Report as of FY2007 for 2006VI78B: "A prospective epidemiological study on the morbidity of bathers exposed to coastal waters in the Caribbean"

Publications

- unclassified:
  - A manuscript based on the date obtained has been prepared and is going through a friendly review. It is expected that the final manuscript will be sent to the Journal of Water and Health, an internationally renowned journal. A second manuscript has been sent for review on the data obtained during the first phase of this project and we are waiting for comments. In addition, several presentations were given at local, national and international meetings.

Report Follows

Problem and Research Objectives

Epidemiological studies in recreational waters have attempted to characterize risks of illness among swimmers as compared to non-swimmers (Cabelli et al. 1982; Kueh et al 1995; Prieto et al. 2001). Studies on this topic are aimed to determine whether the indicators present in water can predict this risk of illness to protect swimmers' health. However, some discrepancies occur in the results obtained after carrying out epidemiological studies to establish the possible relation between bathing and certain infections. There is no agreement as to which indicators are better at predicting the risk of symptoms (Hazen 1988; Fujioka 2002). Some studies found fecal coliforms, enterococci and *E. coli* to be the best predictors of gastrointestinal (GI) symptoms among swimmers in marine waters (Cabelli et al. 1982; Haile et al.1999; Wade et al. 2006).

Others found significant correlations between the prevalence of respiratory and skin symptoms and *Manitol + halophiles* in water (Seyfried et al.1985; Calderon et al. 1991; Cheung et al.1991; Fleisher et al.1998). In addition, some of the bacterial indicators used in the previous studies that predicted the risk of illness associated to recreational water contact can be part of the microbiota of tropical areas (Bermúdez et al. 1988; Rivera et al. 1988; Santiago-Mercado et al. 1987; Hardina et al. 1991). Therefore, the indicator that best predicts this risk in tropical areas is unknown.

The high survival rates of indicators in water and in sand in tropical areas of the world raise the question as to whether these indicators represent recent fecal pollution and whether the indicators are present in the absence of pathogens that may be the etiological agents of diseases (CDC 12001-2002, WHO 2003). To find a good indicator of fecal pollution in tropical areas is an important task to achieve to better protect the health of beach visitors. The latter can only be determined by an epidemiological study.
In the current study, a prospective cohort epidemiological study in the island of Puerto Rico was carried out. A population of beach goers, composed of exposed and non-exposed to bathing waters was followed for a set time to determine the prevalence of symptoms.

Because previous studies have not consider the uniqueness of tropical microbiota, at this moment it is impossible to make sure that those indicators of risk that are protective in other areas of the world work as such under tropical conditions. The objectives of this study were to

- obtain data on the risk of illness that beach visitors may have and to
- determine the correlation between risk and the concentrations of old and new indicators of fecal pollution in water and sand.

Methodology and Principal Findings

Sampling and Epidemiological Methods

Beaches were selected for the study based on the indicator concentrations detected in a two-year period (Sánchez et al. 2006) and because these beaches have a high density of swimmers throughout the year. Subjects selected as swimmers were asked to participate in the study after visual corroboration of their contact with water. A swimmer was defined as anyone with full body contact with water including head immersion (had minutes inside water and had the head or body wet). A non-swimmer was defined as anyone at the beach during the sampling date without any contact with beach water.

The study design was a prospective cohort. For this purpose, we selected swimmers and non-swimmers and then separated subjects based on whether they developed symptoms. Exclusion criteria included subjects having contact with any body of water the week before the study and subjects who completed the beach questionnaire, but had contact with any recreational water body the week after the beach study. This decreased the confounding effect of beach to beach and day to day variability in pollution levels on the illness-pollution relation observed (Cabelli et al. 1982).

Subjects were asked to participate and signed an informed consent form. They were interviewed with the use of an institutional review board (IRB) validated questionnaire about their age, swimming practices, and demographic information. A second (telephone) follow-up interview was performed 8 to 10 days after recruitment. In the latter interview, subjects were asked about the occurrence of any of the following symptoms: stomachache, nausea, vomiting, diarrhea, sore throat, congested nose, runny nose, sneezing, ear ache, itchy skin, skin-rash, headache or fever. These symptoms are the ones more likely to be experienced by bathers after exposure to fecal polluted waters (CDC 2001-2002; WHO 2003; Cabelli et al. 1979; Haile et al.1999). Subjects were also excluded from the study if they did not answer the telephone interview. This reduced the bias of misreporting symptoms.

Collection and analyses of samples for bacterial indicators

Microbial Methods

Previous studies indicated there was no statistically significance in the indicator concentrations within water depths (Sánchez et al. 2006) in the beaches sampled, samples were taken at knee depth at two points (points A and B) within 200 meters wide and these two samples were taken every 3 hours, for a total of 8 water samples per sampling day.
Microbial laboratory methods of water analyses include the membrane filtration technique using MFC, mTEC, and m-Enterococcus for the quantification of thermotolerant coliforms, *Escherichia coli*, and enterococci, Mannitol Salt Agar (MSA) was used to determine total number of manitol + halophilic bacteria (Fujioka 2002). Characteristic colonies were confirmed with lauryl tryptose broth (LTB) for

thermotolerant coliforms, urea substrate for *E. coli*, with Sodium Azide for enterococci. In addition, a coagulase test was performed to yellow colonies on MSA agar for the possible presence of pathogenic strains of *Staphylococcus aureus*. In addition, samples were analyzed for total coliforms and *E. coli* using the Colilert ™ enzymatic method. Methods of analysis of these samples are described elsewhere (APHA 1999). The geometric means of the indicators in water were calculated for each sampling date and for each beach.

Sand samples were collected two feet away from the wave splash zone at two points of 200 meters apart, every three hours. The geometric means of the indicators in these samples were also calculated.

Coliphage concentrations were analyzed in water and in sand by the single layer method using the *E. coli* C3000 (ATCC # 15597) host for both, male and somatic coliphages as described by Grabow et al. (1986). Briefly, sterile soft Tryptic Soy Agar (0.8 % TSA) was prepared, mixed with 50 mL of marine water and with 1 mL of the host (24 h culture, 1.0×10^8 cells/mL) *E. coli* and poured into 5 sterile disposable petri dishes (100 x 15mm). Samples were incubated at 37 ± 0.2°C and counted at 6, 18 and 24 hours. Results were expressed in plaque forming units / 100 mL of water.

Statistical Analyses

Indicator concentrations in water and in sand were tabulated in a database and the geometric means of the overall indicators' concentrations per sampling date were calculated using the Minitab Statistical Program (Version 12.2). Information gathered from the interviews was recorded in categorical variables. Summary statistics were calculated for quantitative variables, while frequency distributions were obtained for qualitative variables. Normality of data was evaluated using the Shapiro-Wilk statistic (Zar 1999).

Since the distribution of data was skewed, a $\log_{10}$ transformation was performed before performing regression analyses. Logistic regression models (multiple regression analyses) were performed using the SAS Statistical Program (Cary, NC), after the appropriate adjustments by variables such as sex, age and beach. Risk analyses were performed using the Odds Ratio Statistic (Gordis 2000) at the 95% confidence level.

Principal Findings

Results of this prospective epidemiological study showed that there is a higher risk of respiratory and skin symptoms in swimmers when compared to non-swimmers in beaches of Puerto Rico. Although not all samples were higher than the Puerto Rico Environmental Quality Board (PREQB) standards, we found high concentrations of all indicators in water samples, but found even higher concentrations of *E. coli* and enterococci in sand samples. The percent of samples exceeding the standards was higher for total coliforms and *E. coli* as analyzed by Colilert, but since *E. coli* can survive for an extended time in sand (Sánchez et al. 2006), its detection in water do not necessarily indicate recent fecal contamination in these beaches.

Prevalence rates of swimmers by beach showed higher prevalence rates in CB beach (Balneario de Carolina) and L (Luquillo beach). Luquillo beach is directly impacted by sewage effluents and by a river (Figure # 6). Isla Verde and Carolina beaches are not directly impacted by a river, but a major WWTP is located near the west side of these two beaches and contributes to the highest effluent from a treatment plant in Puerto Rico (72 MGD – millions of gallons daily). Perhaps marine water currents can move these indicators to the nearby beaches (IV and CB).

On the other hand, the direct source of indicators in the beach with the highest prevalence of symptoms is unknown (Carolina Beach). Since WWTP are supposed to be chlorinated before ending up into the sea, it may be possible that non-treated storm water effluents are causing the high illness rates detected in swimmers from CB beach. Interestingly, all of the beaches included in this study are under the Blue Flag program, an international plan to designate beaches as safe for swimmers based on low concentrations of thermotolerant coliforms and enterococci in water. The blue flag microbiological standards are: 200CFU / 100 mL for thermotolerant coliforms and 35 CFU / 100 mL for enterococci. Our epidemiological study shows that there is still a risk for swimmers even when most of the water samples meet these standards as well as PREQB standards.

Some indicators were slightly related to symptoms. In the analyses by beach, although non-statistically significant due to the small subject sample, coliphages were the indicators with the highest correlation coefficient with gastrointestinal symptoms. This may mean that this indicator is better in predicting the risk of gastrointestinal illness in these three beaches.

For that reason, and since there was not a strong linear relation of indicators and the risk of illness, we believe that indicators in water are not predicting the risk of illnesses. If indicators are surviving in sand, then their presence in water might not be predicting the presence of pathogens present in feces at beaches from Puerto Rico (PR). Furthermore, recreational water indicators that are used in PR (thermotolerant coliforms and enterococci) may not be preventing the swimmer's risk of illness, especially the risk of respiratory and skin symptoms.

Although in the logistic regression analyses no significant linear trend between gastrointestinal illness and indicators was found, the risk of illness was increased in the 3rd and 4th quartile of increasing indicator in water. This shows that there is a risk related to an increase in coliphages in water for each 25% increase. Also, this analysis showed that *E. coli* and coliphages better predict the risk of respiratory symptoms and the risk of ear ache among swimmers with the highest indicator concentration in water (4th quartile). In the categorical analyses skin symptoms were related to the concentrations of *E. coli* in water analyzed by membrane filtration (R^2 = 29.69%).

Our study regressions showed a non-linear trend between illness and indicators concentration in water. This non-linear trend was obtained probably because of the small sample size as compared to studies performed with larger sample sizes (>10,000 subjects. In fact, studies performed in United States, Hong Kong and Egypt recruited as many as 26,000, 18,000 and 23,000 subjects respectively (Cabelli et al. 1982; Kueh et al.1995; Fattal, 1986). In these studies a significant linear trend between prevalence rates of gastrointestinal illness and fecal indicators in water was observed. A lack of a straight linear relation of indicators and prevalence rates in our study may mean that the concentrations of indicators we found are not predicting the presence of the etiological agents of gastrointestinal infections and possibly pathogens in water.

Other smaller epidemiological studies such as the Australia study (Corbett et al. 1993), Israel (Fattal et al. 1986) and South Africa (Von Schirnding et al. 1992) with smaller sample sizes (< 3, 000 subjects) did not find a significant linear relation of gastrointestinal illness and indicators in marine water. In the South Africa study authors postulated that the lack of significance may have been due to the uncertain sources of fecal contamination, as may be the case in Puerto Rico.

High prevalence rates with low indicator concentrations were observed in this study. Similar results were found in a study where illness in swimmers was not necessarily associated with high densities of common fecal indicator bacteria in water but was associated to swimmer to swimmer transmission via the water (Calderón et al. 1991). In fact, other non-sewage sources of fecal indicators can change have an effect in the linear regression analysis needed for a dose response analysis. The prospective trial performed by Von Schirding et al. (N=733) showed a lack of statistical significance due to uncertain sources of fecal contamination (USEPA, 1986).

Sources of fecal contamination in two of our beaches (Isla Verde and Luquillo), are sewage and a river, but in the other (Carolina Beach) fecal sources are unknown. It is important to notice that this beach had the highest prevalence of symptoms even when the source of indicators is unknown. Perhaps resuspension of sand or storm water effluents are the source of indicators at this beach. In fact, since *E. coli* and enterococci were higher in sand than in water samples, indicators found in water may be resuspended from sand sediments and they do not represent recent fecal pollution. Since there was no linear relation between the risk of illness and the presence of *E. coli* and enterococci in water, perhaps these indicators are not predicting the concentrations of pathogens in beach water. Surprisingly, *Manitol + halophiles* were high in water and in sand but it is unknown if these concentrations are dangerous for swimmers because no significant relation between them and the prevalence of symptoms were found.

On the other hand, some correlations between the indicators in water samples were positive and significant ($p<0.05$). This may mean that our methods were successful in the analysis of indicators in water and that common indicators such *as E. coli*, can help us predict alternate indicators such as coliphages and *Manitol + halophiles* in water. Results also showed that at low levels of indicators as well as high concentrations there is a high risk of illness. Haile et al. 1999, got similar results in their epidemiological study where a non-linear trend between indicators and prevalence rates were found. A possible reason for this finding may be that some agents in the beach environment are causing an overestimation of GI illness. Kay (2000), examined some causal agents related to GI illness other than microorganisms in water (confounders) in epidemiological studies. Some of them are: history of migraines, history of stress, frequency of diarrhea current use of prescription drugs, illnesses within 4 weeks prior to the beach study, consumption of certain food associated with diarrhea and alcohol consumption within the 7 day period after the beach study.

We visually corroborated that a high percent of the young beach visitors were drinking alcohol during the beach sampling date. These confounders may have had an effect in the regression analyses. More extensive research has to be performed in order to observe a relation between indicators and risk. Since we did not confirm the symptoms by a medical examination there could be bias in what was reported due to people's perception of the symptoms. Also, the exposure to the sun in swimmers may have harm their immune systems since they were exposed for a high period of time to hot weather temperatures making them more susceptible to other symptoms not caused by the possible pathogens in water.

A larger sample size will help in the development of better linear relationship among prevalence rates and indicators. However, the risk of symptoms among swimmers was well characterized by OR values higher than 1 and by the statistically significant confidence interval values.

SIGNIFICANCE

There is a significant risk of respiratory and skin symptoms among swimmers from marine beaches in PR. This risk exists even at relatively low concentrations of indicators in water as shown by the positive regression coefficients.

Even when waters meet the standards of the PREQB as analyzed by the membrane filtration, there is an increased risk of illness in swimmers when compared to non-swimmers. Moreover, this risk was higher in the beach with unknown sources of fecal indicators in water. As shown by the correlation analyses by beach, it seems that under tropical waters of Puerto Rico, the indicators that better predict the risk of symptoms are the coliphages and the *E. coli* analyzed by the enzymatic method. Results showed that concentrations of *E. coli* in water depend on the method of analysis, and they are present in higher concentrations in marine sand. Therefore, care should be taken when using *E. coli* as an indicator of fecal contamination in tropical recreational waters. Since *E. coli* might not be a good indicator of fecal contamination, coliphages are the indicator that may better predict the risks of illness. Further research has to be done in order to observe if coliphages survive for a long time in water and or in sand in the marine environment.

This is the first epidemiological study to characterize the risk of illness among swimmers from marine bathing beaches in Puerto Rico. Care should be taken when using PREQB indicators since their concentrations in water may not be protecting swimmer's health in our beaches. The use of coliphages as an indicator of fecal pollution will better protect swimmer’s health in beaches from Puerto Rico.