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Bacteria in beach sands: an emerging challenge in protecting coastal water quality and bather health

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Abstract

To protect bather health at recreational beaches, fecal indicator bacterial standards are used to monitor water quality, and waters exceeding the standards are subsequently closed to bathers. However beachgoers are also in contact with beach sands, the sanitary quality of which is not included within beach monitoring programs. In fact, sands and sediments provide habitat where fecal bacterial populations may persist, and in some cases grow, in the coastal zone. Specific pathogens are less well studied in beach sands and sediments, but there is a body of evidence that they too may persist in these environments. This paper reviews the current state of knowledge regarding the abundance and distribution of fecal indicator bacteria and pathogens in beach sands of diverse climatological regions, and at beaches subjected to varied levels of anthropogenic impact. In all regions fecal indicator bacteria are nearly ubiquitous in beach sands, and similar relationships emerge between fecal indicator abundance in dry sand, submerged sands, and water. Taken together, these studies contextualize a potential public health issue and identify research questions that must be addressed in order to support future policy decisions.

Keywords

fecal indicator bacteria; beach sands; pathogens; coastal water quality; beach monitoring

Introduction: Fecal indicators as a proxy for water quality

Every year, bathing in coastal waters polluted with fecal contamination is estimated to cause more than 120 million cases of gastrointestinal illness and 50 million cases of respiratory disease around the world (1). These cases are caused by a diversity of fecal pathogens introduced into the aquatic environment by point sources such as wastewater treatment facilities and combined sewer overflows, or by diffuse nonpoint sources stemming from coastal and shoreline development, leaky septic tanks, urban runoff, agricultural runoff, discharge from boats, from bathers themselves, and from local animal populations (Figure 1). Because it is not feasible to monitor each of the viral, bacterial and protozoan pathogens potentially present, culturable fecal indicator bacteria (FIB) that are correlated with disease in swimmers (usually gastrointestinal) are used as proxies for the presence of sewage-borne pathogens that put bather health at risk (e.g., 2,3). A meta-analysis of twenty-two

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Supporting Information

Table detailing experimental conditions for detection of FIB in sands. This material is available free of charge via the Internet at http://pubs.acs.org.

epidemiological studies conducted between 1953 and 1996 at beaches around the world (4) suggests a causal dose-related relationship between gastrointestinal symptoms and recreational water quality as measured by bacterial indicator counts (including total coliforms, fecal coliforms, enterococci or *E. coli*). Among these studies, *Enterococcus* spp. (ENT) emerge as the indicator bacteria best correlated with health outcomes in marine systems whereas *E. coli* (EC) are best correlated with health outcomes in fresh water systems.

New perspectives on fecal indicators in the environment

Researchers have questioned the efficacy of FIB standards (5) because several assumptions have been proven false, namely, that FIB cannot persist outside their host environment, and that recovery of FIB from the aquatic environment is indicative of the presence of disease-causing pathogens. In many cases, the abundance of FIB in recreational waters does not correlate with specific pathogens (e.g. 5–11) and discrepancies may reflect environmental or persistent FIB populations including bird guano (12,13), FIB growing within vegetation or algal mats on lake shores (14), and FIB in beach sands. As early as 1967, EC and ENT were documented persisting for many days in soils and thereby contributed to "variations in bacterial count of storm-water runoff which have no relation to the sanitary history of the drainage area" (15).

The effects of sunlight and other environmental factors that limit survival of FIB in the water column have been well documented (e.g., 16–20), but the physical, chemical and biotic factors influencing FIB survival in sediments and sands have only recently begun to be assessed (21–24). A comparison of EC and ENT survival as measured in different studies is presented in Table 1. This summary clearly shows that despite differences in methodological and experimental conditions, the loss of cells of both EC and ENT in fresh and seawater supports the assumption that they quickly die in recreational waters. In contrast, studies that examine the loss of EC and ENT cells in wet sand and water find that culturable bacteria persist longer in sand than in water (Table 1, A), and some studies have documented growth of EC and ENT, rather than loss, in beach sands (Table 1, B).

A nuanced relationship between indicators and health outcomes

The selection of FIB as microbiological water quality proxies was supported by strong epidemiological evidence (e.g., 2, 29-31) that FIB are consistently the best predictor of bather health outcomes at beaches affected by point source pollution. Epidemiological studies at beaches with nonpoint source pollution are fewer and have mixed success in correlating FIB abundance to bather health outcomes of enteric illness, respiratory and skin infections. At a beach in California affected primarily by nonpoint source pollution, no association was found between the abundance of traditional FIB and negative bather health outcomes (32), but bathers in the study did have an increased incidence of diarrhea and skin rashes when compared to non-bathing beachgoers. In Florida, Fleisher et al. (33) also documented increased incidence of enteric, respiratory, and skin infections in bathers compared to non-bathers at a beach with nonpoint source microbial pollution; but among the symptoms, only skin rashes increased in a dose-dependent manner with measured ENT. These nonpoint source case studies are important to note because the majority of the approximately 20,000 beach advisories in the U.S. in 2008 were caused by nonpoint sources of bacterial pollution (34). Furthermore, despite increased monitoring and closures over the past decade, the Centers for Disease Control and Prevention concluded in a recent report that the incidence of infection associated with recreational water use has steadily increased over the past several decades as a result of emerging pathogens, increases in aquatic activities, and better disease reporting (35). The lessening of the relationship between FIB and bather health outcomes in cases of nonpoint source pollution suggests that the current water quality

Outside the host: environmental reservoirs and differential survival of enteric bacteria

this lack of correlation between indicator levels and disease symptoms.

FIB are natural residents of the lower intestinal tract of humans and other warm-blooded animals. The host provides a consistently warm and relatively nutrient-replete environment. Once outside their host, fecal bacteria may face osmotic stress, large variations in temperature and pH, limited nutrient availability, and increased predation. Common fecal bacteria such as ENT and EC vary in their ability to deal with these environmental stressors. ENT typically display tolerance to extremes in pH, temperature, salts and detergents (36), and their surface hydrophobicity makes them more successful at utilizing starvation and biofilm modes of growth (37,38). EC have been found to constitute a smaller fraction of particle-associated cells in the aquatic environment (39) and are relatively more sensitive to dessication and inactivation by sunlight (17, 40). Abilities may further vary between bacterial strains or even within a population, due to differences in physiological state or growth stage (41). Nevertheless, non-host environments may broadly be considered to be stressful (i.e., following introduction die-off can be measured) or permissive (characterized by persistence or growth) for enteric bacteria.

It is possible that environmental FIB populations, such as those in beach sands, contribute to

Stressful Environments—Experimental studies have shown that the effects of temperature (42), salinity (e.g., 42,43) and sunlight (e.g. 17,19) in aquatic environments are all factors contributing to the reduction in colony forming unit (CFU) recovery of EC and ENT over time in surface waters, with EC typically more sensitive than ENT to these effects. Thus, aquatic environments may broadly be considered stressful, but the reduction in recovery rates of culturable FIB from environmental waters should be treated cautiously. Enterococcus faecalis, like other nonsporulating bacteria, can respond to environmental stressors by altering its physiology to a starvation state whereby it persists without growing in the environment and is recovered by culture (45), or to a distinctly different viable but nonculturable (VBNC) state whereby cells are vegetative and not culturable, but can be visualized with viable count methods (46). Studies of the viability of E. faecalis in artificial seawater microcosms show that at least 80% of the cells remain viable when colonies can no longer be recovered (47), suggesting that VBNC ENT may persist in a dormant state in the environment. Likewise, in marine waters, enterotoxigenic EC strains have been documented entering the VBNC state upon exposure to sunlight, and subsequently persisting in the environment while retaining toxicity (48). Even when exposed to Antarctic waters, enteric bacteria were able to persist in VBNC states (49). These studies highlight one of the flaws of the culture-based method of indicator bacteria detection -the exclusion of VBNC cells that have the potential to impact health.

Permissive Environments—A survival strategy utilized by many allochthonous bacteria in aquatic environments is sorption to particulate matter. Studies have shown that EC persists longer in seawater and lake microcosms when sand or sediment is present (42, 50). Davies et al. (51) studied seeded EC in marine sediment by enumerating total culturable cells and total viable cells (via acridine orange direct counting) and found that over an experimental period of 68 days the same proportion of total EC remained culturable. Survival in sediment may be enhanced relative to water because of protection from sunlight/UV inactivation, buffered temperatures, and availability of nutrients accumulated from algae, debris and plankton (e.g., 52). Bacteria may also be protected within biofilms on moist sand grains (53). In some geographical regions, highly favorable conditions may be encountered outside the host. In relatively warm, nutrient-rich, pristine tropical soils and waters, EC have been found at densities far exceeding the concentrations found in highly-

In short, although in some cases they are well correlated with health outcomes, FIB and other allochthonous enteric bacteria have mechanisms to survive the stressors frequently found in aquatic environments, and environmentally-adapted strains may establish indigenous populations that are not indicative of recent fecal contamination. Among pathogens, some may be particularly well adapted to life in the nonhost environment (58).

FIB in beach sands within the United States

Recreational waters in America are monitored with standard methods designed to protect human health, even though radical differences in climate, sand type, wave energy, and point and nonpoint sources of pollution may contribute to the bacterial concentrations in the water column. Likewise, the relative importance of beach sands as a reservoir of FIB may also vary at local or regional levels. A few studies have examined the effects of tide, current and groundwater on the movement of FIB between the beach and water (59–63), and these reinforce the likelihood that a combination of coastal parameters effect the distribution and persistence of FIB and pathogens in sands and water. The generation of reactive oxygen species in beach sands and wrack (64) is a possible chemical parameter that may affect FIB persistence as well.

Representative environmental data from subtropical beaches, temperate coastal beaches and estuarine beaches, and Great Lakes beaches, normalized to CFU/100g sand to facilitate comparisons with the units CFU/100mL used in water quality management, are presented in Table 2. Studies reported in this table (23, 52, 65–68) were chosen because the sites vary in their climates and bacterial sources, but the data illustrate that in each of these regions, EC and ENT routinely vary by at least an order of magnitude from ambient water quality measurements and also can vary by an order of magnitude or more in different sand types at the same site. However, directly comparing studies of indicator abundances such as those in Table 2 is complicated by the fact that there is extreme spatial and temporal variability at most sites. For example, at Lover's Point, the Southern California beach whose ENT concentrations are referenced in Table 2, the investigators studied spatial variability over a 24-hour period and found that although in aggregated samples the dry sand had the highest concentrations of ENT, measurements of individual samples varied by three orders of magnitude from below their detection limit of approximately 5CFU/100g, to 4452CFU/100g (95).

Additionally complicating direct comparisons between studies is the lack of a common method for measuring FIB in beach sands. Studies of FIB in beach sands have generally modified the protocol for detection of FIB in recreational waters by suspending sand in water, shaking, and then processing the supernatant as if it were a water sample. As such, there is great variability in how sand studies produce a sample (SI Table 1). There can be major differences in sand sample collection, including holding time before analysis, whether replicate samples were homogenized, whether sands were collected surficially or integrate some depth within the sand, and whether "wet sand" was submerged. The amount of sand actually tested varies from 5g to 200g per sample (studies in Table 2). Also variable is the amount and type of eluant, how long the sands were shaken, whether they were shaken by hand or mechanically, and how long sands were allowed to settle before analysis of the eluant. Both of the EPA-approved methods of detection, membrane filtration to quantify colony-forming units and IDEXX plates to quantify most probable number (MPN), have

been successfully used for detection in of FIB in sands. Only one study to date has compared many of the common methods and reagents used for FIB recovery from sand (69). Overall, most of the methods tested did not produce significant differences in recovery of FIB, but the authors suggested shaking sands suspended in water or PBS in a ratio of 1:10 by hand for two minutes, with one rinse step and a settling time of thirty seconds as the optimal method (69). As studies of sand begin to follow the same method, comparisons between them will become more meaningful.

Subtropical Beaches

In subtropical environments such as Hawaii, FIB are frequently found at extremely elevated concentrations in freshwater streams. However, the source of FIB to these streams is not sewage or human waste but the local soils, which are broadly permissive for the growth of a diversity of fecal bacteria. At Hanauma Bay, a site in Hawaii that experienced declining water quality as it emerged as a tourist destination, transects from submerged sands to inland sands revealed that fecal coliform, EC and ENT concentrations increased steadily and that the highest concentrations of bacteria were found in dry sand where people congregate to sunbathe and eat (70). The authors also recognized that endemic populations of pigeons had increased as the bay developed as a tourist destination. The pigeons were implicated in the contamination of the dry beach sands, whereas further inland, mongoose waste was suggested as a primary source of FIB to soils (70). In Hawaii, it has been shown that the dominant soil microflora have nutrient extraction capabilities superior to those of the EC and ENT, whose growth is limited by competition, but when excess nutrients and moisture become available both EC and ENT quickly respond and grow (40). Mesocosm experiments with tidally-impacted subtropical sediments have also documented significant amounts of regrowth for both ENT and EC with the simulation of tides through wetting, and with the addition of sediment to water (67).

In the coastal environment, some bacterial strains may have the genetic potential to persist longer than others. Mesocosm experiments have tested the persistence of ENT isolates from sand, dog and wastewater sources in subtropical (Gulf of Mexico) sediments and waters (28). In these experiments growth was never observed, but specific decay rates confirmed that ENT persists longer in sediment than in fresh or seawater (28, Table 1).

In 2007, Bonilla et al. (66) published the results of a study at three popular southern Florida beaches, each having different physical and chemical parameters, and found that FIB (both EC and ENT) were recovered, at a 2–23 fold greater concentration in wet sand than in water and at a 30-460 fold greater concentration in dry sands than water (Table 2). No correlations were found between environmental parameters and bacterial concentrations that would explain these results. The highest concentrations of FIB were found at >5m landward from the tidal zone, in beach sand that would be infrequently wet. The inter-sample variability of ENT in sands was consistently high, and a seeding experiment showed that one fecal event from a gull could be spread over $3.1m^2$ of beach sand by pedestrian and natural transport mechanisms. This suggested that small volume/high concentration inputs of bacteria could increase the number of culturable FIB over a fairly wide beach area, and potentially the water column. Other studies in the area (71) have also shown that the highest concentration of bacteria recorded in waters occurs at the high tide. DNA-based identification of ENT species in sands, water, feces and sewage further implicated washout of the sand bacteria into recreational water in the area by showing that assemblages in wet sand and water were more similar (72).

As the Bonilla et al. (66) study found that ENT was statistically elevated in sand relative to water, they considered the potential health risks associated with exposure to sand with a pilot epidemiology study. Preliminary evidence suggested that only time spent in wet sand

and time spent in water were associated with a dose-dependent increase in gastrointestinal illness. The culture methods used to detect both ENT and EC do not differentiate between species from different sources, such as from humans or animals or environmental strains. There may be different health impacts from the presence of non-human indicators, and this may be reflected in the lack of disease associated with dry sand exposure in their study.

Temperate, Coastal Beaches

Especially along the California coast, ENT have been shown to be nearly ubiquitous in beach sands (63) with exposed sands having significantly higher densities of ENT than submerged sands, and the highest densities located near the high tide line. Likewise, intertidal sites have been shown to have higher and more homogeneous concentrations of FIB than submerged marine sediments (73). Sand characteristics such as organic content, moisture content and percentage fines have been shown to affect the densities of EC but not ENT (63), and natural ENT populations in beach sand transported to lab microcosms have been shown to grow in response to simulated tidal rewetting (23, Table 1B).

At Santa Monica Bay, including two open beaches and one sheltered beach, Lee et al. (74) measured FIB (both ENT and EC) levels in water and sediment prior to, during, and following a storm event. At the two open beaches, FIB concentrations in sediment peaked along with water column concentrations during the storm; both sediment and water populations declined after the storm. At the enclosed beach, FIB levels in water and sand were consistently high and did not appear well-correlated with the storm. Further analysis showed that levels of ENT at enclosed beaches were two to three orders of magnitude higher than all of the values observed at eleven open beaches, supporting the hypothesis that the physical environment at enclosed beaches supports environmental reservoirs of ENT in sediments. Using sterilized sediment and water from their study sites, Lee et al. (74) conducted benchtop microcosm studies and found that total culturable ENT remained constant over time in microcosms, and ENT survived even better in water amended with high organic-content sediment.

Temperate, Estuarine Beaches

Sands and sediments at estuarine beaches and coastal wetlands are noteworthy as potential sources of FIB to recreational waters, because particulate matter naturally settles out in these environments and may be resuspended during tidal or high erosional flow conditions (68). Fries et al. (39) examined the proportion of free FIB (both EC and ENT) versus particle-associated FIB in water in a time series at the Neuse River Estuary in North Carolina, and found that 38% of the FIB in water were attached to particulate matter and thus capable of settling out of the water column. In the environment, storm events were correlated to increases in FIB (both EC and ENT) and increases in the amount of particles in suspension in the water column (39).

Evanson and Ambrose (68) examined a tidally influenced wetland in southern California and determined that although sediments were enriched in FIB, they were not a source to surf-zone waters. Sediment and water FIB peaked in conjunction with rain events, but water FIB concentrations always declined quickly after the event whereas sediment-associated FIB populations declined slowly. They concluded that at this location the sedimentary FIB populations likely have population dynamics independent of the water FIB population. Alternatively, other studies in similar systems in Southern California have shown that high concentrations of ENT (possibly stemming from abundant ENT populations identified in sediments, on vegetation, and from birds) in tidal saltwater marshes result in contamination of the surf zone water quality (75). This particular study noted that in the dry season,

surfzone exceedences happen most frequently during the spring tides when tidal transport between the wetlands and surf zone is greatest.

The human health risks associated with ENT derived from wetland effluent are unclear. Although presumably not the same health risks are associated with ENT derived from nonhuman pollution, no epidemiological studies to date have addressed this issue. Some data from mesocosm studies, designed to evaluate efficiency of pathogen removal from wastewater in engineered wetlands, suggest that die-off rates of all bacteria and coliphage are greater in the water column than in the sediment, but that the protozoan pathogen *Giardia* has a greater die-of rate in the sediment than the water (76). These data were generated in an artificial environment, but the study illustrates the potential for sediments to differentially act as ecosystem sources or sinks depending on the microbe.

Temperate Great Lakes Beaches

Many studies at Great Lakes beaches have documented populations of FIB in sands at densities much higher than ambient water concentrations. Wet sands have cultivatable concentrations of ENT and EC that are 4-38 times higher and 3-17 times higher, respectively, than concentrations found in water (65, Table 2). Mesocosm experiments with seeded EC in sterilized local sand show growth and persistence at even higher levels than those in the environment, indicating the probable importance of competition and predation in natural communities (77). Comparisons between wet (intertidal) and dry (foreshore) sands have revealed that dry sand harbors higher concentrations of EC than wet sand or ambient water; these sands are a source of EC to recreational waters in Lake Michigan and support an autochthonous, high density population of indicator bacteria for sustained periods independent of lake, human, or animal input (52). At Lake Superior, Ishii et al. (78) observed the highest densities of EC in nearshore sand and high but extremely patchy populations in far upshore sands; the abundance of EC in sediment, shoreline and nearshore sands increased as temperature increased over the course of the summer. Even throughout the northern Minnesota winter, EC strains were recoverable from sands and source-tracking was unable to connect these strains with known sources (78). Subsequently, in the summer these "naturalized" strains were frequently recovered from water, sand and lake sediments, and were in highest relative abundance in August waters, providing further evidence of environmental populations of FIB (78). In a different study, multilocus enzyme electrophoresis and multilocus sequence typing of EC isolates from soils and sands at freshwater beaches on Lake Huron and the St. Clair river in Michigan revealed great genetic diversity overall, but several distinct genotypes were shared among sites and repeatedly recovered over time (79), which likewise supports the hypothesis of naturalized soil/sand FIB populations. Indeed, the temperate environment may be more hospitable for bacteria at the beach than originally believed; EC isolates have been shown to survive longer at lower temperatures than higher temperatures in soil from the watersheds of this region (80) and in colder temperatures of lake water (42).

Several studies have contextualized the relative contribution of different sources, including beach sands, to ambient water quality in the region. Results from Ishii et al. (78) found beach sands and humans were significant sources of FIB to recreational waters in spring and early summer, whereas the importance of waterfowl as a source of sand and water FIB increased in the late summer and early fall. Haack et al. (81) showed that beach orientation with respect to regional weather patterns such as wind speed and direction as well as regional and local hydrodynamics must be considered in understanding under what conditions beach sands may contribute FIB to local waters.

In addition to the evidence of naturalized populations of EC, there is some evidence of human impact as well. Haack et al. (81) found ENT isolates with phenotypes similar to

human pathogens from beach sands and waters at Grand Traverse Bay in Michigan as well as significant levels of antibiotic resistance. The majority of indicators are not pathogenic; of 3557 strains of EC isolated by Ishii et al. (78), only one could be classified as a potential human pathogen. Analysis of the potential risk of exposure to beach sands contaminated with high concentrations of EC (found at Chicago beaches) showed that after handling sand for 60 seconds, the amount of EC transferred to the hand was correlated to the density of bacteria in the sand rather than the area of hand exposed (82). In this case, using doseresponse estimates developed for swimming water contaminated by human sewage, it was determined that the number of individuals per thousand that would develop gastrointestinal symptoms would be 11 if all EC on the fingertip were ingested (82).

Observations of human pathogens in beach sands

Publication of the first beach sand epidemiological study (83) showed that "sand contact activities," including digging in sand or being buried in sand, were positively associated with enteric illness. This illustrated that for some populations, beach activities may be an overlooked route of exposure to certain pathogens.

To date, most studies investigating human pathogens in beach sands have either not sought or failed to identify a discernible relationship between abundance of indicators and pathogens in beach sands. There is some general evidence pathogens accumulate in sands and sediments. Enteric viruses have been documented at higher concentrations in estuarine sediments than in the water column (84). In freshwater sediment microcosm experiments, culturable EC and the pathogens Klebsiella pneumoniae and Pseudomonas aeruginosa all survived for weeks, though exhibiting linear decay rates (85). P. aeruginosa was isolated more frequently from beach sands than from water in Israel (86) and also has been isolated from tidally influenced beach sands in Portugal (87). Staphylococcus aureus has been found to be enriched in beach sands relative to local waters and S. aureus counts were correlated to the presence of yeasts of human origin as well as the number of swimmers on the beach at the time of sampling, implicating bathers as the source of this bacteria (88, 89). The beach sands along the Gaza Strip, an area of coastline that is heavily polluted with treated, partially treated and untreated sewage, harbor higher concentrations of fecal indicators and higher concentrations of potentially pathogenic Salmonella and Vibrio isolates than local waters (90). The human pathogen Aeromonas hydrophila has been recovered along with pathogenic Vibrio spp. from sands along the Tel Aviv coast in the Mediterranean (86). Additionally, samples taken during a water quality exceedance event at a Florida beach impacted by nonpoint source pollution were positive for the pathogens V. vulnificius and the human Polyomavirus in both sand and water, while sand was exclusively positive for Cryptosporidium spp. and water exclusively positive for Giardia spp. (91); however, with only four sampling events, no significant relationship between indicators and pathogens could be identified.

A study conducted at bathing beaches in England documented pathogenic *Campylobacter jejuni* and *Salmonella* in beach sands, with *Campylobacter* having a higher rate of recovery from wet sand than dry sand (92). At a site receiving sewage effluent and agricultural runoff, campylobacteria and fecal indicators were elevated in surficial sediments but showed no relationship to one another (93). In Brazil, antibiotic resistance in potentially pathogenic ENT isolates has been more frequently observed in the sands of heavily polluted beaches then relatively pristine beaches, and in both cases more frequently in sands than waters (94). Recently, methicillin-resistant *Staphylococcus aureus* (MRSA) has been isolated from beach sand and seawater in southern California (95) and in the state of Washington (96), fueling speculation that public beaches may be a previously overlooked environmental reservoir for the transmission of MRSA.

Summary and Implications

Although the scientific community has long known that diverse bacterial populations exist in beach sands (97) and recognized that soil and sediments may play a role in the survival of FIB in the environment (14, 50), it is only recently that the extent of anthropogenic impact at the beach and the possible public health repercussions have been realized. The studies analysed in this review and especially those presented in Table 2 indicate dry sands that are infrequently wet, where people likely spend time sunbathing and picnicking, generally have the highest FIB concentrations at the beach, and that in comparison to water column bacterial measurements, intertidal sands are also enriched in FIB by an order of magnitude or more. Laboratory and field experiments in subtropical and temperate locales suggest sand rewetting may spur growth of indigenous FIB populations, and thus tidal or precipitation events may directly or indirectly contribute to fluctuations in sand bacterial concentrations. Data from nearly all environments suggest erosional flow conditions generated by storms or tides may flush bacteria out of sediments or sands, resulting in some level of contamination of the water column.

The relative health risk presented by enteric bacteria in sands remains largely unknown. Most epidemiological studies examining water quality at bathing beaches have not excluded bather exposure to sands. Studies that have explicitly tested exposure to sands either did not report FIB abundance (83), or used duration of exposure to sand and water rather than bacterial abundance to test the relationship with negative health outcomes (66). Testing whether there is a dose-dependent response between increasing abundance of FIB in recreational sands and negative health outcomes for beachgoers is necessary in order to understand what level of fecal bacteria in sands constitutes an unacceptable health risk. Furthermore, it would help to clarify whether fecal bacteria in the sand environment should be monitored at all. Although concentrations of FIB in sands seem excessive when normalized to water quality standards, it is important to remember the water quality standards are based on swimmer exposure to, and presumed ingestion of, water. Exposure to sand may be prolonged, but ingestion and other alternative routes of transmission require further study.

In regard to the impact bacteria in sands may have on water quality, the differences in epidemiological studies conducted at bathing beaches with point versus nonpoint source pollution (eg. 2, 32–33) suggest that health outcomes resulting from bathing in waters contaminated with human sewage would be different than from bathing in waters contaminated with bacteria derived from persistent sand populations. Further studies at beaches experiencing varied sources of pollution are needed to determine the conditions when beach sands may be contributing a signal of water pollution via the resuspension of endogenous indicators. For example, based on flow conditions and standard hydrologic relations in a river, one can estimate how frequently sediments near a sewage outfall that are enriched in bacteria would be resuspended into the water (98). Models of sediment and sand resuspension at beaches (eg., 99, 61) can further help to understand when these bacteria may impact water quality and may even contribute to "early warning" models (101).

However, without better characterizing the pathogens in beach sands, their distributions, and the environmental conditions in which they prosper, we cannot characterize the impact these populations may have on water quality or beachgoer health. Although many studies have documented the presence of viral, bacterial, and protistan pathogens in beach sands, we lack basic information about die-off rates, ability to persist, or growth rates of the organisms that may exploit sands or sediments. Collecting this data in a way that facilitates comparisons requires standardized methods of detection which should be experimentally determined and agreed upon by researchers. The recent study comparing methods (69) gives good reference

for culture-based enumeration of FIB in medium to coarse sands from different environments. Similar comparisons of protocols for marsh sediment and fine-grain sands must be conducted, and might require more rigorous treatment to detach bacteria from sand grains. Likewise, as rapid molecular methods of FIB detection are standardized for water quality regulators, these methods will need to be optimized for the detection of FIB in beach sands. If qPCR is used, important questions for moving forward with sand samples will include basic issues related to PCR (see 101, 102, for detailed review), as well as how much sample needs to be used for DNA extraction, how is the recovery of sample DNA from the extraction protocol estimated and whether to correct for this in the final cell estimation, how is inhibition of PCR (which may be highly variable among samples) handled, and what primer sets and standards should be used for the PCR assay. As with water quality samples, it will be important to determine how DNA-based estimates of cells correspond to risk-based analyses that have been based on culturable FIB in epidemiological studies.

In summary, further research into the introduction, distribution and persistence of FIB and pathogens in beach sands, and the public health implications of these findings, is needed before any incorporation of beach sands into a monitoring framework should be considered. With millions of exposures to polluted sand and water every year, the economic burdens associated with negative health outcomes could be substantial (103). But while the relative risks are further explored by scientists and policy makers, there are some relatively low-cost responses that can be employed to better protect human health. Advocating easy preventative measures such as washing hands before eating at the beach, and protecting open wounds at the beach, may effectively reduce illness especially among the populations most vulnerable to opportunistic pathogens - the very young, the old, and those with compromised immune systems. Whitman et al. (82) showed that washing sandy hands effectively removed 92% of EC, which would greatly limit the hand-mouth transfer of bacteria. Likewise, maintaining the general sanitary condition of beach sands, through measures such as cleaning up dog feces and properly disposing of the human-generated garbage that may attract gulls or other animals, may help prevent illness until specific risks can be characterized with greater certainty.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Pathways of fecal indicator bacteria (FIB) into and out of beach sands

(A) *Runoff:* Precipitation causes diffuse land-based runoff that concentrates FIB from urban areas (roadways, parking lots, gutters, lawns, pets), agriculture (overflow of animal waste), or feces from wildlife in the watershed into stormwater. Stormwater flows through local waterways or runs directly over beach sands into the intertidal zone.

(B) *Aging Infrastructure:* In urban areas with combined sewer overflows, heavy precipitation delivers a mix of urban runoff and raw sewage to beach sands and/or coastal waters, depending on outfall location and tidal stage. Leaky sewer infrastructure, failed septic systems and buried drainage pipes in the coastal zone may also be sources of FIB to beach sands.

(C) *Swash zone:* Periodic tidal rewetting enables FIB deposited in dry sands to persist or regrow, and waves may deliver FIB from the water column into the upper intertidal sands. (D) *Exchange:* Resuspension of sand into water by tidal or wind-driven waves may redistribute bacteria from sand to water; humans are then exposed to these bacteria when bathing. Likewise, deposition of particulate matter may introduce or return bacteria to the sand. Accretion of sands could bury FIB-rich sands at the beach, and erosion could alternately expose or relocate contaminated sands along the beach.

(E) *Water:* Residence time of water at the beach may quickly remove or alternately retain bacteria near shore; thus, local hydrography and wind direction contribute to rates of removal or retention.

(F) *Fecal events:* Animals (birds, dogs, wildlife, humans) on the beach may directly introduce FIB to sands, which can subsequently be redistributed over a greater area of beach by pedestrian traffic or weather events.

(G) *Additional Refugia:* Wrack, harboring robust bacterial populations seeded from landbased runoff or surfzone water, may shed FIB to sand or water during high tides.

Table 1

Observations of FIB decay and growth in beach environments

Type of study:	Measured loss of cells (log ₁₀ CFU) over time in:	K _{ENT}	K _{EC}	Reference:
Water mesocosm amended with sewage	Freshwater	0.3387	0.2174	25
	Seawater	0.5262	1.3319	
Water mesocosm amended with sewage	Light seawater	2.21 ^a		26
	Dark seawater	0.907		
Model: best-fit to field observations	Light seawater	7.0 ^a	6.0	27
	Dark seawater	1.3	0.8	
Sediment/water mesocosms amended with untreated	Freshwater	0.27		28
wastewater	Freshwater sediment	0.03		
	Seawater	1.05		
	Seawater sediment	0.22		
Tropical beach sand mesocosms	Sterile sand	0.006 ^b	0.0379 ^b	24
	Sand with phage	0.011 ^b	0.0665 ^b	
	Sand with phage and bacteria	0.0205 ^b	0.337 ^b	
	Sand with phage, bacteria and protozoa	0.0785 ^b	0.3715 ^b	
B . Observed doubling times (day ⁻¹) in marine beach sa	nds			
Type of study:	Sands subjected to:	ENT	EC	Reference
Florida beach sand mesocosms	Varied temperatures, salinities, nutrient and moisture content	1-0.44 ^c	0.36-0.22 °	21
California beach sand mesocosms	Rewetting	1.1–3.5		23

Examples of reported concentrations of FIB	tions of FIB in beach sands				
Site	Organism	Type of sand environment	Concentration (CFU/100g)	Ratio CFU water:sand	Reference
Chicago/Lake Michigan	EC	Submerged sand	7.2×10 ²	1:93	52
		Dry sand	4.0×10 ³	1:17	
Michigan/Lake Huron	EC	Wet sand	1.4-9×10 ²	1:3-17	65
	ENT	Wet sand	2-8×10 ²	1:4–38	
South Florida, marine beaches	ENT	Wet sand	1.9×10 ²	1:2–23	66
		Dry sand	2.429×10 ³	1:30-460	
	Note: this study reports high microscale variability in dry sands	le variability in dry sands			
South Florida river enbankments	EC	Muddy/steep	6.0×10 ³	Not reported	67
		Muddy/flat	1.42×10 ⁶		
		Muddy/dead end	1.2×10^4		
		Sandy	1.5×10^4		
	ENT	Muddy/steep	4.0×10 ³		
		Muddy/f1at	1.17×10 ⁶		
		Muddy/dead end	2.0×10^{3}		
		Sandy	9.0×10 ³		
Southern California Wetland sediments	ENT	"bird" impacted	1.6×10 ⁴	Not reported	68
		"urban" impacted	1.36×10 ⁵		
	EC	"bird" impacted	4.0×10 ³		
		"urban" impacted	3.8×10 ⁴		
	Note: this study reported geometric m	Note: this study reported geometric means in MPN/5g sediment; adjusted here to 100g	20		

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Table 2

Site	Organism	Type of sand environment	Concentration (CFU/100g)	Ratio CFU Reference water:sand	Reference
Southern California coastal sand	ENT	Wet Sand	$3.35*10^{2}$	1:2.5	23
		Dry Sand	$4.5*10^{3}$	1:34	
	Note: values are per 100g dry weight. for purposes of comparison. Many bea 6.2×10 ⁴	Note: values are per 100g dry weight. This table only includes samples collected at one time point from Lover's Point Beach for purposes of comparison. Many beaches were included in this study with EC and ENT levels ranging from undetectable to 5.2×10 ⁴	ne time point from Lover's Point Beach SNT levels ranging from undetectable to		

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