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Evaluation of conventional and alternative monitoring methods for a recreational marine beach with non-point source of fecal contamination

Tomoyuki Shibata^{a,b,c}, Helena M. Solo-Gabriele^{a,e}, Christopher D. Sinigalliano^{a,b}, Maribeth L. Gidley^{a,b}, Lisa R.W. Plano^{a,f}, Jay M. Fleisher^{a,d}, John D. Wang^a, Samir M. Elmir^{a,g}, Guoqing He^{a,e}, Mary E. Wright^{a,e}, Amir M. Abdelzaher^{a,e}, Cristina Ortega^{a,e}, David Wanless^{a,b}, Anna C. Garza^{a,f}, Jonathan Kish^{a,f}, Troy Scott^h, Julie Hollenbeck^a, Lorraine C. Backerⁱ, and Lora E. Fleming^{a,f}

^aNSF NIEHS Oceans and Human Health Center, Rosenstiel School, University of Miami, Miami, FL

^bNOAA Atlantic Oceanographic and Meteorological Laboratory, Miami, FL

^cNorthern Illinois University, DeKalb, IL

^dNova Southeastern University, Fort Lauderdale, FL

^eCollege of Engineering, University of Miami, Coral Gables, FL

^fMiller School of Medicine, University of Miami, Miami, FL

^gMiami Dade County Public Health Department, Miami, FL

^hBCS Laboratories, Miami, FL

ⁱNational Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA.

Abstract

The objectives of this study were to compare enterococci (ENT) measurements based on the membrane filter, ENT(MF) with alternatives that can provide faster results including alternative enterococci methods (e.g. chromogenic substrate (CS), and quantitative polymerase chain reaction (qPCR)), and results from regression models based upon environmental parameters that can be measured in real-time. ENT(MF) were also compared to source tracking markers (*Staphylococcus aureus*, Bacteroidales human and dog markers, and *Catellibacoccus* gull marker) in an effort to interpret the variability of the signal. Results showed that concentrations of enterococci based upon MF (< 2 to 3,320 CFU/100mL) were significantly different from the CS and qPCR methods ($p < 0.01$). The correlations between MF and CS ($r=0.58$, $p<0.01$) were stronger than between MF and qPCR ($r\leq 0.36$, $p<0.01$). Enterococci levels by MF, CS, and qPCR methods were positively correlated with turbidity and tidal height. Enterococci by MF and CS were also inversely correlated with solar radiation but enterococci by qPCR was not. The regression model based on environmental variables provided fair qualitative predictions of enterococci by MF in real-time, for daily geometric mean levels, but not for individual samples. Overall, ENT(MF) was not significantly correlated with source tracking markers with the exception of samples collected during one storm event. The inability of the regression model to predict ENT(MF) levels for individual samples is likely due to the different

Corresponding Author: Tomoyuki Shibata, Ph.D. Assistant Professor School of Nursing and Health Studies Northern Illinois University Wirtz Hall, 209L DeKalb, IL 60115-2828 Phone : 815-753-5696 tshibata@niu.edu.

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sources of ENT impacting the beach at any given time, making it particularly difficult to for environmental parameters to predict short-term variability of ENT(MF).

INTRODUCTION

Recreational water quality has been evaluated based on concentrations of fecal indicator bacteria (FIB) (such as *Escherichia coli* in freshwaters and enterococci (ENT) in marine waters) in order to protect bathers from potential exposures to pathogens in fecally contaminated water, in particular from human-derived sewage. Current recreational water quality guidelines established by the U.S. Environmental Protection Agency (EPA) were based on epidemiological studies conducted about 30 years ago at beaches with known point sources of fecal contamination (1,2). Although the levels of FIB at those beaches have been correlated with gastrointestinal (GI) illness (3), recent studies at marine beaches without point sources of fecal contamination have shown that enterococci levels and GI illness were not statistically associated (4,5,6,7,8). Therefore, identifying whether FIB is of human origin has been one of the major questions when interpreting the results of FIB measurements for routine monitoring. Thus microbial source tracking methods should be included in routine monitoring of recreational marine waters in an effort to better interpret an ENT signal, especially at non-point source beaches.

In the original epidemiological studies, FIB were enumerated by membrane filtration (MF) method, which has been the conventional method for recreational water monitoring. However since this time, alternative methods have emerged, including chromogenic-substrate (CS) method (9) and quantitative polymerase chain reaction (qPCR). The CS method has been approved by the US. EPA and widely used for recreational water monitoring but the qPCR method had not yet been approved, although current efforts are moving in the direction of adding this method to those recommended. The MF method is characterized by a long incubation time (24 hours). The incubation period for the CS method is 25% shorter (18 hours), while qPCR technology is significantly faster with results available typically in 4 hours. Because of the advantages of shorter processing times with respect to the provision of timely beach advisories, more recent epidemiologic studies sponsored by the U.S. EPA (10) and other groups (11,12, John Griffith, SCCWRP, personal communication) have started to include alternative methods for enterococci analysis. Because of the value of understanding the nature of the source, these newer epidemiologic studies have also included measurements of alternative FIB, most notably human-source *Bacteroidales* (13) and *S. aureus* (4).

The ideal scenario would be to issue beach advisories in real-time or to forecast advisories. As currently-available microbial measures require amplification of the microbial signal (either by culture or by PCR), a lag time between sample collection and microbial measures is unavoidable. The only way to accomplish the ultimate goal of real-time beach advisories is to estimate microbial levels through measures that can be obtained in real-time. Real-time assessments of beach water quality could thus be potentially accomplished by developing empirical models of FIB based upon continuous measurements of readily measureable parameters related to climate and the bulk properties of the water (e.g. pH, salinity, turbidity). One class of model that has been utilized in other studies for this purpose is multiple log-linear regression (14,15,16,17,18). Regression models have yet to be evaluated for the prediction of ENT at marine beach sites and at beaches characterized by non-point sources.

The specific objectives of this study included evaluating methods that can provide faster results for ENT(MF) including ENT(CS), ENT(qPCR), and regression models based upon environmental measures. Beach management decisions (open versus closing a beach) between ENT(MF) and alternative methods are compared to evaluate whether the alternative approaches

would provide comparable beach management outcomes. Comparisons were made with source tracking markers specifically Bacteroidales (human and dog markers), *Catelliboccus* (gull marker), and *S. aureus* during wet and dry conditions in an effort to understand the variability of the ENT(MF) signal.

MATERIALS and METHODS

Sample and environmental data collection

Water samples were collected primarily by individual bathers who participated in a pilot epidemiological study (11,12) at a single site located in Miami, Florida (n=668 over 15 days of monitoring from December 2007 to June 2008). Individuals, carrying a pre-sterilized 5-L plastic container, walked out to knee deep water, rinsed their container once with ocean water, then filled the container with surface water. Six water samples associated with a large rainfall event (>10 mm in 24hr) were also collected immediately prior to one of the epidemiologic sampling dates (March 8, 2008). Storm event samples included samples from ditches on the beach (located within 20 meters of the transects used for water sampling), from ankle deep locations, and from knee deep locations; both ankle and knee deep samples were located immediately downstream of the ditches within the beach bathing area.

Physico-chemical parameters measured included pH, salinity, temperature (YSI Model 650, Yellow Springs, OH) and turbidity (Model VWR Model 66120-200, Newark, DE). From the recorded time of sample collection, each sample was also associated with a specific tidal stage, rainfall (6 and 24 hrs), wind direction and speed, and solar radiation from weather stations operated by the University of Miami and the National Oceanic and Atmospheric Administration.

Microbial assays

For the enumeration of enterococci, two culture methods and two qPCR methods were used. The culture methods included the MF method based on the US EPA Method 1600 (19) and the CS method using Enterolert® (IDEXX Laboratories, Inc., Westbrook, Maine) (9). The qPCR methods included those developed by Haugland et al. (20) and by Siefring et al. (21); these qPCR enterococci methods are referred to as qPCR-a and qPCR-b, respectively, and were chosen as the most commonly used qPCR method (20) and an improvement to this method (21) as recommended by the same research group that originally developed the method. Enterococci based on the MF, CS, qPCR-a, and qPCR-b methods were abbreviated as ENT (MF), ENT(CS), ENT(qPCR-a), and ENT(qPCR-b), respectively.

Analysis of source-specific indicator bacteria included two human markers (Bacteroidales UCD, 22, and Bacteroidales-HF8, 23), one dog marker (Bacteroidales-dog, 24), and one seagull marker (*Catelliboccus*-gull, 25). Bacteroidales human specific markers UCD and HF8, Bacteroidales-dog marker, and *Catelliboccus*-gull marker were abbreviated as BAC_{UCD}, BAC_{HF8}, BAC_{dog}, and CAT_{gull}, respectively.

S. aureus was also measured as a potential indicator of bacteria shedding from bathers' skin. *S. aureus* was analyzed by MF using Baird Parker media. Presumptive *S. aureus* grown via MF on Baird Parker media was confirmed based upon a coagulation test followed by PCR analysis and DNA sequencing to confirm speciation and to evaluate methicillin resistance. More details of the analytical methods for all microbes measured in this study are described in the supplemental text.

The concentrations of bacteria based on the MF and CS were expressed in colony forming units (CFU) and most probable number (MPN), per 100 ml, respectively. The concentrations of enterococci qPCR-a and qPCR-b, and Bacteroidales human specific markers were expressed

in genome equivalent units (GEU) per 100mL. The concentrations of animal-specific markers (dog and gull) were expressed in target sequence copies (TSC) per 100 mL. The methodological detection limits of the MF and CS methods were 2 CFU/100mL and 10 MPN/100mL, respectively; samples measuring at below the detection limits (BDLs) were replaced with a value equal to one half of the detection limits for statistical analyses. For the qPCR assays, the concentrations less than 1 GEU and 1 TSC per 100mL were replaced with 0.5 GEU and 0.5 TSC per 100mL, respectively.

Statistical analyses

For consistency with current regulatory guidelines which are based upon single samples and daily geometric means, the data set was analyzed as “individual” samples (IND) corresponding to the samples collected by each individual bather and as “daily geometric means (DGM)” corresponding to the group of samples collected during each of the 15 sampling days.

Microsoft Excel 2007 and SigmaPlot 11 were used for statistical analyses. Prior to the statistical analyses, microbial concentrations were log₁₀ transformed. For normally distributed data, differences of the mean microbial concentrations were evaluated using t-tests. Paired t-tests were used to compare results from the four enterococci measurement methods. The Mann-Whitney rank sum test was used in lieu of the t-test, and Wilcoxon signed rank test was used in lieu of the paired t-test in the cases where normality tests failed. In general, DGM values were normally distributed except for BAC_{HF8} and MRSA (Shapiro-Wilk test, $p < 0.01$). IND were not normally distributed ($p < 0.01$), therefore non-parametric tests were used to evaluate the statistics of the IND. Spearman Rank Order Correlations and Pearson Product Moment Correlation tests were used for IND samples and DGM values, respectively.

Regression analyses for multiple indicators were performed in order to evaluate which environmental parameters were related to the microbial levels and could be used for real-time predictions of recreational water quality. Colinearity of environmental parameters was evaluated based on the Pearson Correlation test (18). Variables were dropped from consideration if they were correlated ($r > 0.60$). To establish regression relations between enterococci by MF and other variables, the number of variables considered in the linear regression was limited to one tenth of sample size. Only environmental parameters with r values greater than positive or negative 0.2 and that provided significant relations ($p < 0.05$) were retained for stepwise regression analysis for IND ($n = 668$). In addition to correlation coefficient (r), adjusted coefficient of determination ($\text{adj } R^2$) and standard error of estimate (SEE) were calculated for the quantitative evaluation of the regression models (18,26).

The conventional enterococci measurement (MF method) was used as the basis of comparison for correctly predicting whether a recreational beach should be opened or closed (thresholds of 104/100mL for the single-sample maximum or geometric mean of 35/100mL for multiple samples). Beach closure scenarios as predicted by the MF method were compared to alternative measurement methods. Preliminary statistical analysis found that enterococci by qPCR were not strongly related to enterococci by MF ($r = 0.37$, $p < 0.01$); therefore, beach closure scenarios focused on comparisons of management decisions that would be obtained from ENT(CS), and from regression models utilizing environmental parameters to predict ENT(MF). Performance for predicting beach closures (using enterococci by MF as the standard) were evaluated based on type I error, type II error, and kappa (See supplemental text).

RESULTS

Environmental monitoring

Average ambient water physico-chemical conditions (pH=8.0, salinity=35.7 PSU, temperature= 26.0°C, and turbidity=12 NTU) and hydrometrologic conditions (tidal stage=0.36 m, wind direction 160°, wind speed=5.2 m/s, and solar radiation=338 W/m²) were typical for this subtropical marine beach site (Supplement Table 1). Of the 15 sampling days, only two days (March 8 – 12 mm and July 13 – 0.5 mm) were impacted by rainfall within the 6 hours prior to sampling (6 hr-rain). Five of the sampling days were characterized by rainfall during the prior 24 hours (24 hr-rain); among these, only 2 days, March 8 and May 10 were characterized by a significant amount (>10 mm) of 24 hr-rain.

Enterococci measurement methods

For the IND the mean ENT(MF) was significantly different from the mean ENT(CS), ENT(qPCR-a), and ENT(qPCR-b) ($p<0.01$) (Figure 1.a and Supplement Table 3). The average ratios of individual enterococci results based on the MF to the CS, qPCR-a, and qPCR-b were 5.5 with 95% confident limits ($\pm$) of 1.2 (Median (Mdn)=1.5), 56 ± 27 (Mdn=7.1) and 53 ± 19 (Mdn=6.9). When the samples with the BDL were excluded, the average ratios of enterococci results were 3.2 ± 0.5 (Mdn=1.4) for CS/MF, 39 ± 31 (Mdn=5.6) for qPCR-a/MF, and 34 ± 18 (Mdn=6.0) for qPCR-b/MF.

Correlation coefficients between culture methods ($r=0.56$, $p<0.01$ for ENT(MF) and ENT(CS)) and between qPCR methods ($r=0.76$, $p<0.01$ for ENT(qPCR-a) and ENT(qPCR-b)) were higher than the values between culture-based analysis and qPCR analyses methods ($r\leq0.42$, $p<0.01$) (Supplement Table 4).

The DGM values of ENT(MF) and ENT(CS) were not significantly different ($p=0.08$) (Figure 1.b, Supplement Table 3). However, in pairwise tests, the DGM's of ENT(MF) and ENT(CS) were significantly different ($p<0.01$). The DGM of ENT(MF) was lower and significantly different than the DGM of ENT(qPCR-a) and ENT(qPCR-b) ($p<0.01$). The DGM of ENT(qPCR-a) and ENT(qPCR-b) were not significantly different from each other based on the t-test and paired t-test. Correlations between enterococci analysis methods increased for the DGM levels (in comparison to the IND) (e.g. $r=0.66$ to 0.71 between the culture versus qPCR methods; $r=0.83$ between MF and CS, and 0.95 between qPCR-a and qPCR-b, $p<0.01$ for all comparisons).

Regression modeling for enterococci

Sampling time was excluded from the linear regression model since the sampling time and solar radiation were colinear ($r=0.81$, $p<0.01$). The variable 6 hr-rain was not included in the regression models because of the lack of variance, as the vast majority of the days were characterized by no 6 hr-rain. For IND, the environmental variables selected for the ENT(MF) regression model included turbidity, tidal stage and wind direction as positively correlated variables and solar radiation as a negatively correlated variable (Table 1). Turbidity and tidal stage were also selected for the other enterococci models (for CS and qPCR predictions). Wind direction was not selected for the other models. Solar radiation was selected for the ENT(CS), but not selected for ENT(qPCR-a and qPCR-b). Wind speed was selected for ENT(qPCR-a and qPCR-b). Although each selected environmental parameter was significant ($p<0.01$) for the regression models, the correlation coefficients were low ($r=0.37$ to 0.51) and the standard error of estimate were relatively large (SEE=0.44 to 0.79). ENT(CS) showed slightly higher correlations with environmental variables than for other enterococci measurement methods.

For modeling DGM enterococci levels, a single linear regression was used instead of multiple linear regression because of the small sample size ($n=15$). The correlation coefficient r and SEE for the DGM of ENT(MF) based on tidal stage ($p=0.07$) were 0.46 and 0.43, respectively. The correlation r and SEE for the DGM of ENT(CS) based on tidal stage ($p=0.03$) were 0.58 and 0.26, respectively. The DGM of ENT(qPCR-a and qPCR-b) showed no significant relationship with the environmental variables ($p>0.10$).

Recreational water ratings

Thirteen percent of ENT(MF), 13% of ENT(CS), 59% of ENT(qPCR-a), and 58% of ENT(qPCR-b) exceeded the EPA's guideline for the single-sample maximum of enterococci (104/100mL) in marine recreational water. The correlations between the two qPCR methods and ENT(MF) were not strong ($r=0.36$, $p<0.01$) and so continued evaluation of enterococci via qPCR (to estimate beach management decisions as predicted by ENT(MF)) was not pursued due to the extremely low ability to predict ENT(MF) levels, especially at a beach with non-point source of fecal contamination. As mentioned earlier, all comparisons were made against predictions of ENT(MF) measurements as this is the method used as the basis for the current EPA's guidelines.

Although the CS method provided the same overall average rating as the MF method (13% of the samples suggesting closures), the ENT(CS) method produced 38% false negative ratings (Type II error) and 5.7% false positive (Type I error) compared to ENT(MF) (Table 2). As compared with the agreement as might be expected by chance, the recreational water quality ratings based on the CS method were moderate ($Kappa=0.56$). The individual water quality rating based on the regression model developed from IND (function of turbidity, tide, wind direction and solar radiation as given in Table 1) exceeded the U.S. EPA guidelines 28% of the time, which was 1.8 times more than MF and CS methods. False positive ratings based upon CS were low (6%) whereas for the regression model was higher at 24%. False negative ratings were the same (38%) based on the regression model and CS method. The regression model provided slight agreement with the MF method ($Kappa = 0.23$).

For the DGM of observed enterococci values, 27% of ENT(MF), 40% of ENT(CS), and 40% of regression model exceeded 35 CFU or MPN/100mL. The DGM of ENT(CS) provided good accuracy ($Kappa = 0.71$) whereas the regression model (function of tide) provided moderate accuracy ($Kappa = 0.47$).

Source tracking markers—For the IND, the levels of human specific markers were significantly different than the enterococci measurements and they lacked correlations with enterococci levels ($p\leq 0.01$). Only BAC_{UCD} showed weak correlations with enterococci ($r=0.23$, $p<0.01$ for ENT(qPCR-a) and $r=0.28$, $p<0.01$ for ENT(qPCR-b)). The human specific markers were characterized by many samples with levels below detection limits (Supplement Table 3). *S. aureus* was detected and confirmed in 37% of the samples, whereas methicillin resistant *S. aureus* (MRSA) was detected in 1% (7/668) of the IND. The mean levels of BAC_{UCD} and BAC_{HF8} were significantly different from each other (Mann-Whitney rank sum test, $p\leq 0.001$) and their correlation was weak ($r=0.28$). The mean levels of *S. aureus* were not significantly different from BAC_{UCD} ($p=0.24$), but were significantly different from BAC_{HF8} ($p<0.01$). *S. aureus* was not correlated with these Bacteroidales markers ($r=-0.10$ for BAC_{UCD} and 0.00 for BAC_{HF8}). Although the median concentrations of BAC_{UCD} and *S. aureus* were low, they were not correlated with each other.

The DGM of BAC_{UCD} showed correlations, although weak, with enterococci levels, especially with ENT(qPCR-b) ($r=0.45$, $p=0.09$). The DGMs of BAC_{UCD} and BAC_{HF8} were statistically different ($p<0.01$) and their correlations increased slightly ($r=0.52$, $p=0.05$) compared with the IND ($r=0.28$, $p<0.01$). The mean of the DGM for *S. aureus* was not significantly different from

BAC_{UCD} ($p=0.62$) but different from BAC_{HF8} ($p<0.01$). The levels of *S. aureus* were not correlated with BAC_{UCD} (-0.17 , $p=0.55$) and BAC_{HF8} (0.07 , $p=0.81$).

No significant correlations were observed between animal markers and enterococci levels. The individual BAC_{dog} and CAT_{gull} levels were significantly different from each other ($p<0.01$) and characterized by weak correlations ($r=0.34$, $p<0.01$). The DGM of BAC_{dog} and CAT_{gull} levels were not significantly different from each other ($p=0.53$) and the correlation was not strong ($r=0.42$, $p=0.12$).

Rain event and comparison of microbial measures—During the March 2008 storm event (6 hr-rain=12 mm), extremely high concentrations of enterococci (over 10^5 CFU/100mL) were observed in the runoff samples (Supplement Table 5). The ratios of enterococci results based on the MF to the CS, qPCR-a, and aPCR-b were 1.5 ± 0.7 (CS/MF), 0.8 ± 0.4 (qPCR-a/MF), and 8.5 ± 1.1 (qPCR-b/MF). These ratios were comparatively smaller than the ratios among IND (i.g. 6 ± 1.2 for CS/MF and 56 ± 27 for qPCR-a/MF) observed during non-storm conditions. During the storm, enterococci levels were strongly correlated with each other ($r=0.82$ to 0.96 , $p<0.05$). The correlation r values between ENT(CS) and the ENT(qPCR) values was 0.71 ($p=0.12$) for ENT(qPCR-a) and 0.51 ($p=0.38$) for ENT(qPCR-b), which were slightly weaker and insignificant compared with correlations between other enterococci measurements.

BAC_{UCD} and BAC_{HF8} were BDL for all storm event samples. The concentrations of confirmed *S. aureus* were BDL in all samples, although presumptive *S. aureus* levels based on Baird Parker agar were approximately 60,000 for the runoff water, 2,000 CFU/100mL for the ankle deep water, and 1,000 for the knee deep water. CAT_{gull} concentrations from the runoff to knee water showed relatively strong correlations with enterococci levels (e.g. $r=0.75$, $p=0.09$, for ENT(MF) and 0.76 , $p=0.08$, for ENT(qPCR-a)). The concentrations of BAC_{dog} and CAT_{gull} in the storm samples were inversely correlated but insignificant ($r=-0.78$, $p=0.12$).

The average concentrations of enterococci for samples collected by the first three bathers within 16 minutes after rainfall stopped were of the same order of magnitude in comparison to the knee deep water samples collected during the storm event. Within 60 minutes after the storm, all enterococci levels were reduced by more than 90% except for ENT(qPCR-b). Similar to enterococci, the CAT_{gull} levels were higher during and shortly after the storm event relative to days characterized by antecedent dry conditions.

DISCUSSION

Data were collected that facilitated the comparison of enterococci using 4 different methods along with comparison of these data with a series of physico-chemical and hydrometeorologic measurements. Results were evaluated for the purpose of establishing the ability of alternative approaches for predicting ENT(MF). For the IND, enterococci based on the CS and qPCR methods were significantly different from the MF method, which was used in the epidemiological studies for establishing current recreational water quality guidelines. Moreover, among the enterococci assay methods, only ENT(qPCR-a) and ENT(qPCR-b) were not significantly different for both individual sample and geometric means (DGMs). These two qPCR assays utilize identical reverse primers but use different probes that are within 5bp of each other, and different forward primers that give different sized products of 139bp for ENT(qPCR-b) and only 89bp for ENT(qPCR-a).

Although the two culture based methods for enterococci were observed to provide significantly different IND values, DGM values were observed to be not significantly different. These results indicate that the total number of beach closures would be similar regardless of whether MF or CS methods were used to evaluate the samples. However, the specific days that the beaches

would be closed would be different depending upon whether MF or CS was used. Thus, an equivalency cannot be established between the two methods, on a sample to sample basis. By averaging the data over the course of the day, this variability is averaged allowing for an equivalency to be established for DGM values.

No significant correlations were observed between source tracking markers and enterococci levels, with the exception of the storm event evaluated. The lack of correlation during non-storm conditions suggests that enterococci may not be related to a single source (human, dog, or gull). During the storm, the enterococci signal coincided with the levels of *Catelliboccus* gull marker. The large storm event evaluated as part of the current study occurred in March, a month characterized by large numbers of birds as documented at the study site using a digital camera (27). In general, the greatest numbers of birds visit the site during the late winter early spring (December through March). Of interest water temperatures were inversely correlated with the concentrations of gull marker, which is consistent with the visitation of birds at the study site during the cooler months of the year. Evaluation of the levels of CAT_{gull} observed during this study on a month to month basis indicated that the levels of this marker correlated strongly and significantly ($r=0.95$, $p<0.01$) with the reported numbers of seagulls on the beach (27,28). Such associations were not observed between the numbers of bathers and enterococci or BAC_{UCD} and between numbers of dogs and BAC_{dog}. Wright et al. (29) documented that the enterococci contribution from dogs for this study site is greater than that of gulls. The significance of birds during the one storm event may reflect an intermittent occurrence as the number of birds at the beach is highly seasonal and the storm event that was sampled as part of this study coincided with a time period characterized by more birds.

Environmental parameters including turbidity, tide, wind, and solar radiation were related to changes in enterococci levels. The impact of tide is likely due to the sand, in particular sand at the extreme upper reaches of the tidal line, serving as a source of enterococci to the water column (26,30,31,32,³³). Turbidity is a measure of the resuspension of sand from the intertidal zone; the correlation of enterococci with turbidity further supports the role of sand within the intertidal zone as serving as a source of the indicator bacteria (16,17,18,32). Enterococci levels based on the culture methods were inversely affected by solar radiation, whereas enterococci based upon the qPCR methods were not affected by solar radiation. This result was consistent with earlier studies that showed inactivation of FIB could be accelerated by solar radiation (26). These earlier studies hypothesized that enterococci by the MF and CS methods reflected microbial inactivation associated with increasing solar radiation, but the qPCR methods, which could measure both viable and non-viable bacteria, were not affected by solar radiation levels (34,35). Wind appears to play a secondary role in impacting enterococci levels, with wind direction serving as a significant variable for ENT(MF). Wind speed served as a negatively correlated parameter with ENT(qPCR). The association between wind direction and speed could be associated with wind-induced circulation and dilution (26).

Rainfall is the main environmental variable that has been used for proactive beach water quality warnings (26). The largest rainfalls observed during the 15-days monitoring period were 12 mm over 6 hours and 28 mm over 24 hours. The levels of enterococci in the runoff water on the beach and water were extremely high (over 10^3 CFU/100 mL) within a few hours after the storm events. This result was consistent with a study conducted at a southern California beach that showed that rainfall greater than 13 mm increased FIB (36).

The primary disadvantage of the culture-based methods is the longer sample incubation times. Enterococci levels could be available within 4 hours by the use of qPCR method. However, the study showed that qPCR methods provided values that were significantly different from ENT(MF). Direct extrapolation of ENT(qPCR) to estimate ENT(MF) was not practical because of weak correlations between the MF and qPCR. Prediction of ENT(MF) based on ENT(qPCR)

in combination with environmental parameters (e.g. solar radiation) was also not successful in this study. In addition to these challenges, limited studies at the study site have demonstrated that recreational water illnesses at the study site were only correlated with the levels of enterococci based on the MF method (11,12).

This study showed that the regression model based on environmental variables could provide reasonable qualitative predictions of ENT(MF) for DGM levels although quantitative predictions were poor. Since regression models based upon environmental measures could potentially provide results even faster than rapid microbiological measurement techniques, there is a potential of utilizing such models to estimate average water quality conditions over a given period of time at this beach. One alternative to current practice would be to couple the use of a regression model with regular ENT(MF) measurements. Ideally the regression model should provide a value for the probability of illness which takes into the account the uncertainties associated with the model in predicting ENT(MF) and the uncertainties of the ENT(MF) in predicting health outcomes. An even more ideal scenario would be to tie the regression models to health outcomes directly (13).

In addition to physico-chemical and hydrometeorologic parameters, results also suggested that bacterial indicator levels were affected by the numbers of animals on the beach which may also have seasonal patterns associated with their numbers and fecal inputs. Thus, levels of enterococci at non-point source beaches are affected by a myriad of environmental factors and input loadings which are very difficult to capture within simple regression models. For marine beach sites characterized by non-point sources, additional factors should be considered to estimate ENT(MF), including the seasonal nature and shorter term variations of different sources of enterococci (e.g. variations in humans, birds, and dogs).

Brief

Alternative methods may be capable of estimating the geometric means of enterococci by membrane filtration, but are not adequate for estimating individual sample results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Microbial concentrations in a) individual samples, and b) DGM levels. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (or error bars) above and below the box indicate the 90th and 10th percentiles. The solid circles outside the whiskers mark results outside the corresponding range.

Table 1Coefficients and significant environmental variables ($p < 0.01$) for the regression models for individual samples

Enterococci				
Parameter	ENT(MF)	ENT(CS)	ENT(qPCR-a)	ENT(qPCR-b)
pH				
Salinity				
Temp ^a				
Turbidity	0.00652	0.00887	0.0243	0.0178
Tide	1.02	0.701	0.592	0.693
6hr-rain				
24hr-rain				
WDIR ^b	0.00125			
WSP ^c			-0.119	-0.130
Solar ^d	-0.000745	-0.000780		
r	0.422	0.514	0.495	0.369
adj R ²	0.173	0.261	0.242	0.132
SEE	0.698	0.433	0.585	0.794

Where r is the correlation coefficient, adj R² is the adjusted coefficient of determination, and SEE is the standard error of estimate. The microbial concentrations were transformed to log₁₀

^aTemp = temperature

^bWDIR = wind direction

^cWSP = wind speed

^dSolar = solar radiation

Table 2

Accuracy of the CS method and regression models in individual samples (IND) and daily geometric mean (DGM) compared with the observed enterococci based on the MF method

	IND (>104/100mL)		DGM (>35/100mL)		
	MF	CS	Model	MF	CS
Exceedance	13%	13%	28%	27%	40%
Type I error ^a		0.057	0.24		0.18
Type II error ^b		0.38	0.38		0.00
Kappa ^c		0.56	0.23		0.71

^aFalse positives

^bFalse negatives

^cKappa value interpretations: <0 no agreement, 0- 0.2 is slight agreement, 0.21 – 0.40 is fair, 0.41 – 0.60 is moderate, and 0.61 – 0.80 is good, and 0.81 -1.0 is very good (Altman 1991).