

Report for 2001VI4421B: Applicable Indicators of Risk for Coastal Waters in Tropical Environments: Phase II

- Water Resources Research Institute Reports:
 - A project completion report is being prepared.
- Conference Proceedings:
 - A paper is being prepared for presentation at a conference in South Africa.

Report Follows:

Summary Report on

APPLICABLE INDICATORS OF RISK FOR COASTAL WATERS IN TROPICAL ENVIRONMENTS: PHASE II

Problem and Research Objectives

The control of water quality in terms of concentrations of microbial pathogens in recreational waters is of great concern for the protection of human health. Epidemiological studies have shown an increase in the risk of morbidity in coastal water swimmers when compared to non-swimmers in waters polluted with total and thermotolerant coliform bacteria (1,2). Also, a significant relationship between bacterial indicators and gastrointestinal symptoms has been established (1). Other symptoms related to exposure to these bacteria are respiratory and skin infections. At risk populations are children playing in the tidal or swash zone, the elderly and people with poor immune system functions.

In the islands of Puerto Rico and St Thomas, in the Caribbean, many beaches are located near high population density areas. Studies have shown that populations near the coast may increase the chance of contamination of coastal waters with urban and industrial wastes and sewage effluents with high levels of pathogenic agents (2). Anthropogenic sources of microbial polluted waters can increase the probability of diseases. However, no formal studies have been conducted to quantify this in St. Thomas or in Puerto Rico. In addition, microbial concentrations in these beaches are not well understood or not well characterized in terms of temporal and spatial characteristics.

Climate conditions favor year-round coastal water activities for both native and tourist populations in these islands. This may increase the chances of contact with polluted waters and a consequent increase in morbidity. On the other hand, the practice of using thermotolerant coliforms, or even *Escherichia coli* to detect fecal pollution in tropical waters is in debate, since they tend to occur naturally in tropical waters (3,4). Also, their survival appears to be longer outside the gastrointestinal tract of blood-warmed animals in tropical environmental conditions (5).

The objectives of this study are to (i) establish temporal and spatial data of microbial indicators of tropical coastal waters, (ii) analyze different microbial indicators of water quality (like total and thermotolerant coliforms and enterococci), to observe their relation with physicochemical characteristics of the water (e.g. pH, temperature, total dissolved solids, salinity, sunlight) and other characteristics (number of people in the water at the time of sampling), and (iii) to investigate a probable pattern of fluctuations of microbial quality indicators in coastal waters year-round.

Methodology

From October to May, 2002, three public beaches (Coki Point, Lindberg Bay, and Magens Bay) from St. Thomas, Virgin Islands were sampled. An area of 200 feet long on the coastline was identified at each beach. Four samples were taken (two at chest depth

and two at ankle depth) with a 200 ft of separation on the beach. Samples were taken in the morning (9:00 am), at noon (12:00pm), afternoon (3:00pm) and in the evening (6:00pm) for two consecutive days during the weekends. This was performed to observe the variation in time that bacterial measurements may have in the days of the week that are most frequented by bathers.

Physical parameters such as temperature, pH and dissolved solids were recorded from each sample before storing them in an ice cooler with cold packs. Samples were then stored at 4 °C in the laboratory for analysis within the next 24 hours. The quantity of swimmers at every hour in the beach was also recorded.

The same procedure was followed to sample five public beaches located mainly on the north coast of Puerto Rico island (Isla Verde, Luquillo, Cataño, Humacao, Manatí) from January to August, 2002. In addition to Colilert and Enterolert tests, we performed Membrane Filtration (MF) analysis (6) were performed in samples from Puerto Rico beaches.

Bacterial Analysis

Colilert and Enterolert tests were performed to analyze total and thermotolerant coliforms, as well as enterococci. The Colilert test is an enzyme-specific test that gives the most probable number (MPN) of total and thermotolerant coliforms in a 100 mL sample. This method consists of use of a reagent which in the presence of the enzyme *B*-galactosidase (present in all coliform bacteria) produces a change in color and in the presence of the enzyme *B*-glucuronidase (present in all thermotolerant bacteria) emits fluorescence under UV light (7). Enterococci can be detected using the Enterolert test, which under UV light shows the MPN of enterococcus bacteria in a 100 mL sample. Marine water samples must be diluted by a factor of 10 for better results as suggested by the manufacturer (Advance Systems Inc.). After mixing the reagent with marine water, samples were poured into a tray having 48 large wells and 48 small wells. The counting of the large and small positive wells for each test, gives us the MPN when compared to a standard MPN table.

Membrane filtration analysis (MF) was performed following the method described by the American Public Health Association (6). Samples analyzed by MF were expressed in colony forming units/ 100 mL of sample.

Statistics

The Minitab Statistical program was used to calculate basic statistics on this preliminary data. A simple linear regression analysis was performed to relate bacterial concentrations with physical parameters of the water. One-way analysis of variance (ANOVA) to observe the differences in bacterial concentrations within methods of analysis was also performed. In addition, another one-way ANOVA was performed to observe any difference in bacterial concentrations within water depth.

Principal Findings and Significance

Descriptive statistics of MPN/100mL of total coliforms, thermotolerant and enterococci in St. Thomas, VI are shown in Table 1. Mean total coliforms were found to be higher in Magens Bay than in Coki Point or Lindberg Bay. However, the highest mean

value of the thermotolerant bacteria and enterococcus bacteria was found in Coki Point. Other ranges bacterial concentration ranges are shown in Table 1.

A one way analysis of variance (ANOVA) to verify the difference in bacterial concentrations within all three beaches in St Thomas showed a statistically significant difference for each bacterial measure as shown by a p value lower than 0.05 (Table 2).

A linear regression analysis showed a statistically significant relation between total coliforms and pH of water ($p=0.000$). This is also true in the relation between thermotolerant coliforms and pH ($p=0.000$). Temperature and total coliforms showed a statistically significant relation ($p=0.001$). Thermotolerant coliforms were also significantly related to temperature of the water sampled. The rest of the B (regression coefficient) and p values of the regression analysis are shown in Table 3.

Mean results of the five beaches sampled in Puerto Rico island with MF are shown in Table 4. The highest mean total coliforms (in CFU/100 mL) value was found at Manatí. For thermotolerants, the highest mean was found in Cataño and for enterococci was in Humacao.

Mean MPN values (analyzed by Colilert and Enterolert) in beaches from Puerto Rico are shown in Table 5. The highest total coliforms value was found in Humacao beach. The highest in thermotolerant coliforms was Manatí beach, and for enterococci the highest value was found in Cataño.

The relation between environmental measurements of the water and the concentration of different bacteria analyzed by Colilert and Enterolert is shown in Table 6. A statistically significant relation was found between total coliforms concentration and the pH. Thermotolerant coliforms and enterococci were significantly related with temperature ($p=0.001$, $p=0.000$) as shown by a p value smaller than 0.05. Additional B values and p values of the simple linear regression analysis are shown in Table 6.

Discussion and Conclusions

Bacterial indicators of recreational waters have been questioned as to their efficiency to indicate risk to human health. Our results indicate that these indicators are present in high concentrations in recreational waters of St Thomas VI, and Puerto Rico. All bacterial measurements vary significantly from beach to beach in both islands but are still high in mean MPN values. The relationship of bacterial concentrations and environmental characteristics of water are not the same for both islands, but the number of swimmers at the time of the sampling was not related to the bacterial concentrations in any of the two islands.

It remains to be seen if the presence of these indicators represent a real public health problem. A prospective epidemiological study is being designed to investigate the morbidity of bathers and its relation to the presence of bacterial indicators of fecal contamination in these tropical waters.

Unfortunately it was not possible to use *Clostridium perfringens* as originally indicated in the proposal, as the difficulty of detecting them (possibly as a result of the low concentrations present) did not permit its use as a possible indicator to be measured.

Thus the original plans to fingerprint the isolates could not be carried out as a result as well. The fingerprinting of the *E. coli* isolates is currently being carried out.

The training aspect of the proposal was only partially met. Several people were trained at the University of Puerto Rico and the University of the Virgin Islands. In Puerto Rico the following students took part in the project:

Johanna Santamaria (M.S. defended her thesis in February, 2003)
Roberto Rodriguez (M.S. defended his thesis in February, 2003)
Elia Enid Sanchez (Ph.D. student)
Astid Huertas (M.S. candidate)
Clarivel Lasalde (M.S. candidate)
Francisco Calderon (Post-doctoral Associate)

All the above students got some support from the project. It should be mentioned that the lack of continuous funding makes it hard to train students at the University of the Virgin Islands. We were, however able to train two persons:

Rebelto Harrigan
Mayra Suarez

The results from the different portions of the project were presented at local, national and international meetings. The last portion of the proposal will be presented at the International Water Quality Conference to be held in Cape Town, South Africa in September 2003. One publication in a peer-reviewed journal is expected from the ongoing portion of the project. This paper will be presented at the South Africa meeting and will be submitted to a journal immediately thereafter.

References

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Table 1. Mean total and thermotolerant coliforms and enterococci MPN (with the range in parenthesis) from beaches in St Thomas VI with Colilert and Enterolert tests.

Beach	N	Total Coliforms*	Thermotolerant *	Enterococcus*
Coki Point	64	571.7 (0.0-1421.0)	357.2 (0.0-1011.1)	111.3 (0.0-1782.0)
Lindberg Bay	64	652.7 (41-1376.0)	291.5 (0.0-1011.1)	35.4 (0.0-1011.1)
Magens Bay	63	933 (52-1011.1)	356.8 (0-2014.0)	98.5 (0.0-1722.0)

* MPN / 100 mL

Table 2. Analysis of Variance (ANOVA) results of bacterial concentrations (MPN / 100 mL) within beaches from St Thomas, V. I. (N=32)

Variable	SS	F value	p value
Total coliforms	5,508	2.92	0.023
Thermotolerant	15,702	3.14	0.016
Enterococcus	329,855	12.68	0.000

Table 3. Simple linear regression analysis for bacterial samples vs. environmental characteristics of water in beaches from St. Thomas VI. *B* (p value)

Bacteria samples	# of bathers	pH	Temperature	TDS
Total coliforms	0.44 (0.85)	495.9 (0.000)	121.30 (0.001)	-10.30 (0.646)
Thermotolerant	0.05 (0.96)	280.92 (0.000)	82.18 (0.000)	-4.21 (0.327)
Enterococcus	0.05 (0.94)	-0.97 (0.983)	13.60 (0.263)	-1.902 (0.345)

p values < 0.05 are in bold

Table 4. Descriptive statistics of mean total coliforms, thermotolerant and enterococci from 5 beaches in Puerto Rico with Membrane Filtration method of analysis

Beach	N	Total Coliforms*	Thermotolerant*	Enterococcus*
Isla Verde	32	8.8	26.66	112.80
Luquillo	32	16.6	15.34	36.59
Cataño	32	17.1	29.40	19.09
Humacao	32	8.0	3.00	128.60
Manatí	32	23.8	10.50	32.34

*CFU / 100mL

Table 5. Mean values of the MPN / 100 ml of samples from PR beaches analyzed by Colilert and Enterolert

Beach	N	Total Coliforms	Thermotolerant	Enterococcus
Cataño	32	1964	461	410.7
Humacao	32	4961	25.9	60.7
Manatí	32	900	140.7	77.8

Table 6. Simple linear regression analysis for bacterial samples (in MPN) vs. environmental characteristics of water in beaches from Puerto Rico *B* (p value)

Bacteria samples	# of bathers	pH	Temperature	TDS
Total coliforms	2.08 (0.954)	2987 (0.012)	-405.5 (0.199)	144.8 (0.683)
Thermotolerant	0.038 (0.997)	199.5 (0.514)	-259.39 (0.001)	114.75 (0.198)
Enterococcus	-4.794 (0.223)	214.3 (0.104)	159.26 (0.000)	82.34 (0.032)