# Environmental Surveillance and Characterization of Antibiotic Resistant *Staphylococcus aureus* at Coastal Beaches and Rivers on the Island of Hawai‘i

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**Abstract**

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*Staphylococcus aureus* are both human commensal bacteria and potential pathogens. The aim of this study was to isolate and characterize methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) from coastal beach and river waters, anchialine pools, sand, and wastewater on the Island of Hawai‘i, Hawai‘i. A total of 361 samples were collected from three regions on the Island from June to December 2020 during the COVID-19 pandemic. From these samples, 98.1% were positive for *Staphylococcus* spp. and 7.2% were *S. aureus* positive. Thirty-six *S. aureus* isolates (nine MRSA and 27 MSSA) were characterized using whole-genome sequencing (WGS). The MRSA isolates were multi-drug resistant ST8 (CC8) SCC*mec* type IVa, carrying 6–9 different antibiotic resistance genes, 16–19 virulence factors, and were clonally related (0–16 SNP differences). The 27 MSSA isolates were grouped into eight clonal complexes (CC) and 12 sequence types (ST). A higher proportion of MRSA was identified in sand compared to water samples (8.6% vs. 1.6%, respectively). Seventy-eight percent of the MSSA isolates carried 1–5 different antibiotic resistance genes with *blaZ* being the most common. All MSSA carried 5–19 virulence factors. Both the MRSA and MSSA STs have previously been associated with human, animal, and/or environmental samples globally. Our

results demonstrate a potential public health hazard at public beaches in Hawai‘i with limited human activity.

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## 1.0 Introduction

*Staphylococcus aureus* is a global, ubiquitous colonizer and pathogen in humans, as well as domestic and wild animals.[1,2] *S. aureus* has also been isolated from a variety of environments.[3–7] While MRSA was initially a healthcare-associated pathogen (HA-MRSA) characterized by multi-drug resistance, a genetically distinct community-associated MRSA (CA-MRSA) emerged in the late 1990's in the United States.[8] These clones typically carry a staphylococcal cassette chromosome *mec* (SCC*mec*) type IV or V element, which mediates resistance to methicillin and often contains the Panton–Valentine leucocidin (*PVL*) gene.[9] However, livestock-associated MRSA (LA-MRSA) has increasingly become recognized since 2005 and has been found in various animal species.[10] In the United States, two dominant MRSA clones are USA300 (ST8) and USA100 (ST5),[11] as well as a predominant animal clone (ST398), which is found globally.[12]

The epidemiology of *S. aureus* continues to evolve. Recent studies comparing MRSA isolates from 2005–2014 indicate that USA300 strains continue to displace USA100 strains.[11,13] A study from 2010–2014 estimated the MRSA- and MSSA-related mortality rates were 14 and 11 deaths per 100,000 hospitalizations, respectively.[14] Over this time period, MRSA-related hospitalizations decreased by 15.8%, whereas MSSA infections increased by 6.6%. A recent study conducted in eight counties and from five states within the United States in 2016 found invasive MSSA incidence (31 cases/100,000 persons) was 1.8 times higher than MRSA (17.5 cases/100,000 persons), with the highest incidence found among African-Americans followed by Native American and Alaska Native racial groups.[15] Based on a national prevalence survey conducted in 2006 and 2010, the State of Hawai'i has a high prevalence rate of MRSA infections (91 and 66 cases per 1,000 inpatients, respectively) compared to the national average (46 and 66

cases per 1,000 inpatients, respectively),[16,17] with Native Hawaiians, Pacific Islanders, and children particularly susceptible to CA-MRSA infections.[8,18–21] Other studies conducted in the early 2000's found an increase in the proportion of MRSA from hospitals, inpatient and outpatient clinics, jails, and nursing homes[18,19,22,23], and is recognized to be a recurring and persistant problem.[24]

Despite many studies suggesting recreational marine waters and beaches as a possible source for *S. aureus* infections, few studies have documented the risk to beachgoers. In 1993, a beach study in Hawai'i found a correlation between swimmer density and staphylococci isolated from seawater.[25] In a subsequent study, it was found that children with a history of seawater exposure had four times the odds of developing staphylococcal skin infections compared to children without exposure to seawater in the previous ten days.[26] An earlier study in Hong Kong found an increase in swimming-associated illness rates for skin, respiratory, and total illnesses at heavily sewage-polluted beaches (staphylococci ≥ 1000/100 mL) compared to relatively unpolluted ones (staphylococci < 1000/100 mL).[27] These studies suggested that recreational coastal waters may serve as a reservoir for *S. aureus*. Since then, there has been limited reports suggesting the potential for linkage between exposure to seawater at recreational beaches in Hawai'i and staphylococcal infections, including MRSA.[24,28] In contrast, a Florida study did not find an association between exposure to *S. aureus* in coastal waters and reported illness.[7] Though, they did find a correlation between *S. aureus* concentrations in the water and number of bathers, with more MSSA (98.4%) isolated then MRSA (1.6%). Other studies have shown that high levels of *S. aureus* are shed by bathers corresponding to $10^5$ to $10^6$ CFU per person per 15 min bathing period,[7,29] implicating crowding at beaches as a possible risk factor for acquiring staphylococcal infections.

Similarly, other studies have looked at the presence of MRSA associated with recreational swimmers in both coastal waters and sand within the United States.[29–31] These studies have identified apparent associations between beachgoers and MRSA and MSSA in recreational areas. MRSA and MSSA has also been identified in streams and freshwater recreational beaches,[4–6,32–34] and may increase in coastal waters following storms.[35]

While *S. aureus* has been readily documented in coastal beach and freshwater environments in the Hawai‘i, less is known about the carriage of antibiotic resistant and virulence genes associated with these strains. The aim of the current study was to isolate *S. aureus* from coastal beach and river waters, anchialine pools, sand, and wastewater on Hawai‘i Island, and to characterize the isolates using whole-genome sequencing (WGS). This is the first study in Hawai'i to characterize MRSA and MSSA isolates using WGS from coastal beaches and rivers. As this project started, the COVID-19 pandemic began closing tourism to Hawai‘i State, which severely reduced beach activities of residents through lockdowns and limitations on public gatherings. Thus, levels measured in this study may be considered close to background levels found in the environment.

## 2.0 Methods and Materials

### 2.1 Study Locations

Water and sand samples were collected multiple times at 36 stations across the three most populous districts in Hawai‘i County, with an emphasis on the largest and most populated area, Hilo (Fig. 1A), and less populated districts of South Kohala and North Kona (Fig. 1B), and Puna (Fig. 1C). Hilo is located on the windward side of Hawai‘i Island. Stations were chosen adjacent to and within the Hilo Bay watershed, spanning ~12 km of coastline, including the Wailuku and Wailoa Rivers and Honoli‘i Stream. A majority of homes in Hilo rely on decentralized wastewater management, where ~8700 cesspools leak ~5.6 million gallons/day into nearshore coastal waters.[36] The districts of S. Kohala and N. Kona and are located on the western side of Hawai‘i Island, dominated by resort developments. Puna is a rapidly developing district located southeast of Hilo. In 2018, an expansive black sand beach and numerous anchialine pools formed at Pohoiki following a volcanic eruption,[37] and is included in this study as it is a popular recreational area.

### 2.2 Shoreline Station Selection and Sampling

A total of 361 samples were collected and processed from coastal beach water (n = 225), river/stream (n = 76), anchialine pools (n = 10), sand (n = 35), and wastewater (n = 15) between June–December 2020 (Fig. 1). In Hilo, 21 stations were selected, which included 14 State and County beaches (station #1–12, station #16, station #20; n = 188 samples) and seven river/stream stations (n = 76 samples) along the Wailoa River (station #13–15), Wailuku River (station #17–19), and Honoli‘i Stream (station #21) (Fig. 1A). In S. Kohala and N. Kona, nine State, County, and private beach stations (station #22–30; n = 27) (Fig. 1B) were sampled three times between August–December 2020. In Puna, two State and County beaches were selected, which included

two beaches (station #31–32; n = 10 samples) and four anchialine pools (station #33–36; n = 10 samples) (Fig. 1C) that were sampled up to five times between June–December 2020. Samples were aseptically collected in sterile, acid-washed polypropylene plastic bottles between 6 AM and noon, stored on ice, and processed within 6 h of collection. A Garmin eTrex® GPS (Olathe, Kansas) was used to collect GPS coordinates at each station (Table S1).

Sand was collected at five stations in Hilo (station #1–2, station #11, station #16, station #20) (Fig. 1A), five times (n = 25 samples), once at four stations in S. Kohala and N. Kona (station #22–23, station #26–27) (Fig. 1B), and two stations in Puna (station #31–32) (Fig. 1C) three times (n = 6 samples) between September–December 2020. Sand samples were collected in 150 mL sterile, polypropylene bottles using a metal spade 1-2" deep, within 1 m of the high tide line, transported on ice, and processed within 6 h. Wastewater influent, effluent, and treated effluent collected from a manhole was collected monthly over five months (n = 15 samples) from the Hilo Wastewater Treatment Plant (HWTP) between August–December 2020 (Fig. 1A).

A time-series experiment was conducted on November 14, 2020, where water samples were repeatedly collected from Richardson's Beach Park in Hilo (station #2) (Fig. 1A) every two h, between 8 AM–6 PM. This was conducted to assess how staphylococci levels may fluctuate with the presence of swimmers as previous research has shown low levels of staphylococci persist during the night, in the absence of swimmers.[25]

**2.3 Isolation and Quantification of Staphylococci**

Water samples were cultured for *Staphylococcus* spp. using broth enrichment in Quanti-Tray 2000® (IDEXX Laboratories, Westbrook, ME). Presumptive *Staphylococcus* spp. were plated onto selective and differential media to screen for MRSA and MSSA and confirmed using biochemical tests, as previously described.[5] Briefly, 50 mL of water samples were combined

with 50 mL of 1.5X m *Staphylococcus* broth (Becton, Dickinson and Company, Sparks, MD), supplemented with 75 µg/mL polymyxin B (TCI America, Portland, OR), 0.01% potassium tellurite (Spectrum Chemical Mfg. Corp, Gardena, CA) in Quanti-Tray 2000® and incubated for 72 h at 37ºC. Positive wells were visually detected via formation of a black precipitate.[5] The number of positive wells were counted and the presumptive *Staphylococcus* spp. MPN/100 mL was calculated using the MPN IDEXX chart and adjusted using a 1:1 dilution ratio. From each Quanti-Tray 2000® sample, up to 1 mL of broth from *Staphylococcus* spp. positive wells (12 for Hilo; 20 for S. Kohala and N. Kona, and Puna) were transferred into a 1.5 mL microcentrifuge and vortexed for 15 s. An aliquot from each positive well was inoculated onto both mannitol salt agar (BD) and Oxacillin Resistance Selective Agar Base (ORSAB) with supplement (Oxoid via Thermo Fisher Scientific, Lenexa, KS) and incubated at 37ºC to screen for MRSA and MSSA, respectively. Presumptive MRSA/MSSA colonies were inoculated onto blood agar (Tryptone Soya agar with sheep blood) (Remel via Thermo Fisher Scientific), incubated at 37ºC for 18–24 h in a candle extinction jar and screened for β-hemolysis. Presumptive MRSA and MSSA isolates were biochemically identified using the Staphaurex™ latex agglutination test kit (Thermo Fisher Scientific Remel Products, Lenexa, Kansas).[38] Presumptive MRSA isolates were biochemically identified using the penicillin-binding protein ($PBP2^{/}$) latex agglutination test kit (Thermo Fisher Scientific Remel Products, Lenexa, Kansas).[39–41] A single MRSA/MSSA isolate from each positive well in every Quanti-Tray 2000® sample was stored on parafilmed blood agar plates until further characterized.

Sand samples were processed using modified methods previously described.[42,43] Briefly, 10 g of wet sand from each station were shaken with 100 mL of 0.15 M NaCl for two min at 100 rpm where 25 mL of the supernatant was transferred into a sterile bottle, where sterile water was

added for a final volume of 50 mL. This volume was then combined with 50 mL of 1.5X m *Staphylococcus* broth, 75 µg/mL polymyxin B, and 0.01% potassium tellurite and processed as described above.[5] Sand samples were dried and converted to MPN/g DW using the proportional dry weight of the sand. For wastewater samples, 0.5–20 mL of water, depending on the sample type, was transferred into a sterile bottle, where sterile water was added for a final volume of 50 mL. Samples were then combined with 50 mL of 1.5X m *Staphylococcus* broth (Becton, Dickinson and Company, Sparks, MD), supplemented with 75 µg/mL polymyxin B (TCI America, Portland, OR), 0.01% potassium tellurite (Spectrum Chemical Mfg. Corp, Gardena, CA) in Quanti-Tray 2000® and processed as previously described.[5]

**2.4 WGS assembly and analysis**

Twenty-seven MSSA and nine MRSA isolates were characterized from 26 samples using WGS. Library preparation was performed using the Nextera XT kit and sequenced on a MiSeq machine (Illumina, San Diego, CA) using a paired end 2 x 250 bp sequencing strategy, following standard Illumina protocols (Illumina, Inc., San Diego, CA). Raw reads were quality filtered and trimmed using Trimmomatic (v0.39.0)[44] with LEADING/TAILING 3 and MINLENGTH 36 used as default parameters. FASTQ files were imported into Geneious Prime v2021.1[45] and *de novo* assembled using the SPAdes genome assembler (v3.13.0)[46] in careful mode. Sequence types were determined using the *S. aureus* MLST 2.0 database,[47] SCC*mec* type elements determined using SCC*mec*Finder 1.2 (https://cge.cbs.dtu.dk/services/SCCmecFinder/). ResFinder 4.1[48,49] and VirulenceFinder 2.0,[50,51] using identity thresholds of 90% for the detection of resistance and virulence genes. The presence of plasmids was assessed using MobileElementFinder, using default settings.[52] Core genome single nucleotide polymorphism (SNP) was assessed using CSI Phylogeny 1.4,[53] using default settings. The following isolates

were used as reference strains for phylogenetic analyses: MRSA ST8 (CC8) (ascession # SAMN18836599), MSSA ST 5 (CC5) (ascession # SAMN18824826), and MSSA ST398 (CC398) (ascession #SAMN18824846). Phylogenetic trees were visualized using Geneious Prime v2021.1[45] (Fig. 4). All WGS data were deposited in NCBI GenBank under project PRJNA721726 with the following ascession numbers: SAMN18824809–SAMN18824812, SAMN18824826, SAMN18824828, SAMN18824831, SAMN18824834–SAMN18824835, SAMN18824839, SAMN18824841, SAMN18824843, SAMN18824846–SAMN18824847, SAMN18824850–SAMN18824852, SAMN18824854–SAMN18824857, SAMN18836577, SAMN18836580–SAMN18836581, SAMN18836590–SAMN18836592, SAMN18836595, SAMN18836599–SAMN18836600, SAMN18836616–SAMN18836620, and SAMN18836622.

**2.5 Statistical Analysis**

Descriptive statistics (geometric mean, standard error, minimum, maximum) were carried out in R (v4.0.5).[54]

## 3.0 Results

### 3.1 Staphylococci, MSSA, and MRSA

A total of 98.1% (354/361) of samples were positive for *Staphylococcus* spp. and 7.2% (26/361) were positive for *S. aureus* and included 44.4% (16/36) of stations (Fig. 2, Table 1). The prevalence of MSSA in coastal beach and river/stream waters, anchialine pools, and sand was 5.3%, 3.9%, 30%, and 5.7%, respectively. The prevalence of MRSA in coastal beach water, anchialine pools, and sand was 1.8%, 10%, and 8.6%, respectively. MRSA was not detected at river/stream stations. Sampling occurred during predominately dry conditions at all sites except for one rainy sampling event in Hilo. Staphylococci MPN/100 mL counts in water varied among stations by 10-fold (Fig. 2). In Hilo, Kealoha Beach Park (station #8) and Honoli‘i Beach (station #20) had the highest MPN/100 mL counts of staphylococci, while the lowest were at Honoli‘i Stream Mouth (station #21). Within the S. Kohala and N. Kona, and Puna districts, Kailua Pier (station #28) and Pohoiki anchialine pool (station #34) had the highest staphylococci counts, while ‘Anaeho‘omalu Beach was lowest (Fig. 2). *S. aureus* isolates obtained from water (beach/river/anchialine pool) were detected at 11 stations in Hilo, one station in S. Kohala and N. Kona, and five stations in Puna (Fig. 2).

*Staphylococcus* spp. was also quantified in beach sands (Table 1). Staphylococci counts in sand was highest at Hāpuna (station #22) and lowest at Hilo Bay (station #16). *S. aureus* isolates were detected in sand three times—once at Lehia Beach Park (station #1), Reed’s Bay (station #11), and Honoli‘i Beach Park (station #20) (Fig. 1, Table 1). Wastewater staphylococci counts in water varied 10–1,000-fold and were higher in influent compared to effluent and manhole samples. However, no MRSA or MSSA was detected in wastewater.

From the time-series experiment at Richardson‘s Beach Park (station #2), staphylococci counts ranged from 52–2827 MPN/100 mL and were lower in the morning (70 MPN/100 mL) and higher in the afternoon (398 MPN/100 mL) (Table S2). MSSA was detected in 14.3% (1/7) of the samples at 2 PM, which corresponded with the highest number of beachgoers counted on the beach during the day (n = 55 people). No MRSA was detected.

**3.2 Genetic characterization of MRSA and MSSA isolates**

Thirty-six *S. aureus* isolates were characterized from 26 Quanti-Tray 2000® samples from 47.2% (17/36) of the stations. *S. aureus* isolates included 12 different STs with 8 CCs (Figs. 3,4; Table 2). The nine MRSA isolates [coastal beach water (n = 5), anchialine pool (n = 1), sand (n = 3)] were ST8 (CC8) SCC*mec* IVa and were isolated from six coastal beach and one anchialine pool station in Hilo and Puna (Fig. 3A, C). Among the 27 MSSA isolates, the most frequent lineages were ST5 (n = 8), ST398 (n = 4), ST72 (n = 3), ST15 (n = 2), ST508 (n = 2), ST97 (n = 2), ST518 (n = 2), and one isolate each of ST6, ST1155, ST3269, and ST1181 (Table 2). Nineteen Quanti-Tray 2000® samples were characterized from Hilo [coastal beach water (n = 13), river water (n = 3), sand (n = 3)], one sample from Kailua Pier (station #28) in S. Kohala and N. Kona, and six from Puna [coastal beach water (n = 2), anchialine pools (n = 4)]. In nine Quanti-Tray 2000® samples, there were multiple *S. aureus* isolates obtained from different wells, 28.6% (2/7) of the water samples contained two different STs, while 100% (2/2) of the sand samples contained multiple (2–3) different STs. From 26 Quanti-Tray 2000® samples, the diversity of STs was lower in water (beach/river/anchialine pool) (8.7%, 2/23) compared to sand (67%, 2/3).

Among the 36 *S. aureus* isolates, one isolate each was characterized from 17 Quanti-Tray 2000® samples and 19 *S. aureus* isolates were characterized from different wells within the same

nine Quanti-Tray 2000® samples. Five trays contained ten genetically related *S. aureus* isolates (0–8 SNP differences). These included one tray with MRSA (isolate #51, isolate #88) and four trays with MSSA: ST5 (CC5) (isolate #30, isolate #69), ST72 (CC8) (isolate #47, isolate #81), ST97 (CC97) (isolate #13, isolate #66), and ST398 (CC398) (isolate #50, isolate #84). Two trays were collected from sand, where one tray had MRSA ST8 (isolate #108) and ST5 (CC5) (isolate #111), and the second tray had MRSA ST8 (isolate #109), ST72 (CC8) (isolate #106), and ST518 (CC5) (isolate #107). The other two trays were isolated from water, where one tray contained ST1181 (CC8) (isolate #79) and ST15 (CC15) (isolate #45), and the other tray contained ST5 (CC5) (isolate #70) and ST518 (CC5) (isolate #32) (Table 2). Water samples (coastal beach and river water/anchialine pools) contained fewer STs (8.7%, 2/23) than the sand (66.7%, 2/3).

All MRSA ST8 SCC*mec* IVa isolates were clonally related based on the SNP analysis (0–16 differences) (Fig. 5A), despite being isolated from two different districts on the island (Fig. 3). The three ST72 (CC8) were also genetically related, with 5–10 SNP differences (Fig. 5A). However, ST1181 was more genetically diverse (~1000 different SNPs). Within CC5 group, there were three STs: ST5, ST6, and ST518 (Fig 5B). Two ST5 isolates were identical, with zero SNP differences (isolate #69, isolate #30), and the other ST5 isolates had 126–631 SNP differences. The two ST518 isolates (isolate #32, isolate #107) had six SNP differences between them, whereas the ST6 (isolate #43) was genetically distinct from the other CC5 isolates (>10,500 SNP differences), compared to the MSSA ST5 (isolate #14). Three of the ST398 (isolate #50, isolate #58, isolate #84) were closely related (1–2 SNP differences), whereas one (isolate #55) had 557–558 SNP differences (Fig. 4). Two isolates from ST15 (CC15), ST508

(CC45), and ST97 (CC97) had SNP differences of 377, 246, and 7, respectively (Fig. 4). The ST3269 and ST1155 isolates were genetically distinct from all other MSSA STs (Fig. 4).

**3.3 Antibiotic Resistance Genes**

The nine MRSA isolates carried 6–9 different antibiotic resistance genes, including the *mecA* gene (Table 2). All isolates carried *aph*(3')-*III* [aminoglycoside resistance], *erm*(C) [macrolide, lincosamide, streptogramin B resistance], *mph*(C) [macrolide resistance], and *msr*(A) [macrolide resistance] genes. Eight (88.9%) of the isolates carried the *blaZ* [β-lactam resistance] gene. Seven (77.8%) isolates carried *ant*(6)-*Ia* [aminoglycoside resistance] gene, two isolates each carried the *tet*(K) [tetracycline resistance] gene (isolate #105, isolate #109) and *qacD* genes [quaternary ammonium compounds] (isolate #88, isolate #89), while one isolate carried one of the following genes: *erm*(T) [macrolide, lincosamide, streptogramin B resistance] (isolate #108), *lnu*(A) [lincosamide resistance] gene (isolate #89), or *qacG* [quaternary ammonium compounds] (isolate #109) (Table 2). Two of three MRSA isolated from sand carried the *tet*(K) gene and were obtained from different stations in Hilo (Fig. 3A, Table 2).

Among the MSSA isolates, 22.2% (6/27) carried no AMR genes, 48.1% (13/27) carried a single AMR gene, 14.8% (4/27) carried two AMR genes, and 14.8% (4/27) carried 4–5 different AMR genes (Table 2). The *blaZ* gene was carried by 70.4% (19/27) of MSSA isolates. Less frequently carried AMR genes included the following; *erm*(T) (22.2%; 6/27), *mph*(C) (11.1%; 3/27), *tet*(K) (11.1%; 3/27), *aph*(3')-*III* (7.4%; 2/27), *ant*(6)-*Ia*, (7.4%; 2/27), *lnu*(A) (7.4%; 2/27), and *msr*(A) (3.7%; 1/27). Four MSSA isolates; ST72 (n = 3) and ST1181 (n = 1) belong to the same clonal complex, CC8, as the MRSA isolates. Both ST72 and ST1181 isolates carried the *blaZ* gene. One of the ST72 isolates also carried the *lnu*(A) gene and the ST1181 (isolate #79) carried four AMR genes: *aph*(3')-*III*, *ant*(6)-*Ia*, *blaZ*, and *tet*(K) genes. Of the eleven CC5

isolates, eight carried ≥ 1 AMR genes; *blaZ* (63.6%; 7/11), *mph*(C) (27.3%; 3/11), *erm*(T) (18.2%; 2/11), *tet*(K) (18.2%; 2/11), *ant*(6)-*Ia*, (9.1%; 1/11), *aph*(3')-*III* (9.1%; 1/11), *lnu*(A) (9.1%; 1/11), and *msr*(A) (9.1%; 1/11). Among the ST5 CC5 MSSA isolates, 62.5% (5/8) carried the *bla*Z gene, 25% (2/8) carried no AMR genes, 12.5% (1/8) carried the *lnu*(A) gene, 12.5% (1/8) (isolate #111) carried four AMR genes [*blaZ*, *erm*(T), *mph*(C), and *tet*(K)] and 12.5% (1/8) (isolate #46) carried five AMR genes [*aph*(3')-*III*, *ant*(6)-*Ia*, *bla*Z, *mph*(C), and *msr*(A)]. The ST6 isolate carried the *blaZ* gene, whereas one ST518 (isolate #107) carried four AMR genes [*blaZ*, *erm*(T), *mph*(C), *tet*(K)] and was isolated from sand and the other ST518 (isolate #32) carried no AMR genes and was isolated from water. All four ST398 MSSA isolates carried the *erm*(T) gene with three isolates also carrying the *blaZ* gene as well. The four ST398 isolates all carried the *erm*(T) gene and were isolated from Hilo, S. Kohala and N. Kona, and Puna, where 75% (3/4) also carried the *blaZ* gene. The ST15 (CC15) (n = 2) and ST508 (CC45) (n = 2) all carried the *blaZ* gene. The ST97 (n = 2), and ST3269 (n = 1) did not carry any AMR genes (Table 2).

**3.4 Toxin Genes**

All MRSA ST8 carried the following toxin genes: *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *lukF-PV*, *lukS-PV*, *sek*, *seq* (Table 2). One MRSA (isolate #109) also carried the *seg*, *sem*, and *sen* enterotoxin genes and was isolated from sand. All MSSA isolates also carried the *hlgA*, *hlgB*, and *hlgC* hemolysin toxin genes. The ST6 (CC8) MSSA (isolate #54) also carried the *sek* and *seq* toxin genes identified in the MRSA isolates, but was not found in the other MSSA isolates. The *lukD* and *lukE* toxin genes were identified in 77.8% (21/27) of the MSSA strains. A variety of enterotoxin genes were detected (Table 2), where 55.6% (15/27) of the MSSA isolates contained ≥ 6 enterotoxin genes. The most common enterotoxin genes detected were *seg*, *sei*,

*sem*, *sen*, *seo*, and *seu,* which were found in 55.6% (15/27) of MSSA isolates and were detected in ST72 (CC8), ST5 (CC5), ST518 (CC5), and ST508 (CC45), but were not present in ST6 (CC5), despite belong to the same clonal complex as ST5 and ST518. The *tst* gene was detected in both ST508 MSSA isolates, which were both identified in Hilo at the Wailuku River Mouth (station #17) and at Waiʻōlena (station #5). No enterotoxin genes were found for ST398, ST15, ST97, ST1155, and ST3269 isolates (Table 2).

**3.5 Exoenzyme genes**

All MRSA isolates contained the following exoenzyme genes: *aur*, *splA*, *splB*, and *splE* (Table 2). The *aur* gene was detected in all 27 MSSA isolates. The *splA* were detected in 77.8% (21/27) of MSSA isolates except for ST398 and ST508. The *splB* were detected in 74.1% (20/27) of the MSSA isolates and was missing from ST1181, ST398, and ST508. The *splE* gene was found in 25.9% (7/27) of MSSA isolates and found in ST72, ST6, ST15, and ST1155.

**3.6 Host immunity genes**

All MRSA isolates contained the following host immunity genes: *ACME*, *sak*, and *scn* (Table 2). The *scn* gene was found in 96.3% (26/27) of MSSA isolates. The *sak* and *scn* genes were found in 74.1% (20/27) of all sequence types, except for ST398, ST15, and ST3269. The *scn* gene was found in 22.2% (6/27) of sequence types and were present on ST398 and ST15. ST3269 was the only MSSA isolate which did not carry any host immunity genes.

**3.7 Plasmids**

3.8 A plasmid classification approach described by Lozano et al.[55] was used which assigned plasmids to replicon families (*rep*-families). Among the 36 *S. aureus* strains, eight *rep* families were detected. The *rep* genes were on the same contig as AMR and/or virulence genes[52] (Table 2). The MRSA isolates contained 2–4 *rep* genes. Among the MSSA isolates, 12 contained no *rep*

families, 14 contained a single *rep* family, and one contained 2 *rep* families. The most dominant *rep* families were $rep_{10}$ and $rep_{19}$ among the MRSA and $rep_{13}$ and $rep_{16}$ among the MSSA. Among the nine $rep_{19}$-positive MRSA isolates, seven carried *blaZ*, and one each carried *msr*(A) and *mph*(C); one $rep_{19}$-positive MSSA isolate carried *mph*(C). All nine $rep_{10}$-positive MRSA isolates carried the *erm*(C) gene. Two $rep_{7a}$-positive MRSA and MSSA isolates each carried *tet*(K) (ST8; ST5, ST1181). One $rep_{21}$-positive MRSA isolate carried *lnu*(A). Five $rep_{13}$-positive MSSA isolates carried the *erm*(T) gene (ST398 and 1 ST5). Three $rep_{16,}$ two $rep_{20}$ and one $rep_{US5}$ -positive MSSA isolates carried *blaZ.* Two ST72 and three MRSA isolates carried the *aur* virulence gene found on the same contig as $rep_{7c}$.0

## 4.0 Discussion

In the United States, *S. aureus* has been isolated from coastal beach water, freshwater, and sand from California,[31] Florida,[7,30,56] Washington State,[4,5,32] Ohio,[6] the Great Lakes,[34] and Hawai‘i.[18,33,35,57] This is the first study in Hawai‘i in which WGS characterization of MRSA and MSSA isolated from environmental reservoirs has been applied. The number of positive water samples for *S. aureus* in our study overall was lower (7.1%) than expected compared to study sites in Florida, which had a 36.2% positive rate for MSSA and 1.0% postive rate for MRSA for coastal waters.[7] However, the proportion of MRSA to MSSA in our study was higher (31.3%; 5/16) compared to the Florida study for coastal waters (2.9%; 7/242). The prevalence of *S. aureus* from sand in this study was also lower compared to recreational beaches in Ohio, where the prevalence of *S. aureus* in freshwater and sand was 30% (21/70) and 20.5% (43/210), respectively.[6] The prevalence of *S. aureus* in rivers was lower in our study (3.9%; 3/76) than previously measured on the Island of O‘ahu, where 50.0% (43/86) of samples were positive and 2.4% (1/42) of MRSA was found.[33] Furthermore, the low prevalence rate of MRSA in coastal and river water stations in our study was lower (1.6%, 0.0%, respectively) compared to similar stations assessed in Hilo Bay (20%, 10–30%, respectively).[35]

In the current study, the MRSA carried 6–9 antibiotic resistance genes and whereas the MRSA SCC*mec* IVa ST8 and ST5 isolates carried less antibiotic resistance and virulence genes in the earlier Florida study.[7] However, the drug resistance levels in the Florida study were not as extensive examined. MRSA has also been characterized from marine beach water in Washington State which identified five multi-drug resistant SCC*mec* I.[32] In our study, all MRSA carried the *ACME* gene, 16–19 virulence factors, and were closely genetically related (0–16 SNP differences) (Fig. 4, Table 2). The Florida study assessed fewer virulence factors and they used

Pulse Field Gel Electrophoresis (PFGE) in their analysis, so we could not directly compare these isolates with ours. While the Florida study assessed fewer virulence factors, a majority of the MRSA isolates contained the *PVL* gene and *ACME*-encoded *arcA* gene which was present in all MRSA in our study.

In our study, the MSSA carried 0–5 AMR genes with 70.4% (19/27) carrying the *blaZ* gene and 14.9% (4/27) of the MSSA were multidrug resistant. Recent findings indicate the ST5 MSSA lineage is hypervirulent in clinical *S. aureus* infections,[58] which is consistent with our findings where the number of virulence genes were similar to those carried in the MSSA clones. Furthermore, ST8 and ST5 was the most abundant sequence types detected in the Ohio study (30.0%, 15.7%, respectively)[6] and in this paper (25.0%, 22.2%, respectively).

A recent study by Challagundla et al., 2018[2] characterized eight MRSA isolates from hospital patients in the State of Hawai‘i in 2011. Isolate data from their study was used to compare to our isolates. A phylogenetic tree was constructed and compared with the environmental MRSA isolates in our study, which differed by 54–133 SNPs (Fig. S1). The MRSA isolates in hospital study carried 2–9 different AMR. The *aac*(6')-*aph*(2''), and *mupA* genes were not detected in the MRSA isolates in our study. The clinical MRSA isolates carried similar virulence genes, except for the *ACME* cluster, which was carried in only 50.0% (4/8) of the clinical MRSA compared to 100% (9/9) of the environmental MRSA isolates in our study. The AMR genes [*erm*(T), *lnu*(A), *qacD*, *qacG*, and *tet*(K)] found in ours were not previously found in clinical ones. An earlier study in Hawai‘i found a wide diversity of environmental *S. aureus* isolates, where 58.1% (18/31) of *S. aureus* isolates were characterized into eight different PFGE types, including: USA100, USA600, USA300, USA200, USA400, USA900, USA1000, and USA1100.[57] However, they found a small proportion of MRSA isolates were USA300

(8.3%), which contrast to the ST8 MRSA clones found in our study. Our findings, plus those from previous studies in Hawai‘i, suggest that MRSA obtained in this study are likely USA300.[2,18,28,57]

The presence of beachgoers and recreational swimmers appears to be the primary factor influencing staphylococci levels in the beach environment. A previous study at freshwater beaches found higher staphylococci concentrations after noon, which corresponded to a greater number of bathers counted at the beach.[34] This is consistent with our study, where staphylococci counts were highest in the afternoon, when the number of beachgoers were the greatest (Table S2). Other studies have found correlations with staphylococci and the number of recreational swimmers/beachgoers[7,34,59] as well as high counts of *S. aureus* shedding from beachgoers into the water.[7,29]

Wastewater may also influence the levels of drug-resistant staphylococci and virulence genes increasing the prevalence of genetic determinants conferring antibiotic resistance which poses a public health threat.[60] While no *S. aureus* was identified in our study, treated effluent from the HWTP ranged up to three orders of magnitude higher compared to coastal beach and river/stream stations. The State of Hawai‘i has nearly 88,000 cesspools with the majority occurring on Hawai‘i Island.[36] The public health risk is unknown but may likely constitute a risk as these systems were not designed to treat sanitary waste. Due to the highly-permeable soils on Hawai‘i Island,[61] it is likely that elevated levels of staphylococci and potentially *S. aureus*, such as those found in wastewater, may reach coastal beaches via submarine groundwater discharge posing a risk to beachgoers and increasing contamination of the environment.

Currently, it is unknown what the level of risk is to the public when visiting staphylococci-contaminated public beaches. In our study, a higher proportion of MRSA was

identified in sand compared to water samples. MRSA was the dominant clone detected in the sand samples which contained more STs compared to the water samples (beach/river/anchialine pool) suggesting the water samples were more homogenous with a single clone compared to the sand samples. Two of the sand samples (isolate #107, isolate #108) contained the *erm*(T) gene, which was also detected in all four of the ST398 isolates found in the districts of Hilo, S. Kohala and N. Kona, and Puna. The *erm*(T) gene has previously been identified in 20% (5/25) of MSSA/MRSA ST398 (CC398) pig-derived food samples[62] and 92.5% (86/105) of CC398 strain isolates of human origin contained *erm*(T).[63] Chroboczek et al.[63] found the *tet*(M) gene was rarely detected in 2.9% (3/105) of human MSSA isolates, whereas the *tet*(K) gene was not detected in the human MSSA isolates, but was present in 33.3% (13/53) of ST398 MRSA isolates. This may potentially complicate treatment of S. *aureus* infections in the general public if they acquire a drug-resistant bacteria. However, more work is required to determine the risk to beachgoers.

A limitation of the current study was that we were not able to obtain *S. aureus* isolates from the hospital. However, the USA300 MRSA identified in clinical samples in Hawai‘i was closely related to our isolates (Fig. S1).[2] The MRSA isolates detected in this study are likely a clonal strain similar to those previously identified in clinical and environmental sources in Hawai‘i.[2,18,28,57] Furthermore, the pandemic drastically reduced the number of visitors to the State of Hawai‘i, including Hawai‘i Island, where visitors arriving in June and December 2020 decreased 98.4% (161,141) and 72.9% (129,778), respectively, compared to visitor arrivals a

year prior.[64,65] Thus, the levels of staphylococci and lower prevalence of *S. aureus* detected in this study is likely to increase with the a rebound in tourism.

Future studies are needed to further examine whether there is a link between recreating in *S. aureus*-contaminated coastal waters and skin infections. Other than the two older publications by Charoenca and Fujioka (1993, 1995),[25,26] there has been limited indication of an increased risk of developing staphylococcal skin infections as a result of recreating in staphylococci-contaminated waters in Hawai‘i. Recreational swimmers in Hawai‘i may be disproportionately affected by *S. aureus* infections, particularly on Hawai‘i Island, due to the presence of rocky beaches and coral reefs where skin abrasions/wounds are common, which have previously been identified as an important risk factor for acquiring staphylococcal infections.[66] *S. aureus* has also been found in rivers during storms,[35] which attracts surfers to beaches who may be disproportionately at risk for these infections, particularly at Honoli‘i (station #20) and Hilo Bay (station #17).

The global dissemination of the MRSA strain USA300, which emerged as the leading cause of both community-associated and hospital-acquired MRSA infections in 2011,[67] has spread globally across North America, South America, Asia, the Middle East, Europe, and Africa by 2017.[68] Therefore, domestic and international travel may represent an increased burden to the environment. Simple public health measures, such as showering before and after swimming may potentially reduce risks of colonization and potential infection of people and the environment. More studies are needed to characterize MRSA and MSSA isolated from coastal shorelines, aquatic environments, and to assess the role rivers play after storms. Assessments comparing environmental and clinical *S. aureus* isolates are needed to better understand the potential risks

with the ongoing evolution, dissemination, and transmission of antibiotic resistant *S. aureus* strains in environmental and community settings.

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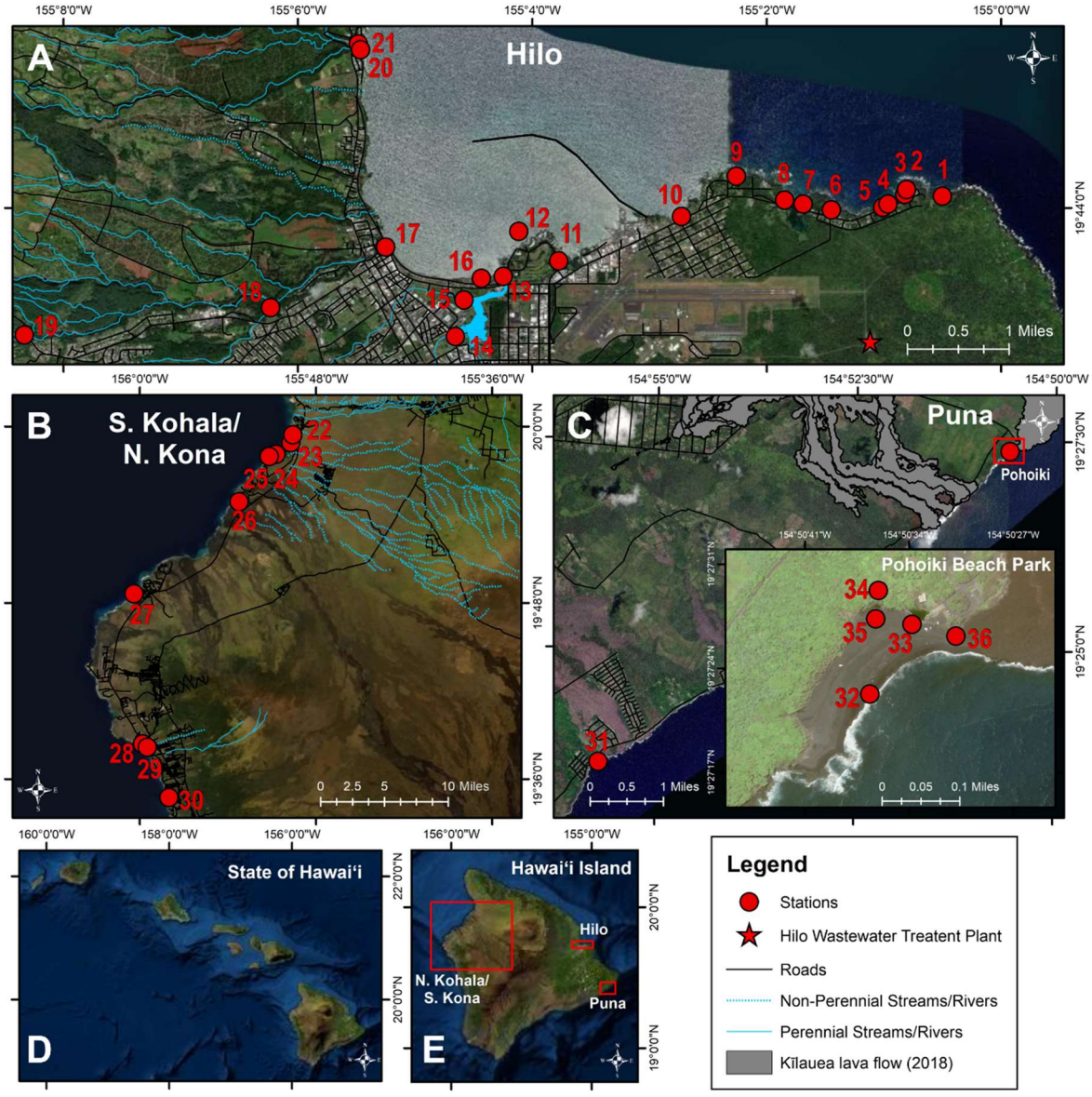
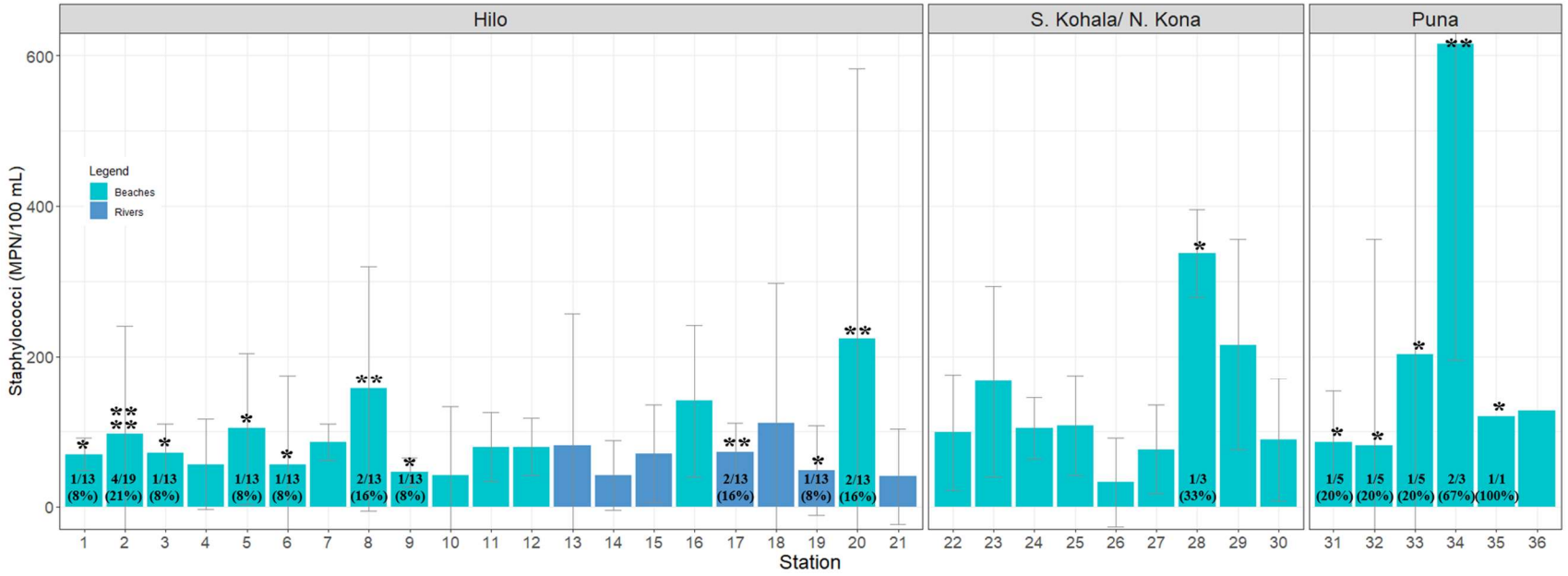
**Figure 1:** Map of sampling stations in the districts of Hilo, S. Kohala and N. Kona, and Puna on Hawai‘i Island, Hawai‘i from July–December 2020



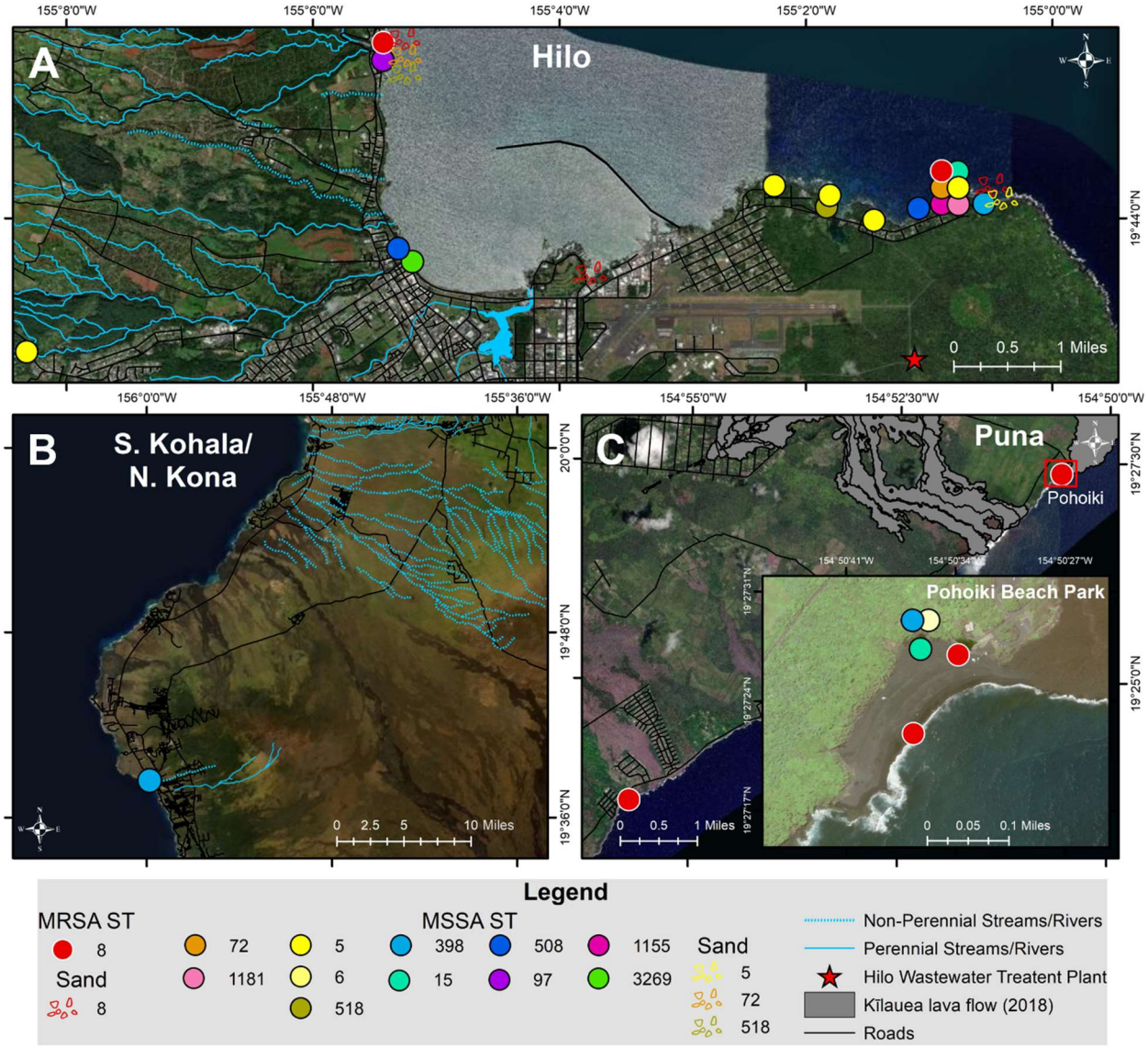

Water sampling stations in the districts of **A**) Hilo (station #1–21), **B**) S. Kohala and N. Kona (station #22–30), and **C**) Puna (station #31–36) on **D**) Hawai‘i Island, **E**) Hawai‘i.

**Figure 2:** Concentration of staphylococci at coastal beach and river/stream stations on Hawai‘i Island, Hawai‘i from July–December 2020




Geometric mean (± SE) of staphylococci in coastal beach and river water stations in the districts of Hilo (station #1–21), S. Kohala and N. Kona (station #22–30), and Puna (station #31–36) from July–December 2020 on Hawai‘i Island, Hawai‘i. Detection of *Staphylococcus aureus* is represented by the *. The prevalence rate of detecting *S. aureus* is located at the base of each station.

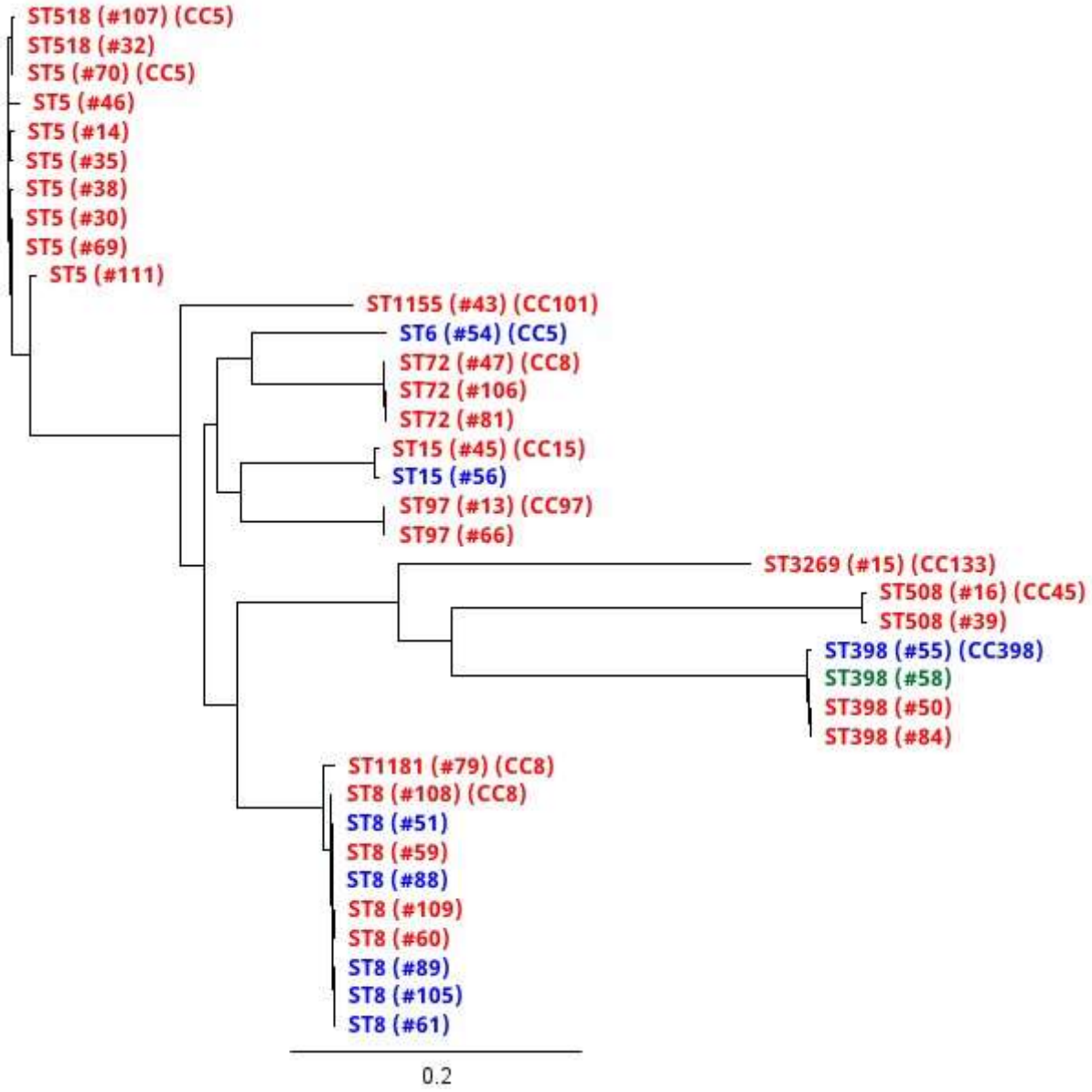
**Figure 3:** Map of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible S. aureus (MSSA) sequence types (ST) found in the districts of Hilo, S. Kohala and N. Kona, and Puna on Hawai‘i Island, Hawai‘i from July–December 2020




Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) sequence types (ST) isolated from coastal beach and river/stream water, anchialine pools, and sand from July–December 2020 in the districts of **A**) Hilo, **B**) S. Kohala and N. Kona, and **C**) Puna on Hawai‘i Island, Hawai’i. The following number of MRSA ST were obtained: ST8 (CC8) SCC*mec*IVa (water) (n = 6) and ST8 (CC8) SCC*mec*IVa (sand) (n = 3). The following number of MSSA ST were found: ST72

(CC8) (water) (n = 3), ST1181 (CC8) (water) (n = 1), ST5 (CC5) (water) (n = 7), ST5 (CC5) (sand) (n = 1), ST6 (CC5) (water) (n = 1), ST518 (CC5) (water) (n = 1), ST518 (CC5) (sand) (n = 1), ST398 (water) (n = 4), ST15 (CC15) (water) (n = 2), ST508 (CC45) (water) (n = 2), ST97 (CC97) (water) (n = 2), ST1155 (CC101) (water) (n = 1), and ST3269 (CC133) (water) (n = 1).

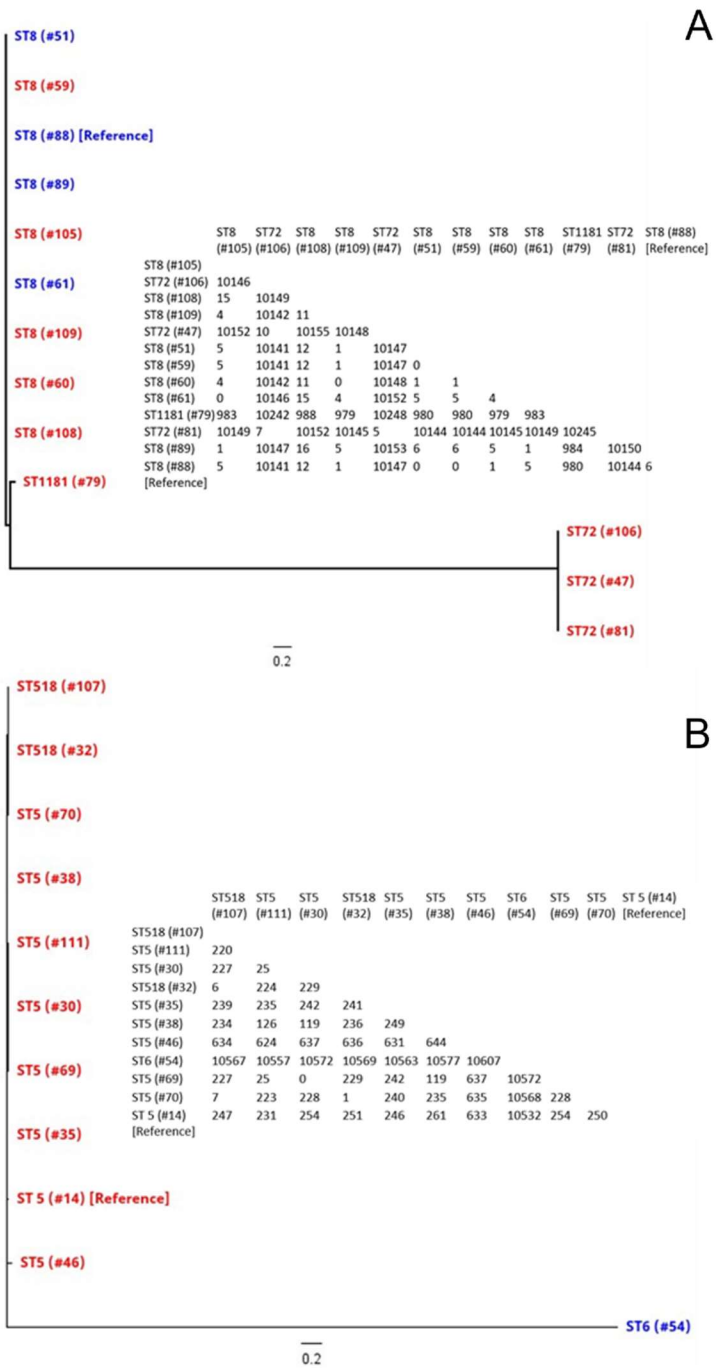
**Figure 4:** Phylogenetic tree of 12 sequence types (ST) and eight clonal complexes (CC) of 36 isolates collected from coastal beach and rivers/stream water, anchialine pools, and sand on Hawai‘i Island, Hawai‘i from July–December 2020.




Phylogenetic tree based on the concatenated alignment of core-genome single nucleotide polymorphism (SNP) created using CSI Phylogeny 1.4. Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) isolates were collected from coastal beach and river water, anchialine pools, and beach sand from July–December 2020 on Hawai‘i Island, Hawai‘i. The scale bar

denotes genetic distance in number of base substitutions per site. Branch tree labels indicate sequence type (ST), isolate #, clonal complex (CC), and are color coded by district: Hilo (blue), S. Kohala and N. Kona (green), and Puna (blue).

**Figure 5:** Phylogenetic tree of CC8 methicillin-resistant *Staphylococcus aureus* (MRSA)/methicillin-susceptible *S. aureus* (MSSA) and CC5 MSSA




Phylogenetic tree of **A**) CC8 MRSA and MSSA (ST8, ST72, ST1181) and **B**) CC5 MSSA (ST5, ST6, ST518) isolates collected from July–December 2020 from from Hawai‘i Island, Hawai‘i. Isolate #88 and isolate #30 were used as the reference strains in **A**) and **B**), respectively. The scale bar denotes genetic

distance in number of base substitutions per site. Matrix of numbers of SNP differences. Branch tree labels indicate ST, isolate #, and are color coded by district: Hilo (red) and Puna (blue).

**Table 1:** Mean (± SE) and [range] of staphylococci in sand and wastewater collected from September–December 2020 on Hawai‘i Island, Hawai‘i. Sand samples were collected five times at five stations in Hilo, once at four stations in S. Kohala and N. Kona, and three times at two stations in Puna.

| **Station** | **staphylococci** (MPN/g DW) | **MSSA** (MPN/g DW) | **MRSA** (MPN/g DW) |
|---|---|---|---|
| **Hilo:** | | | |
| Lehia Beach Park (#1) | 103.8 (193.9) [17.1–1038.3] | + | + |
| Richardson's Beach Park (#3) | 24.6 (198.7) [2.2–1011.5] | ND | ND |
| Reed's Bay (#11) | 5.8 (40.5) [0.4–209.3] | ND | + |
| Hilo Bayfront Beach Park (#16) | 4.2 (9.5) [0.4–45.7] | ND | ND |
| Honoli‘i Beach Park (#20) | 14.3 (37.2) [5.6–193.6] | + | + |
| **S. Kohala and N. Kona**: | | | |
| Hāpuna Beach State Park (#22)[a] | 4,839.2 | ND | ND |
| Waialea Beach (#23)[a] | 12.6 | ND | ND |
| ‘Ānaeho‘omalu Beach (#26)[a] | 49.8 | ND | ND |
| Manini‘ōwali Beach (#27)[a] | 21.2 | ND | ND |
| **Puna:** | | | |
| Kehena (#31) | 44.4 (399.4) [0.5–1259.7] | ND | ND |
| Pohoiki Beach Park (#32) | 4.8 (17.8) [0.5–55.6] | ND | ND |
| | | | |
| **Station** | **staphylococci** (MPN/100 mL) | **MSSA** (MPN/100 mL) | **MRSA** (MPN/100 mL) |
| **Wastewater:** | | | |
| Influent | 65,275.4 (26,527.9) [14,140.0–155,310.0] | ND | ND |
| Effluent | 806.3 (2,960.0) [146.0–15,340.0] | ND | ND |
| Effluent – manhole | 711.0 (528.2) [146.0–3,065.5] | ND | ND |

+ depicts presence/absence

ND: Not detected

[a] a single sample was taken from each station

**Table 2**: Genomic characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) (n = 27) and methicillin-susceptible *S. aureus* (MSSA) (n = 9) isolates collected from coastal beach and river water, anchialine pools, and beach sand from July–December 2020 in the districts of Hilo, S. Kohala and N. Kona, and Puna on Hawai‘i Island, Hawai‘i.

| **Station** | **District** | **Source** | **Date** | **Isolate #** | **MRSA/ MSSA** | **ST[b]** | **CC[c]** | **Antibiotic Resistance[d]** | **Virulence Gene Profile[e]** | | | **Plasmids** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | **Toxin** | **Exoenzyme** | **Host Immunity** | |
| Lehia Beach Park (#1) | Hilo | sand | 12/12/2020 | 108 | MRSA[a] | 8[f] | 8 | *aph*(3')-*III*, *blaZ*, *erm(C)*, *erm(T)*, *mecA*, *mph*(C), *msr*(A) | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *lukF-PV*, *lukS-PV*, *sek*, *seq* | *aur*, *splA*, *splB*, *splE* | *ACME*, *sak*, *scn* | $rep_{10}$, $rep_{19}$ |
| Richardson's Beach Park (#3) | Hilo | beach water | 09/04/2020 | 60 | MRSA[a] | 8 | 8 | *aph*(3')-*III*, *ant*(6)-*Ia*, *blaZ*, *erm(C)*, *mecA*, *mph*(C), *msr*(A) | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *lukF-PV*, *lukS-PV*, *sek*, *seq* | *aur*, *splA*, *splB*, *splE* | *ACME*, *sak*, *scn* | $rep_{10}$, $rep_{19}$ |
| Reed's Bay (#11) | Hilo | sand | 12/14/2020 | 105 | MRSA[a] | 8 | 8 | *aph*(3')-*III*, *blaZ*, *erm(C)*, *mecA*, *mph*(C), *msr*(A), *tet*(K) | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *lukF-PV*, *lukS-PV*, *sek*, *seq* | *aur*, *splA*, *splB*, *splE* | *ACME*, *sak*, *scn* | $rep_{7a}$, $rep_{7c}$, $rep_{10}$, $rep_{19}$ |
| Honoli‘i Beach Park (#20) | Hilo | beach water | 08/15/2020 | 59 | MRSA[a] | 8 | 8 | *aph*(3')-*III*, *ant*(6)-*Ia*, *blaZ*, *erm(C)*, *mecA*, *mph*(C), *msr*(A) | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *lukF-PV*, *lukS-PV*, *sek*, *seq* | *aur*, *splA*, *splB*, *splE* | *ACME*, *sak*, *scn* | $rep_{7c}$, $rep_{10}$, $rep_{19}$ |
| Honoli‘i Beach Park (#20) | Hilo | sand | 12/14/2020 | 109 | MRSA[a] | 8[g] | 8 | *aph*(3')-*III*, *ant*(6)-*Ia*, *blaZ*, *erm(C)*, *mecA*, *mph*(C), *msr*(A), *qacG*, *tet*(K) | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *lukF-PV*, *lukS-PV*, *seg*, *sem*, *sen*, *sek*, *seq* | *aur*, *splA*, *splB*, *splE* | *ACME*, *sak*, *scn* | $rep_{7a}$, $rep_{10}$, $rep_{19}$ |
| Kehena (#31) | Hilo | beach water | 08/20/2020 | 61 | MRSA[a] | 8 | 8 | *aph*(3')-*III*, *ant*(6)-*Ia*, *erm*(C), *mecA*, *mph*(C), *msr*(A) | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *lukF-PV*, *lukS-PV*, *sek*, *seq* | *aur*, *splA*, *splB*, *splE* | *ACME*, *sak*, *scn* | $rep_{7c}$, $rep_{10}$, $rep_{19}$ |
| Pohoiki Beach (#32) | Puna | beach water | 08/20/2020 | 51 | MRSA[a] | 8[h] | 8 | *aph*(3')-*III*, *ant*(6)-*Ia*, *blaZ*, *erm*(C), *mecA*, *mph*(C), *msr*(A) | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *lukF-PV*, *lukS-PV*, *sek*, *seq* | *aur*, *splA*, *splB*, *splE* | *ACME*, *sak*, *scn* | $rep_{10}$, $rep_{19}$ |
| Pohoiki Beach (#32) | Puna | beach water | 08/20/2020 | 88 | MRSA[a] | 8[h] | 8 | *aph*(3')-*III*, *ant*(6)-*Ia*, *blaZ*, *erm*(C), *mecA*, *mph*(C), *msr*(A), *qacD* | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *lukF-PV*, *lukS-PV*, *sek*, *seq* | *aur*, *splA*, *splB*, *splE* | *ACME*, *sak*, *scn* | $rep_{10}$, $rep_{19}$ |
| Pohoiki Harbor (#33) | Puna | anchialine pool | 12/16/2020 | 89 | MRSA[a] | 8 | 8 | *aph*(3')-*III*, *ant*(6)-*Ia*, *blaZ*, *erm*(C), *lnu*(A), | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *lukF-PV*, *lukS-PV*, *sek*, *seq* | *aur*, *splA*, *splB*, *splE* | *ACME*, *sak*, *scn* | $rep_{10}$, $rep_{19}$, $rep_{21}$ |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | *mecA, mph*(C), *msr*(A), *qacD* | | | | |
| Richardson's Beach Park (#2) | Hilo | beach water | 11/14/2020 | 47 | MSSA | 72[i] | 8 | *blaZ* | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *sec*, *seg*, *sei*, *sel*, *sem*, *sen*, *seo*, *seu* | *aur*, *splA*, *splB*, *splE* | *sak*, *scn* | - |
| Richardson's Beach Park (#2) | Hilo | beach water | 11/14/2020 | 81 | MSSA | 72[i] | 8 | *blaZ*, *lnu*(A) | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *sec*, *seg*, *sei*, *sel*, *sem*, *sen*, *seo*, *seu* | *aur*, *splA*, *splB*, *splE* | *sak*, *scn* | $rep_{7c}$ |
| Honoli'i Beach Park (#2) | Hilo | sand | 12/14/2020 | 106 | MSSA | 72[g] | 8 | *blaZ* | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *sec*, *seg*, *sei*, *sel*, *sem*, *sen*, *seo*, *seu* | *aur*, *splA*, *splB*, *splE* | *sak*, *scn* | $rep_{7c}$ |
| Richardson's Beach Park (#2) | Hilo | beach water | 08/21/2020 | 79 | MSSA | 1181[j] | 8 | *aph*(3')-*III*, *ant*(6)-*Ia*, *blaZ, tet*(K) | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *sep* | *aur*, *splA* | *sak*, *scn* | $rep_{7a}$ |
| Lehia Beach Park (#1) | Hilo | sand | 12/14/2020 | 111 | MSSA | 5[f] | 5 | *blaZ*, *erm*(T), *mph*(C), *tet*(K) | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *seg*, *sei*, *sem*, *sen*, *seo*, *seu* | *aur*, *splA*, *splB* | *sak*, *scn* | $rep_{7a}$, $rep_{13}$ |
| Lalakea (#6) | Hilo | beach water | 08/21/2020 | 38 | MSSA | 5 | 5 | - | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *seg*, *sei*, *sem*, *sen*, *seo*, *seu* | *aur*, *splA* | *sak*, *scn* | - |
| Kealoha Beach Park (#8) | Hilo | beach water | 10/31/2020 | 35 | MSSA | 5 | 5 | *blaZ* | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *sed*, *seg*, *sei*, *sej*, *sem*, *sen*, *seo*, *ser*, *seu* | *aur*, *splA*, *splB* | *sak*, *scn* | $rep_{20}$ |
| Kealoha Beach Park (#8) | Hilo | beach water | 07/03/2020 | 70 | MSSA | 5[k] | 5 | *lnu*(A) | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE, seg*, *sei*, *sem*, *sen*, *seo*, *seu* | *aur*, *splA*, *splB* | *sak*, *scn* | - |
| Onekahakaha Beach Park (#9) | Hilo | beach water | 08/21/2020 | 30 | MSSA | 5[l] | 5 | *blaZ* | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *seg*, *sei*, *sem*, *sen*, *seo*, *seu* | *aur*, *splA*, *splB* | *sak*, *scn* | $rep_{20}$ |
| Onekahakaha Beach Park (#9) | Hilo | beach water | 08/21/2020 | 69 | MSSA | 5[l] | 5 | - | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *seg*, *sei*, *sem*, *sen*, *seo*, *seu* | *aur*, *splA*, *splB* | *sak*, *scn* | - |
| Wailuku River – Upper (#19) | Hilo | river water | 10/31/2020 | 14 | MSSA | 5 | 5 | *blaZ* | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *sed*, *seg*, *sei*, *sej*, *sem*, *sen*, *seo*, *ser*, *seu* | *aur*, *splA*, *splB* | *sak*, *scn* | $rep_{20}$ |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Richardson's Beach Park (#3) | Hilo | beach water | 10/31/2020 | 46 | MSSA | 5 | 5 | *aph*(3')-*III*, *ant*(6)-*Ia*, *blaZ*, *mph*(C), *msr*(A) | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *seg*, *sei*, *sem*, *sen*, *seo*, *seu* | *aur*, *splA*, *splB* | *sak*, *scn* | $rep_{19}$ |
| Pohoiki anchialine pool (#34) | Puna | anchialine pool | 09/18/2020 | 54 | MSSA | 6 | 5 | *blaZ* | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *sea*, *seb*, *sek*, *seq* | *aur*, *splA*, *splB*, *splE* | *sak*, *scn* | $rep_{16}$ |
| Kealoha Beach Park (#8) | Hilo | beach water | 07/03/2020 | 32 | MSSA | 518[k] | 5 | - | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *seg*, *sei*, *sem*, *sen*, *seo*, *seu* | *aur*, *splA*, *splB* | *sak*, *scn* | - |
| Honoli'i Beach Park (#20) | Hilo | sand | 12/14/2020 | 107 | MSSA | 518[g] | 5 | *blaZ*, *erm*(T), *mph*(C), *tet*(K) | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *seg*, *sei*, *sem*, *sen*, *seo*, *seu* | *aur*, *splA*, *splB* | *sak*, *scn* | - |
| Lehia Beach Park (#1) | Hilo | beach water | 10/31/2020 | 50 | MSSA | 398[m] | 398 | *blaZ*, *erm(T)* | *hlgA*, *hlgB*, *hlgC* | *aur* | *scn* | $rep_{13}$ |
| Lehia Beach Park (#1) | Hilo | beach water | 10/31/2020 | 84 | MSSA | 398[m] | 398 | *blaZ*, *erm*(T) | *hlgA*, *hlgB*, *hlgC* | *aur* | *scn* | $rep_{13}$ |
| Kailua-Kona Pier (#28) | S. Kohala and N. Kona | beach water | 10/17/2020 | 58 | MSSA | 398 | 398 | *blaZ*, *erm*(T) | *hlgA*, *hlgB*, *hlgC* | *aur* | *scn* | $rep_{13}$ |
| Pohoiki anchialine pool (#35) | Puna | anchialine pool | 12/16/2020 | 55 | MSSA | 398 | 398 | *erm*(T) | *hlgA*, *hlgB*, *hlgC* | *aur* | *scn* | $rep_{13}$ |
| Richardson's Beach Park (#3) | Hilo | beach water | 08/21/2020 | 45 | MSSA | 15[j] | 15 | *blaZ* | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE* | *aur*, *splA*, *splB*, *splE* | *scn* | $rep_{16}$ |
| Pohoiki anchialine pool (#34) | Puna | beach water | 12/16/2020 | 56 | MSSA | 15 | 15 | *blaZ* | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE* | *aur*, *splA*, *splB*, *splE* | *scn* | - |
| Wai'ōlena Beach Park (#5) | Hilo | beach water | 07/31/2020 | 39 | MSSA | 508 | 45 | *blaZ* | *hlgA*, *hlgB*, *hlgC*, *sec*, *seg*, *sei*, *sel*, *sem*, *sen*, *seo*, *seu*, *tst* | *aur* | *sak*, *scn* | - |
| Wailuku Estuary (#17) | Hilo | river water | 12/14/2020 | 16 | MSSA | 508 | 45 | *blaZ* | *hlgA*, *hlgB*, *hlgC*, *sec*, *seg*, *sei*, *sel*, *sem*, *sen*, *seo*, *seu*, *tst* | *aur* | *sak*, *scn* | $rep_{US5}$ |
| Honoli'i Beach Park (#20) | Hilo | beach water | 09/04/2020 | 13 | MSSA | 97[n] | 97 | - | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE* | *aur*, *splA*, *splB* | *sak*, *scn* | - |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Honoli'i Beach Park (#20) | Hilo | beach water | 09/04/2020 | 66 | MSSA | 97[n] | 97 | - | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE* | *aur*, *splA*, *splB* | *sak*, *scn* | - |
| Richardson's Beach Park (#2) | Hilo | beach water | 10/31/2020 | 43 | MSSA | 1155 | 101 | *blaZ* | *hlgA*, *hlgB*, *lukD, lukE* | *aur*, *splA*, *splB*, *splE* | *sak*, *scn* | - |
| Wailuku Estuary (#17) | Hilo | river water | 08/15/2020 | 15 | MSSA | 3269 | 133 | - | *hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE* | *aur*, *splA*, *splB* | - | - |

[a] MRSA: SCC*mec* type: IVa

[b] MLST type determined by the allelic profile of seven *S. aureus* housekeeping genes: *arc*, *aro*, *glp*, *gmk*, *pta*, *tpi*, and *ygi*.

[c] Clonal Complex (CC)

[d] Antibiotic resistant genes (corresponding drug class): *blaZ* & *mecA* (beta-lactam), *aph*(3')-*III* & *ant*(6)-*Ia* (aminoglycoside), *erm*(C) & *erm*(T) (macrolide, lincosamide, streptogrammin B), *lnu*(A) (lincosamide), *msr(*A*)* (macrolide), *tet*(K) (tetracycline), and *qacD* & *qacG* (quaternary ammonium compounds)

[e] Virulence factor genes encoding toxins: *hlgA* (gamma-hemolysin chain II precursor), *hlgB* (gamma-hemolysin component B precuror), *hlgC* (gamma-hemolysisn component C), *lukD* (leukocidin D component), *lukE* (leukocidin D component), *lukF-PV* (Pantom Valentine leukocidin F component), *lukS-PV* (Pantom Valentine leukocidin S component), and enterotoxins (*sea*, *seb*, *sec*, *sed*, *seg*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *seq*, *ser*, *seu*), *tst* (toxic shock syndrome toxin-1); exoenzymes: *aur* (aureolysin), serine proteases (*splA*, *splB*, *splE*); host immunity: *ACME* (arginine catabolic mobile element), *sak* (staphylokinase), *scn* (staphylococcal complement inhibition)

[f] Isolates were chosen from the same Quanti-Tray 2000® sample containing two different STs (ST8, ST5); source: sand

[g] Isolates were chosen from the same Quanti-Tray 2000® sample containing three different STs (ST8, ST72, ST518); source: sand

[h] Isolates were chosen from the same Quanti-Tray 2000® sample containing the same ST (ST8); source: water

[i] Isolates were chosen from the same Quanti-Tray 2000® sample containing the same ST (ST72); source: water

[j] Isolates were chosen from the same Quanti-Tray 2000® sample containing two different STs (ST1181, ST5); source: water

[k] Isolates were chosen from the same Quanti-Tray 2000® sample containing two different STs (ST5, ST518); source: water

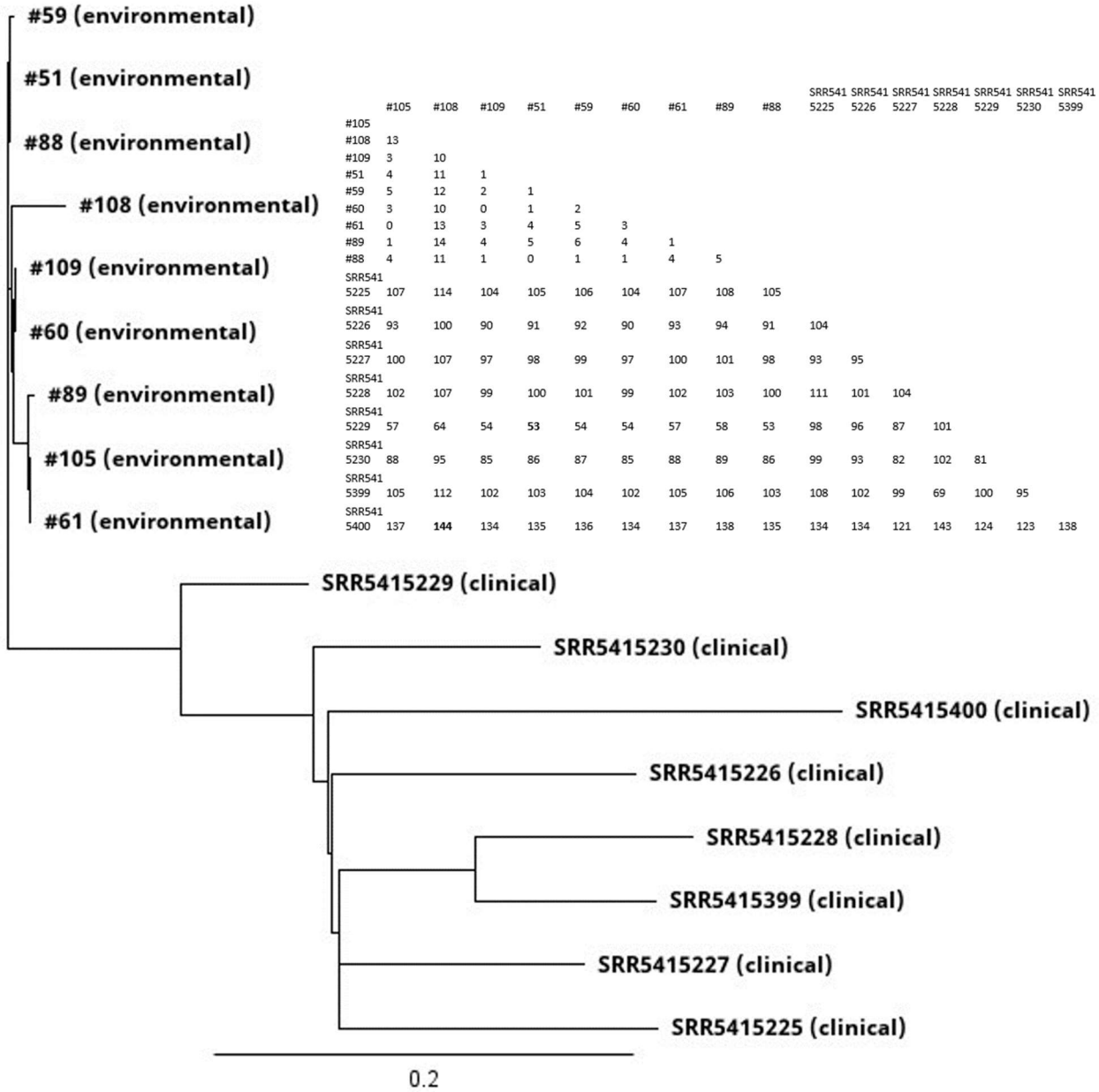
[l] Isolates were chosen from the same Quanti-Tray 2000® sample containing the same ST (ST5); source: water

[m] Isolates were chosen from the same Quanti-Tray 2000® sample containing the same ST (ST398); source: water

[n] Isolates were chosen from the same Quanti-Tray 2000® sample containing the same ST (ST97); source: water

**Supplementary Materials:**

**Figure S1:** Phylogenetic Tree and core-genome Single Nucleotide Polymorphism (SNP) matrix of environmental and clinical MRSA isolates obtained in the State of Hawai‘i




| | #105 | #108 | #109 | #51 | #59 | #60 | #61 | #89 | #88 | SRR5415225 | SRR5415226 | SRR5415227 | SRR5415228 | SRR5415229 | SRR5415230 | SRR5415399 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| #105 | | | | | | | | | | | | | | | | |
| #108 | 13 | | | | | | | | | | | | | | | |
| #109 | 3 | 10 | | | | | | | | | | | | | | |
| #51 | 4 | 11 | 1 | | | | | | | | | | | | | |
| #59 | 5 | 12 | 2 | 1 | | | | | | | | | | | | |
| #60 | 3 | 10 | 0 | 1 | 2 | | | | | | | | | | | |
| #61 | 0 | 13 | 3 | 4 | 5 | 3 | | | | | | | | | | |
| #89 | 1 | 14 | 4 | 5 | 6 | 4 | 1 | | | | | | | | | |
| #88 | 4 | 11 | 1 | 0 | 1 | 1 | 4 | 5 | | | | | | | | |
| SRR5415225 | 107 | 114 | 104 | 105 | 106 | 104 | 107 | 108 | 105 | | | | | | | |
| SRR5415226 | 93 | 100 | 90 | 91 | 92 | 90 | 93 | 94 | 91 | 104 | | | | | | |
| SRR5415227 | 100 | 107 | 97 | 98 | 99 | 97 | 100 | 101 | 98 | 93 | 95 | | | | | |
| SRR5415228 | 102 | 107 | 99 | 100 | 101 | 99 | 102 | 103 | 100 | 111 | 101 | 104 | | | | |
| SRR5415229 | 57 | 64 | 54 | 53 | 54 | 54 | 57 | 58 | 53 | 98 | 96 | 87 | 101 | | | |
| SRR5415230 | 88 | 95 | 85 | 86 | 87 | 85 | 88 | 89 | 86 | 99 | 93 | 82 | 102 | 81 | | |
| SRR5415399 | 105 | 112 | 102 | 103 | 104 | 102 | 105 | 106 | 103 | 108 | 102 | 99 | 69 | 100 | 95 | |
| SRR5415400 | 137 | 144 | 134 | 135 | 136 | 134 | 137 | 138 | 135 | 134 | 134 | 121 | 143 | 124 | 123 | 138 |

**Figure S1:** Phylogenetic tree, generated using a maximum likelihood algorithm and core-genome SNP matrix of MRSA (n = 9) isolates obtained from the environment from July–December 2020 from Hawai‘i Island, Hawai‘i and clinical MRSA isolates collected from hospital patients (n = 8) in the State of Hawai‘i in 2011. Clinical isolate data was obtained from Challagundla et al., 2018[1] under accession

#PRJNA330544. The reference strain used was (isolate #88). The scale bar denotes genetic distance in number of base substitutions per site.

**Table S1**: GPS coordinates for each beach and river station sampled on Hawai‘i Island, Hawai’i.

| Type | District | Station | ID | Latitude | Longitude |
|---|---|---|---|---|---|
| Beach/ sand | Hilo | Lehia Beach Park | 1 | 19.73500 | -155.00835 |
| Beach/ sand | Hilo | Richardson’s Beach Park (back) | 2 | 19.73596 | -155.01344 |
| Beach | Hilo | Richardson’s Beach Park (front) | 3 | 19.73526 | -155.01368 |
| Beach | Hilo | Leleiwi Beach Park | 4 | 19.73389 | -155.01610 |
| Beach | Hilo | Wai‘ōlena Beach Park | 5 | 19.73342 | -155.01683 |
| Beach | Hilo | Lalakea | 6 | 19.73306 | -155.02414 |
| Beach | Hilo | Carlsmith Beach Park (Four Mile) | 7 | 19.73389 | -155.02813 |
| Beach | Hilo | Kealoha Beach Park (Stairs) | 8 | 19.73444 | -155.03077 |
| Beach | Hilo | Onekahakaha Beach Park | 9 | 19.73783 | -155.03764 |
| Beach | Hilo | Puhi Bay | 10 | 19.73213 | -155.04545 |
| Beach/ sand | Hilo | Reed's Bay | 11 | 19.72583 | -155.06292 |
| Beach | Hilo | Moku‘ola (Coconut Island) | 12 | 19.73000 | -155.06859 |
| River | Hilo | Wailoa River estuary | 13 | 19.72361 | -155.07080 |
| River | Hilo | ‘Alenaio Stream entering Wailoa River | 14 | 19.72025 | -155.07640 |
| River | Hilo | Waiakea Stream mouth entering Wailoa River | 15 | 19.71507 | -155.07760 |
| Beach/ sand | Hilo | Hilo Bayfront Beach Park | 16 | 19.72333 | -155.07390 |
| River | Hilo | Wailuku River Mouth | 17 | 19.72778 | -155.08750 |
| River | Hilo | Wailuku River (mid) | 18 | 19.71917 | -155.10390 |
| River | Hilo | Wailuku River (upper) | 19 | 19.71528 | -155.13890 |
| Beach/ sand | Hilo | Honoli‘i Beach Park | 20 | 19.75583 | -155.09110 |
| River | Hilo | Honoli‘i River Mouth | 21 | 19.75667 | -155.09140 |
| Beach/ sand | S. Kohala and N. Kona | Hāpuna Beach Park | 22 | 19.99077 | -155.82627 |
| Beach/ sand | S. Kohala and N. Kona | Waiālea Beach (Beach 69) | 23 | 19.98169 | -155.82865 |
| Beach | S. Kohala and N. Kona | Puakō | 24 | 19.96611 | -155.85305 |
| Beach | S. Kohala and N. Kona | Puakō | 25 | 19.96867 | -155.84546 |
| Beach/ sand | S. Kohala and N. Kona | ‘Anaeho‘omalu Beach | 26 | 19.91472 | -155.88720 |

| | | | | | |
|---|---|---|---|---|---|
| Beach/ sand | S. Kohala and N. Kona | Manini‘ōwali Beach (Kua Bay) | 27 | 19.81028 | -156.00670 |
| Beach | S. Kohala and N. Kona | Kailua Pier (main) | 28 | 19.63992 | -155.99685 |
| Beach | S. Kohala and N. Kona | Kailua Pier | 29 | 19.63964 | -155.99495 |
| Beach | S. Kohala and N. Kona | Kahalu'u Beach Park | 30 | 19.57917 | -155.96690 |
| Beach | Puna | Kehena Beach | 31 | 19.3948 | -154.9289 |
| Beach | Puna | Pohoiki Beach | 32 | 19.4562 | -154.8435 |
| Beach | Puna | Pohoiki anchialine pool (former Pohoiki Harbor) | 33 | 19.4576 | -154.8427 |
| Beach | Puna | Pohoiki ancialine pool | 34 | 19.4581 | -154.8434 |
| Beach | Puna | Pohoiki anchialine pool | 35 | 19.4578 | -154.8434 |
| Beach | Puna | Pohoiki anchialine pool | 36 | 19.45741 | -154.8419 |

**Table S2**: Time-series experiment of staphylococci, methicillin-susceptible *Staphylococcus aureus* (MSSA), and methicillin-resistant *S. aureus* (MRSA) conducted every 2 h at Richardson's Beach Park (station #2) on November 14, 2020 in Hilo, Hawai‘i Island, Hawai‘i. One MSSA isolate was obtained and characterized. No MRSA was detected.

| **Time** | **staphylococci** (MPN/100 mL) | **MSSA** (MPN/100 mL) | **MRSA** (MPN/100 mL) | **Total beachgoers** | |
|---|---|---|---|---|---|
| | | | | **in water** | **at the beach** |
| 8 AM | 91 | 0 | 0 | 1 | 1 |
| 10 AM | 73 | 0 | 0 | 0 | 26 |
| 12 PM | 52 | 0 | 0 | 6 | 18 |
| 2 PM | 156 | + | 0 | 8 | 47 |
| 4 PM | 2827 | 0 | 0 | 14 | 27 |
| 6 PM | 143 | 0 | 0 | 0 | 2 |