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Water quality, weather and environmental factors associated with fecal indicator organism density in beach sand at two recreational marine beaches

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Abstract

Recent studies showing an association between fecal indicator organisms (FIOs) in sand and gastrointestinal (GI) illness among beachgoers with sand contact have important public health implications because of the large numbers of people who recreate at beaches and engage in sand contact activities. Yet, factors that influence fecal pollution in beach sand remain unclear. During the 2007 National Epidemiological and Environmental Assessment of Recreational (NEEAR) Water Study, sand samples were collected at three locations (60 m apart) on weekend days (Sat, Sun) and holidays between June and September at two marine beaches — Fairhope Beach, AL and Goddard Beach, RI — with nearby publicly-owned treatment works (POTWs) outfalls. F^+ coliphage, enterococci, *Bacteroidales*, fecal *Bacteroides* spp., and *Clostridium* spp. were measured in sand using culture and qPCR-based calibrator-cell equivalent methods. Water samples were also collected on the same days, times and transects as the 144 sand samples and were assayed using the same FIO measurements. Weather and environmental data were collected at the time of sample collection. Mean FIO concentrations in sand varied over time, but not space. Enterococci

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CFU and CCE densities in sand were not correlated, although other FIOs in sand were. The strongest correlation between FIO density in sand and water was fecal *Bacteroides* CCE, followed by enterococci CFU, *Clostridium* spp. CCE, and *Bacteroidales* CCE. Overall, the factors associated with FIO concentrations in sand were related to the sand–water interface (i.e., sand-wetting) and included daily average densities of FIOs in water, rainfall, and wave height. Targeted monitoring that focuses on daily trends of sand FIO variability, combined with information about specific water quality, weather, and environmental factors may inform beach monitoring and management decisions to reduce microbial burdens in beach sand.

The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

Keywords

Beach sand; Water quality; Rainfall; Fecal pollution; Enterococci; qPCR

1. Introduction

Seasonal visitation of coastal beaches is a favored pastime in the United States. The National Survey on Recreation and the Environment (NSRE) estimated that during 1999–2000, 43% of the civilian population 16 years and older participated in marine outdoor recreational activities, equivalent to 89 million individuals (Leeworthy et al., 2001). Epidemiologic studies of sand contact among beachgoers suggest there is an increased risk of GI illness in the order of 20–50% (depending on the age range and type of sand exposure) among beachgoers who dig in the sand or are buried in the sand (Heaney et al., 2009). An increased risk of GI illness and diarrhea was also observed among beachgoers digging in sand or who were buried in sand as a function of densities of fecal indicator measures in sand (Heaney et al., 2012). Although the observed risks could be considered modest in magnitude, these findings are of public health importance because of the large numbers of people who recreate at beaches and engage in sand contact activities. Research continues to emerge to advance understanding of hand-to-mouth transfer of FIOs, including *Escherichia coli* and F^+ coliphages, via beach sand contact (Whitman et al., 2009). If the relationship between sand exposure and enteric illness is causal, many cases of illness could be prevented by advancing knowledge of factors that could reduce microbial burdens in beach sand.

The sources of fecal indicator bacteria (FIB), which in this study includes enterococci, *Bacteroidales*, fecal *Bacteroides* spp., and *Clostridium* spp., in sand are numerous, including treated or blended sewage discharges in close proximity to beaches by POTWs (Elmanama et al., 2005; Ghinsberg et al., 1994) and other sources, such as urban and/or agricultural runoff, warm-blooded domestic and wild animals, and beachgoers (Wheeler Alm et al., 2003; Whitman and Nevers, 2003; Whitman et al., 2004, 2006). Recent studies have shown that the fecal pollution of beach sand occurs as a result of poor nearby water quality, when fecal pollution washes-in from the shoreline (Abdelzaher et al., 2010; Phillips et al., 2011). In addition to these diverse sources, field and microcosm experiments using enterococci have shown that autochthonous populations of FIB may persist and/or re-grow in beach sand driven by periodic wetting through wave height and tides, possibly serving as a diffuse

source of FIB in sand and nearby bathing waters (Desmarais et al., 2002; Yamahara et al., 2007; Yamahara et al., 2009). Some have recommended measuring FIOs that cannot grow or multiply in sand (Betancourt and Fujioka, 2006; Luther and Fujioka, 2004; Oshiro and Fujioka, 1995), such as F^+ coliphage (Fujioka, 2001) and anaerobes, such as *Bacteroidales*, fecal *Bacteroides* spp., and *Clostridium* spp. (Converse et al., 2009; Noble et al., 2006; Rinttila et al., 2004). Others still have observed a spatial patchiness of fecal pollution in sand associated with fecal droppings from animals on the beach (Bonilla et al., 2007). In some studies, estimates of FIB density in sand may be greater than FIB estimates observed in recreational waters (Halliday and Gast, 2011; Kinzelman et al., 2004; Skalbeck et al., 2010; Wheeler Alm et al., 2003; Whitman and Nevers, 2003; Whitman et al., 2006).

An expert group at the recent “Microareias 2012” meeting suggested that, by restricting beach monitoring to beach water and excluding sand, a gap may be created in assessing the overall risk to human health in beach areas (Sabino et al., 2014). The objective of this study was to assess factors related to FIO measures in sand, including measures of FIOs in water, and weather and environmental factors. Knowledge of the factors influencing the variability of fecal pollution in sand could inform water quality monitoring and help beach managers understand sources of fecal contamination at their beach.

2. Materials and methods

2.1. Beach sites and sand collection

Sand samples were collected as part of an epidemiologic study of beachgoer exposure to microorganisms in water and sand at two recreational marine beaches affected by treated sewage discharge from nearby POTWs sewage outfalls. This work was part of the 2007 NEEAR Water Study, which is a national survey of beachgoers sponsored by the Environmental Protection Agency (EPA) and the Centers for Disease Control and Prevention (CDC) (Heaney et al., 2009, 2012; Wade et al., 2010). During the NEEAR Water Study, beach sand samples were collected at Fairhope Municipal Park Beach (FB) in Alabama (Lat +30.5269, Long −87.91089) and Goddard Memorial State Park Beach (GB) in Rhode Island (Lat +41.6666, Long −71.43498). These beaches had POTWs discharges within 1.5 miles (2.4 km) of the beach locations and historically showed variability in water quality though were generally in compliance with local and federal water quality guidelines. See Wade et al. (2010) for further description of the maritime sites and water sample collection and analysis. A total of 864 beach water samples were collected from FB (n = 438) and GB (n = 426) over the study period (Wade et al., 2010).

At each beach and on each sampling day, wet sand samples were collected at three transects (lines perpendicular to the shoreline greater than or equal to 20 m apart representing the portion of the beach where most of the beachgoers swam, sample locations 7, 8 and 9 in Fig. S1a–S1b) at 8:00 AM. Approximately 250 g of sand was collected 1 m from the lowest water level (determined when the waves receded from the shoreline) using a rubber mallet and a sterile stainless steel soil auger (2.25 inch diameter by 8 inch, AMS, American Falls, Idaho). If the sand was not wet 1 m from the water, the collection location was moved the shortest possible distance toward the water to a location where the sand was wet. For FIB analyses the samples were filtered within 6 h of collection and for F^+ coliphage analyses the

samples were analyzed within 72 h of collection. Water samples were also collected along these same three transects at both shin (0.3 m) and waist (1 m) depth at three sampling times, 8:00 AM, 11:00 AM, and 3:00 PM (sample locations 1–6 in Fig. S1a–S1b). The samples were stored at 4 °C until analyzed.

2.2. Quantification of FIOs in beach sand and water

Sand and water sampling and FIO analysis protocols have been previously described (Chern et al., 2009; Haugland et al., 2005; Heaney et al., 2012; Siefring et al., 2008a). Briefly, a modification (Love and Sobsey, 2007) of the EPA Method 1601 two-step enrichment procedure for coliphage in water was used to quantify F^+ coliphage in sand using host *E. coli* F_{amp} (ATCC#70089) (EPA, 2001). Three aliquots each of 0.33 g, 3.3 g, and 33 g of FB and GB sand were analyzed in a most probable number (MPN) series. Ten milliliters of 0.5× tryptic soy broth (TSB) with enrichment supplements (4 M magnesium chloride and 100× streptomycin/ampicillin) was added to the 0.33 g and 3.3 g sand samples in 15 ml conical tubes, and 50 ml 0.5× TSB with enrichment supplements was added to the 33 g sand sample in 125 ml Nalgene® (Nalge Nunc, International, Rochester, NY) bottles. These enrichment TSB volumes and containers were selected for optimal wetting and mixing of sand. A positive control with MS2 bacteriophage (ATCC#15597-B1) in 0.5× TSB with enrichment supplements and a negative control with only 0.5× TSB with enrichment supplements were included with each set of samples. The samples were incubated on their sides on a rocking platform at 75 rpm for 1 h at 36 °C ± 1 °C, then held upright without rocking with loosened lids overnight at 36 °C ± 1 °C. After overnight of incubation, 1.0 ml sub-samples were centrifuged in 1.5 ml Eppendorf tubes at 10,000 ×*g* for 10 min to clarify samples. A 10 µl volume from each sand enrichment was spotted onto pre-poured 0.75× tryptic soy agar (TSA) plates containing *E. coli* F_{amp} host, allowed to dry, and incubated inverted at 36 °C ± 1 °C (EPA, 2001; Love and Sobsey, 2007). Lysis zones were scored as positive or negative and used to calculate a three dilution, three replicate MPN expressed per gram dry weight sand (MPN).

To process samples for FIB (enterococci, *Bacteroidales*, fecal *Bacteroides* spp., *Clostridium* spp.), 75 g of each sand sample was distributed into wide-mouth 500 ml bottles, and 300 ml of phosphate-buffered rinse/dilution water (pH 7.2) (APHA, 2006) was added to each bottle aseptically. Culture-based tests of enterococci were performed by distributing 75 g of sand into 300 ml of phosphate buffered rinse/dilution water (pH 7.2), shaking vigorously by hand 50 times, and membrane filtering aliquots of the supernatant following EPA Method 1600 (EPA, 2002). One hundred milliliters of this supernatant was filtered through 0.4 µm polycarbonate membrane filters (catalog #K04CP04700, Osmonics Inc., Minnetonka, MN) within 6 h of sample collection. Polycarbonate filters were archived at −80 °C for subsequent batch qPCR analyses. See the supplementary information to Wade et al. (2010) for further details regarding the qPCR assays and the quantification approaches used (Wade et al., 2010). All qPCR results were estimated as Calibrator Cell Equivalents (CCE) using the delta-delta-cycle threshold method as originally presented by ABI, USER BULLETIN #2, and later described by Haugland et al. (2005). This method included a sample processing control (salmon DNA) which was used to detect sample matrix interference (Haugland et al., 2005). In addition, a 25 g portion of each sand sample was dried at 100 °C to determine the

dry weight of the sand samples. All measured concentrations of FIOs in sand are reported per gram of dry sand.

2.3. Weather and environmental parameters

At the time of sand sample collection (8:00 AM) during the NEEAR Water Study, precipitation (in. or cm) was obtained from the nearest National Oceanic and Atmospheric Association (NOAA) station; mean water temperature (°C) was measured at shin depth water (between 3 and 24 in depth) corresponding to the three sand sampling locations; wind direction (onshore versus offshore) was recorded by a weather station on the beach; and cloud cover was recorded by visual observation on a 5-point scale (0 = sunny to 4 = overcast). Precipitation data were combined into 0–24, 24–48, and 48–72 hour periods prior to the sampling time. Information was also collected about environmental parameters at the beach during the time of sample collection including, wave height (m) using a meter stick, and visual observation of the number of birds on the beach, the number of dogs on the beach, and bather density on the beach on a 5-point scale (0 = none; 1 = 1 to <20; 2 = 20 to <100; 3 = 100 to <200; 4 = ≥200 birds/dogs/humans), and the visible presence of algae on the beach or in wet sand at the water line (0 = absence; 1 = presence). Local tide records were used to create a binary tide phase variable (0 = flood, 1 = ebb). Weather and environmental parameters are summarized in Table S3.

2.4. Statistical analyses

For each $\log_{10}$ measure of FIB (enterococci, *Bacteroidales*, fecal *Bacteroides* spp., *Clostridium* spp.) in sand, generalized linear models were used to calculate F-statistic tests comparing differences in average $\log_{10}$ FIB density (CFU/g; CCE/g) by beach and by transect (within beach). Since F^+ coliphages were detected infrequently in sand, correlations of this FIO with FIB in sand were evaluated by its presence or absence in sand. We used a t-test to assess the differences in average density of $\log_{10}$ FIB when F^+ coliphages were present versus absent in beach sand. We examined correlation coefficients (R) as a measure of the linear (Pearson product-moment) pair-wise dependence between each $\log_{10}$ mean FIB measure (CFU/g; CCE/g) in sand and between all FIO measures in sand and water. A multivariate generalized linear model was used to examine the relation of daily average $\log_{10}$ mean densities of FIOs in water, weather factors (total rainfall in 0–24, 24–48, and 48–72 hour periods prior to the sampling time, water temperature, wind direction, and cloud cover), and environmental parameters (wave height, ebb versus flood tidal phase, and visual observation of number of birds, dogs, and bather density on the beach, and presence of algae on the beach) with daily average $\log_{10}$ mean densities of FIB in sand. Effect estimates of multivariate linear models are reported as β coefficients with standard errors and t-values with degrees of freedom equal to the number of explanatory variables included in the model. Rainfall variables were re-scaled to one-tenth of a cm and wave height was re-scaled to one-tenth of a meter. For all analyses involving continuous measures of FIOs in sand and water, samples below the limit of detection were assigned a value of one-half the lower detection limit (sensitivity analysis using the 10th, 25th, 50th, and 75th percentile did not alter results). For all analyses, we present pooled results (combined by beach, water sampling depth, and sampling time) when the stratified results (by beach, water sampling depth, and sampling time) are similar to pooled results. All analyses were completed using SAS,

version 9.2 (SAS Institute, Inc., Cary, North Carolina) and Stata, version 12 (StataCorp, LP, College Station, Texas).

3. Results

3.1. FIOs in sand

A total of 144 beach sand samples were collected from FB (n = 72) and GB (n = 72) (Table S1). The frequency of occurrence of FIOs in sand with data from both beaches combined (based on percent of samples positive) was *Clostridium* spp. (100%) > enterococci CCE (87%) > enterococci CFU (72%) > fecal *Bacteroides* spp. (68%) > *Bacteroidales* (53%) > F^+ coliphages (17%) (Table S1).

Enterococci CFU estimates were higher at FB (geometric mean = 6.4 CFU/g; min = 0.1 CFU/g; max = 4924 CFU/g) than at GB (geometric mean = 1.5 CFU/g; min = 0.1 CFU/g; max = 1596 CFU/g) (Table S1), but consistent differences in enterococci CCE estimates and other FIOs in sand were not observed by beach (Table S1). There was no consistent difference in FIO densities by sample location (data not shown).

3.2. Correlations between FIOs in sand

Except for *Clostridium* spp. CCE (which were present at lower concentrations when F^+ coliphages were present versus absent) we did not observe differences of FIO densities in sand when F^+ coliphages were present versus absent in sand (Table S2). Although maximum densities of enterococci (CFU/g and CCE/g) were consistently higher when F^+ coliphages were present rather than absent (Table S2), the geometric mean estimates of enterococci within beach were similar.

At both beaches combined, data revealed correlations between densities of FIOs in sand in the following order (from strongest to weakest correlation): Fecal *Bacteroides* spp. CCE with *Bacteroidales* CCE ($R = 0.50$; $p < 0.0005$); followed by fecal *Bacteroides* spp. CCE with enterococci CCE ($R = 0.50$; $p < 0.0004$); followed by *Bacteroidales* CCE with enterococci CCE at ($R = 0.42$; $p < 0.004$) (Table 1). Generally, correlations by beach were similar to those for both beaches combined (Table 1). Enterococci CFU and CCE were uncorrelated in sand at each beach separately and at both beaches combined ($R = 0.11$; $p < 0.48$) (Table 1).

3.3. Relationships between FIOs in sand and water

We observed correlations between densities of FIOs in sand and the corresponding water sampling location in the following order for both beaches combined (from highest to lowest correlation): fecal *Bacteriodes* spp. CCE ($R = 0.72$; $p < 0.0001$); enterococci CFU ($R = 0.51$; $p < 0.0002$); *Clostridium* spp. CCE ($R = 0.42$; $p < 0.0029$); and *Bacteroidales* CCE ($R^2 = 0.30$; $p < 0.0426$). Enterococci CCE ($R = -0.0384$; $p < 0.8025$) and F^+ coliphages ($R = -0.2139$; $p < 0.2478$) were not correlated in sand and water (Table 2). Generally, correlations by beach were similar to those for both beaches combined (Table 2). Geometric mean densities of FIOs in shin depth and waist depth water (per ml) show that FIO density is

generally higher at shin depth than at waist depth (Table 3). The exception to this trend is for F^+ coliphage density in water, which remained the same at both depths.

In multivariate regression models, positive associations between densities of FIOs in water (averaging all water samples taken daily at 8:00 AM) and sand were observed (Tables 4, S4, and S5). The FIOs with the strongest water–sand associations were fecal *Bacteroides* spp., *Clostridium* spp., and *Bacteroidales*. Similar associations were observed between densities of FIOs in water with densities of FIOs in sand when water samples were averaged only for shin depth water over all sampling times (results not shown). A large amount of variation in the multivariate regression models was explained by the water, weather, and environmental factors included, demonstrated by the high R^2 values, which for example were 0.65 for enterococci CFU, 0.61 for fecal *Bacteroides* spp., and 0.55 for *Clostridium* spp. (Table 4).

3.4. Relationship of weather and environmental parameters with FIOs in sand

Wave height, bather density on the beach, and the visible numbers of birds on the beach were similar at both FB and GB (Table S3). Although few dogs were observed at either beach, there were higher maximum counts of dogs on the beach at GB (min = 0; max = 10) compared to FB (min = 0; max = 2) (Table S3). Water temperatures ranged slightly higher at FB with the maximum temperature reaching 28 °C while at GB the maximum temperature was 22.3 °C. Maximum rainfall in the previous 24–48 h at FB was also higher at 2.12 cm compared to the maximum at GB which was 0.75 cm.

In multivariate regression models, positive associations between rainfall and daily average densities of FIOs were observed (Tables 4, S4, and S5). Rainfall in the previous 24–48 h was associated with a 0.04 $\log_{10}$ CFU/g increase in enterococci in sand at both beaches [standard error (SE) = 0.02; p-value = 0.01] (Table 4). Total rainfall in the previous 48–72 h was not consistently associated with a $\log_{10}$ density increase in enterococci at these beaches. Water temperature was positively associated with enterococci (CFU/g; CCE/g) in sand at GB (Table S5), and for enterococci (CCE/g) at both beaches (Table 4). Onshore wind direction and cloud cover were not consistently associated with densities of FIB in sand. Wave height and ebb versus flood tidal conditions were positively associated with enterococci CFU/g densities in sand. The $\log_{10}$ mean density of enterococci CFU/g in sand increased 0.15 for every 0.1 meter increase in wave height at FB (SE = 0.06; p-value = 0.03) (Table S4), increased 0.33 for every 0.1 meter increase in wave height at GB (SE = 0.10; p-value = 0.01) (Table S5) and increased 0.17 for every 0.1 meter increase in wave height when both beaches were combined (SE = 0.05; p-value = 0.001). The $\log_{10}$ mean density of enterococci CFU/g in sand increased 0.54 when the tide was ebbing versus flooding at GB (SE = 0.27; p-value = 0.07) (Table S5).

4. Discussion

4.1. FIO dynamics in beach sand

Overall, our results corroborate other research showing high frequencies of detection and measurable concentrations of FIOs in beach sand (Whitman et al., 2014). *Clostridium* spp. (CCE) was most prevalent in sand, followed by enterococci CCE, enterococci CFU, fecal *Bacteroides* spp. CCE, and *Bacteroidales* CCE. F^+ coliphages (MPN/g) were least abundant

in beach sand. The high prevalence and concentration of *Clostridium* spp. in beach sand may reflect the fact that spores of this bacterium can persist for long periods in beach sands (Cui et al., 2013) and sediments (Davies et al., 1995; Desmarais et al., 2002). Interestingly, few inter-FIO correlations were observed in sand. Notably, we did not observe correlations between CFU/g and CCE/g measures of enterococci in sand. This may be a function of differences between culture-versus qPCR-based measurement methods and differences in the persistence of culturable enterococci versus genetic fragments of viable but non-culturable enterococci in sand.

4.2. Relationships between FIOs in water and sand

Overall at both beaches, several FIOs were positively correlated in water and sand, including (from highest to lowest) fecal *Bacteroides* spp., enterococci CFU, *Clostridium* spp., and *Bacteroidales*. Notable exceptions were the lack of consistent correlations between F^+ coli-phages in sand and water and enterococci CCE in sand and water.

We observed higher FIO density in shin depth water than waist depth water, which could suggest a gradient of declining FIOs in water with increasing depth and distance from the water–sand interface. This declining trend of FIO density with increasing distance from the water–sand interface is consistent with other studies (Enns et al., 2012; Whitman and Nevers, 2003). This trend suggests that FIOs in beach sand may be a source of FIO contamination of water, and that FIOs in water may become more diluted with increasing depth and distance from the sand–water interface.

4.3. Factors associated with FIOs in beach sand

We observed that among the four FIOs that showed a strong positive univariate correlation between water and sand (enterococci CFU/g, *Bacteroides* CCE/g, fecal *Bacteroidales* CCE/g, *Clostridium* spp. CCE/g; Table 2), all but enterococci CFU/g in water were significantly associated with sand FIO concentrations in multivariate models, which included weather and environmental factors (Table 4). This lack of association of water enterococci CFU/g as a predictor of sand enterococci CFU/g in multivariate models could reflect the relative greater importance of weather and environmental factors in driving variability of the culture-based enterococci CFU measure in sand, the non-specific nature of the culture-based enterococci CFU measure as a reflection of recent fecal pollution in sand, or the impact of autochthonous enterococci (regrowth) in beach sand.

The strongest associations with enterococci CFU/g in sand were among factors that involve wetting of sand. For example, short-term rainfall (within the previous 24 h) was negatively associated with enterococci CFU/g in sand, suggesting a washing out of accumulated culturable enterococci in sand in the first 24 h. The enterococci CFU-rainfall association then became positive for one-day prior rainfall (within the previous 24–48 h), which may reflect a temporal lag of re-growth and re-establishment of culturable enterococci in sand after more recent rainfall. This was shown experimentally in microcosm experiments using enterococci in sand (Yamahara et al., 2009). Other studies have shown an association of rainfall with increased levels of fecal indicators in recreational water (Ackerman and Weisberg, 2003; Barbe et al., 2001; Boehm et al., 2002; Love et al., 2010; Noble et al.,

2003; Surbeck et al., 2006) and beach sand (Beversdorf et al., 2007). Rainfall can convey fecal microbial pollution to the beach environment from a diverse number of sources, including POTWs, urban and agricultural run-off, domestic and wild animals, and beachgoers (Baums et al., 2007; Bonilla et al., 2007; Elmir et al., 2007; Ishii et al., 2007; Kinzelman et al., 2004; Noble et al., 2003; Wheeler Alm et al., 2003; Whitman et al., 2003, 2006; Yamahara et al., 2007).

Wave height, another sand-wetting factor, was positively-associated with enterococci (CFU/g) density in sand. Increased levels of FIB during wetting conditions are consistent with other studies (Desmarais et al., 2002; Ki et al., 2007; Rosenfeld et al., 2006; Santoro and Boehm, 2007; Solo-Gabriele et al., 2000; Yamahara et al., 2007). Mika et al. (2009) showed that moisture was the dominant factor controlling *E. coli* inactivation kinetics in sand.

Some sources of fecal pollution in sand (POTWs, runoff, beachgoer shedding, bird or dog droppings) are likely patchy during dry periods and wetting events driven by water, weather, and environmental factors such as precipitation, tides, and wave height may disperse patchy fecal pollution in beach sand, contributing to mean spatial homogeneity. This could suggest a reservoir mechanism of inputs from diverse sources and that certain water, weather, and environmental factors may lead to FIO dispersion across sand. Microcosm experiments have shown that moisture inputs via tidal flow increase concentrations of *E. coli* and enterococci in beach sand (Yamahara et al., 2007). Uncertainty remains regarding over-surface flow and decay of fecal indicator signal in sand. But it is possible that specific factors disperse fecal contamination that is already present in beach sand through wetting events of varying intensity (rainfall, tides, and wave height) (Yamahara et al., 2009). This hypothesis is reinforced by the heterogeneous mean temporal trend in sand FIOs that we observed, which suggests that there are days on which beach sand is on average (across all transects) significantly more contaminated than other days. Furthermore, it is this daily average beach-wide variability in sand FIOs that has been shown to be associated with risk of GI illness and diarrhea among beachgoers in contact with beach sand (Heaney et al., 2009, 2012).

4.4. Study limitations

There were several limitations to this study. First, the sand exposure assessment was added to an epidemiologic study designed to assess the relationship between FIO estimates in water and swimming-associated risk of illness, which placed limitations on the scope and scale of sand sampling. The optimal spatial scale for monitoring FIO levels in sand is difficult to determine given the potential for FIO variability over short distances. A beach sand exposure assessment working group recently convened to develop consensus around these challenges (Sabino et al., 2014) and recommended collection of sand samples at three equidistant transects, representing the beach as a whole (Sabino et al., 2014). We followed this recommendation in our study — sampling sand approximately 60 m apart — as well as another to collect sand and water samples simultaneously along the same transects (Sabino et al., 2014). It is noteworthy that using this sand sampling framework we did not observe consistent differences in the frequency of detection and density of FIOs in sand between sand sampling locations, which indicates that there are not specific sampling locations along

the beach where FIOs are, on average (across the study period), greater than at other sampling locations. Previous studies have employed a finer spatial scale to assess FIO variability corresponding to the size of single animal fecal droppings (Bonilla et al., 2007). Our sand sampling was too coarse to capture fine spatial scales of sand fecal contamination on a given day.

Second, FIO density in water compared to sand should be interpreted with caution due to potential quantification differences in the water versus the sand sample matrix. For example, a recent multi-collaboration effort conducted within the Southern California Coastal Water Research Project has yielded a new approach for quantification of organisms in sand, and if that calibrated approach had been used, a more direct comparison might have been permitted. This could be of concern for molecular-based FIB estimates because qPCR is known to be sensitive to inhibitors present in sand and turbid water samples (Siefring et al., 2008b). Therefore, our analysis reflects relative measurements of FIOs in sand, not estimates based on a sand qPCR microbial indicator methods optimization. Although we employed methods to address the potential influence of qPCR inhibition (Haugland et al., 2005; Siefring et al., 2008b; Sivaganensan et al., 2012), additional validation would be needed to improve the comparability of water and sand FIO estimates.

Third, it is possible that some of the observed differences in sand FIO concentrations by beach were due to differences in sand type. Others have hypothesized that beach sand mineralogy may have an impact on the ability for microorganisms to colonize sand. Although not investigated in our study, sand mineralogy may be an important factor to consider as it may influence moisture retention and biofilm growth (Hernandez et al., 2014; Piggot et al., 2012). Sand at FB had a fine and uniform grain size compared to sand at GB, which was rocky and irregular. Skalbeck et al. (2010), observed that densities of *E. coli* (assessed by a culture-based MPN method) were higher in fine grain beach sands with a uniform distribution compared to large grain sands (Skalbeck et al., 2010), a finding that is somewhat consistent with our results for culture-based enterococci CFU, but not enterococci CCE. In all analyses, beach-specific differences were explored and handled by presentation of stratified estimates or through inclusion of an indicator term in multivariate regression models.

5. Conclusions

Research continues to emerge that is advancing knowledge of hand-to-mouth transfer of fecal pollution via beach sand contact (Whitman et al., 2009) (establishing exposure). Recent studies have linked the human heath outcomes of increased incidence of GI illness and diarrhea among beachgoers in Florida who spent more time in wet sand (Bonilla et al., 2007) and among beachgoers at the beaches under investigation in this study, who were engaged in sand contact activities (digging in sand; buried in sand) (Heaney et al., 2009, 2012). Since beach sand contact is an exposure activity in which a large number of people engage during visitation of coastal and inland beaches, even a small increase in relative risk of illness would have important implications for public health. Further research of factors influencing FIOs in sand could improve information available to beach managers and the public about the predictors of fecal indicators in sand; including enterococci, which is an

indicator used in federal recreational water quality guidelines because of its association with health effects among swimmers (Dufour, 1984; EPA, 2012). Because of the potential for dynamic exchanges of FIOs between water and sand and because FIOs in sand could also be a risk factor for GI illness, it is important to further understand the extent to which people are exposed to microbes in sand, the environmental factors affecting their occurrence and distribution in sand, and their relationship to FIOs measured in water samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- This study aimed to determine the factors associated with fecal pollution in beach sand.
- Bacterial measures of fecal pollution in water and sand were positively correlated.
- Water quality, rainfall, and wave height were related to fecal pollution in sand.
- Water quality and sand wetting factors may inform beach sand microbial burdens.

Table 1

Pearson correlation statistics for $\log_{10}$ concentrations of fecal indicator organisms (FIOs) in sand at Fairhope Beach, AL, Goddard Beach, RI, and both beaches during the 2007 NEEAR Water Study.

	Enterococci (CFU/g) R (p-value[a])	**Enterococci (CCE/g) R (p-value[a])**	***Bacteroidales* (CCE/g) R (p-value[a])**	***Fecal Bacteroides* spp. (CCE/g) R (p-value[a])**	***Clostridium* spp. (CCE/g) R (p-value[a])**
Fairhope Beach, AL	**N = 24[b]**	**N = 21[b]**	**N = 21[b]**	**N = 21[b]**	**N = 24[b]**
Enterococci (CFU/g)	1	-	-	-	-
Enterococci (CCE/g)	0.36 (p < 0.11)	1	-	-	-
Bacteroidales (CCE/g)	0.42 (p < 0.06)	0.70 (p < 0.0005)	1	-	-
Fecal *Bacteroides* spp. (CCE/g)	0.25 (p < 0.27)	0.48 (p < 0.03)	0.61 (p < 0.004)	1	-
Clostridium spp. (CCE/g)	0.45 (p < 0.03)	0.22 (p < 0.33)	0.17 (p < 0.46)	0.09 (p < 0.71)	1
Goddard Beach, RI	N = 24[b]	N = 24[b]	N = 24[b]	N = 24[b]	N = 24[b]
Enterococci (CFU/g)	1	-	-	-	-
Enterococci (CCE/g)	0.18 (p < 0.39)	1	-	-	-
Bacteroidales (CCE/g)	0.24 (p < 0.26)	0.23 (p < 0.27)	1	-	-
Fecal *Bacteroides* spp. (CCE/g)	0.36 (p < 0.08)	0.50 (p < 0.01)	0.41 (p < 0.04)	1	-
Clostridium spp. (CCE/g)	−0.29 (p < 0.16)	−0.21 (p < 0.32)	−0.25 (p < 0.24)	−0.16 (p < 0.47)	1
Both beaches	N = 48[b]	N = 45[b]	N = 45[b]	N = 45[b]	N = 48[b]
Enterococci (CFU/g)	1	-	-	-	-
Enterococci (CCE/g)	0.11 (p < 0.48)	1	-	-	-
Bacteroidales (CCE/g)	0.27 (p < 0.08)	0.42 (p < 0.004)	1	-	-
Fecal *Bacteroides* spp. (CCE/g)	0.19 (p < 0.21)	0.50 (p < 0.0004)	0.50 (p < 0.0005)	1	-
Clostridium spp. (CCE/g)	−0.11 (p < 0.47)	−0.08 (p < 0.60)	−0.12 (p < 0.44)	−0.07 (p < 0.65)	1

[a] p-Values are two-sides.

[b] Number of samples.

Table 2

Pearson correlation between fecal indicator organisms (FIOs) per gram of sand and per 100 ml of water at two marine beaches, Fairhope Beach, AL and Goddard Beach, RI during the 2007 NEEAR Water Study.

	Correlation between each FIO in sand (per g) with the same FIO in water (per 100 ml) at 8:00 AM								
	Fairhope Beach, AL			Goddard Beach, RI			Both beaches[a]		
Fecal Indicator Organism	**R**	**p value**	**n[b]**	**R**	**p value**	**n**	**R**	**p value**	**n**
F^+ coliphages (MPN/g)	−0.6085	0.0358	12	−0.0818	0.7391	19	−0.2139	0.2478	31
Enterococci (CFU/g)	0.2996	0.1550	24	0.3195	0.1280	24	0.5134	0.0002	48
Enterococci (CCE/g)	0.1109	0.6324	21	0.1221	0.5697	24	−0.0384	0.8025	45
Bacteroidales (CCE/g)	0.4336	0.0496	21	0.2289	0.2820	24	0.3036	0.0426	45
Fecal *Bacteroides* spp. (CCE/g)	0.7706	0.0000	21	0.7290	0.0001	24	0.7232	0.0001	45
Clostridium spp. (CCE/g)	0.2512	0.2363	24	0.6044	0.0018	24	0.4209	0.0029	48

[a] Correlations based on samples from both beaches and water samples taken at 8:00 AM average across shin and waist depth.

[b] n, number of samples.

Table 3

Geometric mean densities of fecal indicator organisms (FIOs) in shin depth and waist depth water (per ml) and sand (per g) at two marine beaches, Fairhope Beach, AL and Goddard Beach, RI during the 2007 NEEAR Water Study.

	Shin depth water (8:00 AM) geometric mean (per ml)	Waist depth water (8:00 AM) geometric mean (per ml)	Sand geometric mean (per g)
F^+ coliphage (MPN/ml or g)	5.6	5.6	0.007
Enterococci (CFU/ml or g)	0.2	0.1	3.1
Enterococci (CCE/ml or g)	2.0	1.5	126
Bacteroidales (CCE/ml or g)	14.0	10.7	66
Fecal *Bacteroides* spp. (CCE/ml or g)	4.9	4.3	177
Clostridium spp. (CCE/ml or g)	10.5	7.2	1783

Table 4

Multivariate linear regression model of relationships between water quality, weather and environmental parameters with $\log_{10}$ daily mean densities of fecal indicator organisms (FIOs) in sand at Fairhope Beach, AL and Goddard Beach, RI during the 2007 trials of the NEEAR water study.

	F+ coliphage (MPN/g)	**Enterococci (CFU/g)**	**Enterococci (CCE/g)**	***Bacteroidales* (CCE/g)**	***Fecal Bacteroides* spp. (CCE/g)**	***Clostridium* spp. (CCE/g)**
	N 31[a]	**48[a]**	**45[a]**	**45[a]**	**45[a]**	**48[a]**
	R^2 0.36	**0.65**	**0.30**	**0.42**	**0.61**	**0.55**
	β (SE[c])[b]	**β (SE)[b]**	**β (SE)[b]**	**β (SE)[b]**	**β (SE)[b]**	**β (SE)[b]**
	p-Value	**p-Value**	**p-Value**	**p-Value**	**p-Value**	**p-Value**
Corresponding $\log_{10}$ daily mean densities of FIOs in water at 8 AM (MPN/CFU/CCE per 100 ml)	−0.15 (0.36) 0.68	0.15 (0.12) 0.23	0.16 (0.30) 0.61	0.32 (0.13) 0.02	0.90 (0.15) <0.001	0.48 (0.13) 0.001
Rainfall in previous 24 h (per one tenth of a cm)	0.12 (0.10) 0.25	−0.11 (0.05) 0.02	−0.05 (0.05) 0.34	0.05 (0.06) 0.43	0.05 (0.07) 0.52	−0.07 (0.04) 0.08
Rainfall in previous 24–48 h (per one tenth of a cm)	0.02 (0.04) 0.63	0.04 (0.02) 0.01	0.05 (0.02) 0.02	0.03 (0.02) 0.16	0.02 (0.03) 0.44	0.01 (0.01) 0.31
Rainfall in previous 48–72 h (per one tenth of a cm)	−0.02 (0.06) 0.72	−0.03 (0.02) 0.17	0.03 (0.02) 0.25	0.06 (0.02) 0.02	0.04 (0.03) 0.26	0.02 (0.02) 0.33
Water temperature at shin depth (°C)	0.08 (0.11) 0.48	0.03 (0.04) 0.45	0.12 (0.04) 0.01	0.07 (0.05) 0.15	0.05 (0.06) 0.40	−0.06 (0.03) 0.07
Onshore wind direction (0 = not onshore, 1 = onshore)	0.21 (0.24) 0.40	0.02 (0.14) 0.88	−0.02 (0.16) 0.92	0.001 (0.17) 0.99	−0.14 (0.22) 0.54	0.02 (0.12) 0.88
Wave height (per one tenth of a meter)	−0.05 (0.11) 0.66	0.17 (0.05) 0.001	0.05 (0.06) 0.42	0.05 (0.06) 0.46	0.03 (0.08) 0.70	0.02 (0.04) 0.72
Binary tide phase (0 = flooding; 1 = ebbing)	0.06 (0.31) 0.84	0.30 (0.16) 0.07	0.29 (0.19) 0.14	0.37 (0.20) 0.07	0.08 (0.26) 0.76	0.19 (0.14) 0.17
Cloud cover (0 = sunny, 1 = mostly sunny, 2 = partly cloudy, 3 = mostly cloudy, 4 = overcast)	0.10 (0.14) 0.50	−0.04 (0.06) 0.47	−0.01 (0.07) 0.89	0.09 (0.07) 0.24	0.01 (0.10) 0.95	−0.09 (0.05) 0.07
Number of birds on beach (0, 1 to <20, 20 to <100, 100 to <200, ≥200)	−0.004 (0.004) 0.38	0.003 (0.003) 0.26	−0.003 (0.003) 0.35	0.001 (0.003) 0.80	−0.001 (0.004) 0.75	0.003 (0.002) 0.22
Number of dogs on beach (0, 1 to <20, 20 to <100, 100 to <200, ≥200)	−0.03 (0.05) 0.52	0.07 (0.03) 0.02	−0.05 (0.04) 0.18	−0.007 (0.04) 0.85	0.001 (0.05) 0.99	0.08 (0.03) 0.005
Bather density on beach (0, 1 to <20, 20 to <100, 100 to <200, ≥200)	−0.01 (0.01) 0.46	0.001 (0.006) 0.88	−0.01 (0.01) 0.09	0.002 (0.008) 0.77	0.004 (0.01) 0.68	0.01 (0.01) 0.03
Presence of algae on beach (0 = absent, 1 = present)	0.21 (0.35) 0.56	0.12 (0.14) 0.40	0.06 (0.18) 0.76	−0.45 (0.19) 0.02	−0.14 (0.26) 0.58	0.11 (0.12) 0.38

	F^+ coliphage (MPN/g) N 31[a] R^2 0.36 β (SE[c])[b] p-Value	Enterococci (CFU/g) 48[a] 0.65 β (SE)[b] p-Value	Enterococci (CCE/g) 45[a] 0.30 β (SE)[b] p-Value	*Bacteroidales* (CCE/g) 45[a] 0.42 β (SE)[b] p-Value	*Fecal Bacteroides* spp. (CCE/g) 45[a] 0.61 β (SE)[b] p-Value	*Clostridium* spp. (CCE/g) 48[a] 0.55 β (SE)[b] p-Value
Beach (Fairhope versus Goddard Beach)	0.34 (0.57) 0.56	−0.23 (0.21) 0.27	1.04 (0.28) 0.001	0.74 (0.26) 0.01	0.76 (0.36) 0.04	−0.11 (0.18) 0.53

[a] Number of study days, each representing a daily average of three sand samples.

[b] Estimates are from multivariate linear regression model controlling for variables listed in the table, using 8:00 AM water samples averaged across shin and waist depth.

[c] SE = standard error.