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Traditional and molecular analyses for fecal indicator bacteria in non-point source subtropical recreational marine waters

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ABSTRACT

The use of enterococci as the primary fecal indicator bacteria (FIB) for the determination of recreational water safety has been questioned, particularly in sub/tropical marine waters without known point sources of sewage. Alternative FIB (such as the Bacteroidales group) and alternative measurement methods (such as rapid molecular testing) have been proposed to supplement or replace current marine water quality testing methods which require culturing enterococci. Moreover, environmental parameters have also been proposed to supplement current monitoring programs. The objective of this study was to evaluate the health risks to humans from exposure to subtropical recreational marine waters with no known point source. The study reported symptoms between one set of human subjects randomly assigned to marine water exposure with intensive environmental monitoring compared with other subjects who did not have exposure. In addition, illness outcomes among the exposed bathers were compared to levels of traditional and alternative FIB (as measured by culture-based and molecular-based methods), and compared to easily measured environmental parameters. Results demonstrated an increase in self-reported gastrointestinal, respiratory and skin illnesses among bathers vs. non-bathers. Among the bathers, a dose–response relationship by logistic regression modeling was observed for skin illness, where illness was positively related to enterococci enumeration by membrane filtration (odds ratio = 1.46 [95% confidence interval = 0.97–2.21] per increasing log10 unit of enterococci exposure) and positively

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related to 24 h antecedent rain fall (1.04 [1.01–1.07] per increasing millimeters of rain). Acute febrile respiratory illness was inversely related to water temperature (0.74 [0.56–0.98] per increasing degree of water temperature). There were no significant dose–response relationships between report of human illness and any of the other FIB or environmental measures. Therefore, for non-point source subtropical recreational marine waters, this study suggests that humans may be at increased risk of reported illness, and that the currently recommended and investigational FIB may not track gastrointestinal illness under these conditions; the relationship between other human illness and environmental measures is less clear.

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Abbreviations

BEACHES	Beach Environmental Assessment and Characterization Human Exposure Study
CFU	colony forming units
CS	Chromogenic Substrate
FIB	Fecal Indicator Bacteria
EPA	US Environmental Protection Agency
GEU	genome equivalent units
L	Liter
MF	membrane filtration
mL	milliliter
mm	millimeter
MRSA	Methicillin resistant *Staphylococcus aureus*
MPN	most probable number
PBS	sterile 1X Phosphate Buffered Saline solution
qPCR	quantitative real-time Polymerase Chain Reaction
ROC	receiver operating characteristic
TSC	Target Sequence Copies

1. Introduction

Currently, the levels of enterococci, a fecal indicator bacteria (FIB), as measured by membrane filtration plate counts or by chromogenic substrate method, is the accepted method for regulatory purposes to determine whether water, in particular marine water, is safe for recreational purposes. The current monitoring guidelines are based upon epidemiologic studies at sites impacted by point sources of sewage contamination which have found an increased risk for transmission of infectious diseases, including gastroenteritis, and acute febrile respiratory, skin, eye, and ear illnesses for bathers compared to non-bathers (Wade et al., 2003; Fleisher, 1991; Fleisher et al., 1993, 1996, 1998; Fujioka et al., 1999; Haile et al., 1999; Kay et al., 1994; Prieto et al., 2001; Pruss, 1998; Shuval, 2003).

Increasingly, the use of these fecal indicator bacteria to regulate recreational water bodies, particularly in sub/tropical marine waters without any known sources of sewage, has been questioned (US EPA, 2007; Boehm et al., 2009). In particular, the few epidemiologic studies conducted in sub/tropical environments characterized by non-point sources of fecal indicator microbes have not shown a statistically significant relationship between human health and the current recommended indicator microbes (Fujioka and Byappanahalli, 2001; Dwight et al., 2004; Colford et al., 2007; Prieto et al., 2001).

The US EPA is in the process of developing new Ambient Water Quality Critieria by 2012 for recreational waters (US EPA, 2007; Boehm et al., 2009; WERF, 2009). Evaluation of these new criteria includes both the use of alternative fecal indicators and rapid molecular methods that will give same-day results (within 4 h or less). These alternatives include enterococci by qPCR and the Bacteroidales group by qPCR. Although not-specifically included in the EPA studies, other researchers have also evaluated the relationship between *S. aureus*, a common skin pathogen, and human health outcomes (Fujioka and Byappanahalli, 2001); and, recently markers have been developed to delineate impacts from seabirds relative to mammals which frequent beach sites (Lu et al., 2008). The use of computer predictive modeling (i.e. "Now-Casting") of indicator abundance from real-time measurement of physical environmental parameters (such as salinity, turbidity, water temperature, rainfall, etc.) is also being investigated by the US EPA and other groups (Boehm et al., 2009). However, there remains a number of critical information gaps, including the need for more epidemiologic studies (especially in subtropical environments characterized by non-point sources) to elucidate the relationships between: physical–chemical parameters; the abundance of traditional and alternative indicators as enumerated by both traditional culture-based and rapid molecular-based methods; the detection and presence of pathogens; and the health outcomes of bathers exposed to recreational waters (US EPA, 2007; Boehm et al., 2009; WERF, 2009).

This study was the first prospective randomized exposure study in the US, and the first randomized exposure study in sub/tropical non-point source recreational marine waters globally. The objectives of this study were to evaluate whether exposures at a recreational marine environment with no known point source of sewage result in an increased risk for illness, and to evaluate the use of culture-based and of rapid quantitative PCR methods plus readily measurable