

Fecal Coliforms as Indicators in Tropical Waters: A Review

TERRY C. HAZEN, *Microbial Ecology Laboratory, Department of Biology, University of Puerto Rico, Río Piedras, Puerto Rico 00931, and Environmental Sciences Division, Savannah River Laboratory, E. I. du Pont de Nemours & Company, Aiken, South Carolina 29808*

Abstract

Nowhere is the importance of accurate determination of recent human fecal contamination greater than in the tropics. The diversity of waterborne diseases and their severity is greatest in tropical environments. Since most of the countries in tropical climates are underdeveloped, with large populations that are undernourished, ill-housed, with poor medical services, waterborne diseases may have a much greater effect on public health in the tropics than in temperate areas. Universally, tropical areas accept water maximum contaminant levels developed by temperate nations, despite the obvious differences in tropical climates. High densities of total and fecal coliform bacteria have been detected in pristine streams and in groundwater samples collected from many tropical parts of the world, even in epiphytic vegetation 10 m above ground in the rain forest of Puerto Rico. Nucleic acid (DNA) analyses of *Escherichia coli* from pristine tropical environs has indicated that they are identical to clinical isolates of *E. coli*. Many tropical source waters have been shown to have enteric pathogens in the complete absence of coliforms. Diffusion chamber studies with *E. coli* at several tropical sites reveal that this bacterium can survive indefinitely in most freshwaters in Puerto Rico. An evaluation of methods for the enumeration of fecal coliforms showed that currently used media have poor reliability as a result of large numbers of false positive and false negative results when applied to tropical water samples. Total and fecal coliform bacteria are not reliable indicators of recent biological contamination of waters in tropical areas. Fecal streptococci and coliphages in tropical waters violate the same underlying assumptions of indicator assays as the coliforms. Anaerobic bacteria like *Bifidobacterium* spp. and *Clostridium perfringens* show some promise in terms of survival, but not in ease of enumeration and media specificity. The best course at present lies in using current techniques for direct enumeration of pathogens by fluorescent staining and nucleic acid analysis, and developing tropical maximum contaminant levels for certain resistant pathogens in tropical waters.

SCOPE OF PROBLEM

Over 2 billion people, or half of the world's population, have suffered from diseases due to drinking polluted waters (Barabas, 1986). More than 250 million new cases of waterborne disease are reported each

year, resulting in more than 10 million deaths. Nearly 75% of these waterborne disease cases occur in tropical and subtropical areas. Indeed, nearly 50% of diarrheal disease deaths (4.6 million) occur in children under 5 years of age, living in the tropics (Snyder and Merson, 1982; Bockemühl, 1985). Many investigators and government administrators assume that these high morbidity and mortality rates simply indicate the contaminated nature of water in tropical areas. However, determining the level of biological contamination in tropical source water is much more difficult than most regulatory agencies perceive, even regulatory agencies residing in tropical nations. Yet the need to accurately determine the level of biological contamination is much greater in tropical areas than it is in temperate areas. Tropical areas have a much greater number of waterborne diseases (Tables I and II). In turn, these diseases are exacerbated by the greater reliance on surface water as a drinking water source and the lack of adequate sewage treatment (Feachem, 1977; Esrey *et al.*, 1985).

The literature is sparse with specific references to fecal contamination in the tropics, yet there are some notable exceptions. Evison and James (1973) reviewed literature from Ceylon, Egypt, India, and Singapore, and reported that densities of *Escherichia coli* in water did not seem to coincide with known sources of fecal contamination. Feachem (1974), working in rural areas of New Guinea, found fecal coliform (FC) densities from 0 to 10,000 colony-forming units (CFU) per 100 mL and fecal streptococci (FS) levels from 0 to 6000 CFU per 100 mL, although his mean densities for both indicator bacteria was greater than 100 CFU per 100 mL for nearly all sites. All sites were totally unacceptable for drinking water and Feachem concluded that all sites were grossly contaminated with fecal material because of the high indicator counts. Yet he stated that FC and FS densities were more closely correlated with the number of domestic animals in the watershed than with the number of people. In fact, FC and FS densities closest to the highest human population density were the lowest. In addition, typical of many tropical studies, Feachem did not do complete identification of positive isolates.

In Sierra Leone, Wright (1982) reported densities of fecal coliforms from 40 to 240,000 most probable number (MPN) per 100 mL and FS densities from 7 to 64,000 CFU per 100 mL for water sources used by 29 settlements. However, Wright (1982) found no correlation between any of the fecal indicators that he measured and *Salmonella* spp. Fujioka and Shizumura (1985) found that streams in Hawaii had densities of fecal coliforms and fecal streptococci from 100 to 10,000 CFU per 100 mL, including those not known to be receiving contamination from any fecal source. Oluwande *et al.* (1983) found that rivers in Nigeria

TABLE I
Waterborne infectious bacteria

Organism	Disease	Infectious dose
<i>Acinetobacter calcoaceticus</i>	Nosocomial infections	?
<i>Aeromonas hydrophila</i> ^a	Enteritis, wounds	?
<i>A. sobria</i> ^a	Enteritis, wounds	?
<i>A. caviae</i> ^a	Enteritis, wounds	?
<i>Campylobacter jejuni</i> ^a	Enteritis	?
<i>C. coli</i> ^a	Enteritis	?
<i>Chromobacterium violaceum</i> ^a	Enteritis	?
<i>Citrobacter</i> spp. ^a	Nosocomial infections	?
<i>Clostridium perfringens</i> type C ^a	Enteritis	?
<i>Enterobacter</i> spp. ^a	Nosocomial	?
<i>Escherichia coli</i> serotypes ^a	Enteritis	>10 ⁶ CFU ^c
<i>Flavobacterium meningosepticum</i>	Nosocomial, meningitis	?
<i>Francisella tularensis</i> ^a	Tularemia	10 CFU
<i>Fusobacterium necrophorum</i>	Liver abscesses	10 ⁶ CFU
<i>Klebsiella pneumoniae</i> ^a	Nosocomial, pneumonia	?
<i>Leptospira icterohaemorrhagiae</i> ^a	Leptospirosis	?
<i>Legionella pneumophila</i> ^a	Legionellosis	>10 CFU
<i>Morganella morganii</i> ^a	Urethritis, nosocomial	?
<i>Mycobacterium tuberculosis</i> ^a	Tuberculosis	?
<i>M. marinum</i>	Granuloma	?
<i>Plesiomonas shigelloides</i> ^a	Enteritis	?
<i>Pseudomonas pseudomallei</i> ^a	Melioidosis	?
<i>Salmonella enteritidis</i> ^a	Enteritis	>10 ⁶ CFU
<i>S. montevideo</i> B ^a	Salmonellosis	?
<i>S. paratyphi</i> A&B ^a	Paratyphoid fever	?
<i>S. typhi</i> ^a	Typhoid fever	10 ⁶ CFU
<i>S. typhimurium</i> ^a	Salmonellosis	>10 ⁵ CFU
<i>Serratia marcescens</i> ^a	Nosocomial	?
<i>Shigella dysenteriae</i> ^a	Dysentery	?
<i>Staphylococcus aureus</i> ^a	Wounds, food poisoning	?
<i>Vibrio alginolyticus</i> ^a	Wounds	?
<i>V. cholerae</i> ^a	Cholera dysentery	10 ⁸ CFU
<i>V. fluvialis</i> ^a	Enteritis	?
<i>V. mimicus</i> ^a	Enteritis	?
<i>V. parahaemolyticus</i> ^a	Enteritis	10 ⁵ CFU
<i>V. vulnificus</i> ^a	Wound infections	?
<i>Yersinia enterocolitica</i> ^b	Enteritis	10 CFU

^a Found in the tropics.

^b Found exclusively in the tropics.

^c CFU: colony-forming units; see Hazen *et al.* (1987), Dufour (1986), Hutchinson and Ridgway (1977), and Hawkins *et al.* (1985).

TABLE II
Other waterborne pathogens

Organism	Disease	Infectious dose
Viruses		
Adenovirus ^a	Enteritis, pharyngitis	1-10 PFU ^c
Calicivirus ^a	Enteritis	1-10 PFU
Norwalk virus ^a	Enteritis	1-10 PFU
Coronavirus	Enteritis	1-10 PFU
Coxsackie virus A & B	Meningitis	1-10 PFU
Echo virus	Enteritis, meningitis	1-10 PFU
Hepatitis A virus ^a	Hepatitis	1-10 PFU
Poliovirus ^a	Poliomyelitis	1-10 PFU
Rotavirus ^a	Enteritis	1-10 PFU
Astrovirus	Enteritis	1-10 PFU
Cyanobacteria		
<i>Cylindrospermopsis</i> spp. ^b	Hepatoenteritis	Bloom
Fungi		
<i>Candida</i> spp. ^a	Candidiasis	?
<i>Rhinosporidium seebertii</i> ^b	Rhinosporidiosis	?
Protozoa		
<i>Balantidium coli</i> ^a	Balantidiasis	?
<i>Cryptosporidium</i> spp. ^a	Cryptosporidiosis	?
<i>Giardia lamblia</i> ^a	Giardiasis	1 cyst
<i>Entamoeba histolytica</i> ^a	Dysentery	1 cyst
<i>Naegleria fowleri</i> ^a	Meningoencephalitis	?
<i>Acanthamoeba</i> spp. ^a	Meningoencephalitis	?
Helminths		
<i>Schistosoma mansoni</i> ^b	Schistosomiasis	1 cercariae
<i>S. haematobium</i> ^b	Schistosomiasis	1 cercariae
<i>S. japonicum</i> ^b	Schistosomiasis	1 cercariae
<i>S. intercalatum</i> ^b	Schistosomiasis	1 cercariae
<i>S. mekongi</i> ^b	Schistosomiasis	1 cercariae
<i>Fasciola hepatica</i> ^a	Fascioliasis	1 metacercariae
<i>Fasciolopsis buski</i> ^b	Fasciolopsiasis	1 metacercariae
<i>Paragonimus westermani</i> ^a	Paragonimiasis	1 metacercariae
<i>Clonorchis sinensis</i> ^a	Chinese liver fluke	1 metacercariae
<i>Diphyllobothrium latum</i>	Pernicious anemia	1 pleurocercoid
<i>Dracunculus medienensis</i> ^b	Guinea worm	1 larvae
<i>Ascaris lumbricoides</i> ^a	Ascariasis	1 larvae

^a Found in the tropics.

^b Found exclusively in the tropics.

^c PFU: plaque-forming units; see Hazen *et al.* (1987), Dufour (1986), Hutchinson and Ridgway (1977), and Hawkins *et al.* (1985).

had total coliform counts from 8 to 100,000 CFU per 100 mL. Oluwande *et al.* (1983) also assumed that the high densities of fecal indicators meant that these waters were heavily contaminated with human feces, even though their own data showed that densities of total coliforms were often higher upstream from known contamination sources. Thomson (1981) also found that *Salmonella* spp. in drinking water from wells in Botswana were not correlated to densities of total coliforms, fecal coliforms, or *E. coli*. He observed that *Salmonella* spp. could be found in the complete absence of *E. coli*, or fecal coliforms, and with either high or low densities of total coliforms. Lavoie and Viens (1983) reported that 95% of the traditional water sources in the Ivory Coast, West Africa, had unacceptably high densities of fecal coliforms (11,421 CFU per 100 mL), yet fewer than 55% of the positive total coliform isolates were actually *E. coli* and fewer than 66% of the FC isolates were actually *E. coli* (Lavoie, 1983). However, when he examined the feces of local inhabitants he found that 92% of the total coliform isolates were *E. coli* and 89% of the FC isolates were *E. coli*. Few, if any, of these studies have carefully examined and tested the underlying assumptions of the fecal-pathogen indicator being used. Bonde (1977) eloquently enumerated these criteria as follows:

1. The indicator must be present whenever pathogens are present.
2. It must be present only when the presence of pathogenic organisms is an imminent danger.
3. It must occur in much greater numbers than the pathogens.
4. It must be more resistant to disinfectants and to aqueous environments than the pathogens.
5. It must grow readily on relatively simple media.
6. It must yield characteristic and simple reactions enabling as far as possible an unambiguous identification of the group or species.
7. It should preferably be randomly distributed in the sample to be tested.
8. Its growth on artificial media must be largely independent of any other organism present.

In the late 1800s Houston proposed the idea of using three groups of bacteria (i.e., coliforms, fecal streptococci, and the gas-producing clostridia that are commonly found in the feces of warm-blooded animals) as indicators of fecal pollution of waters (Hutchinson and Ridgway, 1977). He argued that since these groups could only come from fecal sources their presence would indicate recent fecal pollution (Hutchinson and Ridgway, 1977). For nearly 80 years the coliform group of bacteria has been used as such indicators. The indicator used

universally to assess biological contamination of water is *E. coli*, in both tropical and temperate countries (Barbaras, 1986).

The first drinking water regulation for microbial contamination in the United States was published in 1914. This was the first Public Health Service Drinking Water Standards regulation. Subsequently, this regulation was replaced by the U.S. Public Service Acts of 1915 and 1962. The current U.S. regulation comes from the Safe Drinking Water Act (Public Law 93-523, 1974). The U.S. Environmental Protection Agency proposed changes that are now being implemented (*Federal Register* 48:45502-45521, October 1983). The law was approved in July 1986 and is currently in its first phase of implementation. The new regulation requires that there be 0 coliforms/100 mL by any method for any sampling frequency for drinking waters. WHO (Barbaras, 1986) only allows 10 coliforms/100 mL for small community water sources. For tropical nations and tropical parts of the United States, even the old regulations may be unrealistic. As observed in Botswana (Thomson, 1981) and Sierra Leone (Wright, 1982), no correlation could be found between *Salmonella* spp. and *E. coli* in tropical source water. In Puerto Rico densities of pathogenic yeast (Valdés-Collazo *et al.*, 1987), *Klebsiella pneumoniae* (López-Torres *et al.*, 1987), *Legionella pneumophila* (Ortiz-Roque and Hazen, 1987), *Vibrio cholerae* (Pérez-Rosas and Hazen, 1988), *Yersinia enterocolitica* (Elías *et al.*, 1988), and *Aeromonas hydrophila* (Hazen *et al.*, 1987) were also found to be unrelated to densities of *E. coli* in source water. Thus pathogens could be present in the absence of *E. coli* in tropical source water. Indeed, it has been well demonstrated that high densities of enteric viruses, a dominant cause of waterborne disease in both temperate and tropical areas, may be found in the complete absence of fecal coliforms or *E. coli* (Berg and Metcalf, 1978).

In Nigeria (Oluwande *et al.*, 1983), Hawaii (Fujioka and Shizumura, 1985), New Guinea (Feachem, 1974), Puerto Rico (Carrillo *et al.*, 1985), Sierra Leone (Wright, 1982), and the Ivory Coast (Lavoie, 1983), high densities of *E. coli* were found in the complete absence of any known fecal source, i.e., no pathogens. Monitoring of Puerto Rican waters by the U.S. Geological Survey reported that 54 out of 67 water sampling stations on rivers in Puerto Rico exceeded the recommended maximum contaminant levels (MCL) for recreational waters (i.e., <1000 fecal coliforms per 100 mL) during 1984 (Curtis *et al.*, 1984). Thus only 19% of all sites sampled met the recommended MCL for recreational waters, and none of these waters could meet raw source water standards (<2 fecal coliforms per 100 mL). These findings have resulted in condemnation of sewage treatment facilities in Puerto Rico as a source of fecal pollution of natural waters (Hazen *et al.*, 1987). Yet

sampling sites upstream from sewage treatment plants in Puerto Rico had FC densities that were just as high as most downstream sites.

Enumeration of Coliforms in Tropical Waters

Even in temperate waters coliforms have been shown to have limitations using viable count media. Burlingame *et al.* (1984) found bacterial interference with coliform colony green-metallic sheen production on membrane filters on m-Endo medium [American Public Health Association (APHA), 1985] by noncoliform (*Pseudomonas aeruginosa* and *A. hydrophila*) as well as coliform bacteria (*E. coli* and *Enterobacter cloacae*). Means and Olson (1981) reported the formation of products by noncoliforms antagonistic to the growth or biochemical activity of coliforms. Thus high densities of background bacteria, typical of tropical waters, could result in significant underestimation of coliform bacteria.

Recent studies by Xu *et al.* (1982), Colwell *et al.* (1985), Baker *et al.* (1983), and Roszak *et al.* (1984) have shown that enumeration by complex media like those used to grow *E. coli* may not be reliable. These studies have demonstrated that *E. coli* and pathogens like *Salmonella enteridis*, and *V. cholerae* may be able to survive and remain pathogenic, and yet be unculturable.

Pagel *et al.* (1982) compared four standard (APHA, 1985) FC assays in various types of freshwaters in southern Canada. They found that while these assays were somewhat variable in their abilities to detect fecal coliforms from environmental samples, they were all acceptable in terms of their specificity and selectivity. In similar studies in our laboratory (Santiago-Mercado and Hazen, 1987; Hazen *et al.*, 1987), using the same methodology to detect fecal coliforms from freshwaters in Puerto Rico, we found that the specificity of the media (determined by the ability of the medium to restrict growth of organisms other than the target bacterium) was at least 20% less than the specificity claimed by the Canadian investigators (Table III). Thus, all the methods gave significantly higher false positive and false negative errors. Controls using known strains of *E. coli* indicated the accuracy of the methods to be the same in both studies (Table III). Identification of more than 300 FC isolates from various freshwater sites around Puerto Rico showed that less than 40% of these isolates were actually *E. coli* (Santiago-Mercado and Hazen, 1987; Hazen *et al.*, 1987). Similar studies using the same methods in the continental United States (Dufour *et al.*, 1981), South Africa (Grabow *et al.*, 1981), Canada (Pagel *et al.*, 1982), and England (Evison and James, 1973) have demonstrated that more than 90% of FC positive isolates are identified as *E.*

TABLE III
Comparison of fecal coliform assay methods in tropical and temperate source waters^a

Performance Parameter	MMB ^g		M-FC1 ^h		M-FC2 ^h		mTEC ^g	
	Temperate	Tropical	Temperate	Tropical	Temperate	Tropical	Temperate	Tropical
Accuracy ^b	59	67	89	84	100	105	94	93
Specificity ^c								
False positive	11	39	16	30	18	19	13	36
Undetected	4	25	1	20	2	11	2	21
Selectivity ^d	88	66	85	72	90	82	86	66
Comparability ^e								
FC recovery	26	41	41	75	48	94	45	73
Non-FC recovery	4	30	11	29	15	19	7	18
Overall rank ^f	2	4	3	2	1.5	1	1.5	3

^a All data labeled temperate is from Pagel *et al.* (1983); all data labeled tropical is from Santiago-Mercado and Hazen (1987).

^b Mean percentage number of colonies on test medium/[mean number of colonies on a nonselective medium] × 100, using *E. coli* (ATCC 10798 and ATCC 23848).

^c Percentage false positive error = false positive error + number of presumptive target colonies - number of verified target colonies/[total presumptive target colonies] × 100. Percentage false negative error = false negative counts/[verified + undetected target counts] × 100.

^d Selectivity index = presumptive typical colonies/[presumptive typical or target colonies + presumptive nontypical or nontarget colonies] × 100.

^e Percentage FC recovery and percentage nonfecal coliform (NFC) recovery.

^f Best overall efficiency of the method is given by the lowest overall rank.

^g MMB = MacConkey Membrane Broth medium, M-FC1 = MFC medium with no preincubation, M-FC2 = MFC medium with preincubation for 2 h at 35°C, mTEC = mTEC medium.

coli. Wright (1982) found that in Sierra Leone waters fewer than 10% of the positive isolates from fecal coliform assays were *E. coli*. Lavoie (1983) found that less than 66% of the FC isolates from Ivory Coast well waters were actually *E. coli*. It is not surprising that the thermotolerant *E. coli* encounters more mesophilic and thermotolerant background flora in the tropics given the higher and more constant temperatures and productivities. Santiago-Mercado and Hazen (1987) in Puerto Rico and Jen and Bell (1982) in Singapore showed that high densities of mesophilic and thermophilic background flora in tropical source waters were significantly reducing the numbers of *E. coli* on the standard FC media.

In general, the microbial diversity of tropical waters is greater than temperate source water, as are the floral and faunal communities in the watershed. Thus, not only is there a greater array of autochthonous microorganisms, but a greater array of allochthonous microbes and substances entering tropical waters from the surrounding milieu (Hill and Rai, 1982). Barrell and Roland (1979) showed that rainfall in Gambia significantly increased bacterial densities in well water. Fujioka and Shizumura (1985) also found that densities of all bacteria were significantly greater in Hawaiian streams after a rainfall. Carrillo *et al.* (1985) demonstrated that after a torrential rainfall even densities of total anaerobes in Puerto Rico stream water exceeded the most polluted temperate waters for total anaerobes (Daily *et al.*, 1981). Thus, the tropical background microbial flora, environmental characteristics, and survival and activity characteristics of allochthonous microbes are quite dissimilar from temperate waters.

Survival of Coliforms in Tropical Waters

Gordon and Fliermans (1978) demonstrated that *E. coli* could survive for much longer periods of time in a temperate lake receiving thermal effluent. Thus, environmental factors in the tropics might also significantly affect the recovery of fecal coliforms in environmental samples. Bigger (1937) first reported the growth of coliforms in tropical waters, while in India, Ragavachari and Iver (1939) showed that coliforms can survive for several months in natural tropical river waters. In Puerto Rico (Carrillo *et al.*, 1985; López-Torres *et al.*, 1987; Valdés-Collazo *et al.*, 1987; Hazen *et al.*, 1987) showed that *E. coli* not only survived in rain forest streams but also grew (Table IV). Once introduced, *E. coli* could remain and/or become part of the normal flora. Under *in situ* conditions in a tropical rain forest *Y. enterocolitica* (Elías *et al.*, 1988), *K. pneumoniae* (López-Torres *et al.*, 1987), *Candida albicans* (Valdés-Collazo *et al.*, 1987), *A. hydrophila* (Hazen *et al.*, 1982), *Salmonella*

TABLE IV
Comparison of tropical and temperate *in situ* survival of *E. coli*

	Initial density	Survival (h) ^a	Reference
Temperate	10 ⁹	50	Gordon and Fliermans
	10 ⁸	30.6	McFeters <i>et al.</i> (1974)
	10 ⁶	24	Sjogren and Gibson (1981)
Tropical	10 ⁷	∞	Carrillo <i>et al.</i> (1985)
	10 ⁷	294	López-Torres <i>et al.</i> (1987)
	10 ⁶	206	Valdes-Collazo <i>et al.</i> (1987)
	10 ⁶	∞	Ragavachari and Iver (1939)

^a Survival time: time to reach 90% (1 log or T₉₀) reduction of initial cell density.

typhimurium (Jiménez *et al.*, 1988), and *V. cholerae* (Pérez-Rosas, 1983) were all shown to have different survival rates from *E. coli*, their indicator. The inability of *E. coli* to survive in a manner similar to pathogens would further impede its ability to indicate those pathogens. A few temperate investigators have also suggested that coliforms are an inadequate index of water quality in temperate waters (Dutka, 1973; Evans *et al.*, 1981). Temperate survival of *E. coli* in source waters has been shown under numerous circumstances to be only a matter of days, with densities decreasing by more than 90% every 60 min (Bonde, 1977). Regrowth of *E. coli* extraenterally in temperate areas has only been rarely observed (Bonde, 1977). As seen in Table IV *E. coli* always survives significantly longer *in situ* in tropical waters as compared to temperate waters. The increased survival of *E. coli* in tropical waters alone suggests that temperate MCLs based on fecal coliforms cannot be applied to tropical waters.

Natural Origin of FC Bacteria in Tropical Waters

Recent studies have shown that *E. coli* can be isolated from pristine areas of tropical rain forests in Puerto Rico (Rivera *et al.*, 1988; Bermúdez and Hazen, 1988). Plasmid profiles, antibiotic sensitivities, coliphage susceptibility, and physiological and biochemical characteristics confirm that even *E. coli* isolated from epiphytes in trees 15 m above the ground are very similar to clinical isolates of *E. coli* (Rivera *et al.*, 1988). These environmental isolates have identical mol% G + C of the isolates DNA and more than 85% DNA homology with *E. coli* B (Hazen *et al.*, 1987; Bermúdez and Hazen, 1988). Thus tropical source waters may not only have high densities of *E. coli* in the absence of pathogens,

or fecal sources, but *E. coli* may be naturally occurring in some tropical areas. This observation is further suggested by the high densities of *E. coli* in diverse tropical countries in the absence of identifiable fecal sources.

Alternative Indicators for Tropical Waters

Escherichia coli, the indicator used by most developed nations for determining biological contamination of source waters, does not fit most of the underlying assumptions of a good indicator in tropical waters. In fact, only Criteria 7 of Bonde (1977); see above, seems suitable for tropical waters. It is not surprising, therefore, that developing nations in tropical areas are finding it difficult if not impossible to meet their own legislated standards for biological contamination. Since the problems with the FC assay are in part due to *E. coli* being the target organism, changes in media and incubation would not correct these problems for tropical systems. Coliphages have been suggested as being more specific and not subject to the cultivation problems of *E. coli* (Berg and Metcalf, 1978). However, since the host organism for these viruses is *E. coli*, if *E. coli* survives and grows in tropical source water, then likewise the viruses that infect coliforms would also survive and grow. Therefore, coliphages would be no better than *E. coli*, their host, in the tropics.

Several investigations of tropical source water have looked at FS densities. Feachem (1974) found that streams in the New Guinea highlands had densities of fecal streptococci from 0 to 6000 per 100 mL, and the FC/FS ratio ranged from 0.20 to 1.31. Feachem interpreted these results to mean that these waters were 25 times more likely to be contaminated by pigs than humans, based upon the studies of Geldreich and Kenner (1969). However, if *E. coli* is able to survive in these waters the ratio could be inflated. Because of their high densities the fecal streptococci would also seem to be indicating fecal contamination when none may be present. Wright (1982) found that source water in Sierra Leone had densities of fecal streptococci (7–64,000 per 100 mL) that were an order of magnitude lower than densities of either fecal coliforms (40–240,000 per 100 mL) or presumptive *E. coli* (30–120,000 per 100 mL). When FS positive isolates were identified, Wright (1982) found that anywhere from 14 to 100% were confirmed as *Streptococcus faecalis*, the target organism for the assay. In addition, Wright (1982) could find no correlation between the incidence of *Salmonella* spp. in these source waters and the densities of fecal streptococci. Fujioka and Shizumura (1985) also found 100–10,000 CFU per 100 mL of fecal streptococci in streams in Hawaii, even though many of

these streams were not known to have any fecal source of contamination. Densities of fecal streptococci in Hawaiian streams were quite similar to densities of fecal coliforms. Studies in Puerto Rico have demonstrated that *S. faecalis* can survive and remain active for long periods of time in diffusion chambers in a tropical rain forest stream (Muñiz *et al.*, in preparation). Even tropical marine waters with petroleum contamination could support high densities of *S. faecalis* (Santo Domingo *et al.*, in press). Monitoring of Puerto Rican waters by the U.S. Geological Survey has shown that like fecal coliforms, fecal streptococci exceeded recommended MCL for recreational waters more than 80% of the time (Curtis *et al.*, 1984). Yet like fecal coliforms, densities of fecal streptococci were only slightly lower and occasionally higher at sampling stations upstream from sewage treatment plants. Evison and James (1973) suggested that *E. coli* in feces from tropical countries is in much lower proportions to *S. faecalis*. This would also invalidate the use of the FC/FS ratio for determining the origin of the fecal contamination, as would the higher survival rates of both *E. coli* and *S. faecalis* in the tropics. Although even fewer studies have been done in tropical water with the fecal streptococci than with fecal coliforms, the fecal streptococci also only seem to satisfy one of Bonde's eight criteria for an ideal indicator.

Evison and James (1975) suggested that *Bifidobacterium* spp. might be a more appropriate indicator for the tropics. Bifidobacteria are obligate anaerobes, and thus could not grow in most extraenteral environments. In addition, they are always found in the human gut, quite often in higher densities than *E. coli*, and yet are seldom found in other animals (Resnick and Levin, 1981). With the advent of the YN-6 medium (Resnick and Levin, 1981), bifidobacteria would seem to be an excellent candidate for fecal indicator in tropical source waters. Carrillo *et al.* (1985) showed that *Bifidobacterium adolescentis* decreased one order of magnitude each day during *in situ* exposure in a tropical rain forest stream in Puerto Rico. Gyllenberg *et al.* (1960) and Evison and James (1975) showed that densities of bifidobacteria were always greater than densities of *E. coli* in contaminated waters in temperate areas. In a Puerto Rico rain forest, densities of bifidobacteria were greater than *E. coli* at all sites except those at a sewage outfall (Carrillo *et al.*, 1985). In addition, identification of isolates from the rain forest stream using the YN-6 medium showed that less than 80% were actually bifidobacteria. Bifidobacteria show promise as an indicator of recent fecal contamination in terms of lack of survival *in situ* and specificity as a human fecal indicator. Unfortunately, the currently available medium for enumeration (YN-6) is hampered by a lack of

specificity and insufficient resolution when background bacterial densities are high (Carrillo *et al.*, 1985).

Clostridium perfringens has also been suggested as a suitable alternative indicator to fecal coliforms and fecal streptococci in tropical source waters (Fujioka and Shizumura, 1985). They found that in uncontaminated streams, densities of *C. perfringens* were from 0 to 46 CFU per 100 mL, while discharge sites had from 56 to 2100 CFU per 100 mL. Fujioka and Shizumura (1985) found that 91% of Hawaiian isolates from mCP medium were confirmed as *C. perfringens*; this compares favorably to the temperate studies with *C. perfringens* of Bisson and Cabelli (1980). However, densities of *C. perfringens* in human fecal material from the tropics are much lower than any of the previously mentioned indicators (Wright, 1982), and this raises serious doubts as to the ability to easily detect *C. perfringens* when pathogens are an eminent danger. Carrillo *et al.* (1985) found that sites receiving heavy rainfall had high densities of total anaerobes (>500,000 per 100 mL). Fujioka and Shizumura (1985) made the observation that densities of *C. perfringens* increased significantly in uncontaminated sites after rainfall. This suggests that under some conditions *C. perfringens* might have an extraenteral source. Wright (1982) found that source waters in Sierra Leone had densities of *C. perfringens* from 40 to 1500 per 100 mL, that they compared favorably with densities of fecal streptococci, and like the fecal streptococci they were unrelated to the isolation of *Salmonella* spp. *C. perfringens* seems to satisfy more of Bonde's criteria for an ideal indicator than the fecal coliforms or the fecal streptococci, yet it falls short on at least three points.

RECOMMENDATIONS

Because few other studies have reported the use of fecal indicators other than *E. coli* in tropical source water, objective evaluations of the efficacy of these alternate indicators is difficult. At present, obligate anaerobes seem the best candidates for a better indicator for tropical source water, primarily due to their inability to survive extraenterally. However, all of these indicators have the inherent difficulty that under some conditions they may survive and that the media used for their enumeration may allow false positive background flora. In addition, the viable but nonculturable phenomena reported for many pathogens in both temperate and tropical waters suggests that indicators may only rarely be correlated with disease risk in source waters (Colwell *et al.*, 1985; Hazen *et al.*, 1987; López-Torres *et al.*, 1987). The best indicator may be no indicator, i.e., directly enumerate selected

resistant pathogens. This would allow a more realistic estimation of health risk. Immunofluorescent staining can detect densities of pathogenic bacteria as low as 10 cells per mL, a density that may give no culturable counts (Colwell *et al.*, 1985). Nucleic acid probes can detect enteric viruses at densities as low as one virus particle per liter. DNA probes have also been developed for enterotoxigenic *E. coli* (Bialkowska-Habrzanska, 1987; Hill *et al.*, 1983; Moseley *et al.*, 1982) and *Salmonella* spp. (Fitts *et al.*, 1983). Thus direct enumeration of pathogens is currently possible. Common enteric pathogens that might be enumerated could be polio virus, or *Salmonella typhimurium*. Detection of either one of these in tropical source water would indicate risk of human disease. Instead of enumeration, MCLs could be based on detection only. The public health of people living in the tropics and the economic development and respect of their countries in the world community is dependent upon a more suitable standard being developed for tropical source waters. The currently used FC assays are unacceptable for indicating biological contamination of tropical source waters.

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