

Environmental Surveillance and Characterization of *Staphylococcus aureus* at Beaches and Rivers on Hawai'i Island

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Introduction

Staphylococcus aureus is a ubiquitous colonizer and pathogen in both humans, domestic, and wild animals around the world

> In Hawai'i, Native Hawaiians and Pacific Islanders, and children are particularly susceptible to community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) infections

> Previous studies have found a correlation between swimmer density and staphylococci isolated from seawater in Hawai'i and beachgoers with a history of seawater exposure were four times more likely to develop staphylococcal skin infections compared to beachgoers without exposure to seawater in the previous 10 days^{1,2}

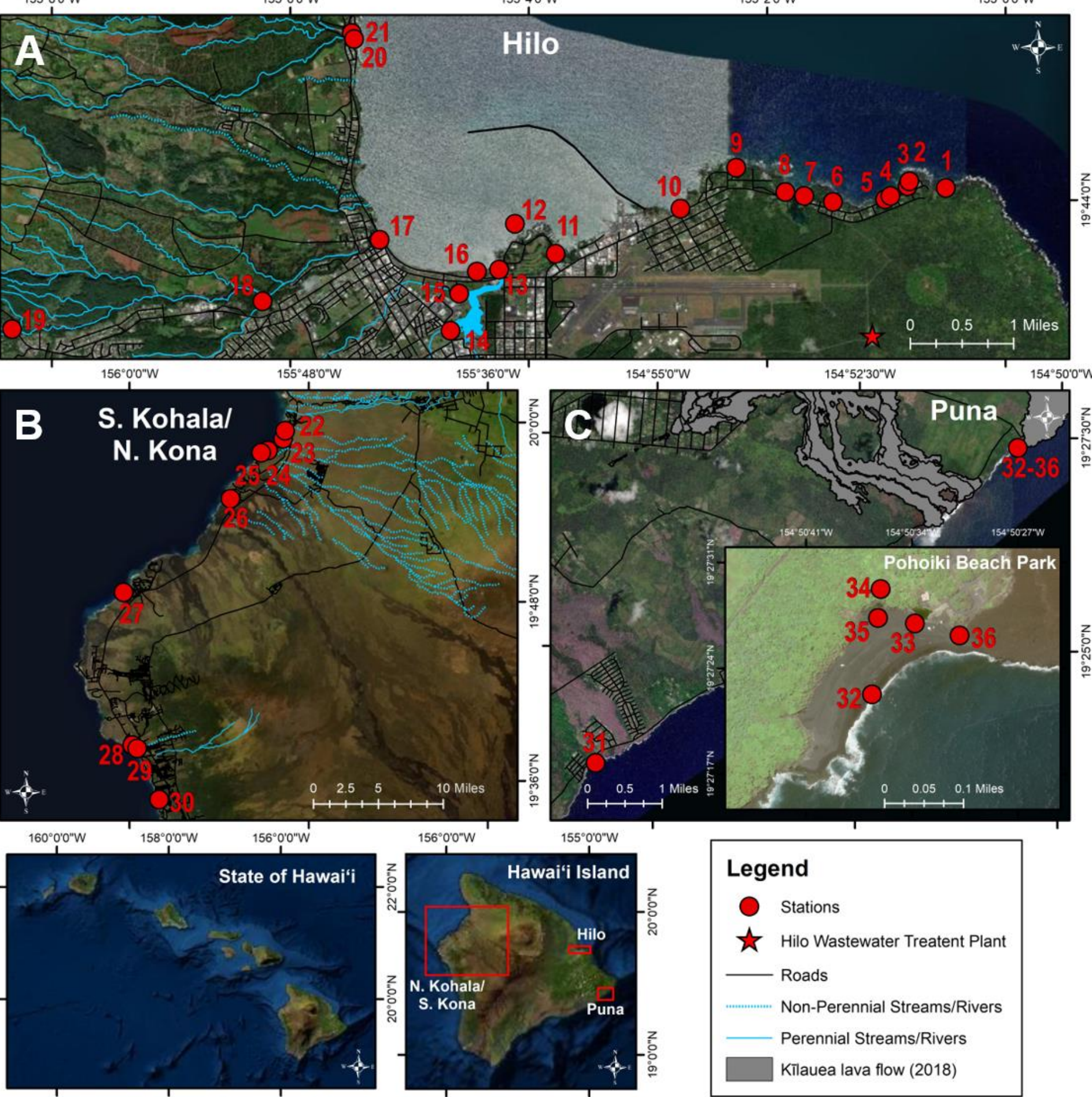
> Limited studies in Hawaii have molecularly characterized MSSA and MRSA for the carriage of antibiotic resistant and virulence genes from recreational marine, river, and sand reservoirs in Hawai'i

> The aim of this study was to isolate *S. aureus* from shoreline beach water, river water, sand, and wastewater stations on Hawai'i Island, Hawai'i (U.S.A.) and to characterize the *S. aureus* isolates using whole-genome sequencing. However, the pandemic hit as the project started closing tourism to the State of Hawai'i, reducing the beach activities of the local population. Therefore, the levels of staphylococci and presence of MSSA/MRSA measured in this study can be considered close to background levels in the environment with limited visitors during the sampling period

Study Locations

> 361 water samples were collected between 6AM–12PM from 36 stations between July–Dec. 2020

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

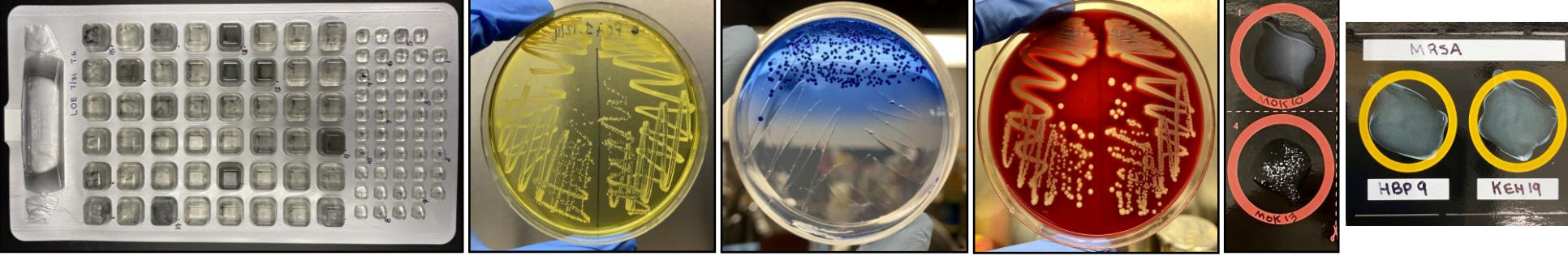


Methods

Isolation and quantification of staphylococci, MSSA/MRSA

- > Water samples were processed using broth-enrichment in Quanti-Tray 2000®
- > An aliquot of broth from up to 20 wells were plated onto mannitol salt agar and Oxacillin-resistance Selective Agar Base (ORSAB) with supplement, followed by screening for β -hemolysis
- > Biochemical confirmation was performed using latex agglutination test kits

Figure 2: Quanti-Tray/2000, Mannitol salt agar, ORSAB, Blood agar, Staphaurex™ and Oxoid™ PBP2' latex agglutination test kits

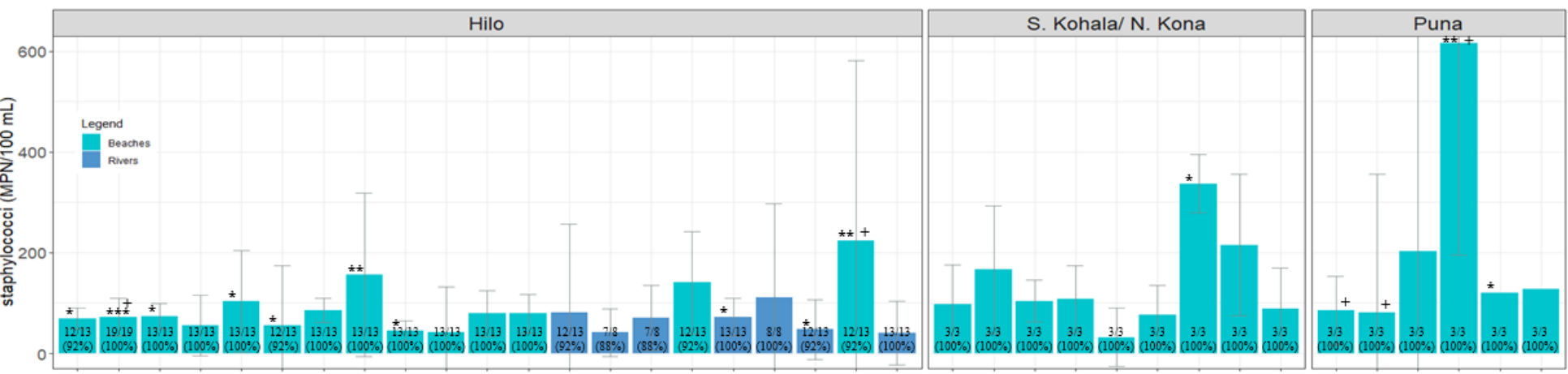


Whole Genome Sequencing Assembly and Analysis

- > Thirty-six (9 MRSA, 27 MSSA) *S. aureus* isolates were characterized using whole genome sequencing
- > Assembled contigs were uploaded to the Center for Genomic Epidemiology to assign the *Staphylococcus* multi-locus sequence type (ST), identify the *SCCmec* elements in the MRSA isolates, and determine the carriage of antibiotic resistance and virulence genes
- > Phylogenetic trees and a matrix of single-nucleotide polymorphism (SNP) differences were constructed

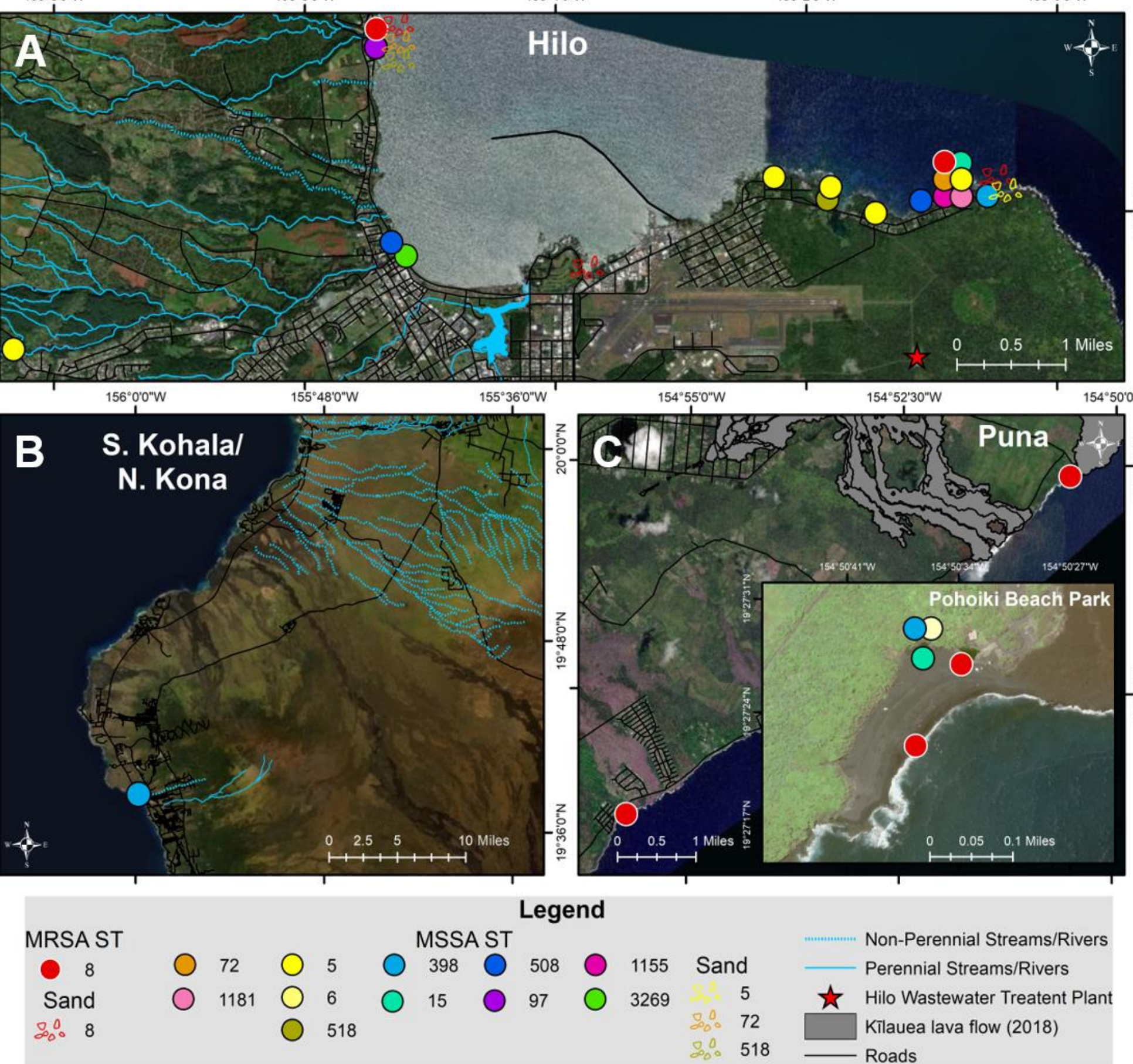
Results

Figure 3: Concentration (geometric mean \pm SE [range]) of staphylococci at beaches and rivers on Hawai'i Island, Hawai'i. * and + depicts the presence of MSSA and MRSA, respectively.



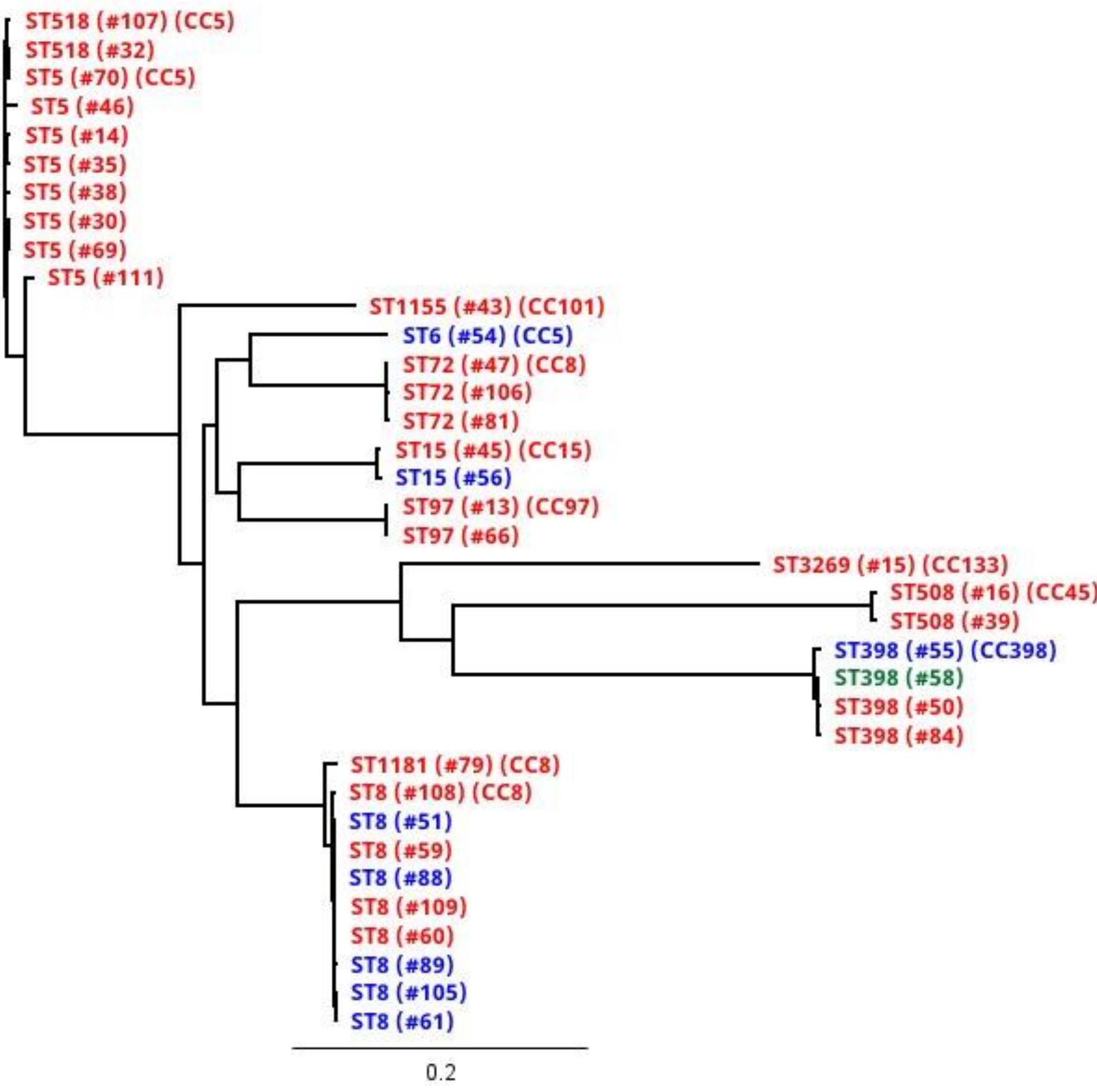
> Staphylococci levels were up to an order of magnitude different among sites and was highest at Pohoiki anchialine pool (#34) (616 ± 420 [192–1632] MPN/100 mL

Figure 4: Map of MSSA and MRSA sequence types (ST) found in the districts of A) Hilo, B) S. Kohala, N. Kona, and C) Puna on Hawai'i Island, Hawai'i



- > All 9 MRSA were ST8 (CC8) *SCCmec* IVa USA300
- > 11 lineages were found from 27 MSSA including: ST5 (n = 8), ST398 (n = 4), ST72 (n = 3), ST15 (n = 2), ST508 (n = 2), ST97 (n = 2) ST518 (n = 2), and one each of ST6, ST1155, ST3269, and ST1181

Figure 5: Phylogenetic tree of MRSA/MSSA isolates, generated using a Maximum likelihood algorithm. Branch tree labels indicate ST, isolate #, clonal complex (CC), and is color coded by district: Hilo (blue), S. Kohala, N. Kona (green), and Puna (blue). Reference strain used was (isolate #88)



Results

- > **98% (354/361)** of samples were positive for *Staphylococcus* spp.
- > **26 (7.2%)** samples were positive for MSSA/MRSA
- > **5 (1.6%)** water samples were positive for MRSA
- > **3 (8.6%)** of sand samples were positive for MRSA
- > No MSSA/MRSA was detected in wastewater
- > The **9 MRSA** were ST8 (CC8) *SCCmec* type IVa and carried 6–9 AMR genes, 16–19 virulence genes, and were closely related (0–16 SNPs)
- > The **27 MSSA** identified included 11 ST, eight CCs, and carried 0–5 AMR genes, and 5–19 virulence genes

Conclusion

- > The MRSA in this study are genetically closely related to the USA300 MRSA previously characterized in the Hawaiian Islands in 2011³
- > MRSA was commonly detected at shoreline beach stations with visibly high counts of swimmers and beachgoers
- > This may be considered a background study due to limited human activity along the beaches
- > Future studies should examine the risk of swimming in *S. aureus* contaminated waters and the development of skin infections

References:

- Charoenc N, Fujioka RS. Assessment of *Staphylococcus aureus* Bacteria in Hawaii's Marine Recreational Waters. *Water Sci Technol* 1993; **27**: 283–9.
- Charoenc N, Fujioka R. Association of Staphylococcal Skin Infections and swimming. *Water Sci Technol* 1995; **31**: 11–7.
- Challagundla L, Luo X, Tickler IA, *et al*. Range Expansion and the Origin of USA300 North American Epidemic Methicillin-Resistant *Staphylococcus aureus* Barbour AG, ed. *mBio* 2018; **9**: e02016-17.

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