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A multi-beach study of *Staphylococcus aureus*, MRSA, and enterococci in seawater and beach sand

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ABSTRACT

Incidences of *Staphylococcus aureus* and methicillin resistant *S. aureus* (MRSA) have risen worldwide prompting a need to better understand routes of human exposure and whether standard bacterial water quality monitoring practices adequately account for this potential threat. Beach water and sand samples were analyzed during summer months for *S. aureus*, enterococci, and MRSA at three southern California beaches (Avalon, Doheny, Malibu Surfrider). *S. aureus* frequently was detected in samples of seawater (59%, $n = 328$) and beach sand (53%, $n = 358$). MRSA sometimes was detected in seawater (1.6%, $n = 366$) and sand (2.7%, $n = 366$) at relatively low concentrations. Site specific differences were observed, with Avalon Beach presenting the highest concentrations of *S. aureus* and Malibu Surfrider the lowest in both seawater and sand. *S. aureus* concentrations in seawater and sand were correlated to each other and to a variety of other parameters. Multiple linear regression on the combined beach data indicated that significant explanatory variables for *S. aureus* in seawater were *S. aureus* in sand, water temperature, enterococci in seawater, and the number of swimmers. In sand, *S. aureus* concentrations were related to *S. aureus* in seawater, water temperature, enterococci in seawater, and inversely to surf height classification. Only the correlation to water temperature held for individually analyzed beaches and for *S. aureus* concentrations in both seawater and sand. To provide context for these results, the prevalence of *S. aureus* in sand was compared to published fomite studies, and results suggested that beach prevalence was similar to that in homes.

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1. Introduction

Microbial contamination of marine waters worldwide is estimated to cause millions of gastrointestinal and acute respiratory infections (ARIs) (Shuval, 2003) and numerous skin infections (Yau et al., 2009) every year. Marine-borne pathogens in the US cost over $900 million per year, with $300 million from gastrointestinal illness from beach recreation (Ralston et al., 2011). Marine-related food-borne illness from *Staphylococcus aureus* was estimated at less than $500,000 per

Abbreviations: CHROMagar™ Staph aureus (SCA), fecal indicator bacteria (FIB).

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year; unfortunately, data were not available to consider costs due to skin infections.

S. aureus is an opportunistic pathogen carried by 20–40% of people (Al-Zu'Bi et al., 2004 Kluytmans and Wertheim, 2005; Kuehnert et al., 2006) with an estimated ~0.8% of the US population (~2.3 million people) colonized by MRSA (Kuehnert et al., 2006). Incidence of infection from hospital- and community-onset *S. aureus* and MRSA is on the rise worldwide (Chambers, 2001; Zetola et al., 2005). Unlike typical hospital-associated strains, some strains of community-associated *S. aureus* can cause infections in healthy people with no traditional risk factors for infection (Chambers, 2001; Baba, 2002; Eguia and Chambers, 2004; Mulvey et al., 2005; Gorwitz, 2008). Even though most patients are treated as outpatients, hospitalization rates remain substantial (Kuehnert et al., 2005; Jarvis et al., 2007). The proportion of skin and soft tissue infections caused by MRSA has risen substantially (Moran et al., 2005), and drug resistant infections place an additional $5 billion burden on the United States health care system annually (Zhang et al., 2011).

Beaches have been suggested as a potential source of community-acquired *S. aureus* infection (Charoenca and Fujioka, 1995; Soge et al., 2009). Support for this suggestion derives from concentrations of *S. aureus* and total staphylococci being correlated to GI illness and to skin, eye and ear infections among bathers (Seyfried et al., 1985; Calderon et al., 1991; Gabutti et al., 2000). *S. aureus* and MRSA are shed by swimmers (Robinton and Mood, 1966; Hanes and Fossa, 1970; Smith and Dufour, 1993; Elmir et al., 2007; Plano et al., 2011), and both are found in beach seawater and sand (Goodwin and Pobuda, 2009; Soge et al., 2009; Sinigalliano et al., 2010; Shah et al., 2011; Enns et al., 2012). *S. aureus* concentrations have been correlated to bather density and attributed to human activity (Calderon et al., 1991; Charoenca and Fujioka, 1995; Papadakis et al., 1997; World Health Organization, 2003).

It has been suggested that human activity is the source of *S. aureus* at the beach (El-Shenawy, 2005). However, *S. aureus* also is found in stormwater (Selvakumar and Borst, 2006) and in coastal streams that drain to the coast (Viau et al., 2011), and wastewater may be another source of *S. aureus* to the environment. Although some studies have not found viable cells or genetic signatures in treated municipal wastewater (Volkmann et al., 2004; Shannon et al., 2007), other studies have found viable *S. aureus* and MRSA in raw (Ahtiainen et al., 1991; Rusin et al., 2003; Börjesson et al., 2009, 2010; Goldstein, 2010) and secondary treated wastewater (Goldstein, 2010). In addition to human inputs, domestic pets can be reservoirs for *S. aureus* and MRSA (Malik et al., 2006; Weese et al., 2006; Nuttall et al., 2008; Baptiste et al., 2009), and dogs can be significant contributors of fecal indicator bacteria (FIB), to the beach (Wright et al., 2009; Zhu et al., 2011).

In an effort to reduce human exposure to microbial contaminants, recreational waters are monitored for FIB, such as enterococci (USEPA, 2004; Dorman and Stoner, 2007). In turn, beach closures can be costly; for example, a 4-month closure of a Southern California beach resulted in millions of dollars of lost revenue, and almost 2 million dollars was spent in closure investigation fees (Dwight et al., 2005). Despite the investment in FIB monitoring, there are concerns that it may overlook pathogens that are not primarily associated with feces (such as *S. aureus*), and that a complementary indicator may be warranted (Cheung et al., 1990). Staphylococci have been suggested as an alternative or complementary indicator for marine water quality (Seyfried et al., 1985; Cheung et al., 1990; Gabutti et al., 2000).

An additional concern is that current FIB guidelines do not monitor the concentrations of bacteria in beach sands, and concern is growing with regard to this exposure route (Heaney et al., 2009; Yamahara et al., 2009; Hartz et al., 2010; Halliday and Gast, 2011; Phillips et al., 2011; Shibata and Solo-Gabriele, 2012). There also is concern that even dead and injured cells deposited to the environment from treated wastewater may pose a threat because antibiotic resistance genes can be taken up by live bacteria (Ahtiainen et al., 1991; Martinez, 2009; Börjesson et al., 2009, 2010). The transfer of *mec*A genes, which confers resistance to methicillin and other beta-lactam antibiotics, is thought to be relatively rare; however, as the abundance of *mec*A DNA has increased in the environment, the chance of transfer has increased (Chambers, 2001).

In this study, the prevalence and concentration of *S. aureus* and MRSA were studied in both seawater and beach sand. Results were compared to enterococci seawater concentrations and environmental parameters. Correlation, regression and multivariate statistical analyses were performed to identify parameters descriptive of the measured bacterial concentrations and to explore whether FIB monitoring might reflect concentrations of *S. aureus* (a non-fecal pathogen) and whether FIB and pathogens might share conditions suggestive of environmental persistence. The study represents a large collection of samples analyzed for *S. aureus* and MRSA from beach water (>320) and sand (>350) taken on 89 different sampling days.

2. Materials and methods

2.1. Sample collection, processing, and bacterial identification

Samples of sand and seawater were collected from beaches at Avalon, Doheny, and Malibu Surfrider beaches in California. Bulk water samples were collected as part of the Pacific Coast Water Study and processed as described previously in Converse et al. (2012). Processing of samples for *S. aureus* analysis was as described in Goodwin and Pobuda (2009) for Avalon and Doheny beaches. In addition, samples of seawater (40–300 ml) or sand (~120 g) were collected from Malibu Surfrider beach from May to September in 2009. A total of 12 beach sites, excluding sites in the lagoons at Malibu and Doheny, were tested on 89 different sampling days during the summers of 2007–2009. At Avalon (Fig. 1) samples were collected on 46 different days; sites A, B, and C were tested in both 2007 and 2008, whereas site D (Descanso Beach; separate watershed with less commercial development) was tested only in 2008. For Malibu Beach (Fig. 1), samples were collected on 38 different days. Malibu sites A and B were near the lagoon outlet, and Site A also was near a housing development on septic system. Only a small number of samples were successfully processed for *S. aureus* from Doheny Beach (5