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Using Rapid Indicators for *Enterococcus* to Assess the Risk of Illness after Exposure to Urban Runoff Contaminated Marine Water

John M. Colford Jr.[a,*], **Kenneth C. Schiff**[b], **John F. Griffith**[b], **Vince Yau**[a,1], **Benjamin F. Arnold**[a], **Catherine C. Wright**[a], **Joshua S. Gruber**[a], **Timothy J. Wade**[c], **Susan Burns**[d], **Jacqueline Hayes**[d], **Charles McGee**[e], **Mark Gold**[f], **Yiping Cao**[b], **Rachel T. Noble**[g], **Richard Haugland**[h], and **Stephen B. Weisberg**[b]

[a]University of California, Berkeley, School of Public Health, 101 Haviland, MC# 7358, Berkeley CA, 94720-7358, USA

[b]Southern California Coastal Water Research Project, 3535 Harbor Blvd., Suite 110 Costa Mesa, CA 92626, USA

[c]United States Environmental Protection Agency, National Environmental Health Effects Research Laboratory, Chapel Hill, NC 27711, USA

[d]University of California, Berkeley, Survey Research Center, 2538 Channing Way #C, Berkeley, CA 94720-5101, USA

[e]Orange County Sanitation District, 10844 Ellis Avenue, Fountain Valley, CA 92708

[f]Heal the Bay, 1444 9th Street, Santa Monica, CA 90401, USA

[g]University of North Carolina at Chapel Hill, Institute of Marine Sciences, 3431 Arendell Street, Morehead City, NC 28557, USA

[h]United States Environmental Protection Agency, National Exposure Research Laboratory, Cincinnati, OH, 26 W. Martin Luther King Dr., Cincinnati, OH 45268, USA

Abstract

Background—Traditional fecal indicator bacteria (FIB) measurement is too slow (>18 hr) for timely swimmer warnings.

Objectives—Assess relationship of rapid indicator methods (qPCR) to illness at a marine-beach impacted by urban-runoff.

Methods—We measured baseline and two-week health in 9525 individuals visiting Doheny Beach 2007-08. Illness rates were compared (swimmers vs. non-swimmers). FIB measured by traditional (*Enterococcus* spp. by EPA Method 1600 or Enterolert™, fecal coliforms, total coliforms) and three rapid qPCR assays for *Enterococcus* spp. (Taqman, Scorpion-1, Scorpion-2) were compared to health. Primary bacterial source was a creek flowing untreated into ocean; the

*__Corresponding Author__: John M Colford, Jr., 101 Haviland, MC# 7358, Berkeley, CA 94720-7358; Ph:510.642.9370, Fax: 510.666.2551, jcolford@berkeley.edu.
[1]Prestent Affiliation: Kaiser Permanente Division of Research, 2000 Broadway Oakland, CA 94612, USA

creek did not reach the ocean when a sand berm formed. This provided a natural experiment for examining FIB-health relationships under varying conditions.

Results—We observed significant increases in diarrhea (OR1.90, 95% CI 1.29-2.80 for swallowing water) and other outcomes in swimmers compared to non-swimmers. Exposure (body immersion, head immersion, swallowed water) was associated with increasing risk of gastrointestinal illness (GI). Daily GI incidence patterns were different: swimmers (2-day peak) and non-swimmers (no peak). With berm-open, we observed associations between GI and traditional and rapid methods for *Enterococcus;* fewer associations occurred when berm status was not considered.

Conclusions—We found increased risk of GI at this urban-runoff beach. When FIB source flowed freely (berm-open), several traditional and rapid indicators were related to illness. When FIB source was weak (berm-closed) fewer illness-associations were seen. These different relationships under different conditions at a single beach demonstrate the difficulties using these indicators to predict health risk.

Keywords

Gastrointestinal illness; recreational water quality; diarrhea; indicator organisms; qPCR

1. INTRODUCTION

Current methods for monitoring beach water quality involve the enumeration of fecal indicator bacteria (FIB) using culture-based methods, such as membrane filtration or defined substrate kits. These methods are widely accepted because of relative ease of use, low cost, and demonstrated relationship to health risk (Wade et al. 2003; Zmirou et al. 2003). However, the time required for FIB enumeration ranges from 18 to 24 hr, with confirmation steps adding 1+ days. Each beach is unique, but FIB concentrations can change substantially on time scales of less than a day (Boehm et al. 2002). Thus, contaminated beaches remain open during the enumeration period and the contamination event may have passed by the time warnings are posted (Leecaster and Weisberg 2001).

Technological advances provide opportunities to measure bacterial water quality more rapidly (Bushon et al. 2009; Haugland et al. 2005; Noble et al. 2010; Noble and Weisberg 2005). Whereas current EPA-approved methods rely on bacterial growth and metabolic activity, these new rapid molecular methods directly quantify intracellular molecules, such as ATP, DNA, or RNA. Eliminating the enrichment and incubation steps associated with culture-based methods reduces assay time to as little as two hours and provides the opportunity for public health warnings to be issued on the same day that samples are collected (Griffith and Weisberg 2011). The best developed of these methods is quantitative polymerase chain reaction (qPCR), such as the *Enterococcus* spp. (herein referred to as *Enterococcus*) assay developed by Haugland et al. (2005).

Quantitative PCR has been found to correlate with traditional culture-based methods (Griffith et al. 2009; Haugland et al. 2005; Lavender and Kinzelman 2009; Noble et al. 2010), even though the measurement endpoint is different. Given the inherent differences between the two classes of methods, epidemiology studies are needed to establish health-risk relationships before establishing qPCR-based standards. Several studies have developed this relationship for waters affected by wastewater effluent (Wade et al. 2008; Wade et al. 2006; Wade et al. 2010), but few have assessed it for beaches affected by urban runoff (Sinigalliano et al. 2010). Here we report results from an epidemiologic study comparing health-risk relationships between qPCR-based (three different assays) and culture-based quantification of *Enterococcus* at a marine recreational beach affected by urban runoff.

2. MATERIALS AND METHODS

2.1 Study Site

The study was conducted at Doheny State Beach in Dana Point, California, USA. Based on the frequency and magnitude of FIB water quality standards exceedances, Doheny Beach is chronically listed as one of the most polluted beaches in California (www.healthebay.org). Several potential sources of beach FIB exist including an adjacent small craft harbor and a 21 MGD secondary treated wastewater outfall 2.1 km offshore, but modeling and current measurement studies suggest that these sources are too distant to have a consistent effect on water quality at this beach (Jones 2009). The largest and most direct FIB source to Doheny State Beach is San Juan Creek, which drains the adjacent 347 km^2 watershed. However, southern California has a Mediterranean climate and San Juan Creek does not flow to the ocean year-round because a sand berm forms and effectively dams the creek when creek flow is low. When the berm is open, the untreated creek-flow discharges directly to the surf zone and dramatically increases FIB concentration; when closed, water quality generally improves. There was no measurable rain during this 12-week study, as is typical in the summer, and the berm was open for three weeks.

2.2 Study Design

The study was designed as a prospective cohort, similar to prior studies (Coford et al. 2007; Wade et al. 2008; Wade et al. 2006; Wade et al. 2010). Participants were recruited each sampling day with current health and degree of water exposure recorded. Ten to 14 days later interviewers contacted participants by phone and recorded illness occurring after their visit. We used regression models to evaluate the association of illness between swimmers and non-swimmers and between FIB and illness.

2.3 Water Quality Data Collection and Analysis

Surface water samples were collected in sterilized containers at 0.5 m depth on incoming waves. We collected samples three times (8 AM, 12 Noon, 3 PM) at each of five beach sites, three of which were within 400 m of the creek mouth (sites A,B,D), one that was in the creek (site C), and one that was a reference site located about 3000 m to the south (sites E; see Supplemental Materials, Figure 1). Samples were analyzed for traditional culture-based FIB (*Enterococcus,* fecal coliforms, total coliforms) and three qPCR assays for *Enterococcus.* Total and fecal coliform bacteria were enumerated by membrane filtration on m-Endo and m-FC media, respectively (APHA 2009). Culture methods for *Enterococcus* included EPA Method 1600 (USEPA 2006) and Enterolert™ (IDEXX, Westbrook ME; APHA 2009) a defined substrate technology . All culture methods were processed immediately, while filters for the three qPCR methods were frozen for later processing. Two of the qPCR methods, here referred to as TaqMan and Scorpion-1 targeted the same broad species range of the genus *Enterococcus*, but differed in their probe chemistries and the manner in which final quantitative results were calculated (Haugland et al. 2005; Noble et al. 2010). The third method, here referred to as Scorpion-2, was identical to Scorpion-1 except that the primer-probe complex was slightly modified for more specific amplification of *E. faecium* and *E. faecalis*, two of the more common *Enterococcus* spp. commonly found in human fecal contamination (Layton et al. 2010). Taqman qPCR results were reported as calibrator cell equivalents per 100 ml based on the delta-deltaCt method described in Haugland et al (2005), whereas Scorpion-1 and Scorpion-2 results were reported in cell equivalents (CE) per 100 ml using the deltaCt method outlined in Pfaffl (2001) and used by Noble et al (2010).

2.4 Beach Recruitment and Follow-up Interviews

The Committee for Protection of Human Subjects at the University of California, Berkeley approved all protocols. Eligibility criteria included: 1) no previous participation in the study; 2) at least one household member at the beach ≥18 years old; 3) home address in United States, Canada, or Mexico; and 4) verbal consent. Interviewers recorded the closest water-sampling site to the recruit. Participants were given an incentive (beach ball) and a questionnaire to complete prior to departure. The questionnaire assessed possible confounding exposures at the beach and exposures/illnesses experienced the previous three days. Participants failing to complete the beach survey on-site were contacted within 3 days by telephone. Ten to 14 days following their visit, participants were telephoned for a 10–15 minute interview. This interview collected demographic information, swimming and exposures since the beach day, pre-existing health problems (e.g., chronic diarrhea), and acute health conditions since the beach visit. The head of household answered questions on behalf of the family.

2.5 Health Outcomes

Health outcomes included gastrointestinal, respiratory, dermatologic symptoms, and non-specific symptoms. Gastrointestinal outcomes included nausea, vomiting, diarrhea, and stomachache or abdominal cramping. Diarrhea was defined as ≥3 loose or watery stools in 24 hours (Baqui et al. 1991). Highly credible gastrointestinal illness (HCGI) was defined as: i) diarrhea; or ii) vomiting; or iii) nausea and stomach cramps; or iv) nausea and missed daily activities due to gastrointestinal illness, or (v) stomach cramps and missed daily activities due to gastrointestinal illness (identical to “GI illness” defined in Wade et al 2010). Respiratory outcomes included cough and sore throat symptoms. Highly credible respiratory illness was defined as any 2 of the following symptoms: cough, runny nose, sore throat, fever or cold. Dermatologic outcomes included skin rashes and infected cuts. Non-specific symptoms included fever, ear infection, allergies, watery eyes, eye infection, and urinary tract infection. Respondents who reported a symptom at baseline (within 72 hours before the beach visit) were excluded from analysis for that outcome, but not other outcomes.

2.6 Definition of Swimming

We used four graded definitions of “swimmer” based on an individual's reported minimum exposure: i) any water contact; ii) body immersion; iii) head immersion; and iv) swallowed water. We defined body immersion as water contact above the waist, head immersion as head below the water line, and swallowed water as ingestion of any ocean water.

2.7 Statistical Methods and Data Analysis

For swim exposure analyses, we modeled the probability of illness, p, with a logistic regression:

$$\ln\left[p/(1-p)\right]=\alpha+\beta_1 A+\beta_2 S+\gamma\mathbf{X} \tag{1}$$

where A is an indicator variable for any water contact, S is a dichotomous indicator variable for exposure greater than or equal to some level of water contact (body immersion, head immersion, swallowed water), and $\mathbf{X}$ is a vector of potentially confounding covariates (see below). We estimated the relative risk of illness due to swim exposure using the odds ratio (OR), estimated as $\text{OR}=\exp\left(\widehat{\beta}_1+\widehat{\beta}_2\right)$. Thus the comparison group for these analyses was non-swimmers: individuals who had no contact with ocean water during their day at the beach.

In our analyses of the relationships between FIB concentrations and health outcomes, our goal was first to identify a set of conditions under which the traditional indicators appeared to have the expected relationships to health outcomes, especially gastrointestinal symptoms, as reported in prior studies (Wade et al. 2008; Wade et al. 2006; Wade et al. 2010). The conditions we examined included berm status (open, closed, and all days combined), level of participant exposure to water (body immersion, head immersion, swallowed water), specific health symptoms (detailed in 2.5 above) and indicator averaging method. Based on these exploratory analyses, we chose to use a site-specific daily average (one of nine averaging methods that we considered). We estimated site-specific daily averages by calculating the geometric mean of the indicator concentration levels over the 8:00 AM, 12 Noon, and 3 PM samples for each of the five sampling sites. Each swimmer was assigned the average indicator value for the sampling site nearest to where the individual reported swimming.

FIB concentrations were $\log_{10}$ transformed for the analysis because they were right-skewed. When indicator values were below the level of detection (LOD) for a given assay results were set equal to 10 per 100 ml. We also explored other imputation methods by substituting the LOD, the LOD/2, and LOD/SQRT(2). We restricted the population for each analysis to swimmers with a defined level of water contact. The probability of illness, *p*, was modeled for all berm days combined using logistic regression:

$$\ln\left[p/(1-p)\right]=\alpha+\beta I+\gamma\mathbf{X} \tag{2}$$

where I is a continuous measure of the site-specific daily average for the indicator of interest and **X** is a vector of potentially confounding covariates. All ORs were estimated as $\text{OR}=\exp\left(\widehat{\beta}\right)$ and, thus estimate the increase in risk for a one unit change on the $\log_{10}$ scale of the indicator concentration among swimmers with a defined water exposure level.

The probability of illness, *p*, on berm-open and berm-closed days was modeled using logistic regression with a berm-indicator interaction term:

$$\ln\left[p/(1-p)\right]=\alpha+\beta I+\gamma\mathbf{X}=\delta B+\varphi\left(I^{*}B\right) \tag{3}$$

where I and **X** are equivalent to equation (2), B is a dichotomous indicator of berm status (open=1, closed=0) and $I*B$ is an interaction term between indicator concentration and berm status. ORs for berm closed days were estimated from equation (3) as $\text{OR}=\exp\left(\widehat{\beta}\right)$ and for berm open days as $\text{OR}=\exp\left(\widehat{\beta}+\widehat{\varphi}\right)$, and estimate the increase in risk for a one unit change on the $\log_{10}$ scale of the indicator concentration among swimmers with a defined water exposure level. The coefficient $\left(\widehat{\varphi}\right)$ and associated p-value were used to test whether the interaction term differed from 0, and thus whether the association between an indicator concentration and health differed by berm status. Both models (2) and (3) assume that the association between indicators and illness is linear on the log-odds scale.

Models were adjusted for covariates, **X**, that were associated with the outcome or judged to be potential confounders: study year, age, sex, race, swimming on multiple days, allergies, contact with animals, contact with other sick people, frequency of beach visits, digging in the sand, and consumption of raw or undercooked eggs or meat. All covariates, except age and frequency of beach visits, were categorized as 1 or 0. Race was dichotomized as white or nonwhite. Consistent with prior recreational water analyses (Coford et al. 2007; Wade et al. 2008; Wade et al. 2006; Wade et al. 2010), we selected a subset of these covariates for each model using a change in estimate algorithm, which retains covariates that change the estimated OR by at least 5% when removed from a multivariable specification (Rothman 1998). We estimated the 95% confidence intervals (CI) for the ORs using robust standard errors (Freedman 2010) that allow for correlated observations within household, but assume that households are independent. The decision to examine the health-indicator relationships stratified by berm status (berm-open, berm-closed, and all days combined) was planned prior to the initiation of the study. The "berm-open" analyses provide estimates of indicator-health relationships under poor water quality conditions; the "combined" analyses provide estimates of the indicator-health relationships averaged over the mix of berm conditions as would be typical for use of FIB at this beach.

3. RESULTS

3.1 Water Quality

A total of 481 water samples were collected and analyzed. Overall, *Enterococcus* concentrations by EPA 1600 ranged from < 2 to 41,000 colony forming units (CFU)/100 mL. Overall, 17% of the samples exceeded the single sample marine water quality standard (WQS) of 104 CFU/100 ml for *Enterococcus* as determined by EPA Method 1600. At least 10% of the samples exceeded the standard at each of the three sampling sites located near the creek (see sites A, B, and D in Supplementary Material, Figure 1). Water quality at Doheny Beach differed significantly when the sand berm restraining San Juan Creek was closed compared to when open and the creek flowed untreated into beach waters (see Supplemental Material, Figure 2). Examining the site directly in front of the creek, median *Enterococcus* concentrations as measured by EPA1600 were 316 CFU/100 mL on berm-open days compared to 10 CFU/100 mL on berm-closed days. Similarly, 5% of samples from the same site exceeded single sample WQS on berm-closed days compared to 71% on berm-open days (data not shown).

3.2 Population Characteristics

We approached 6,686 eligible households. Of these, 4,499 households (67%) agreed to participate and completed the beach interview, and 3,587 households completed the two-week follow-up interview. Of 9,525 individuals completing the study, 62% were swimmers (Table 1). Among individuals completing the study, 21% failed to complete beach interviews on-site (while at the beach) and were contacted by phone within 3 days of their visit, consistent with Colford et al. (2007). No differences were found in reported swim exposures by beach interview format (on-site vs. phone) or in the basic demographics of the two groups (data not shown). We collected limited data on those who enrolled but could not be located for follow-up; we did not observe notable differences ("lost to follow-up" in Tables 1-2).

3.3 Health Outcomes for Swimmers compared to Non-Swimmers

Among the 3,585 non-swimmers at Doheny Beach, 3.49% had an episode of diarrhea in the 10-14 days following their visit (Table 3); this is comparable to the estimated 3.26% endemic 12-day prevalence of diarrhea in the United States (Scallan et al. 2005). The incidence of diarrhea following the beach visit was significantly higher for body immersion (4.58%), head immersion (4.59%) and those who swallowed water (6.13%) than among those with no contact. The adjusted odds ratio (aOR) for diarrhea among swimmers compared to non-swimmers increased with increasing water exposure: body immersion (aOR = 1.38, 95% CI 1.03-1.86); head immersion (aOR 1.46, 95% CI 1.07-1.99); and swallowed water (aOR 1.90, 95% CI 1.29-2.80). Similar patterns were observed for HCGI. We also collected information on non-gastrointestinal outcomes (see Supplemental Material, Tables 1,2,3,4,6,7,8 and 9). Generally these symptoms were less frequently observed than diarrhea and HCGI.

3.4 Associations of indicators with diarrhea and HCGI

The strongest associations between levels of FIB and diarrhea among swimmers were seen among those with highest level of water exposure ("swallowed water") on berm-open days (Table 4). For example, $\log_{10}$ increases in *Enterococcus* CFU measured by EPA Method 1600 were associated with an aOR of 2.50 (95% CI 1.52-4.11), fecal coliforms had an aOR of 2.30 (95% CI 1.48-3.59) and TaqMan qPCR had an aOR of 2.34 (95% CI 1.13-4.84) when swimmers swallowed water on berm-open days. Berm-open ORs were consistently higher than berm-closed and berm-combined ORs. For each indicator, we report *P*-values for a test of interaction between indicator concentration and berm-status (comparing open and closed estimates from the interaction model). The tests of interaction suggest that indicator-health associations differ by berm-status, in particular among swimmers that swallowed water. Similar patterns (stronger, significant effects on berm-open days, among those who swallowed water) were seen for the association of traditional and rapid measurements of FIB with gastrointestinal illness (Table 5). Alternate LOD imputation methods were explored for indicator analyses, but did not alter conclusions (see Supplemental Material, Tables 10,11 and 12 for LOD/2; other results not shown.)

3.5 Lagged analysis (EPA 1600)

In current beach monitoring practice, the 24 hr incubation time needed for culture-based methods means that water quality results are not available until the day following collection. We therefore repeated our epidemiological analyses lagging culture-based exposure by one day to account for laboratory processing time (i.e. measuring the association between FIB on a given day and illness among swimmers the following day). In these analyses (Supplemental Material, Table 13) we found no significant associations between prior-day FIB and illness. For example, with berm-open the aOR for diarrhea was 1.30 (95% CI, 0.66-2.52) among swimmers with head immersion.

3.6 Dichotomized analysis (EPA 1600)

In current practice, single samples measuring EPA 1600 are typically reported as values above or below 104 CFU/100ml. As a further check on the internal consistency of our findings, we dichotomized site-specific daily average values for EPA 1600 at 2.017 for the geometric mean, corresponding to a concentration of 104 CFU/ml. We then took this dichotomized variable and measured the association with diarrhea and HCGI. We found strong associations between exposure and illness when specifying *Enterococcus* in this manner (see Supplemental Material, Tables 14 and 15). For example, among the small subsample of those who swallowed water (N=181) on berm-open days, the aOR for diarrhea was 8.66 (95% CI 1.89-39.81) for those exposed to EPA 1600 levels above 104 CFU/100ml compared to those exposed to levels below 104 CFU/100ml.

3.7 Indicator-illness associations among non-swimmers: "negative controls"

Our *a priori* assumption was that there should be only random associations between FIB concentrations and gastrointestinal illness among the non-swimmers. Because our study was observational rather than randomized and involved a multiplicity of analyses (i.e. multiple hypothesis testing), we carried out an additional step to investigate the robustness of the associations we observed. We used non-swimmers as "negative controls" (Lipsitch et al. 2010): we explored the association between average FIB concentrations at the beach for a given day and gastrointestinal illness among non-swimmers who visited the same day (who did not contact water, and were unlikely to be exposed to waterborne pathogens). In comparison with the indicator-illness associations seen among swimmers (Tables 4 and 5) there appear to be no patterns in the associations between FIB concentrations and gastrointestinal outcomes among non-swimmers (see Supplemental Material, Table 16).

This suggests that health associations with FIB concentrations (both traditional and rapid) observed among *swimmers* are unlikely to be an artifact of unmeasured confounding, or our estimation approach.

3.8 Daily incidence of diarrhea

Swimmers reported a markedly different pattern of diarrhea incidence than non-swimmers following their beach visit (see Supplemental Material, Figure 3). Among swimmers, diarrhea rates were strongly elevated two days post-exposure relative to non-swimmers. Furthermore, these increases among swimmers were consistent with a dose-response relationship; the greatest elevation seen among swimmers who swallowed water, followed by swimmers with head immersion, and finally swimmers with body immersion.

3.9 Morning vs. afternoon sampling

As described in Methods (section 2.7), we assigned indicator values to swimmers using the site-specific daily average of all morning and afternoon sample-values for the site nearest to the swimmer's area of immersion. To evaluate the impact of the timing of water sampling on indicator-health relationships, we analyzed the morning and afternoon samples separately (see Supplemental Material, Tables 17,18,19,20). Across all point estimates for the indicators, there appeared to be a stronger relationship to health when analyzing the morning rather than the afternoon samples.

3.10 Associations of indicators with other (non-gastrointestinal) symptoms

Although the principal goal of our study was to measure associations between FIB concentrations and gastrointestinal illnesses, we also measured associations between FIB concentrations and non-gastrointestinal health outcomes, including respiratory, eye, ear, and skin complaints. Because these outcomes were less frequently reported, we show only the data for swimmers who placed their heads under water (see Supplemental Material, Tables 21,22,23,24,25,26,27). Unlike associations seen for the indicators with diarrhea and highly credible gastrointestinal illness, there were no clear patterns of indicator-illness associations.

4. DISCUSSION

4.1 Summary

We found that swimmers at Doheny Beach in the summers of 2007 and 2008 experienced diarrhea at a significantly increased rate compared to non-swimmers. Additionally, although it was not a primary focus of our study, we found increased rates of eye infections and earaches among swimmers. We found strong associations between several FIB quantification approaches and diarrhea, with evidence that these associations differed by berm-status. Additionally, the data suggest an increasing dose-response relationship; the strongest associations were seen for those who reported swallowing water, especially on berm-open days. The associations of the FIB concentrations, using rapid molecular assays, with gastrointestinal health outcomes were similar to those of the traditional culture-based assays when examined under the same berm conditions. The pattern of time to diarrhea onset among swimmers (strong peak at 2 days) appears to be different from that seen among non-swimmers. Using non-swimmers as “negative controls” we saw no relationship between FIB and diarrhea among individuals with no water contact, further strengthening the suggestion that the associations observed between traditional and rapid indicators and illness among swimmers were not spurious findings related to our observational design.

4.2 Berm status: Open, Closed and all days combined

Our observation of a large difference in the associations between measures of *Enterococcus* and illness when the berm was open compared to berm-closed days, and all days combined could indicate a different FIB source between the different conditions. Boehm et al. (2004) suggested that FIB can transport through sand, but the transport of contaminated material to the beach is more rapid when the berm is open, reducing time for degradation and inactivation of FIB and pathogens alike. Additionally when the berm is closed, sand can filter out pathogens and *Enterococcus*, and appears to be impacting the association between *Enterococcus* densities and adverse health effects often seen among swimmers proximate to direct, flowing sources. More research is needed on the differential fate and transport impacts of pathogens and FIB through sand, and the potential cause of the breakdown of FIB density-illness relationships.

4.3 Lagged Analyses

The associations we observed were similar between the culture-based and qPCR methods, but this is based on analyses assessing health relationships with samples collected on the same day that swimmers were in the water. We found that the indicator-health associations for the culture-based methods were no longer significant (nor was there a pattern of increasing odds ratios with increasing swimmer exposure) when the results were lagged by one day, typical of current beach monitoring practice. Thus, while these methods theoretically provide comparable levels of health protection, qPCR could provide a substantial advantage in practical application if rapid results were used to make decisions about health-risk management on the same day.

4.4 Morning vs. afternoon results

The processing lag for qPCR is less than for culture-based methods, but there is still about six hours from morning sample collection to when warnings are issued in the afternoon. Our results suggest that the effect of this six-hour lag would be minimal, though, as we found that the odds ratios for samples collected in the morning were more likely to be statistically significant than those for samples collected in the afternoon (see Supplemental Material, Tables 17,18,19,20). This is in apparent contrast with rapid changes in bacterial concentrations that have been observed at some beaches (Boehm et al. 2002). A likely explanation is that the morning samples better represent the average swimmer's exposure compared to afternoon samples. This may be due to solar inactivation, which alters the relationship between FIB and the pathogens with which they co-occur (Davies-Colley et al. 1994; Noble et al. 2004; Sinton et al. 2007). This is consistent with our observation of consistently lower *Enterococcus* concentrations in the afternoon samples compared to morning samples (data not shown).

4.5 Differences in rapid indicators

We evaluated three qPCR assays that utilize primer-probe sets specific for *Enterococcus* and found little difference in their associations with illness. Two (TaqMan and Scorpion-1) used primer-probe sets targeting a gene sequence similar to that of Ludwig and Schleiffer (2000) and are intended to quantify the broad range of *Enterococcus* species enumerated by EPA 1600 (Moore et al. 2008). The similarity in odds ratios between these two methods is consistent with several studies finding they yield similar *Enterococcus* concentrations (Griffith et al. 2009; Noble et al. 2010). The third primer-probe set (Scorpion-2) was a modified design intended to more specifically amplify *E. facaelis* and *E. faecium*, species thought to be important in human fecal contamination. The lack of difference in health relationships for this third method may result from the fecal sources at the site already having high concentrations of these species. Alternatively, it may result from the Scorpion-2 primer-probe design not being exclusionary and still amplifying a wide array of species, as suggested by the concentration correlations with the other two methods observed over a range of sample types (Noble et al. 2010).

4.6 Previous studies

Most bathing water epidemiology studies investigating municipal wastewater effluent-impacted waters, and studies examining the health risks from exposure to land-based runoff are equivocal. Schoen and Ashbolt (2010) used quantitative microbial risk assessment to show that non-point source runoff-affected beaches present considerably less health risk than those affected by wastewater, which is consistent with several studies that found no relationship between GI illness and increasing levels of *Enterococci* at beaches without known sources of sewage (Calderon et al. 1991; Coford et al. 2007; Fleisher et al. 2010). In contrast, McBride et al. (1998) found health risk from human and animal fecal material were not substantially different. Similarly, Haile et al. (1999) found increased health risk for several health outcomes (including fever, chills, cough, ear discharge and respiratory disease although not for HCGI-1 and HCGI-2) from swimming in proximity to urban runoff sources; these runoff source were known to contain human sources of fecal contamination based on the presence of human enteric viruses. Despite the separation of sanitary from storm-water runoff pipes and conduits in southern California, our study also provides an equivocal answer. When the berm was open, we observed associations between *Enterococcus* and health outcomes that were consistent with those seen in studies conducted near wastewater effluent (Wade et al. 2010). In contrast, these associations were weak when the berm status was not taken into account. The United States Environmental Protection Agency has committed to a new water quality standard by October 2012. Boehm et al. (2009) noted that some have suggested the potential establishment of different standards for beaches without direct impact from human fecal sources. Findings from our study suggest that while this option may be possible, the contamination source and delivery must be well understood, as FIB-illness relationships can vary between conditions even within a beach.

4.7 Limitations

There are potential limitations when evaluating our results. Although multiple attempts were made to contact all participants, 22% of participants could not be reached. We have no data to suggest that this introduced a systematic bias into our findings as the baseline enrollment characteristics of those who completed the study and those who did not are similar. The final number of participants completing the study (9,525) was less than the 12,230 we had initially hoped to enroll. Enrollment was impacted by weather conditions that reduced beach usage during the months of our study and conceivably could have limited our ability to detect indicator-health associations for less frequently observed outcomes. We assigned exposure to each participant based on the FIB concentrations collected at the site closest to where that participant swam. Although this may not represent each individual's actual exposure, the internal consistency of the results (increased illness when water quality was poor during open-berm conditions, markedly different daily incidence pattern for swimmers and non-swimmers, increasing illness with increasing exposure) does not suggest a systematic bias. Although indicator exposure was not randomly assigned in our study, neither participants nor investigators had knowledge of water quality results during water exposure. Finally, our results must be interpreted with the understanding that we estimated and report numerous (indicator and health outcome) associations.

5. CONCLUSIONS

Our data suggest an increased risk of swimming-associated gastrointestinal illness at this urban runoff contaminated beach. When the source of FIB consistently exceeded water quality standards (berm-open), traditional and rapid methods for *Enterococcus* were both strongly related to illness. When the source of FIB was diffuse (berm status not adjusted for), fewer significant associations were measured. These differences in relationships between FIB and illness, even at a single beach, demonstrate that it can be difficult to consistently predict FIB-health associations at urban runoff impacted beaches using currently available indicators.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

FIB	fecal indicator bacteria
qPCR	quantitative polymerase chain reaction
GI	gastrointestinal illness
HCGI	highly credible gastrointestinal illness
UTI	urinary tract infection
HCRESP	highly credible respiratory illness
CI	confidence interval
OR	odds ratio
CFU	colony forming unit
WQS	water quality standard
LOD	level of detection
MGD	million gallons per day

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Highlights for: Rapid Indicators for *Enterococcus* and the Risk of Illness after Exposure to Urban Runoff Contaminated Marine Water: A "Natural Experiment"

- Cohort design evaluated swimmer health-fecal indicator bacteria (FIB) relationships
- Rapid (qPCR) and traditional (culture-based) methods used to enumerate FIB
- Swimming, and increased water exposure, associated with negative health outcomes
- Health-FIB relationship depended on beach conditions and swimmers' water exposure
- Demonstrated difficulties using FIB to predict health outcomes at marine beach

Table 1

Doheny Beach Demographics by Swimmer Exposure Status, Individual Level

Variable	Lost to Follow Up	Completed Follow Up	Non-Swimmers	Body Immersion	Head Immersion	Swallowed Water
Individuals	2194	9525	3585	4335	3290	1219
Households	912	3587	913	2159	1784	769
Age (years)						
0-5	12.7%	12.5%	9.7%	13.3%	10.3%	16.5%
5.1-10	13.6	14.2	2.9	24.1	25.2	29.1
10.1-20	15.6	15.2	7.5	23.3	26.1	26.2
20.1-30	13.9	9.1	10.2	7.9	7.7	7.5
30.1-40	18.0	18.4	24.2	12.6	11.9	9.4
40.1-50	16.8	18.2	26.1	12.1	12.0	7.2
> 50	9.4	12.0	19.0	6.3	6.5	3.8
Missing	0.4	0.4	0.4	0.3	0.3	0.4
Sex						
Male	45.9%	47.4%	38.0%	58.3%	62.1%	59.8%
Female	54.1	52.2	61.8	41.3	37.4	39.7
Missing	0.4	0.3	0.3	0.4	0.5	0.5
Race/Ethnicity						
White	58.0%	66.8%	68.8%	66.8%	67.4%	66.9%
White, Hispanic	0.0	4.4	4.4	4.3	4.4	5.7
Non-White, Hispanic	0.0	10.8	10.5	10.3	9.9	8.9
Black	2.4	1.3	1.3	1.2	1.1	1.0
Asian	7.3	4.9	5.4	4.3	3.6	3.7
Indian	0.0	0.5	0.6	0.5	0.4	0.3
Multiple	0.0	7.1	5.3	8.9	9.8	10.2
Other	8.8	2.0	1.9	1.9	1.7	1.9
Missing	23.5	2.0	1.9	1.8	1.6	1.4

Table 2

Doheny Beach Demographics, Household Level

Variable	Lost to Follow Up	Completed Follow Up
Number of household residents	%	%
1	14.9	9.5
2	17.7	19.8
3	24.6	22.3
4	26.1	27.9
5	11.9	13.4
6	3.3	5.2
7	1.3	1.0
8	0.4	0.8
Household income		
< $10,000 - $25,000	-	5.50
$25,001 – $50,000	-	10.90
$50,001 - $75,000	-	14.50
$75,001 - $100,000	-	15.80
$100,001 - $150,000	-	22.70
> $150,000	-	19.10
Missing	-	11.50
Citizenship		
US	99.5	99.6
Canada	0.2	0.03
Mexico	0.3	0.4

Table 3

Associations between Gastrointestinal Illness and Swimming for Various Levels of Water Exposure and Different Berm Conditions.

	No Contact (N=3585)	Body Immersion (N=4335)		Head Immersion (N=3290)		Swallowed Water (N=1219)	
Health Outcome	**% Ill**	**% Ill**	**Adjusted OR[a] [95% CI]**	**% Ill**	**Adjusted OR[a] [95% CI]**	**% Ill**	**Adjusted OR[a] [95% CI]**
Berm-Combined							
Diarrhea	3.49	4.58	1.38 [1.03-1.86]	4.59	1.46 [1.07-1.99]	6.13	1.90 [1.29-2.80]
HCGI	5.37	6.82	1.16 [0.90-1.50]	6.92	1.25 [0.96-1.63]	8.07	1.32 [0.96-1.79]
Berm-Open							
Diarrhea	3.65	4.13	1.27 [0.64,2.51]	4.71	1.61 [0.82,3.16]	6.28	1.92 [0.77,4.78]
HCGI	6.41	6.80	1 [0.59,1.67]	7.50	1.21 [0.72,2.01]	8.97	1.31 [0.67,2.56]

HCGI, highly credible gastrointestinal illness

[a]Odds Ratio calculated using non-swimmers as the reference group

Table 4

Associations between Diarrhea and Exposure to Specific Indicators for Various Levels of Water Exposure and Berm Conditions.

	Berm Combined	Berm Closed	Berm Open	
Indicators[a]	**Adjusted OR(95%)**[b]	**Adjusted OR(95%)**[c]	**Adjusted OR(95%)**[c]	**Test of Interaction *P*-value**[d]
Body Immersion				
Traditional				
EPA 1600	1.33 (1.07,1.64)	1.20 (0.94,1.53)	1.70 (1.17,2.46)	0.12
Enterolert	1.25 (1.03,1.50)	1.20 (0.99,1.46)	1.46 (0.94,2.26)	0.42
Fecal coliform	1.14 (0.93,1.40)	1.02 (0.82,1.28)	1.52 (1.05,2.19)	0.07
Total coliform	1.11 (0.93,1.31)	1.08 (0.9,1.29)	1.40 (0.81,2.41)	0.38
Rapid				
Taqman qPCR (delta delta)	1.03 (0.78,1.35)	0.92 (0.69,1.22)	1.50 (0.92,2.44)	0.09
Scorpion-1 qPCR	1.05 (0.82,1.33)	0.99 (0.74,1.33)	1.20 (0.76,1.91)	0.34
Scorpion-2 qPCR	1.03 (0.82,1.30)	1.01 (0.79,1.29)	1.15 (0.71,1.88)	0.64
Head Immersion				
Traditional				
EPA 1600	1.33 (1.03,1.73)	1.12 (0.83,1.51)	1.87 (1.28,2.72)	0.04
Enterolert	1.29 (1.02,1.62)	1.20 (0.95,1.51)	1.54 (0.97,2.45)	0.35
Fecal coliform	1.18 (0.92,1.52)	1.04 (0.79,1.38)	1.61 (1.12,2.31)	0.06
Total coliform	1.12 (0.91,1.37)	1.03 (0.84,1.28)	1.49 (0.85,2.59)	0.23
Rapid				
Taqman qPCR (delta delta)	1.05 (0.76,1.45)	0.87 (0.62,1.22)	1.66 (1.02,2.68)	0.03
Scorpion-1 qPCR	1.12 (0.84,1.49)	1.07 (0.75,1.53)	1.24 (0.74,2.06)	0.65
Scorpion-2 qPCR	1.04 (0.79,1.36)	0.93 (0.67,1.27)	1.30 (0.82,2.04)	0.23
Swallowed Water				
Traditional				
EPA 1600	1.74 (1.25,2.43)	1.42 (0.93,2.18)	2.50 (1.52,4.11)	0.09
Enterolert	1.38 (0.99,1.93)	1.07 (0.77,1.49)	2.17 (1.35,3.49)	0.02
Fecal coliform	1.29 (0.89,1.87)	0.96 (0.65,1.43)	2.30 (1.48,3.59)	0.00
Total coliform	1.29 (0.93,1.80)	1.13 (0.82,1.56)	2.15 (0.91,5.13)	0.17
Rapid				
Taqman qPCR (delta delta)	1.28 (0.82,2.01)	0.90 (0.56,1.44)	2.34 (1.13,4.84)	0.03
Scorpion-1 qPCR	1.34 (0.89,2.03)	1.16 (0.72,1.87)	2.02 (0.73,5.60)	0.34
Scorpion-2 qPCR	1.49 (1.14,1.95)	1.25 (0.90,1.73)	2.30 (1.46,3.61)	0.03

[a] Indicator exposure assigned based on site-specific daily average

[b] Odds Ratio for diarrhea associated with a 1 unit increase in the log10 indicator concentration using non-interaction model

[c] Odds Ratio for diarrhea associated with a 1 unit increase in the log10 indicator concentration using interaction model

[d] *P*-value associated with interaction term comparing open to closed berm conditions

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Table 5

Associations between HCGI and Exposure to Specific Indicators for Various Levels of Water Exposure and Berm Conditions.

Indicators[a]	Berm Combined Adjusted OR(95%)[b]	Berm Closed Adjusted OR(95%)[c]	Berm Open Adjusted OR(95%)[c]	Test of Interaction *P*-value[d]
Body Immersion				
Traditional				
EPA 1600	1.16 (0.97,1.39)	1.08 (0.88,1.32)	1.36 (0.98,1.89)	0.24
Enterolert	1.10 (0.94,1.30)	1.09 (0.92,1.29)	1.15 (0.79,1.66)	0.79
Fecal coliform	1.11 (0.95,1.31)	1.03 (0.87,1.23)	1.36 (1.00,1.84)	0.13
Total coliform	1.10 (0.96,1.27)	1.09 (0.94,1.27)	1.19 (0.83,1.72)	0.66
Rapid				
Taqman qPCR (delta delta)	0.97 (0.79,1.20)	0.90 (0.71,1.13)	1.23 (0.80,1.91)	0.21
Scorpion-1 qPCR	1.02 (0.84,1.24)	1.00 (0.79,1.28)	1.06 (0.75,1.50)	0.80
Scorpion-2 qPCR	0.96 (0.79,1.16)	0.95 (0.77,1.17)	0.98 (0.66,1.45)	0.91
Head Immersion				
Traditional				
EPA 1600	1.16 (0.94,1.45)	1.01 (0.79,1.29)	1.54 (1.10,2.16)	0.04
Enterolert	1.13 (0.93,1.36)	1.07 (0.87,1.30)	1.26 (0.85,1.86)	0.45
Fecal coliform	1.15 (0.94,1.39)	1.03 (0.83,1.29)	1.49 (1.09,2.03)	0.06
Total coliform	1.16 (0.99,1.36)	1.09 (0.91,1.31)	1.38 (0.95,2.01)	0.27
Rapid				
Taqman qPCR (delta delta)	0.94 (0.74,1.21)	0.83 (0.63,1.09)	1.26 (0.78,2.03)	0.14
Scorpion-1 qPCR	1.11 (0.89,1.39)	1.02 (0.77,1.36)	1.25 (0.85,1.82)	0.41
Scorpion-2 qPCR	1.00 (0.80,1.23)	0.93 (0.73,1.18)	1.12 (0.75,1.67)	0.42
Swallowed Water				
Traditional				
EPA 1600	1.52 (1.12,2.06)	1.29 (0.88,1.88)	1.94 (1.23,3.05)	0.18
Enterolert	1.20 (0.88,1.63)	0.93 (0.69,1.26)	1.75 (1.16,2.64)	0.02
Fecal coliform	1.15 (0.84,1.59)	0.89 (0.63,1.27)	1.95 (1.29,2.97)	0.00
Total coliform	1.32 (1.01,1.72)	1.16 (0.88,1.53)	2.01 (1.06,3.83)	0.12
Rapid				
Taqman qPCR (delta delta)	1.21 (0.83,1.75)	0.95 (0.65,1.39)	1.95 (1.05,3.59)	0.05
Scorpion-1 qPCR	1.28 (0.92,1.77)	1.17 (0.79,1.71)	1.55 (0.80,3.00)	0.46
Scorpion-2 qPCR	1.35 (1.03,1.75)	1.19 (0.88,1.61)	1.70 (1.10,2.63)	0.18

HCGI, highly credible gastrointestinal illness

[a]Indicator exposure assigned based on site-specific daily average

[b]Odds Ratio for HCGI associated with a 1 unit increase in the log10 indicator concentration using non-interaction model

[c]Odds Ratio for HGCI associated with a 1 unit increase in the log10 indicator concentration using interaction model

[d]p-value associated with interaction term comparing open to closed berm conditions