Prospective epidemiological pilot study on the morbidity of bathers exposed to tropical recreational waters and sand

Elia E. Sánchez-Nazario, Tasha M. Santiago-Rodriguez and Gary A. Toranzos

ABSTRACT

A prospective cohort epidemiological pilot study was performed at three tropical beaches with point- and non-point-sources of fecal pollution to characterize the risk of illness among swimmers and non-swimmers. There was an increased risk of illness in swimmers as compared to non-swimmers, even when waters met current microbial standards for recreational water quality. Illnesses included gastrointestinal (GI), skin and respiratory symptoms, earache and fever. Odds ratios (ORs) ranged from 0.32 to 42.35 (GI illness), 0.69 to 3.12 (skin infections), 0.71 to 3.21 (respiratory symptoms), 0.52 to 15.32 (earache) and 0.80 to 1.68 (fever), depending on the beach sampled. The indicators that better predicted the risks of symptoms (respiratory) in tropical recreational waters were total (somatic and male-specific) coliphages (OR = 1.56, $p < 0.10$, $R^2 = 3.79\%$) and *Escherichia coli* (OR = 1.38, $p < 0.10$, $R^2 = 1.97\%$). The present study supports the potential of coliphages as good predictors of risks of respiratory illness in tropical recreational waters. This is the first study that has determined risks of illness after exposure to tropical recreational waters with point- and non-point sources of fecal contamination. The results give an opportunity to perform epidemiological studies in tropical recreational waters in Puerto Rico which can include more participants and other indicators and detection techniques.

Key words | coliphages, *Escherichia coli*, thermotolerant coliforms, tropical recreational waters

Elia E. Sánchez-Nazario
Tasha M. Santiago-Rodriguez (corresponding author)
Gary A. Toranzos
Biology Department,
Environmental Microbiology Laboratory,
University of Puerto Rico,
Rio Piedras Campus,
San Juan,
Puerto Rico 00931
E-mail: *tsantiagoro@gmail.com*

INTRODUCTION

Epidemiological studies can help to prevent health-related risks resulting from the exposure to possibly fecally contaminated recreational waters. Three epidemiological study designs have been used to determine the possible risks to bathers: prospective and retrospective cohorts, and randomized trials. The advantages and drawbacks of each experimental design have been reviewed elsewhere (Pruss 1998; Wade *et al.* 2003). Information gathered from epidemiological studies can help to determine the associations of microbial indicators with illnesses exhibited by swimmers and non-swimmers (Cabelli *et al.* 1982; Kueh *et al.* 1995; Prieto *et al.* 2001). However, there is still no consensus on which microbial indicator(s) better predict symptoms after exposure to fecally contaminated recreational waters (Fujioka 1997).

Fecal coliforms, enterococci and *Escherichia coli* have all been proposed as the most appropriate predictors for gastrointestinal (GI) symptoms among swimmers in recreational marine waters (Cabelli *et al.* 1982; Haile *et al.* 1999; Wade *et al.* 2006). Although most pioneer studies focused on culture methods, molecular techniques have also shown promising results. For instance, detection of *Enterococcus* by polymerase chain reaction (PCR) and quantitative PCR (qPCR) have been associated with GI illnesses among bathers in temperate regions (Wade *et al.* 2006, 2010). Predictors of health-related risks are not

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restricted to bacterial indicators, as bacteriophages, such as male-specific coliphages, have also been associated with GI illnesses. However, these associations have been considered cautiously since male-specific coliphages have not been detected regularly when bathers are exposed to waters contaminated by non-point sources of fecal contamination (Colford *et al.* 2007). Associations of microbial indicators with GI illnesses are the most common, but predictors of other health risks, including skin infections have also been identified, with *Staphylococcus* spp. being one of the most promising (Seyfried *et al.* 1985; Cheung *et al.* 1991).

However, an increasing concern with currently used microbial indicators in the USA and other geographical regions is that these may not reliably signal fecal contamination and the possible presence of enteric pathogens in tropical recreational waters (Santiago-Rodriguez *et al.* 2012). Traditional microbial indicators may be part of the environmental microbiota of tropical waters and non-fecal sources may also contribute to their input. Moreover, currently used microbial indicators may replicate outside of their host, especially in tropical regions (Santiago-Mercado & Hazen 1987; Bermudez & Hazen 1988; Rivera *et al.* 1988). Another important issue with traditional indicators is that these usually cannot identify the source of the fecal input (Byappanahalli *et al.* 2003). Identifying the source of fecal pollution is important in order to remediate or eliminate the contamination which could lead to health-related risks. Information gathered from indicators used for microbial source tracking may be used to improve monitoring of microbial water quality in recreational waters with point-and non-point sources of fecal contamination (Shah *et al.* 2011). Thus, there is still the need to determine which microbial indicator(s) may be more appropriate to determine possible risks to public health in tropical regions. In the present study, a preliminary prospective cohort epidemiological study was performed in Puerto Rico, in which the association between risk and the concentrations of traditional microbial indicators (thermotolerant coliforms, *E. coli*, enterococci and *Staphylococcus* spp.) and alternate indicators (coliphages) of fecal pollution was determined in recreational waters and sand.

MATERIALS AND METHODS

Study sites

The pilot study was performed from August 2005 to March 2006 at three beaches located in northeastern Puerto Rico. Studies were performed at weekends (Friday to Sunday) and holidays. Beaches were selected based on the prevalence of microbial indicators in a 2-year period (data not shown) and for convenience will be referred as IVB, BC and LB. These beaches are heavily used throughout the year and are under the Blue Flag program, an international plan to designate beaches as safe for swimmers based on thermotolerant coliforms (200 colony forming units (CFU) /100 mL) and enterococci (35 CFU/100 mL) in water.

Various possible sources of fecal contamination may impact the study beaches. Small creeks (in turn impacted by domestic wastewater treatment plants, septic tanks and animal feces) discharge into IVB and LB. IVB and LB are located 5 to 10 km from a wastewater treatment plant and thus could also be impacted by point-sources of fecal contamination. For BC, the exact source of microbial indicators remains unknown.

Epidemiological design

The study design was a prospective cohort, in which a 'healthy' population is followed for a fixed period of time after a beach visit to observe any illness. Subjects selected as swimmers were asked to participate in the study after their contact with water was visually corroborated. A swimmer was defined as an individual with full-body contact with water, including head immersion. A non-swimmer was defined as an individual without any contact with water. Exclusion criteria included subjects having contact with any waterbody during the week prior to or following the present study. This decreased the potential of misclassification of exposure resulting from multiple contacts with beach water (Cabelli *et al.* 1982).

Subjects were asked to participate and sign an informed consent form. They were interviewed with the use of a validated questionnaire by the Institutional

Review Board of the Centers for Disease Control and Prevention about their age, swimming practices and demographic information. A follow-up telephone interview was performed 8 to 10 days after beach recruitment. Subjects were asked about the occurrence of any of the following symptoms: stomach ache, nausea, vomiting, diarrhea, sore throat, congested nose, runny nose, sneezing, earache, itchy skin, skin rash, headache or fever. Gastroenteritis is the most commonly reported symptom following exposure to fecally contaminated waters. Additional symptoms were included as they may be associated with respiratory or dermal infections or irritations and, in some cases, have been shown to be associated with bathing exposures (Cabelli *et al.* 1979; Haile *et al.* 1999). Subjects were also excluded from the study if they could not be contacted by telephone 8 to 10 days after the beach recruitment.

Bacterial indicators and coliphage detection

Water samples were collected at knee depth, between two sample sites, separated by 200 meters. Sand samples were collected within the drier sand 60 cm above the wave splash zone, between two sample sites, separated by 200 meters. Water and sand samples were collected every 3 h, for 24 h, totaling eight water and sand samples per day. Samples were kept at 4–7 °C and processed within 3 h. One hundred grams of sand samples were eluted in 100 mL of phosphate buffer solution and 0.1% Tween 20. Microbial methods included the membrane filtration technique using MFC agar, mTEC agar, mannitol salt agar and m-Enterococcus agar for the quantification of thermotolerant coliforms, *E. coli*, *Staphylococcus* spp. and enterococci, respectively (Fujioka 1997). Typical thermotolerant coliform, *E. coli* and enterococci colonies were confirmed with lauryl tryptose broth (Difco), urea substrate and sodium azide medium (Difco), respectively. A coagulase test was performed for presumptive *Staphylococcus aureus* yellow colonies growing on mannitol salt agar. Samples were also analyzed for total coliforms and *E. coli* using the Colilert™ system (APHA 1992). The geometric means of the indicators in water and sand samples were calculated for each sampling date and for each sampled beach.

Coliphage concentrations were analyzed in water and sand using the single layer method and *E. coli* C3000 (ATCC 15597) as the host for both F-male specific and somatic coliphages as described previously (Grabow & Coubrough 1986). Briefly, trypticase soy broth (Difco) and agar (0.8%) was mixed with 50 mL of marine water and with 1 mL of a fresh culture of the bacterial host (approximately 1.0×10^8 cells/mL) and poured into Petri dishes. Samples were incubated at 37 ± 0.2 °C and viral plaques were counted at 6, 18 and 24 h. Results were expressed as plaque forming units (PFU) per 100 mL of water.

Statistical analyses

Indicator concentrations per sampling date were calculated using the Minitab Statistical Program (Version 12.2). Information gathered from the interviews was recorded as categorical variables. Summary statistics were calculated for quantitative variables, while frequency distributions were obtained for qualitative variables. Normality of health and microbiological data were evaluated using the Shapiro–Wilk statistic. Data were log-transformed prior to the regression analyses. Logistic regression models (multiple regression analyses) were performed using the SAS Statistical Program (Cary, NC, USA), after the appropriate adjustments by variables including sex, age and beach. Risk analyses were performed using the odds ratio (OR) statistic (Gordis 2000) at the 95% confidence level.

Regression analyses were performed with the daily geometric mean of the indicator, in which the OR is the risk for each log increase in the indicator's concentration. In the logistic regression analyses the log n value of the OR equals the regression coefficient. The square of the regression coefficient equals the variation in the prevalence rates of symptoms that is explained by the indicator concentration in water. To observe the relation of the prevalence of symptoms among swimmers and the concentrations of indicators in all beaches, a logistic multivariate regression analysis was performed. This analysis is performed when a categorical variable is included in a model and when the distribution of the variable is not normal. All regression models were adjusted by age, sex and by beach variability.

RESULTS

Recruitment and enrollment

A total of 705 subjects were recruited. Sixty-four were excluded because of contact with a water body the week before ($n = 13$; 20%) or after the recruitment ($n = 10$; 16%), incorrect ($n = 23$; 37%) or out of service ($n = 19$; 30%) telephone numbers. A total of 641 subjects successfully completed the questionnaires (response rate of 90.1%); 64% of the subjects were females and and 36% males. A high percentage of recruited subjects (94.4%) were residents of Puerto Rico and the others were visitors from continental USA. Of the 641 recruited subjects, 552 were selected as swimmers and 89 as non-swimmers, based on the visual confirmation of their appearance (Table 1). Results showed that 60.2% of the subjects had their body and heads wet at the moment of recruitment and 6.1% were dressed.

Table 1 | Quantitative variables of the subjects in the epidemiological study

Variable	No. (%)
Number of recruited subjects	641 (90.9)
Swimmers	552 (86.1)
Non-swimmers	89 (13.9)
Females	413 (64.4)
Male	228 (35.6)
Swimmers' mean time in water (min)	33 ± 35.2
Number of subjects from Puerto Rico	605 (94.4)
Number of subjects from USA	36 (5.6)

Microbial water quality

Indicator concentrations in water samples are shown in Table 2. Presumptive *Staphylococcus* spp. showed the highest density per volume, while the thermotolerant coliforms and enterococci did not exceed current standards for recreational waters (200 and 33 CFU/100 mL, respectively). Environmental variables including temperature, pH and total dissolved solids were measured in this study, but none were significantly related to indicator concentrations in water or sand (data not shown). For this reason, no further analyses were performed with the physico-chemical parameters. Statistics on indicator concentrations in sand samples are also shown in Table 2. *E. coli* concentrations were higher in sand than in water when analyzed by Colilert and MF. Enterococci concentrations were also higher in sand than in the water samples.

Health statistics

Incidence proportion rates (proportion of symptoms among those interviewed) were calculated as: (no. of individuals that reported a symptom at follow up interview/no. of swimmers interviewed that day) × 1,000.

Results of the incidence proportion rates of GI, respiratory, skin and other symptoms by beach sampled are shown in Table 3. Rates of over 19 represent a risk of illness for swimmers (Stone *et al.* 2008). In the present study, incidence proportion rates vary according to the beach sampled. Results of the risk analysis in all beaches are shown in Table 4. Significant ORs were mainly observed at BC and

Table 2 | Descriptive statistics on indicator's geometric mean (GM) and standard deviation (SD) in water ($n = 50$) and in sand ($n = 50$). Results show CFU/100 mL or 100 g of sand

	GM ± SD		Range	
Indicator	Water	Sand	Water	Sand
Coliphages	220 ± 221.8	80 ± 179.2	4–468	2–876
Total coliforms (Colilert)	445 ± 548.7	436 ± 390.1	0–2419	0–1011
E. coli (Colilert)	51 ± 81.3	130 ± 108.2	4–403	3–344
Staphylococcus spp.	100,440 ± 23,957	27,209 ± 35,750	0–1,410,000	1200–127,000
Thermotolerant coliforms	16 ± 22.9	13 ± 21.2	1–110	0–71
E. coli (mTEC)	12 ± 30.4	145 ± 227.9	1–200	0–1011
Enterococci	39 ± 67.5	97 ± 142.7	1–378	4–800

Table 3 | Incidence proportion rates among swimmers × 1,000 population for all illness by beach

Beach (no of swimmers per beach)	GI	Respiratory	Skin	Earache	Headache	Fever
BC (131)	203.6	598.0	112.0	13.7	94.2	38.2
IVB (220)	36.4	157.6	28.8	1.7	25.8	21.2
LB (201)	49.8	315.9	32.3	4.0	52.2	14.9

Table 4 | Illness rates in swimmers (S) and non-swimmers (NS) and OR by beach. Symptoms include GI and respiratory illnesses, skin infections and ear and headache

		IVB ($n = 220$)			LB ($n = 201$)			BC ($n = 131$)		
Symptoms		Ill	Not ill	OR	Ill	Not ill	OR	Ill	Not ill	OR
GI	S	24	196	1.96[a]	20	181	0.32	80	51	42.35[a]
	NS	2	32		7	20		1	27	
Respiratory	S	104	116	0.71	127	74	2.15[a]	109	22	3.21[a]
	NS	19	15		12	15		17	11	
Skin	S	19	201	3.12[a]	13	188	1.80[a]	10	121	0.69
	NS	1	33		1	26		3	25	
Earache	S	5	215	0.79	8	193	0.52	14	117	15.32[a]
	NS	1	34		2	25		1	128	
Headache	S	17	203	2.76[a]	21	180	3.03[a]	37	94	5.12[a]
	NS	1	33		1	26		2	26	
Fever	S	14	206	2.24[a]	6	195	0.80	15	116	1.68[a]
	NS	1	33		1	26		2	26	

S = swimmers; NS = non-swimmers.
[a]Significant OR based on the 95%CI.

swimmers are at risk for most of the symptoms. Interestingly, this beach had the highest geometric means of *E. coli*, enterococci and coliphages. These indicators are shown to be good predictors of GI symptoms in other epidemiological studies, based on a linear dose-response relation of illness and indicators concentrations in water (Fattal *et al.* 1986). Although this correlation was not statistically significant, a Pearson's correlation analysis showed a positive association in the prevalence of GI symptoms and coliphages in waters.

A non-linear relation was found between indicators and the daily geometric mean of indicators by beach (Figure 1). A significant linear dose–response relation between an indicator and the OR or the incidence proportion rate is needed to recommend an indicator of illness in water (Cabelli *et al.* 1979, 1982). A total of 249 (45%) swimmers reported at least one symptom 8 to 10 days after the beach recruitment. Congested nose (15%) and sneezing (12%) were the highest reported symptoms among swimmers, followed by sore throat (12%) and runny nose (11%). The case rate of the

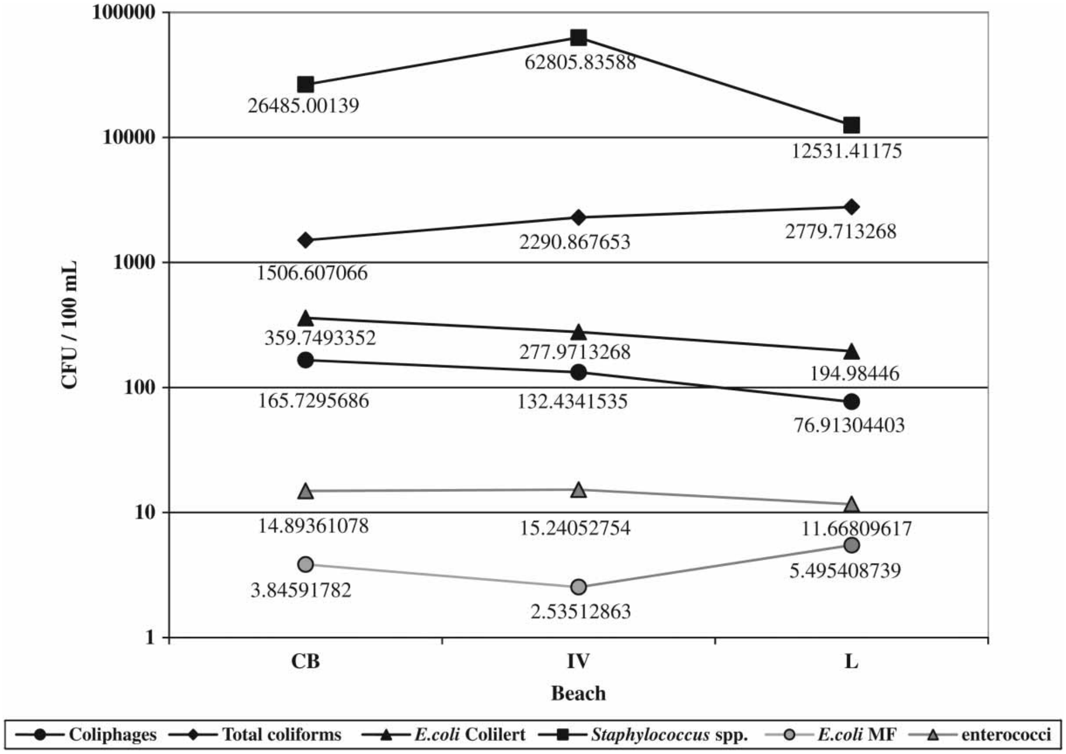

Figure 1 | Daily geometric mean of indicators by beach. Results show a non-linear relation between the coliphages, total coliforms, *E. coli* (Colilert), *E. coli* (MF), *Staphylococcus* spp. and enterococci.

GI symptoms was lower (from 1 to 9%). We found an OR higher than 1 in all symptoms, but respiratory symptoms and skin symptoms were statistically significant with a confidence level of 95%. These OR values were adjusted by beach, age and sex. Statistics on the OR by group of symptoms are shown in Table 5. A higher prevalence of symptoms was observed in subjects from 10 to 30 years old (Figure 2), but the distribution of symptoms among the recruited subjects was similar to the age distribution in the sample. Eighty-five percent of people reporting symptoms were 22 years old (Figure 2). There was a slight increase in the reported symptoms in male (43.9%) when compared with females (42.9%), but this difference was not statistically significant. In addition, although there was no statistically significant relationship of indicator concentrations and time of the day, the trend of the reported symptoms was significant with the time of the day the swimmers had contact with seawater. Specifically, a higher number of swimmers reported symptoms at 12 pm (115 swimmers), followed by 10 am (95 swimmers), 3 pm (34 swimmers) and 8 am (eight swimmers).

The OR and the R^2 values of regression analyses between the daily geometric mean of indicators and the prevalence of symptoms are shown in Table 6. In this continuous analysis, respiratory illness was significantly associated with *E. coli* detected by Colilert ($OR = 1.4$, $p = 0.06$) and coliphages ($OR = 1.6$, $p = 0.1$). No other statistically significant correlations were noted, although several OR were >1.0.

In the first categorical water quality model, each daily average exposure was divided into quartiles. The OR is related or referenced to non-swimmers with zero minutes in water for each daily quartile of indicator in water. Two of the indicators that may better predict the risk of GI symptoms were the coliphages and *E. coli,* as shown by the OR values higher than 1. The categorical models performed for coliphages are shown in Table 7. The exposure variable means a 25% increase in coliphage concentration that is related to an increased risk of GI illness in swimmers. Although not statistically significant, GI illnesses seemed to increase with an increase in coliphage concentrations in water in the first, third and fourth quartiles. *E. coli*, detected

Table 5 | Number of sub-symptoms reported by swimmers and non-swimmers and adjusted OR* for all beaches

Symptoms	No. of swimmers/non-swimmers that reported the symptom	OR (CI 95%)
Overall GI symptoms	76/10	1.61* (0.73–3.55)
Stomach ache	22/2	
Vomiting	11/4	
Nausea	23/1	
Diarrhea	20/3	
Overall respiratory symptoms	466/50	1.43* (1.93–2.22)
Sore throat	96/10	
Cough	47/8	
Congested nose	116/11	
Runny nose	87/10	
Sneezing	93/9	
Overall skin symptoms	80/5	1.75* (1.05–3.60)
Rash	32/1	
Itchy skin	48/4	
Headache	75/6	2.18 (0.92–5.16)
Earache	27/2	1.08 (0.42–2.78)
Fever	35/4	1.44 (0.49–4.15)

Results are adjusted by beach, age and sex. No. of swimmers = 552; no. of non-swimmers = 89.

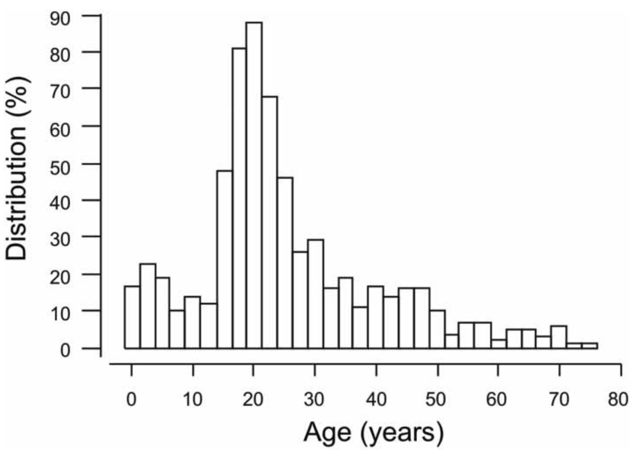

Figure 2 | Distribution of subjects (%) that reported symptoms (by age).

by Colilert, showed a similar trend when related to the GI symptoms (OR = 1.9, 0.8, 1.2 and 1.8 for first, second, third and fourth quartiles, respectively). Similarly, *Staphylococcus* spp. were related to an increased risk in skin symptoms, as seen in the first, second, third and fourth quartile (OR = 1.9, 0.4, 1.5 and 2.1, respectively). In terms of the coliphages, the risk of respiratory symptoms was significantly increased in water with an increase from 345 to 846 PFU per 100 mL. In this categorical analysis, the risk of having any of the symptoms screened showed a similar trend (a significant OR in only three of the quartiles). The risk of any symptom was better predicted by coliphage concentrations in water as shown in Table 7.

DISCUSSION

This is among the first epidemiological pilot studies to identify the risk(s) of illness among swimmers in tropical recreational waters with point- and/or non-point-sources of fecal contamination. The present prospective epidemiological pilot study showed a high risk of symptoms in swimmers when compared to non-swimmers. Not all water samples exceeded *E. coli* and enterococci concentrations as stated by the Puerto Rico Environmental Quality Board (PREQB) standards. Our epidemiological pilot study shows that there is still a risk for swimmers even when water samples met these criteria. Similar outcomes have been reported elsewhere (Haile *et al.* 1999), suggesting that microbial standards should be carefully considered, at least in tropical regions (Santiago-Rodriguez *et al.* 2012). More interesting, perhaps, is that the number of swimmers that reported any symptom was the highest at 12 pm, the time of day when the number of swimmers is usually one of the highest, at least at beaches in Puerto Rico. It is possible that the high number of swimmers at midday contributes to a higher number of microbial indicators and pathogens. Our study is not consistent with another study which found that the number of swimmers that reported any symptom was the highest in the morning (Colford *et al.* 2012). A possible explanation for this is that sunlight inactivates microbial indicators and pathogens in water. During the morning, the effect of sunlight in inactivating microorganisms is less. This may have to be evaluated in

Table 6 | Logistic regression analysis (continuous model)*. Results show the correlation of traditional indicators and coliphages and GI, respiratory illnesses, skin infections and earaches

Indicators	GI OR	R^2 (%)	Respiratory OR	% R^2	Skin OR	% R^2	Earache OR	% R^2
Coliphages	0.7	1.6	1.6[a]	3.8	0.8	1.1	2.3	13.6
E. coli	0.8	0.6	0.9	0.1	3.5	29.7	0.1	0.9
Staphylococcus spp.	1.1	0.1	1.2	0.8	1.0	0.0004	1.0	0.008
Total coliforms (Colilert)	0.9	0.4	1.2	0.5	0.8	0.5	1.1	0.07
E. coli (Colilert)	0.9	0.1	1.4[a]	2.0	0.9	0.5	1.3	1.5
Enterococci	0.5	9.1	1.0	0.006	0.6	5.5	1.0	0.04

[a]Significant OR 95% CI, with a p-value < 0.1.

Table 7 | Risk of GI symptoms vs. $\log_{10}$ of coliphages in water. Results are divided into quartiles, adjusted by sex, age, and beach variability

OR (95% CI/*p*-value)

	GI	Respiratory	Any symptom
First quartile	2.0	1.3	2.0**
X = 59	0.8 (5.0/0.1)	0.8 (2.1/0.3)	1.2 (3.3/0.01)
Second quartile	0.9	1.0	1.3
X = 120	0.2 (3.0/0.8)	0.5 (2.1/1.0)	0.7 (2.2/0.4)
Third quartile	1.2	1.7	1.4
X = 345.5	0.4 (3.4/ 0.7)	0.9 (2.9/0.1)	0.9 (2.4/0.2)
Fourth quartile	1.8	2.0*	2.0*
X = 868	0.6 (5.1/0.3)	1.0 (3.9/0.04)	1.1 (3.5/0.02)

Statistically significant OR: * $p < 0.05$; ** $p < 0.01$.

parallel with future epidemiological studies in tropical waters in Puerto Rico.

The present pilot study is also among the few performed regarding indicator bacteria in tropical sand and their possible association with health risks. One concern is that the presence of microbial indicators in beach sand shows that non-swimmers, as well as swimmers, may be exposed to enteric microorganisms (Yamahara *et al.* 2012). In the present study, higher concentrations of *E. coli* (as analyzed by Colilert) and enterococci were detected in the sand samples. These results are consistent with studies performed on beaches in Hawaii and California, where higher levels of indicator bacteria were detected in sand (Yan *et al.* 2011). Although further studies are still needed, it remains feasible that traditional indicators may replicate in sand. If this is the case for sand in proximity to the splash zone, it is possible that their presence in the water samples may not be necessarily reflecting a fecal input (Hartz *et al.* 2008). Thus, it is possible that the presence of microbial indicators in water in contact with sand may not predict risks of illness. Since *E. coli* and enterococci were detected in higher concentrations in sand as compared to water samples, it is feasible that indicators found in water may be resuspended from sand.

A non-linear trend between illness and the microbial indicators in water was noted in the present study. This suggests that the presence of *E. coli* and enterococci in water may not be suitable for predicting risks of illness in Puerto Rico. However, the non-linear trend may also be due to the small sample size as compared to other studies with >10,000 subjects. Studies performed in the USA, Hong Kong and Egypt recruited 26,000, 18,000 and 23,000 subjects, respectively (Cabelli *et al.* 1982; Fattal *et al.* 1986; Kueh *et al.* 1995), and found a significant linear trend between the prevalence rates of GI illness and fecal indicators. Thus, a larger sample size may result in a better linear relationship among prevalence rates and indicators; although, in the present study, the risk of symptoms among swimmers was well characterized by OR values higher than 1 and by the statistically significant confidence interval (CI) values. Similar small epidemiological studies with <3,000 subjects, such as those performed in Australia (Corbett *et al.* 1993), Israel (Fattal *et al.* 1986) and South Africa (von Schirnding *et al.* 1992; von Schirnding & Yach 1992), did not find a significant linear relation of GI illness and microbial indicators in marine water. In these studies, it was suggested that a lack of significance may have been due to the uncertain sources of fecal contamination. The non-sewage sources of fecal indicators in the sampled beaches may have an effect in the linear regressions required for a dose response analysis (von Schirnding et al. 1992; von Schirnding & Yach 1992). Similar outcomes have been reported recently, in which the presence of traditional indicators in sand correlates with increased health risks (Heaney et al. 2012).

In the analyses by beach, although no statistically significant associations were noted, coliphages exhibited the highest correlation coefficient with GI symptoms. The risk of illness was increased in the 3rd and 4th quartiles, showing that there may be a risk related to an increase of coliphages in water for each 25% increase in their concentrations. Our results are comparable with a study in California, with 22,085 subjects, which showed an increase in the risk of respiratory symptoms with an increase in viruses in waters (Haile et al. 1999). In studies performed in Miami, F-specific coliphages also associated with increased risks of GI symptoms (Abdelzaher et al. 2011). In the present study, coliphages were related to an increased risk in GI symptoms, but in the continuous analyses, coliphages were more related to respiratory symptoms and earache. Similar studies in California suggested that male-specific coliphages were a good indicator of GI illness, respiratory symptoms, skin infections, to mention a few; however, one of the major drawbacks of coliphages is these are not detected regularly in recreational waters (Colford et al. 2007).

CONCLUSIONS

This is among the first prospective epidemiological pilot studies to identify the risk(s) of illness among swimmers in tropical recreational waters. Compliance with Puerto Rico Environmental Quality Board standards is highly dependent on the method of water analysis. Yet, current standards may not necessarily protect swimmers from respiratory and skin symptoms. Even in waters meeting the Puerto Rico Environmental Quality Board water quality standards, there was an increased risk of symptoms in swimmers when compared to non-swimmers. Results support the potential of coliphages as good predictors of risk of illness in swimmers in tropical recreational waters, but this should certainly be confirmed in other tropical regions. Notably, this is a pilot study that opens the opportunity to perform similar epidemiological studies with more subjects and other indicators and techniques in tropical recreational waters.

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