Comparison of total coliform, fecal coliform, and enterococcus bacterial indicator response for ocean recreational water quality testing

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ABSTRACT - In July 1999, California's ocean recreational bacterial water quality standards were changed from a total coliform (TC) test to a standard requiring testing for all three bacterial indicators: TC, fecal coliforms (FC), and enterococci (EC). To compare the relationship among the bacterial indicators, and the effect that changing the standards would have on recreational water regulatory actions, three regional studies were conducted along the southern California shoreline from Santa Barbara to San Diego, California. Two studies were conducted during dry weather and one following a large storm event. In each study, samples were collected at over 200 sites. Sites were selected using a stratified random design, with strata consisting of open beach areas and rocky shoreline, and areas near freshwater outlets that drain land-based runoff. During the dry-weather studies, samples were collected once per week for five weeks. For the storm event study, sampling occurred on a single day approximately 24 h following the storm. The three indicator bacteria were measured at each site and the results were compared to the single sample standards (TC >10,000; FC>400 and EC>104 MPN or cfu/100mL). EC was the indicator that failed the single sample standards most often. During the wet-weather study, 99% of all standard failures were detected using EC, compared with only 56% for FC and 40% for TC. During the summer study, EC was again the indicator that failed the single sample standards most often, with 60% of the failures for EC alone. The increased failure of the EC standard occurred consistently regardless of whether the sample was collected at a beach or rocky shoreline site, or at a site near a freshwater outlet. Agreement among indicators was better during wet weather than during dry weather. During dry weather, agreement among indicators was better near freshwater outlets than along open shoreline. Cumulatively, our results suggest that replacement of a TC standard with an EC standard will lead to a five-fold increase in failures during dry weather and a doubling of failures during wet weather. Replacing a TC standard with one based on all three indicators will lead to an eight-fold increase in failures. Changes in the requirements for water quality testing have strong implications for increases in beach closures and restrictions.

INTRODUCTION

The concentration of indicator bacteria in ocean waters has been used for decades to measure recreational water safety. Indicator bacteria are not necessarily pathogenic, but are found abundantly in wastes with human contributions where pathogenic organisms, such as viruses, are likely to exist. The levels of indicator bacteria in bathing waters have been shown to correlate with the incidence of illness in swimmers from Santa Monica Bay, California (Haile *et al*. 1999). Recreational water quality programs world-wide collect water samples; test for indicator bacteria; and post, close, or otherwise restrict access to recreational waters based on the concentrations of indicator bacteria present. Governmental and environmental organizations use these monitoring data to take regulatory actions or to grade the recreational water quality at a given beach.

While the use of bacterial indicators to measure water quality is widespread, there is not universal

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agreement on which indicator organism(s) is most useful; nor do federal regulations mandate a single standard for bacterial indicators. Thus, different indicators and different indicator levels identified as standards are used by water quality programs in different states, countries, and regions. Today, the most commonly measured bacterial indicators are TC, FC, and EC. The threshold limits for each of the three indicators were established using different procedures. TC was the first to be used, and one of the ways that the threshold was developed was by extrapolation of technological limits developed for drinking water. The FC thresholds were developed in the late 1960s. The U.S. Public Health Service used an epidemiology study and observed detectable swimming-associated health effects with TC levels of 2300 cfu/100 mL (Dufour 2001). By extrapolating the fraction of TC that was FC, a threshold of 200 cfu/100 mL was developed for FC. More recently, *E. coli* (a subset of the FC group) and EC were established as preferred indicators, and thresholds were based on a series of epidemiological studies that were carried out in sewage-impacted recreational waters (Cabelli 1983a, Cabelli 1983b, Cabelli *et al.* 1982, Dufour 1984). These studies demonstrated that the concentration of EC and *E. coli* correlated best with bather illness, while TC did not correlate well. As a result of these studies, the U.S. Environmental Protection Agency (U.S. EPA) recommended in 1986 that EC be used as the sole indicator for ocean water bacterial monitoring (U.S. EPA 1986). This recommendation has not been universally implemented, although an increasing number of states have adopted or are planning to adopt it.

The selection of an indicator organism has important consequences for management of recreational water resources and perceived water quality of the resource. The indicator organism and concentration and the responses of the indicators to different sources of fecal pollution will directly affect the number of ocean water recreational sites that pass or fail water standards. California recreational water standards changed in July 1999 from a single TC standard, which had been used since 1958, to a standard requiring measurement of three indicator organisms: TC, FC, and EC. The new requirements have now been implemented for ocean recreational water monitoring along the entire stretch of the southern California coastline, from Santa Barbara to San Diego. This heavily populated area is world famous for its coastline, with beach and recreational ocean water usage by an estimated 175 million visitors annually (NRC 1990). It is also one of the most intensively monitored coastlines in the world, with $3 million spent annually by local agencies to evaluate the microbiological quality of the water (Schiff *et al.* 2001). Here, we present the results of three large-scale shoreline microbiology monitoring studies that were conducted along the coastline of the Southern California Bight. These studies examined the relationships among the three bacterial indicators over a regional scale, including multiple types of shoreline and areas impacted by stormwater runoff, during various weather conditions (dry versus wet). As part of these studies, we provide a comparison of the bacterial indicator responses and assess the implications of the new regulatory standards on water quality management.

MATERIAL AND METHODS

Three studies of shoreline microbiological water quality were performed along the 700 km coastline between Point Conception, California, and the United States-Mexico international border. The first was conducted between August 1 and September 7, 1998 (Summer Study), which was a dry-weather period in southern California during which there was no rain. The second was conducted between February 1 and March 3, 1999 (Winter Study), during which there was less than 2 cm of rain. The third study took place on February 22, 2000 (Storm Study), 24 h after a storm that produced at least 5 cm of precipitation over the entire region. Samples were taken at 224 sites (Summer and Storm studies) and 211 sites (Winter Study). Sites were selected using a stratified random design, with strata consisting of open beach areas and rocky shoreline and areas near freshwater outlets that drain land-based runoff to the ocean. Samples were collected once a week for five weeks during the Summer and Winter studies, while the Storm Study involved collection on the single rain-affected date.

The TC and FC testing was conducted at all sites during all three studies. The EC testing was conducted at 70% of the sites during the Summer Study, and was conducted at all of the sites during the Winter and Storm studies. Samples were collected and processed by a consortium of 21 organizations that conduct routine monitoring of southern California’s beaches. Each of the laboratories used their standard methods, including membrane filtration (MF), multiple tube fermentation (MTF), and the defined substrate technology test kits Colilert® and

Enterolert® (IDEXX Laboratories, Inc., Portland, ME). All analyses were performed using techniques as outlined in Standard Methods (APHA 1995), or according to the manufacturer's instructions. Comparability among laboratories and among methods was confirmed prior to the study through a series of quality control studies (Noble *et al.* 2003), although cross-laboratory comparison was of minor importance since samples from a site were tested for the different indicators by the same laboratory.

Results for each bacterial indicator were compared to the California single sample standards, which set a failure level at >10,000 MPN or cfu/100 mL for TC, >400 MPN or cfu/100 mL for FC, and >104 MPN or cfu/100 mL for EC. When the Colilert® method was used, *E. coli* results were treated as FC for data analysis. Correlation analysis was also used to compare the log-transformed bacterial indicator concentrations.

RESULTS

Median concentrations for all three of the bacterial indicators were 4 to 50 times higher during the Storm Study than during either the Summer Study or the Winter Study (Table 1). For the Storm Study, the median concentration of EC exceeded the single sample standard of 104 MPN or cfu/100 mL, regardless of the type of shoreline that was sampled (Table 1). Median indicator concentrations for all of the other studies were well below the standards (Table 1). During the Storm Study, 36.4% of the samples exceeded at least one bacterial indicator standard, compared to 5.0% for the Summer Study and 6.5% for the Winter Study. During the Summer Study for all sites, the proportion of all failures that were due to exceedence of either the FC or EC standard was nearly equal, at 47.1% and 41.3%, respectively (Table 2). However, during the Storm Study, EC was responsible for 51% of the failures at all sites, as opposed to 29.1% of the total failures due to violation of the FC standard (Table 2).

The TC and FC concentrations were strongly correlated in all three studies (r = 0.85-0.93, Table 3). The TC/EC and FC/EC were strongly correlated in the Storm Study (r = 0.83-0.86), less well correlated during the Winter Study, and poorly correlated during the Summer Study. Correlations among indicators were similar regardless of whether samples were taken at beaches or near freshwater outlets (Table 3).

In all three studies, EC was the indicator that exceeded the standard most frequently (Figure 1). During the Storm Study, 99% of all standard failures were detected using EC, compared with only 56% for FC and 40% for TC. During the Summer Study, less than 70% of the failures included an EC failure, but 60% of the failures were for EC alone (Figure 1). During the Winter Study, 64% and 71% of the standard failures were for EC alone along the entire shoreline and at freshwater outlets, respectively (Figure 1). The increased failure of the EC standard occurred consistently regardless of whether the sample was collected at a beach or near a freshwater outlet. During wet weather, there was much greater concordance among failures by the three indicators, as evidenced by the overlap in Figure 1. During dry weather (Summer and Winter studies), there was generally poor agreement among failure of the water quality standards, with concordance among indicator failures only when samples were taken near the freshwater outlets (Figure 1).

DISCUSSION

EC was the bacterial indicator that exceeded the single sample standards most often in our studies. During the dry Summer Study, more than 60% of the water quality failures were for EC alone. During the Storm Study, EC was associated with 99% of the observed water quality standard failures. This finding of greater numbers of EC standard failures is not unique to southern California (Nuzzi & Buhrans 1997), but is of interest because the bacterial source material in southern California differs from that in other parts of the country. Southern California is one of the few areas in the country that have independent storm drain and sewage conveyance systems. As a result, the primary source material is not weather-induced sewerage overflows, but urban runoff that drains directly to the ocean without treatment.

One possible explanation for the consistently higher rate of EC standard failures is that EC survive longer in the marine environment than TC or FC. Hanes & Fragala (1967) found that *E. coli* survival in marine water was 0.8 d while EC survival was 2.4 d. Sieracki (1980) found that *E. coli* degraded more rapidly with increased sunlight intensity than did EC, a finding that was recently confirmed for bacterial samples from southern California (Noble *et al.* 2001). Southern California has few cloudy days, particularly during the summer dry period, which would enhance sunlight effects on survival.

This differential survival hypothesis seems to be supported by the greater consistency in standard

Table 1. Comparison of the three studies, including sample size, percent failure of standards, and median bacterial concentrations.

Study	Number of Samples	Percent of Samples Failing Any Bacterial Standard[a]	Median Bacterial Concentration[b]					
			Total Coliforms		Fecal Coliforms		Enterococci	
			All Sites	Freshwater Outlets	All Sites	Freshwater Outlets	All Sites	Freshwater Outlets
Summer	1,120	5.0%	14	40	4	20	2	9
Winter	1,105	6.5%	20	63	10	20	10	10
Storm	224	36.4%	961	1,450	130	85	185	230

[a] Standards used: Total coliforms >10,000; fecal coliforms >400, and enterococci >104 colony forming units (cfu) or most probable number (MPN)/100 mL.
[b] cfu or MPN/100 mL.

Table 2. Percentage of indicator failures by indicator, study, and sample type.

Study	Summer Study[a]			Winter Study[a]			Storm Study[a]		
Shoreline Type Indicator	All Sites	Freshwater Outlets	Shoreline[b]	All sites	Freshwater Outlets	Shoreline[b]	All Sites	Freshwater Outlets	Shoreline[b]
Total Coliforms	11.6	12	0	11.5	12.3	8.7	19.7	15.7	25.5
Fecal coliforms	47.1	51.1	36	22.6	24.6	15.2	29.3	26.8	33
Enterococci	41.3	36.9	64	65.9	63.1	76.1	51.5	57.5	41.5

[a] Represented as a percentage of all standard failures for that study.
[b] Includes sandy beaches and rocky shoreline sites, but not sites near freshwater outlets.

Table 3. Spearman rank correlation (r-value) between log-transformed concentrations of total coliforms, fecal coliforms, and enterococci for the three studies.

Indicators	Total Coliforms/ Fecal Coliforms		Total Coliforms/ Enterococci		Fecal Coliforms/ Enterococci	
Study	All Sites	Freshwater Outlets	All Sites	Freshwater Outlets	All Sites	Freshwater Outlets
Summer / Dry	0.93	0.93	0.29	0.28	0.29	0.30
Winter / Dry	0.85	0.84	0.64	0.79	0.70	0.73
Storm / Wet	0.85	0.81	0.86	0.81	0.83	0.81

Figure 1.

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failures among indicators in the Storm Study than in the dry-weather studies. During wet weather, land-based runoff is distributed to the beach more quickly and represents a "fresher" source of contamination, providing less time for differential degradation to occur. Similarly, in the dry-weather studies, greater consistency was observed among indicators near freshwater outlets than on open beaches away from outlets, consistent with the fresher source of contamination coming from freshwater outlets.

The U.S. EPA has promoted the use of a single bacterial indicator (U.S. EPA 1986), EC, in its national guidance documents for marine waters. Our results, consistent with those of other researchers (Cabelli 1983b, Dufour 1984, Kay *et al.* 1994), support the use of EC if a single indicator must be selected, as this study found that most of the coliform standard failures coincided with EC failures, while the reverse was not true. An increased number of standard failures alone do not make EC a better indicator; but combined with a demonstrated correlation with illnesses at the threshold levels, EC may be the most appropriate single indicator (Cabelli 1983b, Cabelli 1983c, Dufour 1984, Haile *et al.* 1999).

The U.S. EPA's recommendation of a single indicator contrasts with current California recreational water regulations, which require that health departments measure three bacterial indicators (TC, FC, and EC) at high-use beaches between April and October. Our findings tend to support California regulations to measure three indicators, as we found poor agreement among indicators in the summer; and insufficient scientific evidence exists at the present time to select one indicator over the others (Noble *et al.* 2000). Focusing extra public health protection measures on the high usage period in light of uncertainty associated with individual indicators appears warranted.

The case for using three indicators during the winter months, when storms are more frequent and fewer swimmers use the beach for recreation, is less clear. During the Storm Study, 99% of the TC and FC failures were also identified by failure of the EC standard (Figure 1). While this storm was slightly larger than a typical rainstorm in southern California, it was not a worst-case scenario for bacteriological contamination as the storm was preceded by antecedent rainfall. Even during dry winter periods (Winter Study), there was a higher level of consistency among indicators than in the summer, possibly due to lower levels of UV irradiation and lower rates of degradation than in the summer. Naturally, measuring all three indicators would be preferable; but if budgets are limited, the effort expended in monitoring three indicators during the winter months might be more cost-effectively expended by sampling more beach sites or sampling at more frequent intervals (Schiff *et al.* 2001).

Addressing which, and how many, indicators should be measured will ultimately require additional research to understand how the bacterial indicators relate to the presence of pathogens that directly impact public health. Investigators have shown that EC and coliphage have similar survival characteristics in receiving lake waters (Rajala and Heinonen-Tanski 1998). If the etiology of swimming-associated gastroenteritis is viral, and if coliphage react to physical and environmental stressors in a manner similar to human enteric viruses, then EC alone might be a better predictor of adverse health outcomes from exposure to fecal contamination. Cabelli *et al.* (1982) and Dufour (1984) showed that EC correlated better with swimming-associated gastroenteritis at marine and freshwater bathing beaches with wastewater influences. This relationship between EC and swimming-associated gastroenteritis has been more recently examined by Kay *et al.* (1994), who demonstrated a significant dose-response relationship between gastroenteritis and fecal streptococci (of which EC is a subgroup) concentrations. However, recent work has demonstrated that the presence of viral pathogens is not necessarily related to levels of bacterial indicators (Jiang *et al.* 2001, Noble and Fuhrman 2001, Schvoerer *et al.* 2001). Also, different indicators may be predictors of specific types of diseases. Haile *et al.* (1999) found that the relative risk differed by indicator when its particular threshold was exceeded. The most appropriate indicator will be that which is most similar in occurrence, numbers, and rates of degradation to pathogens of concern. It may be that appropriate indicators can only be defined to limited areas because of changes in environmental parameters (sunlight, salinity, temperature, levels of suspended solids, types of wastewater inputs, etc.). Studies to address this issue will improve the quality of public warning systems, as well as the cost efficiency of monitoring, by more closely relating existing measures of ocean water quality to public health risk.

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