. aureus* or MRSA from their skin into seawater or sands, as individuals can shed  $10^5$ – $10^6$  CFU/100 ml of *S. aureus* over a 15-min period fully submerged (Akanbi et al., 2017; Plano et al., 2011). In our study, MRSA concentrations in sands may have been influenced by human shedding at beaches, especially at popular beaches like Richardson's Beach Park, which observed the highest MRSA concentrations in sand across all beaches (Akanbi et al., 2017; HTA, 2023). Gerken et al. (2021) detected *S. aureus* in only 7.2% of their samples collected in 2020, a notable decrease compared to our detection rate of 67% during 2016–2019. This decline in *S. aureus* detection on Hawai'i Island aligns with the timeline of the COVID-19 pandemic in 2020, during which there were approximately 8 million fewer visitors compared to 2019 (HTA, 2019, 2020). This suggests the potential significance of human shedding as a source of *S. aureus* and MRSA.

Since *S. aureus* and MRSA are opportunistic pathogens, any open wound or abrasion exposed to water with these pathogens can increase the risk of infection (Early & Seifried, 2012). Recreational water activities, such as swimming, paddling, and surfing were the primary activities engaged in at our sites and have been associated with an increased risk of *S. aureus* infections (Charoenca & Fujioka, 1995; Thapaliya et al., 2017; Viau et al., 2011). During activities like paddling, water accumulates and sloshes around the bottom of canoes, potentially carrying *S. aureus* and MRSA from the water or from the paddlers' skin. Thousands of beaches across the State of Hawai'i and the U.S. require beachgoers to walk across sharp rocks to access the waterbody, which can cause cuts and scratches that may lead to a *S. aureus* infection. The longevity of time spent in the ocean during

these activities also increases infection likelihood (Charoenca & Fujioka, 1995). This survey on beach activities also demonstrated the importance of studying bacterial pathogens and fecal indicators in coastal waters and sands since these beaches are human-populated beaches with local communities and tourists from around the globe. This is especially vital for populations like Native Hawaiians and Pacific Islanders who engage in water activities for cultural significance, like surfing and paddling, which were some of the most common beach activities in this study.

With multiple respiratory epidemics frequently overloading clinics and hospitals, it is imperative to reduce infections from pathogens like *S. aureus* and MRSA, especially during high-risk conditions like elevated turbidity and heavy rainfall events. The number one survey comment in our study was, “I feel more at risk for an infection after heavy rains and near river mouths.” These community concerns are valid as increased turbidity and heavy rainfall events are projected to increase with climate change and 80% of worldwide diseases and 50% of child deaths are a result of poor water quality (Lin et al., 2022). A study on surfers in the U.S. Pacific Northwest reported higher incidents of ear infections, sore throats, and diarrhea during rain events (Harding et al., 2015). Given the frequent detection of *S. aureus* and MRSA in environmental waters and sands, it is imperative to conduct epidemiological studies to establish worldwide water quality standards and thresholds for these pathogens. Incorporating these findings into the WHO’s Guidelines on Recreational Water Quality would be crucial in mitigating potential health risks associated with these pathogens (WHO, 2021). Moreover, while there are established guidelines for intestinal enterococci in seawater, such as the WHO’s recommendation of 200 CFU/100 ml, no specific guidelines exist for *S. aureus* and MRSA. Therefore, epidemiological studies are essential to determine appropriate thresholds for these pathogens and ensure the safety of recreational water activities.

## Application of Predictive Models

A potential alternative to minimize health hazards linked with pathogens without direct measurements of bacteria is utilizing data collected by real-time water quality sensors like salinity, water temperature, tides, and turbidity, to input into models for rapid predictions of safe water quality (Goodwin et al., 2012). Despite no formal monitoring of pathogens or FIB in coastal sands, the incorporation of sand-specific predictive models could provide an

additional layer of information for assessing health risks associated with recreational activities on beaches without the use of direct bacterial measurements. Notifications like the HDOH “brown water advisory” - a beneficial warning to the public of compromised water conditions—are often based on anticipated heavy rainfall, and high wave or tidal action, rather than direct bacterial or physicochemical measurements. Our study shows that elevated pathogen levels exist independent of these warnings therefore real-time turbidity monitoring with water quality remote sensors and tidal buoys may offer a cost-effective and efficient way to issue timely brown water advisories based on turbidity, and tidal heights. The statistically robust predictive models developed in this study for *S. aureus*, MRSA, and FIB can utilize these real-time or forecasted measurements. Regarding seawater *Enterococcus* spp. models, the  $\Omega_0^2$  value for the best-fit model in this study ( $\Omega_0^2 = 0.53$ ) is comparable to previous studies reported adjusted  $r^2$  values (0.11–0.61) (Feng et al., 2015; Frick et al., 2008; Hou et al., 2006; Thoe et al., 2014; Wiegner et al., 2017). The  $\Omega_0^2$  for the highest ranked models for *C. perfringens* seawater concentrations ranged from 0.21–0.30, which is lower than previous studies adjusted  $r^2$  value in Hilo Bay (0.55) (Wiegner et al., 2017). Coefficients of determination are built for linear models, which were not used and may not accurately represent the proportion of variance explained by models in this study, possibly explaining the moderate  $\Omega_0^2$  values for FIB seawater models. These models can be readily utilized for predictions of *S. aureus*, MRSA, and FIB in seawater and sand by community members, land managers, and public health officials (Supporting Information). However, there are limitations to the application of these predictive models. Models in this study were developed utilizing culture-based techniques without precise identification (such as through molecular or analytical tools), which may impact the reliability of the model predictions. Still, these models may function as a baseline for future studies to utilize and build upon for broader application. For example, future work can employ this model framework to expand and apply different combinations of predictors or for predictions of other pathogens not measured in this study to improve the U.S. EPA current software without data transformation, predictor collinearity, or overdispersion concerns (U.S. EPA, 2010). The increased risk of infection due to climate change, heavy rainfall, rising sea levels, and heightened flood risks, stresses the need for improved monitoring through robust modeling approaches for accurate water quality predictions (Mora et al., 2022). Utilizing and expanding seawater and sand predictive models in this study could be a valuable step in mitigating risks to beachgoers.

## CONCLUSION

*S. aureus*, MRSA, and FIB in seawater and sand were detected at all beaches in this study, with the highest concentrations observed at beaches with surface or groundwater discharge, and during high turbidity conditions. To reduce the risk of contracting a disease from one of these bacteria, especially *S. aureus*, beachgoers should be aware of potentially high-risk conditions such as high turbidity and excessive freshwater discharge after rain events. Based on our observations, monitoring water turbidity in real-time will increase the predictive power of the abundance of *S. aureus*, MRSA, and FIB in coastal waters, which do not require direct bacterial measurements. Educational outreach on precautions to limit pathogen exposure and treatment of infections may help reduce the number of community-acquired infections among recreational water users in Hilo and elsewhere. Further surveillance, specifically isolation and identification of pathogens like *S. aureus*, MRSA, and FIB, of coastal sands is required to better understand risks to human health. It is unclear whether sand may be a source or sink of FIB or pathogens into recreational waters, but relationships between seawater and sand concentrations are likely driven by tidal forces. Models created in this study can be utilized to improve or expand the already existing predictive FIB modeling framework and are readily available for use to model *S. aureus*, MRSA, and FIB in seawater and sand. Still, the most effective way to reduce *S. aureus* and MRSA infections resulting from recreational water use would be to update worldwide water quality guidelines with developed standards and proper monitoring in seawater and potentially even sand. This could be done with an epidemiological study linking seawater *S. aureus* and MRSA concentrations to infection rates from recreational water use.

## AUTHOR CONTRIBUTIONS

**Maria Steadmon:** Conceptualization; methodology; software; validation; formal analysis; investigation; data curation; writing—original draft; writing—review and editing; visualization; supervision; funding acquisition.

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**Tracy N. Wiegner:** Conceptualization; methodology; data curation; validation; resources; writing—original draft; writing—review and editing; funding acquisition; project administration; formal analysis; supervision; visualization. **Mikayla Jones:** Conceptualization; methodology; investigation; data curation; writing—original draft; supervision; funding acquisition; writing—review and editing. **Louise M. Economy:**

Writing—review and editing; investigation; data curation. **Jazmine Panelo:** Investigation; data curation. **Lynn A. Morrison:** Conceptualization; methodology; writing—review and editing. **Matt C. I. Medeiros:** Software; formal analysis; writing—review and editing; visualization. **Kiana L. Frank:** Resources; writing—review and editing; visualization; supervision; funding acquisition.

## ACKNOWLEDGMENTS

We are grateful for Tyler Gerken, Leilani Abaya, Carmen Garson-Shumway, Amy Olsen, Adel Sharif, Leilani Sablan, Chad Shibuya, Scott Laursen, Sharon Ziegler-Chong, Noelani Puniwai, Barb Bruno, Debbie Beirne, Edith Karth and the University of Hawai'i at Hilo Analytical Lab for their assistance in the field and laboratory, as well as student support. The authors also thank Craig Nelson, Margaret McManus, Tamara Ticktin, Andrea Kealoha, and other anonymous reviewers who helped improve this manuscript. Publication number #11797 of the University of Hawai'i School of Ocean and Earth Science and Technology.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. The sponsors had no role in the design, execution, interpretation, or writing of the study.

## DATA AVAILABILITY STATEMENT

The environmental and bacterial data and analysis that support these findings are openly available in “Hilo\_STAPH” at “[https://github.com/mstead/Hilo\\_STAPH](https://github.com/mstead/Hilo_STAPH)”.

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## SUPPORTING INFORMATION

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**How to cite this article:** Steadmon, M., Takakusagi, M., Wiegner, T. N., Jones, M., Economy, L. M., Panelo, J., Morrison, L. A., Medeiros, M. C. I., & Frank, K. L. (2024). Detection and modeling of *Staphylococcus aureus* and fecal bacteria in Hawaiian coastal waters and sands. *Water Environment Research*, 96(5), e11037. <https://doi.org/10.1002/wer.11037>