Hand–mouth transfer and potential for exposure to *E. coli* and F+ coliphage in beach sand, Chicago, Illinois

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ABSTRACT

Beach sand contains fecal indicator bacteria, often in densities greatly exceeding the adjacent swimming waters. We examined the transferability of *Escherichia coli* and F+ coliphage (MS2) from beach sand to hands in order to estimate the potential subsequent health risk. Sand with high initial *E. coli* concentrations was collected from a Chicago beach. Individuals manipulated the sand for 60 seconds, and rinse water was analysed for *E. coli* and coliphage. *E. coli* densities transferred were correlated with density in sand rather than surface area of an individual's hand, and the amount of coliphage transferred from seeded sand was different among individuals. In sequential rinsing, percentage reduction was 92% for *E. coli* and 98% for coliphage. Using dose-response estimates developed for swimming water, it was determined that the number of individuals per thousand that would develop gastrointestinal symptoms would be 11 if all *E. coli* on the fingertip were ingested or 33 if all *E. coli* on the hand were ingested. These results suggest that beach sand may be an important medium for microbial exposure; bacteria transfer is related to initial concentration in the sand; and rinsing may be effective in limiting oral exposure to sand-borne microbes of human concern.

Key words | beach sand, coliphage, *Escherichia coli*, hand to mouth transfer, recreational waters, risk assessment

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INTRODUCTION

Freshwater and marine beach sand often contains appreciable amounts of fecal indicator bacteria (Ghinsberg *et al.* 1994; Whitman *et al.* 1994; Alm *et al.* 2003; Whitman & Nevers 2003; Yamahara *et al.* 2007). Fecal indicator bacteria elutriated from beach sand often exceed the amount in an equivalent volume of adjacent lake water regardless of whether comparisons are made between nearshore lake water concentration and pore water, bulk wet, or dry weight sand (Whitman & Nevers 2003). The relationship between the concentration of fecal indicator bacteria in beach water and the potential risk of exposure to pathogens has been well studied (Van Donsel & Geldreich 1971; Burton *et al.* 1987; Bolton *et al.* 1999; Elmanama *et al.* 2005; Ishii *et al.* 2007). Fewer studies have correlated enteric viral occurrence in beach water and risk of exposure to pathogens. Only pilot studies have been conducted (Heaney *et al.* 2006; Bonilla *et al.* 2007) that relate the concentration of fecal indicator bacteria and viruses in beach sand to risk of exposure to illness (Calderon *et al.* 1991; Kueh *et al.* 1995), despite the potential close association of shoreline recreational contact and high-risk groups such as children, the elderly and immuno-compromised individuals.

Epidemiological studies have related fecal indicator bacteria levels and viruses to beach contamination and

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exposure through beach activities (e.g. swimming, water sports) (Wade *et al.* 2006). Exposure is assumed to be predominantly through the oral route, with waterborne pathogens ingested by swallowing during swimming activities. For beach sand, estimating dose is problematic. Studies on recreational beach use and sand exposure are limited, and only preliminary epidemiological studies on beach sands have been conducted (Bonilla *et al.* 2007).

Beach sand may provide a more significant risk of gastrointestinal disease than the water. For many beach users, more recreational time is spent on the beach sand than in the water. Time on the beach sand provides opportunities for passive exposure (e.g. through walking, sports, sunbathing) and active exposure (e.g. excavating, burying, throwing, building sand castles) to fecal indicator bacteria-contaminated sand. Additionally, beach closings designed to protect users from adverse effects may actually provide increased opportunity for illness due to gastrointestinal disease, as closing may result in more time spent on the sand where there may be elevated concentrations of fecal indicator bacteria as well. Previous research supports this by identifying a significant correlation between *Escherichia coli* concentrations in foreshore sand and lake water at the study beach (Whitman & Nevers 2003).

Currently, the potential health risk of sand to beach visitors is unknown. In this paper we explore the transferability of two pathogen indicators–*E. coli* and an F^+ coliphage (MS2)–from beach sand to hands as well as the rate of removal through rinsing. The experiments were designed to estimate the amount of fecal indicator bacteria and viral particles that could be accumulated on the hands with active exposure to beach sand and also the potential ingestion rate due to sand–hand–oral contacts. Finally, the effectiveness of rinsing to avoid ingestion and pathogen exposure was explored.

MATERIALS AND METHODS

Site description

Sand used in this study was collected from 63rd Street Beach, which is located on the southern shore of Lake Michigan in Chicago, Illinois. Historically, the beach has frequent swimming advisories due to high *E. coli* concentrations, and the sand contains much higher concentrations of *E. coli* than the beach water. The study area, sample locations and sand characteristics have previously been described in detail (Whitman & Nevers 2003).

Sample collection

Sand samples were collected 2–3 m from the shoreline on three occasions: 11 June, 11 July and 24 July 2007. On 11 June, approximately 5–7 kg of sand was collected for *E. coli* analysis at each of five locations (100 m apart) along the shoreline and placed into sterile buckets. Samples were collected from a 25–30 cm diameter area (10 cm depth) using a sterile garden spade; between locations the spade was sterilized thoroughly with 70% alcohol and rinsed several times with sterile, distilled water. Additionally, a lake water sample was collected in 45-cm deep water adjacent to each of the five locations. On two occasions, 11 and 24 July, sand samples were collected for MS2 coliphage analysis. Five subsamples (approximately 1 kg each) were collected from two locations 2–3 m from the shoreline and were pooled in a sterile bucket (total approximately 11–14 kg). After each sampling occasion, sand was transported to the laboratory on ice and used within 48–72 hours.

Sanitation techniques

Prior to sand rinsing experiments, the sanitizing effect of alcohol-based hand sanitizer (Johnson & Johnson, New Brunswick, New Jersey) on *E. coli* and antimicrobial soap on MS2 coliphage were determined. Hands were cleaned with alcohol-based hand sanitizer or antimicrobial soap, followed by a thorough rinsing with sterile water. Hands were then submerged to the wrist into individual sterile polyethylene bags containing 150 ml (*E. coli* analysis) or 200 ml (MS2 coliphage assay) of phosphate-buffered water (PBW) (pH 6.8) and rinsed for 1 min; the rinse water was analysed for *E. coli* or MS2 coliphage. No *E. coli* or MS2 coliphage were detected in the rinse water. Participants sanitized their hands before the beginning of a test as well as between discrete sand samples. Antimicrobial soap was used for the MS2 coliphage component of the study because soap is a better antiviral than alcohol-based products (Sickbert-Bennett *et al.* 2005).

Sand-to-hand transfer

Escherichia coli

Four individuals, two male (M1, M2) and two female (F1, F2), participated in the *E. coli* sand-to-hand transfer study. Initially, an individual participant manipulated (i.e. dug/kneaded/gently mixed) sand in a large container for 60 s. Each hand (left and right) was then submerged to the wrist into individual sterile polyethylene bags containing 100 ml of PBW and rinsed for 60 s. Hand rinsing resulted in virtually all of the sand and debris being removed from the hand, as determined by sight. This procedure was repeated by each study participant for sand collected at each beach location. Appropriate volumes of resulting rinse water (40 samples) were analysed for *E. coli*. A representative sub-sample of sand from each location was analysed for background *E. coli* density.

MS2 coliphage

Initial analysis of the sand revealed a non-detectible level of MS2 coliphage; sand was subsequently spiked to establish a concentration of 100,000 MS2 coliphage particles/g sand. The initial cell concentration of a laboratory MS2 coliphage titre was determined by preparation and analysis of serial dilutions. A series of sterile polyethylene bags containing 300 g of sand was inoculated with the appropriate dilution and volume of titre; each sand sample was then gently but thoroughly mixed for even distribution.

Six subjects, three male (M1, M2 and M3) and three female (F1, F2 and F3), took part in the MS2 coliphage component of the study. Initially, an individual submerged one hand to the wrist into a polyethylene bag containing MS2 coliphage spiked sand; another participant held the bag in place and manipulated the sand onto the hand surface for 60 s. The hand was then submerged to the wrist in a sterile polyethylene bag containing 200 ml of PBW and rinsed for 60 s. The procedure was repeated with four additional bags; the right and left hands were alternated between replicate samples to avoid overwashing. Resulting rinse water (30 samples) was analysed. Controls ((1) uninoculated sand and (2) inoculated but unmanipulated sand) were also analysed.

Sequential rinsing

This experiment examined the reduction of residual sand-borne *E. coli* and MS2 coliphage densities on an individual's hand through the course of sequential rinses. For *E. coli*, sand from location 1 was used. Again, individuals manipulated sand for 60 s. After the right hand was initially submerged and rinsed in a PBW-filled polyethylene bag, it was sequentially submerged and rinsed for 60 s in a series of polyethylene bags containing 100 ml of PBW. Both experiments were performed by five study participants (F1, F2, M1, M2 and M3); participant F1 performed four sequential rinses, while the remaining participants performed two sequential rinses.

Sample analysis

For determining background *E. coli* and MS2, each sand sample was well homogenized, then 100 (*E. coli*) or 300 (MS2 coliphage) grams of sand was added to a sterile 500-ml bottle or sterile polyethylene bag, followed by 200 ml of PBW. The mixture was shaken for 2 min, and appropriate volumes were used for analysis of *E. coli* or MS2 coliphage. *E. coli* samples were elutriated and analysed using Colilert-18 system (IDEXX, Inc., Westbrook, Maine). Rinse water was also analysed using Colilert-18. Densities rinsed into water from hand transfer experiments are expressed as most probable number (MPN)/cm^2 of the hand; and densities for lake water are expressed as MPN/100 ml. Elutriated MS2 coliphage were analysed according to EPA method 1602 (US EPA 2001) using host *E. coli* F_{amp}; concentrations rinsed into water from hand transfer experiments are expressed as plaque forming units (PFU)/cm^2 of the hand; concentrations for beach sand are expressed as PFU/volume sand.

In order to express organismal densities per cm^2 of the hand, the total hand surface was covered with duct tape, and area was calculated using a piece of duct tape with a known area and mass. *E. coli* concentration recovered from the rinse water for each study participant was divided by their hand surface area, resulting in the unit of MPN/cm^2. The surface area of the index finger and thumb were calculated by multiplying the circumference (cm)

and length (cm) of each participant's digit, assuming a cylinder, while the entire hand was estimated by weighing tape coverage.

Statistical analysis

Statistical analyses were performed using SPSS, version 12.0 (SPSS 2003). For *E. coli* data, statistical procedures were performed on $\log_{10}$-transformed data to meet parametric assumptions of normality. There was no significant difference between *E. coli* densities of participants' left and right hands; therefore, the arithmetic mean was used in further analyses.

RESULTS

Transfer of *E. coli* and MS2 coliphage

Background *E. coli* densities in sand collected from the five locations were variable, ranging from 3.52 to 5.27 (log MPN/100 g). Sand with the highest *E. coli* densities were at locations 1 (5.22) and 3 (5.27), and location 2 had the lowest *E. coli* density (3.52). *E. coli* densities in water samples collected adjacent to the five locations were lower than in sand, ranging from 1.24 to 2.34 (log MPN/100 ml). The highest and lowest *E. coli* densities were recovered from lake water collected at locations 5 and 2, respectively.

Mean *E. coli* densities (log MPN/cm^2 ± SE) transferred from the sand to study participants' hands for each location were 0.99 ± 0.06 (location 1), 0.37 ± 0.07 (location 2), 1.84 ± 0.32 (location 3), 0.47 ± 0.12 (location 4), and 0.53 ± 0.10 (location 5). *E. coli* densities transferred to participants' hands were not correlated with hand surface area but rather with the background *E. coli* densities of the sand at each location ($N = 20$, Pearson $R = 0.774$, $P < 0.0001$); matching the pattern of background sand densities, more *E. coli* were recovered from hand rinses for locations 1 and 3, significantly more at location 3. The minimum and maximum amount of *E. coli* transferred from the beach sand to the rinse water was calculated for components of the hand based on surface area: 0.48 and 2.45 (log MPN/cm^2 ± SE) cells for the fingertip, 2.06 and 4.07 for the index finger and thumb, and 2.50 and 4.50 for the palm.

For sand seeded with 10^5 MS2 phage particles/300 g sand, 1.5 (±0.06) to 2.1 (±0.05) PFU/cm^2 (±SE) were recovered from study participants' hands. For all five replicate samples, there was high variability in MS2 coliphage counts (33.74 ± 4.87 to 119.44 ± 14.33 mean PFU/cm^2 ± SE). Transfer of MS2 coliphage was significantly higher for participants M1 and F3 than for the remaining study participants ($P < 0.05$).

Sequential rinsing

Sequential rinsing resulted in substantial decreases in *E. coli* densities for all study participants. Initial concentration was obtained by rinsing the beach sand (collected at location 1) from each participant's hands after sand manipulation; this resulted in a mean of 1.03 ± 0.08 log MPN/cm^2 (range = 0.77 to 1.25) transferred from the hand to the rinse water. In the first subsequent rinse, *E. coli* concentration decreased by 86.4 to 97.5% from the initial concentration, with an overall mean of 91.8%. Two rinses resulted in a decrease of 92.9 to 99.9% from the initial concentration, with an overall mean of 96.8%. For person F1, *E. coli* decreased by 98.5% after three rinses and 99.4% after four rinses (Table 1).

MS2 coliphage was undetectable in the sand collected from the beach, so for sequential rinsing, 1.56×10^6 PFU were added to 300 g of sand. Sand was elutriated, and 5×10^5 MS2 PFU/300 g were recovered from the inoculated sand (32%). Initial recovery concentration as obtained by rinsing each participant's hand after sand manipulation resulted in a mean of 1.96 ± 0.09 log PFU/cm^2 (range = 1.64 to 2.20) transferred from the hand to the rinse water. During sequential rinsing, MS2 coliphage counts decreased by 96.4 to 98.9% after the first rinse and by 99.6 to >99.9%, after the second rinse (Table 1).

DISCUSSION

Recreational beach sand is not monitored for indicator bacteria or associated pathogens. Although *E. coli* concentrations in sand often far exceed the regulatory standard established for water, based on comparable volumes, studies have not directly linked *E. coli* in sand with ingestion

Table 1 | Sequential rinsing of hands exposed to *E. coli* or MS2 coliphage. Results indicated that a single aqueous rinse removed a large proportion of both, and four rinses removed almost all *E. coli* or MS2 coliphage

Rinse number	MS2 coliphage Mean % reduction	Range reduction (%)	*E. coli* Mean % reduction	Range reduction (%)
1	98.3	96.4–98.9	91.8	86.4–97.5
2	98.3	99.6–100	96.8	92.9–99.9
3	99.9	99.9	98.5	98.5
4	>99.9	>99.9	99.4	99.4

and potential risk of illness from sand-borne pathogens. Preliminary epidemiological studies suggest that more time spent in the sand is associated with increased gastrointestinal illness (Bonilla *et al.* 2007). Beach use for recreational activities is not limited to the water; however, contact with beach sand is inevitable, and both passive and active ingestion of sand are likely.

Using the guidelines for health effects water criteria for *E. coli* developed by the USEPA, we compared illness rates and consumption of *E. coli* in water with our sand results. In epidemiological studies, it was determined that eight individuals would develop illness after swimming in water with a geometric mean of 126 *E. coli*/100 ml (Dufour 1984; US EPA 1986). Further, it has been determined that in any given swimming activity, an adult individual will swallow an average of 16 ml of water (Dufour *et al.* 2006). Given this dose-response relationship (ingesting 20 ml of water with a geometric mean concentration of 126 *E. coli*/100 ml results in 8 illnesses per 1,000 individuals), we determined that ingesting the amount of sand and *E. coli* on an individual's fingertip, using the sand in this study, would result in 11 individuals per 1,000 developing gastrointestinal symptoms (Figure 1). Ingesting all of the sand and *E. coli* on the hand would result in the rate of 33 per 1,000 individuals with swimming-associated gastrointestinal symptoms. Risk associated with beach activities in sand has not been previously studied in any depth beyond a pilot investigation that did not include microbial density measurements (Heaney *et al.* 2006). In the absence of an epidemiological investigation, surveys of pathogens in sand and rates of ingestion would be useful in determining the need for health guidelines for sand.

The risk of infection for ingested enteric viruses is 10 to 10,000-fold greater than for pathogenic bacteria at similar exposures (Haas *et al.* 1993). Viruses can remain infective on hands for significant periods of time and continue to be transferred from hands and fingers to other surfaces (Boone & Gerba 2007). Viruses also persist longer in the environment than enteric bacteria, and some can remain infectious for up to 100 days in soil at temperatures of 20–30°C (Jiang *et al.* 2001). Discharge of raw or untreated sewage into the lake may result in sewage-associated pathogens accumulating in sand, which could enhance their survival in the environment (see review in WHO 2003). In one study, beach water was sampled down-current from a river outfall into which sewage had been discharged; MS2 coliphage concentrations in the beach water were as high as 297 PFU/100 ml (Byappanahalli *et al.* 2008). In the present study, MS2 coliphage, which resembles many human enteric viruses, attached to the human skin; on the fingertip alone (approximate 2 cm^2), there was an average of 151 PFU; if this were predictive of the amount of pathogenic viruses present, it would suggest a health risk, since as few as 10–100 virions can cause infection (Boone & Gerba 2007). It should be noted that transfer from the sand to hands could be considerably different for naturally

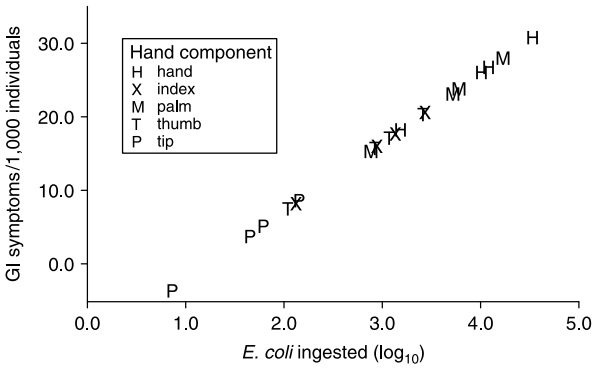

Figure 1 | Number of illnesses associated with ingesting *E. coli* bacteria, as calculated by dose-response relationship established in two studies (Dufour 1984; Dufour *et al.* 2006). Mean amount of *E. coli* accumulated on specific components of individuals' hands are indicated.

occurring MS2 coliphage than for a spiked sample, perhaps because of differential adhesion to the sand and sediment particles.

Hand rinsing results demonstrated that simply rinsing hands before eating or leaving the beach could enhance disease avoidance assuming the beach water was relatively clean. In one study, tap water and non-medicated soap reduced virus titres by 83.6 and 72.5% and bacterial titres by 90 and 68.7%, respectively (Ansari *et al.* 1989); rinsing was even more effective in this study. We presume that this is a consequence of the fact that relatively clean, well-sorted sand is easier to rinse than finer soils and because of the affinity of microbes for clay particles (Wong *et al.* 2008). Children might be advised to rinse repeatedly because of recurrent exposure.

Children are likely to be more vulnerable to sand-borne pathogens; they play in the sand more frequently near areas of greater microbial sources, and children display far more hand-to-mouth activity (Freeman *et al.* 2001), which could potentially lead to more likely exposure to potential pathogens in sand than for adults. More importantly, since children have undeveloped immune systems they are at greater risk for enteric illnesses. Epidemiological studies show that children are more prone to gastrointestinal illness from recreational water contaminated with fecal indicator bacteria than adults (Wade *et al.* 2008).

Beach sand has increasingly been recognized as a source of pathogens, such as *Campylobacter* and *Salmonella* (Obiri-Danso & Jones 2000; Jones 2001). This study represents the first step in determining the necessity for establishing a public health criterion for sand concentrations of fecal indicator bacteria and associated pathogens. More information is needed to assess the risk and variation of illness associated with beach sand, including the types and concentrations of pathogens in beach sand, their survival rates, and *in situ* transferability of pathogens from sand to mouth.

CONCLUSIONS

1. Human pathogen surrogates used in this study–*E. coli* and MS2 coliphage–were transferred to the human skin through contact with contaminated beach sand, and the amount transferred was strongly associated with initial concentration in the sand.
2. More gastrointestinal illness may be associated with sand than with beach water.
3. Both *E. coli* and MS2 coliphage could be rinsed from hands after exposure to the beach sand.

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