

Fecal Indicators in Sand, Sand Contact, and Risk of Enteric Illness Among Beachgoers

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Background: Beach sand can harbor fecal indicator organisms and pathogens, but enteric illness risk associated with sand contact remains unclear.

Methods: In 2007, visitors at 2 recreational marine beaches were asked on the day of their visit about sand contact. Ten to 12 days later, participants answered questions about health symptoms since the visit. F⁺ coliphage, *Enterococcus*, *Bacteroidales*, fecal *Bacteroides*, and *Clostridium* spp. in wet sand were measured using culture and molecular methods.

Results: We analyzed 144 wet sand samples and completed 4999 interviews. Adjusted odds ratios (aORs) were computed, comparing those in the highest tertile of fecal indicator exposure with those who reported no sand contact. Among those digging in sand compared with those not digging in sand, a molecular measure of *Enterococcus* spp. (calibrator cell equivalents/g) in sand was positively associated with gastrointestinal (GI) illness (aOR = 2.0 [95% confidence interval (CI) = 1.2–3.2]) and diarrhea (2.4 [1.4–4.2]). Among those buried in sand, point estimates were greater for GI illness (3.3 [1.3–7.9]) and diarrhea (4.9 [1.8–13]). Positive associations were also observed for culture-based *Enterococcus* (colony-forming units/g) with GI illness (aOR digging = 1.7 [1.1–2.7]) and diarrhea

(2.1 [1.3–3.4]). Associations were not found among nonswimmers with sand exposure.

Conclusions: We observed a positive relationship between sand-contact activities and enteric illness as a function of concentrations of fecal microbial pollution in beach sand.

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High densities of *Escherichia coli*, *Enterococcus*, and other microbial indicators of fecal pollution are present in beach sand, sometimes in higher concentrations than in nearby bathing waters.^{1–6} Halliday and Gast⁷ reviewed studies that measured densities of *E. coli* and *Enterococcus*—fecal indicator bacteria used to indicate the degree of fecal contamination at recreational beaches across the United States—and found that fecal indicator bacteria density in sand was 2 to 38 times higher than in water. Fecal indicator bacteria such as *E. coli* and *Enterococcus* indicate the potential presence of pathogenic microorganisms. Although the density of *E. coli* and *Enterococcus* in water has been shown to predict gastrointestinal (GI) illness among swimmers,⁸ the illness risks associated with fecal pollution in beach sand remain unclear. Whitman et al⁹ demonstrated potential for hand-to-mouth transfer and exposure to *E. coli* and the fecal indicator virus F⁺ coliphage (MS2) among persons in contact with beach sand at a Chicago, IL, beach. In addition to fecal indicator organisms, enteric pathogens have been detected in beach sand.^{4,10–14} Factors influencing the level of fecal contamination of beach sands are numerous; these may include publicly owned treatment-works discharges in close proximity to beaches,^{15,16} nonpoint sources such as agricultural and urban runoff, warm-blooded domestic and wild animals, bathers,^{3,4,17} and potential regrowth of autochthonous *E. coli* and *Enterococcus* in sand.^{18,19}

The occurrence of fecal indicator organisms in sand has led to questions about whether beach sand can transmit illness associated with fecal contamination—a matter of increasing concern to beach managers, public health officials, and beachgoers. Despite knowledge of the presence of fecal indicator organisms and pathogens in beach sand, dose-response relationships between fecal indicator densities in

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sand and specific illnesses have not been well characterized. Because the beach-going public may spend more time on the beach than in the water, and because young children typically spend time at the water's edge playing in sand, it is important to understand the relation of fecal indicator densities in sand with the risk of enteric illness.

Using data from beachgoers participating in the 2007 trials of the National Epidemiological and Environmental Assessment of Recreational water study,^{20–22} we explored whether increased daily average estimate of fecal indicator organisms (F^+ coliphage, *Enterococcus*, *Bacteroidales*, fecal *Bacteroides*, and *Clostridium* spp.) in wet sand were associated with an increased risk of enteric illness among beachgoers engaged in sand-contact activities.

METHODS

Study Design/Participant Sampling

The National Epidemiological and Environmental Assessment of Recreational Water Study evaluated microbial water quality and followed cohorts of visitors to freshwater and marine beaches in the United States. The data collection methods have been described previously.^{20–22} In brief, we interviewed beachgoers as they arrived and as they were leaving the beach regarding their contact with beach sand, swimming behaviors, and other beach activities. Ten to 12 days later, one adult in the household was interviewed by telephone about symptoms experienced by participating household members. Because of the acute nature and short duration of illnesses considered during this study, reenrollment in the study was allowed 28 days after a previous enrollment.

Beach Descriptions

Two recreational marine beach sites with a nearby publicly owned treatment-works outfall were chosen for the 2007 studies. Fairhope Municipal Park Beach is located on Mobile Bay in Fairhope, Alabama, and Goddard Memorial State Park Beach is located on Greenwich Bay near Warwick, Rhode Island. These beaches had publicly owned treatment-works discharges within 1.5 miles (2.4 km) of the beach location.

Beach Sand Collection and Analysis

Wet beach sand samples were collected at 8:00 AM along with water samples each day of the study. Sand samples were collected using a 2.25-inch diameter stainless steel soil auger at a distance of 1 meter perpendicular to the lowest point of the water level (when the waves receded to their lowest point on the shoreline) at the same 3 sampling points where water samples were collected on each beach. No dry sand samples were collected. Sampling points were located at least 60 m apart to encompass the length of the beach. Beach sand samples were tested for fecal indicator bacteria *Enterococcus*, *Bacteroidales*, fecal *Bacteroides* (which targets the

most common human *Bacteroides* spp.), and *Clostridium* spp. using a quantitative polymerase chain reaction (qPCR) calibrator cell equivalent method.^{23–26} Culture-based tests of *Enterococcus* were performed by local laboratories within 6 hours of collection following EPA Method 1600²⁷ with some modifications (eAppendix 1, <http://links.lww.com/EDE/A532>). Samples for the F^+ coliphage analysis were sent on ice at 4°C by overnight express and processed by a modification²⁸ of EPA Method 1601²⁹ to accommodate the analysis of sand instead of water samples (eAppendix 1, <http://links.lww.com/EDE/A532>). Fecal indicator organism concentrations are reported as qPCR calibrator cell equivalents per gram of dry weight sand for *Enterococcus*, *Bacteroidales*, fecal *Bacteroides*, and *Clostridium* spp. Calibrator cell equivalents were calculated according to the comparative delta-delta cycle threshold method reported by Wade et al.²⁰ *Enterococcus* measured by EPA Method 1600²⁷ are reported in colony-forming units per gram of dry weight sand, and F^+ coliphage as a most probable number per gram of dry weight sand following a modification²⁸ of Method 1601.²⁹ Results below detection and potential inhibition for qPCR-based data were handled as described previously.²⁰

Definition of Sand Contact

On leaving the beach, participants reported whether they had dug in the sand or built sand castles (digging in the sand), or had their body buried in the sand during their beach visit. Participants also reported whether they got sand in their mouth, ate or drank after playing in the sand, and washed their hands before eating or drinking after playing in sand. Participants were asked to report whether they had contact with wet or dry sand. In addition to sand exposures on the day of enrollment, participants reported other beach activities such as any water contact (swimming), or eating raw meat, runny eggs, or shellfish in the 3 days before enrollment.

Health Assessment

Ten to twelve days following the beach visit, participants were contacted by phone and asked if they had experienced any physical symptoms of enteric illness since the day of enrollment. The GI illness definition followed previously published research on waterborne illness^{20,30} and was considered as any of the following: diarrhea (≥ 3 loose stools in a 24-hour period), vomiting, nausea and stomach ache, and nausea or stomach ache plus interference with regular activities (missed time from work or school, or missed regular activities as a result of the illness). Diarrhea was also considered as a separate outcome. In addition, participants were asked about activities since the enrollment-day interview, including number of times they went to the same beach, went swimming at another beach, went swimming in a pool, or ate raw or undercooked foods (eg, red meat, fish, shellfish, eggs).

Statistical Analysis

Participants with complete data for the exposure (sand contact), outcome (enteric illness), and potential confounding

covariates (age, sex, race/ethnicity, swimming status, and beach) were included in analyses. Participants with prevalent enteric illness at enrollment were excluded from follow-up analyses. Logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for enteric illness (GI illness, diarrhea) and associations with beach sand-contact activities and densities of fecal indicators in beach sand.

Measurements of fecal indicator organism densities in beach sand were \log_{10} transformed. Fecal indicator organism samples below the lower detection limit were assigned a value of one-half the lower detection limit and subsequently \log_{10} transformed. We analyzed the daily average of the three 8:00 AM sand samples collected on each beach as a reflection of sand quality each day of the study.

Because a large proportion of F^+ coliphage data was below the detection limit, we considered only categorical classifications involving simple presence/absence and above

versus below the median concentration (most probable number/g). For fecal indicator bacteria, we evaluated categorical classifications of above versus below the median, as well as tertiles (colony-forming units/g; calibrator cell equivalents/g). Categorical models involved comparisons of those in contact with sand on days when a fecal indicator was present at a specific average density (eg, highest tertile) with those who were not in contact with sand (did not dig; were not buried in sand). For these models, the adjusted odds ratio (aOR) can be interpreted as the risk (odds) of enteric illness (GI illness; diarrhea) among those who dug in the sand on days when average sand quality was at a specific level (eg, highest tertile) divided by the risk of enteric illness among those who did not have contact with sand. Tests of linear trend were completed by the ranks of each categorical fecal indicator exposure variable in a logistic regression model as a linear term.

TABLE 1. Characteristics of Those Who Did or Did Not Dig in the Sand, and Those Who Were or Were Not Buried in the Sand

Characteristic	Digging in the Sand		Body Buried in the Sand	
	No (n = 3586) No. (%)	Yes (n = 1362) No. (%)	No (n = 4730) No. (%)	Yes (n = 218) No. (%)
Age (years)				
0–4	183 (5)	287 (21)	429 (9)	50 (23)
5–10	219 (6)	423 (31)	550 (12)	92 (42)
11–19	356 (10)	172 (13)	507 (11)	21 (10)
20–54	2186 (61)	435 (32)	2568 (54)	53 (24)
55+	642 (18)	36 (3)	676 (14)	2 (<1)
Sex				
Male	1505 (42)	603 (44)	2009 (42)	99 (45)
Female	2081 (58)	759 (56)	2721 (58)	119 (55)
Race/ethnicity				
White	2315 (65)	870 (64)	3061 (65)	124 (57)
Black	468 (13)	222 (16)	654 (14)	36 (17)
Asian	60 (2)	24 (2)	82 (2)	2 (1)
American Indian	7 (<1)	5 (<1)	9 (<1)	3 (1)
Hispanic/Latino	636 (18)	186 (14)	778 (16)	44 (20)
Multiethnic/other	100 (3)	55 (4)	146 (4)	9 (4)
Conditions in the 3 days prior to the beach visit				
Vomiting	50 (1)	24 (2)	69 (1)	5 (2)
Diarrhea or loose bowels	102 (3)	33 (2)	131 (3)	4 (2)
History of chronic GI illness	124 (3)	21 (2)	145 (3)	0 (0)
Water contact status				
No	2154 (60)	257 (19)	2387 (50)	24 (11)
Yes	1432 (40)	1105 (81)	2343 (50)	194 (89)
Contact with animals 48 hours prior to beach visit or between beach visit and phone interview	2192 (61)	970 (71)	3011 (64)	151 (69)
Consumption of raw meat 48 hours prior to beach visit or between beach visit and phone interview	508 (14)	113 (8)	607 (13)	14 (6)
Beach				
Fairhope Beach	1249 (35)	766 (56)	1905 (40)	110 (50)
Goddard Beach	2337 (65)	596 (44)	2825 (60)	108 (49)

To evaluate the impact of multiple fecal indicators in sand, we created an index variable defined as the number of fecal indicators present in sand at each of the 3 sample locations. The potential range of this fecal indicator index variable was from 0 to 18 and was examined in tertiles. This variable was created as an alternative method to describe the burden of fecal contamination in the sand.

Robust variance estimates were used to account for the nonindependence of observations within household.^{31,32} We considered covariates strongly associated with beach sand contact and illness, or those regarded by investigators to be potential confounding factors, for inclusion in regression models. Information on other covariates was collected for the larger study (eg, contact with animals, contact with other persons with diarrhea, number of other visits to the beach, asthma, chronic GI illnesses, eating any food or drink while at the beach, eating raw or undercooked meats, shellfish, and fish). If there was a substantial difference between fully adjusted and reduced model results, we used a backward elimination approach. For each analysis, the set of covariates was reduced through a change-in-estimate procedure.³³ Adjusted odds ratio (full [aOR_{full}] and reduced [aOR_{reduced}])

values were compared by using the formula: $\ln \text{aOR}_{\text{full}} / \text{aOR}_{\text{reduced}} \times 100$. A criterion of 5% change was used; results from fully adjusted models are presented if the reduced model resulted in a change in estimate of greater than 5%. At a minimum, age, sex, race/ethnicity, swimming (defined as any contact with water which includes wading, body immersion, head immersion, and swallowing water), and beach were included in all models. Data management was completed using SAS version 9 (SAS Institute Inc., Cary, NC), and statistical analyses were completed using Stata version 11 (StataCorp LP, College Station, TX).

RESULTS

A total of 7041 beachgoers were offered enrollment. Of these, 1280 (18%) declined to participate. Of those who agreed to participate, 4999 (87%) completed the telephone interview 10 to 12 days after visiting the beach and were eligible for analysis. Of these interviews, 4948 (99%) respondents provided complete information on age, sex, race/ethnicity, contact with beach sand, and any swimming.

Respondents at the 2 beaches differed by age, race/ethnicity (defined as white/nonwhite), miles traveled to the

TABLE 2. Relation of F⁺ Coliphage in Sand With GI Illness and Diarrhea by Status of Sand Contact and by Categorical Classification

	GI Illness		Diarrhea	
	No. (%) ^a	aOR (95% CI) ^b	No. (%) ^a	aOR (95% CI) ^b
Digging in sand		(n = 3835) ^c		(n = 3864) ^c
Not digging ^d	142 (5)	1.00	86 (3)	1.00
Digging				
F ⁺ coliphage				
Absent	57 (9)	1.57 (1.04–2.39)	38 (6)	1.81 (1.12–2.93)
Present	26 (8)	1.19 (0.69–2.06)	19 (6)	1.43 (0.75–2.71)
Test for trend ^e		P = 0.17		P = 0.07
≤ Median (0.014 MPN/g ^f)	72 (9)	1.51 (1.04–2.20)	48 (6)	1.72 (1.12–2.65)
> Median	11 (8)	1.03 (0.43–2.50)	9 (7)	1.43 (0.55–3.73)
Test for trend ^e		P = 0.17		P = 0.05
Buried in sand		(n = 3838) ^c		(n = 3867) ^c
Not buried ^d	208 (6)	1.00	130 (4)	1.00
Buried				
F ⁺ coliphage				
Absent	9 (8)	1.39 (0.63–3.09)	6 (6)	1.51 (0.56–4.05)
Present	8 (16)	2.29 (0.74–7.07)	7 (14)	3.32 (0.94–11.7)
Test for trend ^e		P = 0.11		P = 0.05
≤ Median (0.014 MPN/g ^f)	14 (10)	1.52 (0.76–3.05)	10 (6)	1.78 (0.74–4.29)
> Median (0.014 MPN/g ^f)	3 (30)	4.20 (0.34, 51.1)	3 (30)	6.99 (0.60–81.3)
Test for trend ^e		P = 0.12		P = 0.06

aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and any contact swimming.

^aThe numbers are participants reporting new symptoms, among those without baseline symptoms.

^bRobust variance estimates clustering on household.

^cThe numbers are participants with complete information on age, sex, race/ethnicity, beach, and any contact swimming, among those without baseline symptoms.

^dReference category.

^eP value from a linear trend test across the non-sand-exposed to the highest sand-exposed category.

^fMPN/g indicates most probable number per gram of dry weight sand.

beach, and proportion of persons who reported contact with sand (Table 1). Overall, respondents were 64% white and 57% female, with a median age of 30 years. Factors associated with sand contact have been described previously.³⁴ Similar to previous observations,³⁴ those who dug in the sand were younger than those who did not (median age, 10 and 36 years, respectively), but at baseline, similar proportions of both groups reported vomiting and other GI symptoms (Table 1). Digging in the sand was strongly associated with swimming, as 81% of swimmers reported digging in the sand compared with only 19% of nonswimmers. This large difference in the proportion of persons who swam was also seen for those buried and not buried in the sand (Table 1). Participants at Fairhope Beach were more likely to have reported digging in the sand (766/2015; 38%) and being buried in the sand (5%) (Table 1).

The frequency of detection and densities of fecal indicators in sand at Fairhope Beach and Goddard Beach are summarized in eAppendix 2 (<http://links.lww.com/EDE/A533>). The detection of fecal indicators in sand ranged from 100% for

qPCR-based *Clostridium* spp. to 87% for qPCR-based *Enterococcus*, 72% for culture-based *Enterococcus*, 68% for qPCR-based fecal *Bacteroides* spp., 53% for qPCR-based *Bacteroidales*, and 17% for culture-based F⁺ coliphage (eAppendix 2). We did not observe spatial variability in fecal indicator measures in sand.

Relationships Among Sand Contact, Measures of Fecal Indicators in Sand, and Enteric Illness

The incidence of GI illness and diarrhea was 6.3% and 4.2%, respectively, during the 10–12-day follow-up period. GI illness and diarrhea incidence was highest among children younger than 5 years (9.5% and 5.2%, respectively) and lowest among those aged 55 and older (5.5% and 4.3%, respectively).

Because there were few days when F⁺ coliphage was present in sand (17% of samples positive), we examined only presence versus absence and above versus below the median value of samples with results above the detection limit (Table 2). There was no increase in risk of GI illness or diarrhea among those digging in sand when F⁺ coliphage was

TABLE 3. Relation of *Enterococcus* (Colony Forming units/g) in Sand With GI Illness and Diarrhea by Status of Sand Contact and by Categorical Classification

	GI Illness		Diarrhea	
	No. (%) ^a	aOR (95% CI) ^b	No. (%) ^a	aOR (95% CI) ^b
Digging in sand		(n = 4672) ^c		(n = 4706) ^c
Not digging ^d	182 (5)	1.00	115 (3)	1.00
Digging				
<i>Enterococcus</i>				
< Median 13 CFU/g ^e	47 (7)	1.21 (0.82–1.77)	30 (4)	1.26 (0.80–1.99)
≥ Median 13 CFU/g ^e	65 (11)	1.65 (1.10–2.47)	51 (8)	2.06 (1.33–3.18)
Test for trend ^f		P = 0.02		P = 0.001
Tertile 1: ≥0–5 CFU/g ^e	28 (7)	1.30 (0.81–2.10)	16 (4)	1.22 (0.68–2.19)
Tertile 2: 5–27 CFU/g ^e	41 (9)	1.26 (0.79–2.02)	33 (7)	1.64 (0.98–2.74)
Tertile 3: >27 CFU/g ^e	43 (11)	1.74 (1.11–2.72)	32 (8)	2.07 (1.27–3.38)
Test for trend ^f		P = 0.01		P = 0.002
Buried in sand		(n = 4675) ^c		(n = 4709) ^c
Not buried ^d	274 (6)	1.00	181 (4)	1.00
Buried				
<i>Enterococcus</i>				
< Median of 13 CFU/g ^e	9 (7)	0.95 (0.47–1.90)	6 (4)	0.98 (0.42–2.29)
≥ Median of 13 CFU/g ^e	11 (15)	2.18 (0.85–5.60)	9 (13)	2.61 (0.87–7.89)
Test for trend ^f		P = 0.18		P = 0.14
Tertile 1: ≥0–5 CFU/g ^e	6 (8)	1.32 (0.54–3.22)	4 (5)	1.40 (0.49–4.06)
Tertile 2: >5–27 CFU/g ^e	6 (8)	1.04 (0.33–3.33)	5 (7)	1.31 (0.34–4.99)
Tertile 3: >27 CFU/g ^e	8 (14)	1.87 (0.68–5.16)	6 (10)	2.06 (0.58–7.32)
Test for trend ^f		P = 0.18		P = 0.23

aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and any contact swimming.

^aThe numbers are participants reporting new symptoms, among those without baseline symptoms.

^bRobust variance estimates clustering on household.

^cThe numbers are participants with complete information on age, sex, race/ethnicity, beach, and any contact swimming, among those without baseline symptoms.

^dReference category.

^eCFU/g indicates colony forming units per gram of dry weight sand.

^fP value from a linear trend test across the non-sand-exposed to the highest sand-exposed category.

TABLE 4. Relation of *Enterococcus* (Calibrator Cell equivalents/g) in Sand With GI Illness and Diarrhea by Status of Sand Contact and by Categorical Classification

	GI Illness		Diarrhea	
	No. (%) ^a	aOR (95% CI) ^b	No. (%) ^a	aOR (95% CI) ^b
Digging in sand	(n = 4331) ^c		(n = 4364) ^c	
Not digging ^d	174 (5)	1.00	110 (3)	1.00
Digging	<i>Enterococcus</i>			
< Median 156 CCE/g ^e	47 (7)	1.12 (0.75–1.68)	32 (5)	1.27 (0.78–2.06)
≥ Median 156 CCE/g ^e	54 (11)	1.67 (1.10–2.55)	39 (8)	1.96 (1.23–3.12)
Test for trend ^f		P = 0.02		P = 0.005
Tertile 1: ≥0–107 CCE/g ^e	36 (9)	1.15 (0.70–1.86)	27 (7)	1.39 (0.80–2.40)
Tertile 2: >107–324 CCE/g ^e	28 (7)	1.12 (0.67–1.89)	17 (4)	1.13 (0.61–2.09)
Tertile 3: >324 CCE/g ^d	37 (11)	1.98 (1.23–3.19)	27 (8)	2.44 (1.41–4.22)
Test for trend ^f		P = 0.02		P = 0.006
Buried in sand	(n = 4334) ^c		(n = 4367) ^c	
Not buried ^d	256 (6)	1.00	167 (4)	1.00
Buried	<i>Enterococcus</i>			
< Median 156 CCE/g ^e	7 (7)	0.91 (0.41–2.02)	3 (3)	0.61 (0.19–1.97)
≥ Median 156 CCE/g ^e	12 (15)	2.32 (0.97–5.52)	11 (14)	3.43 (1.32–8.90)
Test for trend ^f		P = 0.11		P = 0.05
Tertile 1: ≥0–107 CCE/g ^e	4 (7)	0.74 (0.27–2.04)	2 (3)	0.57 (0.14–2.31)
Tertile 2: >107–324 CCE/g ^e	4 (6)	0.95 (0.33–2.79)	2 (3)	0.77 (0.18–3.22)
Tertile 3: >324 CCE/g ^d	11 (18)	3.25 (1.33–7.92)	10 (16)	4.90 (1.79–13.4)
Test for trend ^f		P = 0.06		P = 0.03

aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and any contact swimming.
^aThe numbers are participants reporting new symptoms, among those without baseline symptoms.
^bRobust variance estimates clustering on household.
^cThe numbers are participants with complete information on age, sex, race/ethnicity, beach, and any contact swimming, among those without baseline symptoms.
^dReference category.
^eCCE/g indicates qPCR calibrator cell equivalents per gram of dry weight sand.
^fP value from a linear trend test across the non-sand-exposed to the highest sand-exposed category.

present (Table 2). Among those buried in sand, 14% (7/50) had diarrhea when *F*⁺ coliphage was present compared with 4% (130/3709) among those who were not buried in sand, and there was some evidence of an increasing trend of aORs across exposure categories (Table 2). However, due to small numbers of cases, overall results were not suggestive of a consistent association of *F*⁺ coliphage with the sand exposures considered and GI illness and diarrhea (Table 2).

Enterococcus measured by Method 1600 (colony-forming units/g) showed positive associations with GI illness and diarrhea among those digging in the sand, and associations among those buried in the sand were stronger. Compared with people who did not dig in sand, the aORs of illness among people who reported digging in sand with *Enterococcus* levels in the highest tertile were 1.74 (95% CI = 1.11–2.72) for GI illness and 2.07 (95% CI = 1.27–3.38) for diarrhea (Table 3). Similar associations (but less precise) were observed for GI illness and diarrhea among people buried in sand (Table 3). There was a trend of increasing risk

for *Enterococcus* colony-forming units/g in sand (assessed as above vs. below the median or in tertiles) with enteric illnesses among both sand-exposure groups; however, the increase in aORs was not monotonic (Table 3).

Enterococcus measured in sand by a rapid molecular method (calibrator cell equivalents/g) was also associated with GI illness and diarrhea (Table 4). The aORs of illness among people who reported digging in sand with *Enterococcus* in the highest tertile were 1.98 (1.23–3.19) for GI illness and 2.44 (1.41–4.22) for diarrhea compared with people who did not dig in sand (Table 4). The aORs of GI illness and diarrhea among people buried in sand with the highest tertile of *Enterococcus* were 3.25 (1.33–7.92) and 4.90 (1.79–13.4), respectively, compared with people who reported not being buried in sand (Table 4). The lower precision of aORs for being buried in the sand reflects smaller numbers of participants with that exposure. There was a trend of increasing *Enterococcus* calibrator cell equivalents/g in sand (above vs. below the median and tertiles) with both enteric illnesses among both sand-exposure groups (Table 4).

TABLE 5. Relation of *Bacteroidales* in Sand With Enteric Illness by Status of Sand Contact and by Categorical Classification

	GI Illness		Diarrhea	
	No. (%) ^a	aOR (95% CI) ^b	No. (%) ^a	aOR (95% CI) ^b
Digging in sand	(n = 4331) ^c		(n = 4364) ^c	
Not digging ^d	174 (5)	1.00	110 (3)	1.00
Digging				
<i>Bacteroidales</i>				
< Median 165 CCE/g ^e	49 (8)	1.23 (0.83–1.82)	32 (5)	1.32 (0.84–2.06)
≥ Median 165 CCE/g ^e	52 (10)	1.51 (0.98–2.33)	39 (7)	1.86 (1.14–3.05)
Test for trend ^f	P = 0.06		P = 0.01	
Tertile 1: ≥0–54 CCE/g ^e	39 (10)	1.38 (0.88–2.16)	27 (7)	1.53 (0.92–2.54)
Tertile 2: >54–390 CCE/g ^e	27 (7)	1.21 (0.72–2.02)	19 (5)	1.45 (0.78–2.68)
Tertile 3: >390 CCE/g ^e	35 (10)	1.48 (0.88–2.47)	25 (7)	1.73 (0.97–3.07)
Test for trend ^f	P = 0.12		P = 0.04	
Buried in sand	(n = 4334) ^c		(n = 4367) ^c	
Not buried ^d	256 (6)	1.00	167 (4)	1.00
Buried				
<i>Bacteroidales</i>				
< Median 165 CCE/g ^e	6 (6)	0.77 (0.33–1.79)	3 (3)	0.61 (0.19–1.96)
≥ Median 165 CCE/g ^e	13 (17)	2.55 (1.10–5.90)	11 (14)	3.44 (1.31–9.04)
Test for trend ^f	P = 0.09		P = 0.05	
Tertile 1: ≥0–54 CCE/g ^e	2 (3)	0.37 (0.09–1.44)	1 (2)	0.30 (0.04–2.12)
Tertile 2: >54–390 CCE/g ^e	9 (16)	2.63 (1.05–6.57)	6 (10)	2.72 (0.85–8.70)
Tertile 3: >390 CCE/g ^e	8 (13)	1.94 (0.71–5.29)	7 (11)	2.75 (0.88–8.63)
Test for trend ^f	P = 0.07		P = 0.05	

aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and any contact swimming.
^aThe numbers are participants reporting new symptoms, among those without baseline symptoms.
^bRobust variance estimates clustering on household.
^cThe numbers are participants with complete information on age, sex, race/ethnicity, beach, and any contact swimming, among those without baseline symptoms.
^dReference category.
^eCCE/g indicates qPCR calibrator cell equivalents per gram of dry weight sand.
^fP value from a linear trend test across the non-sand-exposed to the highest sand-exposed category.

Positive associations were observed between *Bacteroidales* with GI illness and diarrhea for each of the sand exposures (Table 5). Positive associations were observed between *Bacteroidales* and diarrhea for the above versus below the median for both digging in the sand (1.86 [1.14–3.05]) and being buried in sand (3.44 [1.31–9.04]). Positive associations of *Bacteroidales* tertiles with diarrhea were also observed for both sand-contact groups, although numbers of events in some *Bacteroidales* tertiles were small (Table 5). There was a trend of increasing *Bacteroidales* calibrator cell equivalents/g in sand (above vs. below the median and tertiles) with diarrhea, among both sand-exposure groups (Table 5).

We observed less consistent relationships of fecal *Bacteroides* with illness among both sand-contact groups (Table 6). With one exception, all comparisons of fecal *Bacteroides* in sand at densities above the median value showed smaller aORs than for densities below the median value (Table 6). Adjusted ORs for fecal *Bacteroides* tended to be higher in the middle than in the highest tertiles (Table 6).

We also observed inconsistent relationships of *Clostridium* spp. estimates (calibrator cell equivalents/g) in sand with GI illness and diarrhea among both sand-contact groups (Table 7). Overall, categorical comparisons of *Clostridium* spp. density (median and tertiles) showed smaller point estimates at the higher *Clostridium* spp. density categories compared with lower density categories (Table 7).

A composite index variable suggested that a larger number of fecal indicators in sand was positively associated with GI illness and diarrhea. There was some evidence of an increasing trend of aORs across fecal-index tertiles, particularly for digging in sand and diarrhea (Table 8).

The fecal indicator-enteric illness associations were also present among the subset of people who reported getting sand in their mouth. Among this group, 16% (14/90) had GI illness symptoms when *Enterococcus* (measured as calibrator cell equivalents/g) was in the highest tertile compared with 6% (242/4074) among those who did not get sand in their mouth (data not shown); the aOR was 2.53 (95% CI = 1.27–5.04).

TABLE 6. Relation of Fecal *Bacteroides* in Sand With Enteric Illness by Status of Sand Contact and by Categorical Classification

	GI Illness		Diarrhea	
	No. (%) ^a	aOR (95% CI) ^b	No. (%) ^a	aOR (95% CI) ^b
Digging in sand		(n = 4331) ^c		(n = 4364) ^c
Not digging ^d	174 (5)	1.00	110 (3)	1.00
Digging				
Fecal <i>Bacteroides</i>				
< Median 563 CCE/g ^e	61 (9)	1.40 (0.96–2.06)	43 (7)	1.62 (1.04–2.51)
≥ Median 563 CCE/g ^e	40 (8)	1.29 (0.81–2.04)	28 (6)	1.49 (0.89–2.51)
Test for trend ^f		<i>P</i> = 0.15		<i>P</i> = 0.05
Tertile 1: ≥0–89 CCE/g ^e	37 (8)	1.07 (0.67–1.69)	26 (6)	1.23 (0.74–2.03)
Tertile 2: >89–1088 CCE/g ^e	39 (12)	2.11 (1.33–3.36)	26 (8)	2.27 (1.33–3.90)
Tertile 3: >1088 CCE/g ^e	25 (7)	1.08 (0.63–1.84)	19 (6)	1.40 (0.74–2.63)
Test for trend ^f		<i>P</i> = 0.09		<i>P</i> = 0.03
Buried in sand		(n = 4334) ^c		(n = 4367) ^c
Not buried ^d	256 (6)	1.00	167 (4)	1.00
< Median 563 CCE/g ^e	12 (11)	1.55 (0.72–3.33)	7 (6)	1.40 (0.46–4.29)
≥ Median 563 CCE/g ^e	7 (10)	1.38 (0.47–4.04)	7 (10)	2.29 (0.78–6.76)
Test for trend ^f		<i>P</i> = 0.30		<i>P</i> = 0.12
Tertile 1: ≥0–89 CCE/g ^e	5 (7)	0.90 (0.35–2.36)	1 (1)	0.27 (0.04–1.94)
Tertile 2: >89–1088 CCE/g ^e	7 (13)	2.12 (0.77–5.85)	6 (11)	2.96 (0.89–9.83)
Tertile 3: >1088 CCE/g ^e	7 (12)	1.71 (0.58–5.11)	7 (12)	2.88 (0.96–8.65)
Test for trend ^f		<i>P</i> = 0.16		<i>P</i> = 0.04

aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and any contact swimming.

^aThe numbers are participants reporting new symptoms, among those without baseline symptoms.

^bRobust variance estimates clustering on household.

^cThe numbers are participants with complete information on age, sex, race/ethnicity, beach, and any contact swimming, among those without baseline symptoms.

^dReference category.

^eCCE/g indicates qPCR calibrator cell equivalents per gram of dry weight sand.

^f*P* value from a linear trend test across the non-sand-exposed to the highest sand-exposed category.

CCE indicates calibrator cell equivalents and are reported as per dry weight of sand.

DISCUSSION

Estimates of fecal contamination in wet sand measured by molecular methods (*Enterococcus* and *Bacteroidales*) and culture-based methods (*Enterococcus*) were positively associated with enteric illness among those digging in sand and being buried in sand at 2 recreational beach sites. This association was observed for a definition of enteric illness based on composite symptoms (GI illness) and a more narrow definition (diarrhea alone). Although there was some evidence of positive associations between sand-contact activities and enteric illness for culture-based F⁺ coliphage and molecular fecal *Bacteroides* and *Clostridium* spp. estimates, there was inconsistency across the exposure classifications considered (above vs. below the median and tertiles). Our ability to make conclusions for the F⁺ coliphage measure was limited because of its low frequency of detection in beach sand samples (17%).

This is one of the first studies to show an association between beach sand contact and enteric illness as a function of microbial sand quality. One previous study observed a relationship between sand contact and GI illness, but expo-

sure was assessed as a function of time spent in contact with wet sand, not as a function of an objective measure of microbial sand quality.⁵ Two other studies of beach sand exposure and health effects (which included objective measures of beach sand fecal indicator densities) did not show consistent relationships between fecal contamination in beach sand, sand-contact activities, and illness (including GI illness).^{35,36} Another study,³⁴ which lacked objective measures of fecal contamination of sand, observed an increased risk of enteric illness associated with beach sand-contact activities (digging in sand and being buried in sand).

Investigators have observed manyfold higher concentrations of fecal microbial indicators (including *E. coli* and *Enterococcus*) in beach sand compared with nearby bathing water.^{35,36} Halliday and Gast⁷ recently completed a comprehensive review of studies of fecal microbial pollution of beach sand and observed that densities of fecal indicator bacteria in wet sand were up to 38 times higher than in nearby bathing waters. It has been hypothesized that sand could serve as a source of fecal contamination for bathing water, especially the surf zone along the shoreline.^{3,4,37} Others have

TABLE 7. Relation of *Clostridium* spp. in Sand With GI Illness by Status of Sand Contact and by Categorical Classification

	GI Illness		Diarrhea	
	No. (%) ^a	aOR (95% CI) ^b	No. (%) ^a	aOR (95% CI) ^b
Digging in sand	(n = 4672) ^c		(n = 4706) ^c	
Not digging ^d	182 (5)	1.00	115 (3)	1.00
Digging				
<i>Clostridium</i> spp.				
< Median 2468 CCE/g ^e	63 (9)	1.46 (1.00–2.13)	53 (8)	1.98 (1.31–2.99)
≥ Median 2468 CCE/g ^e	49 (8)	1.36 (0.89–2.09)	28 (5)	1.25 (0.77–2.04)
Test for trend ^f	P = 0.08		P = 0.10	
Tertile 1: ≥0–1723 CCE/g ^e	23 (6)	1.04 (0.62–1.75)	18 (5)	1.35 (0.74–2.49)
Tertile 2: >1723–3579 CCE/g ^e	50 (11)	1.62 (1.06–2.48)	40 (9)	2.02 (1.30–3.16)
Tertile 3: >3579 CCE/g ^e	39 (9)	1.53 (0.96–2.44)	23 (5)	1.47 (0.84–2.55)
Test for trend ^f	P = 0.02		P = 0.01	
Buried in sand	(n = 4675) ^c		(n = 4709) ^c	
Not buried ^d	274 (6)	1.00	181 (4)	1.00
< Median 2468 CCE/g ^e	13 (12)	1.57 (0.69–3.56)	12 (11)	2.22 (0.92–5.38)
≥ Median 2468 CCE/g ^e	7 (7)	1.12 (0.50–2.49)	3 (3)	0.73 (0.22–2.38)
Test for trend ^f	P = 0.40		P = 0.50	
Tertile 1: ≥0–1723 CCE/g ^e	4 (6)	0.80 (0.22–2.87)	4 (6)	1.28 (0.35–4.70)
Tertile 2: >1723–3579 CCE/g ^e	9 (17)	2.17 (0.78–6.01)	8 (15)	2.80 (0.90–8.70)
Tertile 3: >3579 CCE/g ^e	7 (8)	1.30 (0.58–2.90)	3 (3)	0.84 (0.26–2.78)
Test for trend ^f	P = 0.21		P = 0.32	

aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and any contact swimming.

^aThe numbers are participants reporting new symptoms, among those without baseline symptoms.

^bRobust variance estimates clustering on household.

^cThe numbers are participants with complete information on age, sex, race/ethnicity, beach, and any contact swimming, among those without baseline symptoms.

^dReference category.

^eCCE/g indicates qPCR calibrator cell equivalents per gram of dry weight sand.

^fP value from a linear trend test across the non-sand-exposed to the highest sand-exposed category.

demonstrated that sand can serve as both a source and a sink of fecal microbial contamination.¹⁴ Debate continues about the applicability of fecal indicator bacteria (eg, *E. coli* and *Enterococcus*) as a measure of beach water quality and fecal contamination of beach sand.^{4,38,39} Several studies have examined potential for regrowth of *E. coli* and *Enterococcus* in sand.^{18,19} Beach sand may serve as a source of autochthonous fecal indicator bacteria (*E. coli*; *Enterococcus*) to nearby bathing water in the absence of inputs from point sources of fecal contamination (and associated pathogens). Beach sand has been implicated as contributing unnecessarily to beach advisories based on results of water-quality fecal indicator bacteria tests.^{4,14} EPA guidelines for monitoring fresh and marine recreational waters are based on *E. coli* and *Enterococcus*^{40,41}; however, no guidelines exist for fecal contamination of sand.

Our results suggest that *Enterococcus* density in sand increases the rate of GI illness and diarrhea among beachgoers who have contact with sand. The positive association of a qPCR-based measure of *Enterococcus* in sand with enteric illness was more consistent than that observed for a traditional culture-based measure of *Enterococcus* and several

alternative measures of sand fecal pollution (culture-based F⁺ coliphage, and qPCR-based *Bacteroidales*, fecal *Bacteroides*, and *Clostridium* spp.). These alternative measures have been considered by some to be more specific indicators of human sewage sources of fecal contamination.⁹ Previous research has demonstrated a consistent positive association between *Enterococcus* calibrator cell equivalents and swimming-associated illness among both adults and children.^{21,22} Culturable fecal indicator bacteria cells (eg, total and fecal coliforms, *E. coli*, and *Enterococcus*) are considered a better measure of viable bacteria associated with fecal pollution than qPCR-based measures, which reflect the genetic material of bacterial cells and may have differential environmental fates.^{42–46}

The 2 beaches were located near publicly owned treatment-works sewage outfalls. It is possible that fecal contamination from municipal sewage reached recreational beaches through tidal flow, wave action, on-shore wind direction, or currents. However, some evidence suggests that diffuse sources (including coastal birds, other animal populations, bather density, and run-off) may contribute most of the fecal contamination to beach sand.^{47,48} It is unclear whether the observed relationships of *Enterococcus* with enteric illness

TABLE 8. Relationship Between a Presence-absence Index of All 6 Fecal Indicators in Sand and Enteric Illness by Sand Contact Type

	GI Illness		Diarrhea	
	No. (%) ^a	aOR (95% CI) ^b	No. (%) ^a	aOR (95% CI) ^b
Digging in sand		(n = 4672) ^c		(n = 4706) ^c
Not digging ^d	182 (5)	1.00	115 (3)	1.00
Digging				
Fecal index				
Tertile 1	38 (9)	1.32 (0.83–2.10)	27 (6)	1.48 (0.89–2.47)
Tertile 2	41 (9)	1.59 (1.01–2.50)	28 (6)	1.76 (1.07–2.90)
Tertile 3	33 (8)	1.33 (0.82–2.13)	26 (7)	1.69 (0.98–2.93)
Test for trend ^e		P = 0.06		P = 0.01
Buried in sand		(n = 4675) ^c		(n = 4709) ^c
Not buried ^d	274 (6)	1.00	181 (4)	1.00
Buried				
Fecal index				
Tertile 1	4 (5)	0.59 (0.21–1.65)	2 (2)	0.44 (0.11–1.74)
Tertile 2	7 (11)	1.89 (0.81–4.41)	5 (8)	2.14 (0.72–6.34)
Tertile 3	9 (16)	2.16 (0.75–6.19)	8 (14)	2.92 (0.91–9.37)
Test for trend ^e		P = 0.12		P = 0.07

aOR estimated from logistic regression model adjusted for age, sex, race/ethnicity, beach, and any contact swimming.

^aThe numbers are participants reporting new symptoms, among those without baseline symptoms.

^bRobust variance estimates clustering on household.

^cThe numbers are participants with complete information on age, sex, race/ethnicity, beach, and any contact swimming, among those without baseline symptoms.

^dReference category.

^eP value from a linear trend test across the non-sand-exposed to the highest sand-exposed category.

can be generalized to beaches not influenced by municipal sewage outfalls (ie, nonpoint source pollution beaches), to freshwater beaches, or to tropical beaches where the population dynamics of fecal indicators in sand may be different.

Swimming was strongly associated with sand contact; 81% of swimmers versus 19% of nonswimmers reported digging in sand, and 89% of swimmers versus 11% of nonswimmers reported being buried in sand. As a result, our study has little power to evaluate associations between sand contact and illness among nonswimmers because there were very few sand-exposed cases among nonswimmers. For example, among nonswimmers who dug in sand with *Enterococcus* in the highest tertile, there were no exposed diarrhea cases and one exposed GI illness case. Therefore, although we adjusted for swimming in all analyses of sand contact, it is possible that associations between sand contact and illness reflect exposure to contaminated water as well as sand. In our previous study,³⁴ which included more beaches but lacked measures of fecal contamination of sand, digging in sand was associated with GI illness (adjusted incidence proportion ratio = 1.26 [95% CI = 1.03–1.54]) and diarrhea (1.26 [0.98–1.62]) among nonswimmers, suggesting that sand may be an additional route of exposure to pathogens.

Beachgoers' contact with sand was assessed by questionnaire and included a question asking whether they had contact with wet or dry sand. However, only wet sand was

collected and analyzed for microbial measurements. We used microbial quality in wet sand as a proxy for quality of all sand. To examine the potential influence of misclassification of exposure by wet versus dry sand contact, we examined associations restricted to participants with wet sand contact only. The results were similar to those among all participants. For example, among tertiles of the qPCR-based *Enterococcus* measure, the aORs of associations with GI illness among those digging in wet sand only were 1.02 (95% CI = 0.58–1.80), 1.31 (0.71–2.43), and 2.48 (1.46–4.21), respectively.

Overall, associations were stronger among those buried in sand (which represents a more intense form of sand exposure) than among those digging in sand. Defining sand exposure as any sand contact, including either digging in the sand or buried in the sand, did not alter our conclusions (data not shown).

Research on health effects among beachgoers has focused largely on swimming-associated illness and microbial water quality. We show a relation of sand-contact activities with GI illness and diarrhea as a function of objective measures of fecal pollution of beach sand. Limitations of the study include a small sample size for investigation of associations among subgroups (children, nonswimmers), use of wet sand as a proxy for exposure to dry sand-contact activities, lack of tests of specific enteric pathogens in beach sand, lack of analyses taking account of water quality, and lack of

objective measures of enteric illness self-reports by microbial or immunologic tests of biospecimens. Laboratory confirmation of infection with pathogens via tests of saliva, blood, or stool could improve the classification of incident symptoms.

Further investigation of sand exposure and its association with enteric and nonenteric illness appears warranted based on these results. It is unknown whether the relation of *Enterococcus* in sand with GI illness and diarrhea can be extended to nonenteric illnesses (eg, skin rash, upper respiratory illness, eye irritation, earache, infected cuts/wounds). Further studies at a broader geographic range of beach sites, including nonpoint source run-off, freshwater, and tropical beaches, may advance understanding of sand exposures associated with illness risk and the association of densities of fecal-indicator organisms in beach sand with illness risk among beachgoers.

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