Proposal Subject:	Refinement of Fecal Colliform Sources
Specific NSSP Guide Reference:	2009 NSSP Guide for the Control of Molluscan Shellfish Section II Model Ordinance Chapter IV Shellstock Growing Areas @.02 Bacteriological Standards.
Text of Proposal/ Requested Action	Add the following statement to Note: "Where there is evidence that the fecal coliform strategy for sampling is effected by false positives from decaying vegetation or other bacteria (within 1000 feet of shoreline) that do not indicate a risk to consumer health, the authority is required to perform adequate source testing. The authority shall subtract these false positive results from the fecal coliform result to get an accurate reading of the actual bacteriological quality of the test station."
Public Health Significance:	None. This additional source testng is to refine the source of fecal in a non-point source remote site where there is no other evidence of human pathogens. There is substantial evidence that the bacteria that is involved in the decay of vegitation does test positive for the fecal coliform in the test that is currently the standard. Three documents are attached to provide adequate and sufficient rationale for this change to the NSSP.
Cost Information (if available):	Unkown. It is expected that cost of sampling will be reduced as more accurate sampling will result in less sampling required.
Action by 2011 Task Force I	Recommended no action on Proposal 11-110. Rationale: Adequately addressed in the Model Ordinance.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 11-110.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-110.

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Infectious Diseases Associated with Molluscan Shellfish Consumption

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INTRODUCTION

Raw and partially cooked molluscan shellfish (clams, oysters, and mussels) have a long history as vectors of infectious agents and marine biotoxins. Illnesses associated with these food sources originate principally from bacterial and viral pathogens and from toxin-producing dinoflagellates concentrated by shellfish during the filter-feeding process. Infectious disease outbreaks have been reported in the United States since the late 1800s; since then, more than 400 outbreaks and 14,000 cases have been reported (Table 1). These illnesses are attributed to bacterial and viral agents that are associated either with human wastes (delivered to estuarine and marine environments in sewage effluents that have received variable levels of treatment) or to bacterial pathogens indigenous to coastal marine environments (e.g., *Vibrio* spp.).

Before the 1950s, the most common illness associated with the consumption of raw molluscan shellfish was typhoid fever (Fig. 1). After several large outbreaks of typhoid in the mid-1920s (15), when more than 1,500 cases and 150 deaths were reported in several U.S. cities, the U.S. Public Health Service convened a committee to establish regulations for the sanitary control of shellfish. This committee, a forerunner of the National Shellfish Sanitation Program, made the following recommendations (7). (i) Shellfish should be marketed from growing areas that, on careful examination, are free from any suspicion of dangerous contamination with disease-producing organisms or from any deleterious or offensive substances. (ii) After their removal from the water, shellfish should be handled in a manner that would safeguard them from contamination with pathogenic microorganisms or nonpathogenic agents (e.g., toxins, heavy metals, and organics), deterioration, or alteration that would render them unfit for consumption, either hygienically or aesthetically. (iii) Epidemiological studies should be conducted for all outbreaks (epidemics) that implicate shellfish so that the sources of the shellfish can be promptly and accurately traced and measures can be initiated to prevent further infection.

It was understood at that time that the inappropriate disposal of raw and partially treated sewage was a principal reason for the increasing incidence of shellfish-borne illness, particularly typhoid fever. In addition, the process of "fattening" oysters, whereby the animals absorb water through osmosis when placed in tanks of low salinity, was also of significant public health concern. Under poor sanitary conditions, these tank waters (and shellfish) may have been contaminated with pathogenic microorganisms, including *Salmonella typhi*.

As the National Shellfish Sanitation Program recommendations gradually gained acceptance, the incidence of typhoid began to decline for at least two reasons. First, the technology for treating sewage wastes improved, particularly with regard to the removal of pathogen-associated particulates and disinfection. Second, a water quality standard was developed for classifying shellfish-growing areas on the basis of densities of the total coliform bacterial indicator group. This early classification system was used to determine whether or not shellfish could be harvested from given waters, depending on the levels of the indicator group found therein. The standard, as one aspect of the National Shellfish Sanitation Program, appears to have been effective, since no shellfish-associated typhoid cases have been reported in the United States in almost 40 years (Fig. 1).

In the past two decades, however, the nature of shellfish-vectored illness has changed. This report considers infectious diseases from a historical perspective, leading up to current public health issues associated with consumption of raw shellfish. It deals with problems that result from the contamination of molluscan shellfish resources by infectious agents from human and/or animal fecal wastes, treated and untreated wastewaters, and the marine environment.

Data are presented for outbreak (defined as two or more cases of illness resulting from a common exposure), incident (a report of infectious disease resulting from a given exposure, involving usually one person and an etiological agent of *Vibrio* spp.), and case reports primarily from the United States. Information used for this report was obtained from federal,

TABLE 1. Cases and outbreaks of infectious disease (all agents) resulting from the consumption of molluscan shellfish (1898–1990)

Decade	Outbreaks		Cases	
	No.	% of total	No.	% of total
1900	11	2.6	364	2.5
1910	7	1.7	208	1.4
1920	17	4.0	2,161	14.8
1930	31	7.4	567	3.9
1940	40	9.5	1,840	12.6
1950	6	1.4	134	0.9
1960	48	11.4	1,726	11.9
1970	44	10.5	871	6.0
1980	217	51.5	6,687	45.9

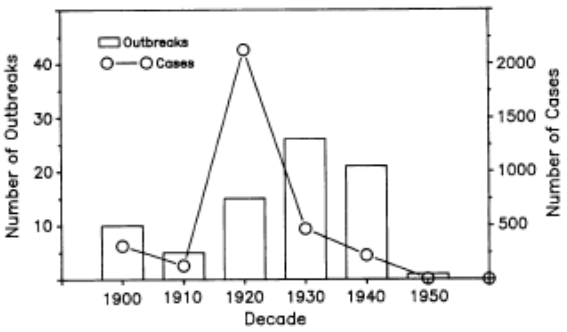

FIG. 1. Number of outbreaks and cases of shellfish-vectored typhoid fever reported since 1894.

state, and local government agencies, research reports, news accounts, and personal communications and does not represent an active, prospective investigation to identify cases of shellfish-associated disease. The data reported here probably represent only a small portion of the actual number of cases that occur annually (10). The true incidence of shellfish-vectored infectious disease may be underestimated as much as 20-fold or more (2). This is true for several reasons. First, because there are no mandatory federal requirements for reporting gastroenteritis of an unspecified nature (i.e., it is not a reportable illness), physicians and state health departments are generally under no obligation to forward case reports to federal authorities. Second, many reported illnesses are cases of relatively mild gastroenteritis; thus, few victims ever seek treatment by a physician. Those reported often describe outbreaks in which relatively large groups of people are affected (e.g., company picnics or gatherings at restaurants). Third, when only a limited number of people are infected, it is very difficult to ascribe the illness to one particular food source. For these reasons, the data may not accurately reflect the true magnitude of the social and economic consequences of illnesses that result in death, that require extended physician and/or hospital care, or, if moderately acute, that prevent individuals from pursuing normal daily activities (2).

ILLNESS ASSOCIATED WITH DISPOSAL OF FECAL WASTES AND SEWAGE INTO THE AQUATIC ENVIRONMENT

Wastewater Disposal Practices and Shellfish-Vectored Illnesses

The association of shellfish consumption and infectious disease has been known or suspected for many years. In 1816, more than 40 years before Pasteur advanced his germ theory of disease, the French physician Pasquier described typhoid fever in a group of people who had consumed oysters harvested from a coastal area contaminated by raw sewage (9, 18). In the United States, infectious bacterial disease associated with molluscan shellfish consumption was first reported in 1894 with two cases of typhoid fever described in Connecticut from shellfish harvested from its coastal waters. No documented cases of infectious disease were reported in the United States before that time, although other types of shellfish-associated illnesses (caused by marine biotoxins) were reported in the late 1700s (16). There are several reasons for this. The construction of storm water or sewerage systems, which began during the mid- to late 1800s in urban centers, resulted in the consolidation of human-derived wastes in collection systems and their eventual release into near coastal environments (8). This practice resulted in the progressive contamination of commercial and recreational shellfish-growing areas and outbreaks of enteric disease associated with shellfish harvested from them. Also, unlike the case for illnesses caused by marine biotoxins, the association between consumption of sewage-contaminated shellfish and infectious disease was not established until the late 1800s. Marine biotoxins, which are produced by dinoflagellates and are a naturally occurring and often highly visible phenomenon, are not associated with sewage contamination of coastal environments. In addition, the occurrence of algal blooms coupled with shellfish-associated human intoxications (generally occurring within several hours of ingestion) establishes an immediately evident relationship. With enteric infections, the relationship is not as clear, since there is no visible measure of water quality and the onset of illness after consumption of contaminated shellfish can be days to weeks.

Until the 1980s, the number of shellfish-associated infectious disease outbreaks was less than 50 outbreaks per decade. Outbreaks and cases by decade as a percentage of the total reported are presented in Table 1. More than 45% of the total historical cases were reported in the 1980s, although there are no obvious reasons for this dramatic increase. However, in the past decade, certain states have adopted aggressive procedures for identifying and describing shellfish-associated disease outbreaks. New York and Florida alone account for more than 50% of the total number of incidents reported nationwide (Table 2). This fact, coupled with increasing consumer awareness of health problems associated with seafood consumption,

TABLE 2. Incidents and cases of shellfish-associated disease (all agents) by principal reporting states

State	Incidents		Cases	
	No.	%[a]	No.	%[b]
Florida	197	26.5	735	5.0
New York	195	26.3	6,611	45.3
Louisiana	48	6.5	195	1.3
Massachusetts	41	5.5	665	4.6
Connecticut	37	5.0	517	3.5
Texas	31	4.2	452	3.1
California	26	3.5	323	2.2
New Jersey	22	3.0	1,989	13.6
Alabama	20	2.7	291	2.0
Georgia	12	1.6	37	0.3

[a] Percentage of the total number of incidents reported nationwide (see text for definitions).
[b] Percentage of the total number of cases reported nationwide.

TABLE 3. Shellfish-borne disease agents occurring in and transmitted by sewage and/or wastewater (1898–1990)

Agent	No. of cases	No. of incidents	No. of outbreaks
Unknown[a]	7,978	277	256
Typhoid	3,270	93	78
Hepatitis A	1,798	51	42
Norwalk virus	311	7	7
Salmonella spp.	130	8	3
Snow Mountain virus	116	4	4
Shigella spp.	111	9	4
Hepatitis[b]	47	5	2
Campylobacter spp.	27	12	1
Plesiomonas spp.	18	3	1
Aeromonas spp.	7	1	1
Staphylococcus aureus	5	1	1
Bacillus cereus	4	1	1
Escherichia coli	2	1	1

[a] No agent isolated or identified.
[b] Type unspecified.

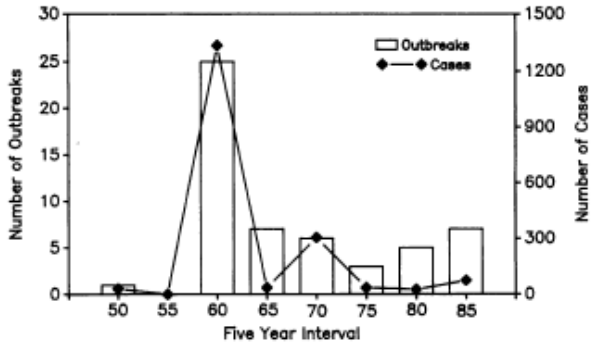

FIG. 2. Shellfish-vectored outbreaks and cases of hepatitis A from 1950 to 1989.

may partially explain the abrupt increase in outbreak and case reports. In addition, shellfish, and particularly oysters, are becoming an increasingly scarce resource as the total acreage of estuarine and marine environments approved or conditionally approved for harvest for direct human consumption decreases with increasing inputs of human-associated contaminants to those areas (14). Also, large areas of potentially productive shellfishing grounds remain closed because they have not been subjected to the sanitary survey work required for proper classification. As a result, there is a strong economic incentive for the illegal harvesting of shellfish from closed but productive growing areas where contaminant loads exceed a generally accepted safe level. This criminal activity is certainly a factor that affects public health, as sewage-contaminated shellfish enter the marketplace. Finally, the rise in case reports may be attributed to deficiencies in current sewage treatment practices (e.g., sewage treatment plants may exceed their design capacity or may have periodic breakdowns which result in inadequate particle removal or disinfection), coupled with the increasing volumes of wastes disposed of in our coastal waters. The use of chlorine to disinfect wastewater effluents is a particular problem in this regard. Certain human enteric viral pathogens (e.g., Norwalk virus) are resistant to the elevated chlorine levels (12) that effectively inactivate vegetative bacterial cells, including the total and fecal coliform indicator groups. Thus, the fecal coliform group, which is the principal indicator of the sanitary quality of most state shellfish-growing waters, may not reliably index the quality of waters that receive chlorine-disinfected effluents. Waters presently considered to be safe for the harvest of molluscan shellfish may, in fact, be contaminated with enteric viral pathogens, and shellfish harvested from those areas may pose an unacceptably high risk of viral illness.

There is no conclusive evidence of an association between contamination derived from animal fecal wastes and the occurrence of shellfish-vectored human illnesses (22). Current assumptions are that illnesses occur primarily from shell stock that accumulate waste from human-associated sources.

Agents of Viral and Bacterial Gastroenteritis

The etiological agents associated with the consumption of raw and lightly cooked molluscan shellfish are listed in Table 3. Most illness reports are ascribed to gastroenteritis, with no causative agent isolated or identified. However, in most cases, the symptoms of disease are very similar. Many reports describe a relatively "mild" gastroenteritis with a typical onset time of 24 to 48 h and a duration of about 2 days. Rarely is a physician's care required. The symptoms, onset, and duration are characteristic of viral gastroenteritis (Norwalk virus has often been implicated). However, since methods for identifying some of these viruses in stools have only recently been developed, and since a limited supply of antigen (obtained from fecal samples of infected individuals) has previously been available only for serological work, these viral pathogens have rarely been identified in shellfish-associated outbreaks. Moreover, there are presently no methods for isolating and culturing viruses from the Norwalk family of agents, including many of the small round viruses.

Bacterial illnesses associated with molluscan shellfish consumption have been infrequently reported since the last case of shellfish-vectored typhoid fever in 1954. Among these bacterial agents (e.g., *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Plesiomonas* spp., *Aeromonas* spp., and *Escherichia coli*), most are found in sewage wastes and are readily inactivated by chlorine disinfection. *Aeromonas* spp. and *Plesiomonas* spp. also occur naturally in freshwater and estuarine environments but appear to present a minimal public health hazard, especially compared with that associated with the environmental *Vibrio* spp. Illnesses caused by *Staphylococcus aureus* or *Bacillus cereus* are most likely a result of postharvest contamination. The recently recognized *E. coli* O157 biotype associated with outbreaks from improperly cooked beef has yet to be associated with a shellfish vector. However, its frequent occurrence in livestock indicates a potential public health problem with shellfish harvest areas affected by farm runoff.

Hepatitis A Infections

Hepatitis A is one of the most serious illnesses associated with shellfish-vectored disease, causing debilitating and chronic infection and even death. The first documented outbreak of shellfish-borne hepatitis occurred in Sweden (19) in 1956, when 629 cases associated with raw oyster consumption were reported. Subsequent to that, hepatitis A cases were reported in the United States (Fig. 2). In 1961, several large outbreaks were reported among consumers of raw oysters in Mississippi and Alabama and consumers of raw clams in New Jersey and Connecticut. In 1964, 20 outbreaks and 743 cases were reported among consumers of both oysters and hard clams and other, unspecified shellfish in several states. In most instances, shellfish harvest areas were not identified for several

reasons. First, the onset time of this illness is 2 to 8 weeks or more, and by then, the implicated shellfish were no longer in the distribution system. Second, the tagging systems used to identify original shellfish harvest sites were, and still are, often unreliable. (The tagging system involves labeling sacks of shellfish with specified information, including harvest area. Tags remain attached to the sacks throughout the distribution network until a specified time after their retail distribution.) There is no standard, nationally accepted tagging system for confidently determining the original harvest area of a given lot of shellfish. Third, the economic incentive for "bootlegging" shellfish (i.e., illegally harvesting animals from unapproved or prohibited areas) is quite compelling. The state patrol procedures needed to deter this illegal activity are often compromised by the lack of financial resources and manpower needed for active and suitable enforcement. In addition, the penalties for these offenses are often not a sufficient deterrent. Thus, shellfish that are not suitable for raw consumption can, and do, enter the marketplace. The magnitude of this problem is not known.

The percentage of hepatitis A virus outbreaks is lower than that caused by certain other infectious agents, and most outbreaks that are reported usually involve a large number of cases. Underreporting of a shellfish-vectored hepatitis A virus outbreak is due to the extended onset period following consumption of the contaminated food and the corresponding difficulty in determining a common food source when only a limited number of individuals are involved. Outbreaks of hepatitis A have been reported consistently since the early 1960s (Fig. 2), and the illness continues to be a public health concern today. Worldwide, the illness is reported frequently. The most disturbing recent incident occurred in China in 1988 (1), when more than 292,000 cases (nine deaths) of hepatitis A (associated with the consumption of uncooked, contaminated cockles) were reported in the urban areas around Shanghai. This outbreak clearly demonstrated the need for effective sanitation programs to prevent the introduction of contaminated shellfish into the marketplace and what can happen when the system breaks down or when there are no effective programs in place.

Seasonality of Illness Reports

Gastroenteritis of an unknown or viral etiology seems to occur more frequently at certain times of the year. When grouped by month, both the outbreak (Fig. 3) and case (Fig. 4) data reveal two periods of increased illness: late spring and late fall. These incidents roughly coincide with times when bioaccumulation rates in shellfish are high. During certain times in spring (4) and fall (5) in temperate waters, hard clams (*Mercenaria mercenaria*) accumulate viruses (and other microbial indicators) at a significantly higher rate than at other times of the year and thus can be periodically contaminated with high levels of sewage-associated microorganisms, including microbial pathogens. This phenomenon is subsequently reflected in the human health effects data. The increased consumption of raw shellfish (particularly hard clams) during these periods may also be coincident with higher illness rates. Although data are lacking on the seasonal incidence of Norwalk and Norwalk-like viruses, they probably show a seasonal occurrence much like that of other viral groups that are epidemic only at certain times of the year. Their input into the marine and estuarine environment would then be intermittent and unpredictable as they pass through the infected individual into the wastewater stream.

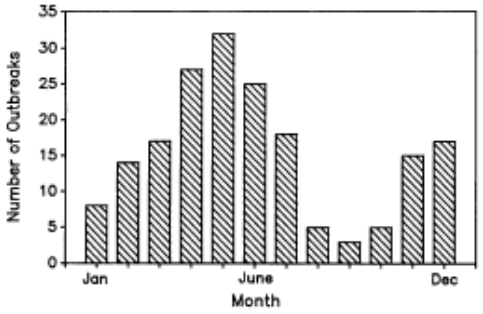

FIG. 3. Number of outbreaks by month of illness ascribed to viral pathogens or to illnesses of undetermined etiology (1894 to 1989).

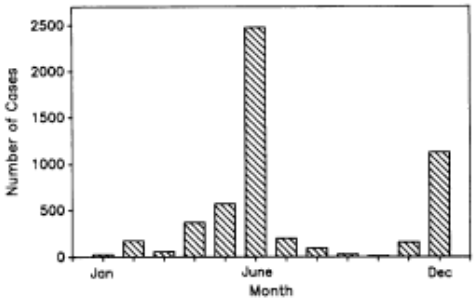

FIG. 4. Shellfish-associated illness cases ascribed to viral pathogens or to illnesses of undetermined etiology (1894 to 1989).

Relative Incidence of Allocthonous Microbial Agents Involved in Disease Outbreaks

The bacterial agents of shellfish-associated disease (Table 4) represent a small proportion of the outbreak (4.0%) and case (3.8%) reports. This may be because the indicator organisms used to assess and classify the sanitary quality of shellfish-growing areas (as open, restricted, or prohibited) effectively protect the health of the shellfish-consuming public against diseases of an allocthonous bacterial origin or because the etiological agents of gastroenteritis associated with shellfish outbreaks are infrequently isolated and identified.

Compared with bacteria, viral agents of shellfish-vectored disease represent a significantly greater proportion (Table 4) of the totals reported. However, those cases in which no agent was isolated represent the bulk of illness reports (more than 75% of the cases and 79% of the outbreaks). If the presumption is correct that most of these "unknowns" can be ascribed

TABLE 4. Shellfish-vectored disease outbreaks and cases by class of agent for sewage- and wastewater-associated pathogens (1905–1990)[a]

Class of agent	Cases		Outbreaks	
	No.	% of total	No.	% of total
Unknown	7,978	75.7	256	79.0
Viral	2,272	21.5	55	17.0
Bacterial	304	3.8	13	4.0

[a] Typhoid fever is not included in this table. The last reported shellfish-vectored case was in 1954.

TABLE 5. Incidents, outbreaks, and cases of shellfish-associated illnesses associated with members of the *Vibrio* genus (1967–1990)

Vibrio species	No. of cases	No. of incidents	No. of outbreaks
V. parahaemolyticus	159	60	14
V. vulnificus	160	133	8
V. cholerae non-O1	143	57	14
V. cholerae[a]	5	3	2
V. cholerae O1	14	14	
V. fluvialis	8	6	1
V. hollisae	15	15	
V. mimicus	14	14	
V. alginolyticus	1	1	
Vibrio spp.	6	4	1

[a] No serotype specified.

to a viral agent (symptomatically), enteric viral pathogens present the principal concern to the public health.

AUTOCHTHONOUS MARINE BACTERIA AS AGENTS OF SHELLFISH-VECTORED ILLNESSES

Several *Vibrio* spp., native to both marine and estuarine environments, have been identified as the causative agents of shellfish-vectored illnesses (Table 5). These halophilic, nonsporeforming bacteria occur in saline aquatic environments in densities that are related, at least in part, to water temperatures and salinity. Other factors that influence their occurrence and distribution in the aquatic environment are not well understood. The severity of human disease caused by the different species (Table 5) varies considerably. *Vibrio vulnificus* infections can result in septicemia with a high mortality rate; cholera has been reported among consumers of raw shellfish for the past two decades. All of the *Vibrio* spp. listed in Table 5, except *V. vulnificus*, are associated with gastroenteritis of varying severity. Among this group, the O1 serogroup of *Vibrio cholerae* is the most serious and debilitating. In general, all of these agents produce a much more severe gastroenteritis than that caused by enteric viral pathogens.

V. vulnificus

Oyster-associated *V. vulnificus* septicemia and death were first reported in 1975 (3). Since then, about ten cases (five deaths) of oyster-borne infections attributable to this species have been reported annually in the United States. The population at risk of developing this illness is well defined because they have certain health problems, such as liver cirrhosis, diabetes, hemochromatosis, and immunosuppressive disorders (17), which predispose them to infectious disease. The case fatality rate averages about 50% among this group. Given the numbers of individuals at risk and the frequency of raw shellfish consumption in certain areas of the United States (13), it is surprising that the number of cases and deaths is not higher. Clearly, the mechanisms of pathogenesis of this organism need further investigation. In addition, temperature abuse (i.e., the holding of shellfish at temperatures in excess of 45°C for prolonged periods of time in transit or in the marketplace) may contribute to illness associated with *Vibrio* spp. (or other bacteria) by providing a condition that would allow these pathogens to multiply in the molluscan shell stock. The significance of the role of temperature abuse in human morbidity or mortality is unknown.

V. vulnificus case reports show a seasonal pattern, with the highest frequencies occurring from midsummer through late

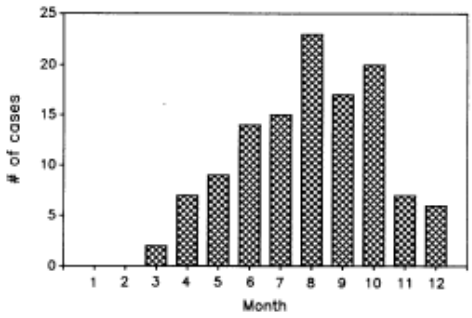

FIG. 5. Cases of shellfish-vectored *V. vulnificus* infections reported by month (1975 to 1989).

fall (Fig. 5). No shellfish-associated cases have been reported in the United States in January or February. Because of its temperature sensitivity, *V. vulnificus* is found in highest densities when water temperatures exceed 15°C (23); below this temperature, environmental densities decline rapidly. *V. vulnificus* is commonly found in all U.S. coastal waters and presumably in all species of near-coastal shellfish in densities that fluctuate with the season. However, case reports associate illness from this organism only with consumption of raw oysters and with shellstock harvested from waters of the U.S. Gulf Coast. The reason for this remains unexplained.

V. cholerae

Cholera was first identified in the United States in 1832, and the illness, involving several large food- and waterborne epidemics (20), was reported periodically until 1911. After that, it was believed to have been eradicated from this country. However, in 1973, a Texas fisherman was diagnosed with the illness (24), although the source of the organism could not be determined. Since that time, *V. cholerae* cases (and deaths), although rare, have been reported sporadically among shellfish consumers (Fig. 6). Both the O1 and other serotypes have been isolated from individuals with relatively severe gastroenteritis. Non-O1 serotypes are reported most frequently, and although the illness caused by them is generally less severe than that caused by O1 serotypes, these organisms have been associated with several oyster-vectored deaths. Non-O1 biotypes are indigenous to marine environments; however, there is no conclusive evidence for an autochthonous marine O1 popula-

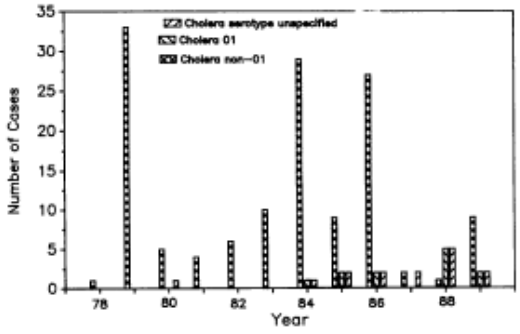

FIG. 6. Shellfish-associated cases of cholera (*V. cholerae* O1, non-O1 cholera, and unspecified serotypes) reported from 1978 to 1989.

TABLE 6. Shellfish-associated outbreaks and cases of infectious illness by type of shellfish (1898–1990)

Species	Agents other than *Vibrio* spp.[a]		*Vibrio* spp.[b]	
	No. of incidents	No. of cases	No. of incidents	No. of cases
Hard clams	232	6,201	4	4
Oysters	155	4,959	279	362
Soft clams	10	43	0	0
Mussels	14	174	1	1
Scallops	1	2	0	0

[a] Includes all illnesses from agents other than those in the *Vibrio* genus and of unknown etiology.

[b] Includes *V. parahaemolyticus*, *V. cholerae* O1 and non-O1, *V. vulnificus*, *V. fluvialis*, *V. mimicus*, and *V. hollisae*.

tion, although this possibility has been advanced (21). No cases of shellfish-vectored, domestically acquired cholera associated with the serotype responsible for the current South American epidemics (6) have been reported in the United States.

Other Vibrios

A number of other *Vibrio* spp. have been associated with shellfish-vectored illness outbreaks (Table 5). *Vibrio parahaemolyticus* cases are reported as frequently as *V. vulnificus* cases. However, illness caused by this bacterium is generally confined to gastroenteritis, although it can be severe and of relatively long duration. A problem in clearly establishing *Vibrio* spp. as etiological agents is that they are all native to marine waters (and presumably shellfish) and may be simply passing through the human gut after the ingestion of uncooked or lightly cooked shellfish. Classical epidemiological methods (e.g., isolation of the organism from the human host followed by reinfection) cannot be used to demonstrate the causality of a suspect organism in a foodborne outbreak. Therefore, the relationship between the isolation of a *Vibrio* sp. from a fecal sample and its role as the causative agent in a case report may be generally assumed but not conclusively established.

SPECIES AND SOURCES OF SHELLFISH FROM ILLNESS REPORTS

Most illness reports in which no causative agent was identified, or the agent was not identified as a *Vibrio* spp., have historically been associated with consumption of hard clams (Table 6). More than 56% of the outbreaks and 54% of the case reports identify hard clams (*M. mercenaria*) as the vector. Oysters (*Crassostrea virginica* and *Ostrea edulis*) are also significant vehicles for infectious illness, associated with 38% of outbreaks and 44% of cases. Soft clams, mussels, and scallops are of minimal public health concern with regard to infectious disease because they are usually cooked before consumption, or only the adductor muscle is usually consumed (scallops). The sources of shellfish (i.e., the original growing areas or last point of water immersion, such as a depuration facility or wet storage area) implicated in these illnesses are predominantly unknown. Of the 412 total outbreaks associated with species other than *Vibrio* spp. (Table 6), 317 (66%) were from shellfish of unknown or questionable origin. New York and Florida, the states most frequently reported as the sources of shellfish implicated in outbreaks, also reported the largest number of outbreaks and cases (Table 2). This association is probably not coincidental.

A completely different history is evident for infections

TABLE 7. Incidents of *Vibrio*-caused illnesses by source of shellfish (1898–1990)

Source of shellfish	No. of incidents[a]
Unknown	198
Louisiana	41
Florida	40
Texas	5
Alabama	5

[a] Incidents are defined as one or more cases from a common source.

associated with certain *Vibrio* spp., particularly *V. vulnificus*, the bacterial species of primary public health concern today. For illnesses caused by these autochthonous bacteria, oysters (principally *C. virginica*) are the predominant vector. More than 98% of the incident reports and 99% of the case reports (Table 6) are associated exclusively with this shellfish species. Most reports involve oysters whose original harvest (or wet storage) sites could not be reliably determined (Table 7); however, positively identified harvest areas were exclusively in Gulf Coast waters. This very interesting fact remains unexplained in light of the limited information available on the marine and estuarine ecology of these aquatic bacteria, particularly since their densities vary widely in the saline environments of all continental U.S. coasts (11, 23). In addition, there is little information on the mechanisms of pathogenesis of these organisms, although the predisposing factors that affect the susceptibility and morbidity of the human host are generally well described.

CONCLUSION

Infectious diseases attributable to the consumption of raw and lightly cooked molluscan shellfish are caused by bacterial agents that are native to the marine environment and by viral and bacterial agents from sewage effluents and other sources that contaminate environmental waters. As filter-feeding organisms, shellfish magnify public health problems associated with environmental contamination because they accumulate microbial pathogens, including viruses, manyfold over the densities found in overlying waters.

The current public health problems of greatest concern to consumers of molluscan shellfish are associated with viral, and suspected viral, pathogens. The numbers of cases and outbreaks caused by these pathogens far exceed those of all other infectious diseases. In terms of the severity of human illness and death, the *Vibrio* genus (specifically *V. vulnificus*) presents a serious problem. Although the number of cases reported yearly is quite low, the high mortality rates involved are of significant public health concern.

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Rate of Occurrence of False-Positive Results from Total Coliform Most-Probable-Number Analysis of Shellfish and Estuaries

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The incidence of confirmed test, false-positive coliform most-probable-number results was compared with environmental parameters and was found to be inversely related to water temperature. It is concluded that the completed coliform test must be done when water temperatures drop below 15°C.

Shellfish harvested from estuarine waters are examined for total numbers of coliforms, along with water and sediment samples from the harvesting areas. The most-probable-number (MPN) analysis (1, 3, 13) is routinely employed and is carried through the presumptive, confirmed, or completed sequence of tests. The completed tests are not always done when the sanitary quality of water is being assessed, notably in the cases of bathing and potable waters (2). To establish a balance between efficiency and accuracy, the incidence of false-positive and false-negative results at each stage of the analysis should be known.

It has been well documented that the presumptive test alone may be of limited reliability (11), historically because of those noncoliforms which may be present and capable of fermenting lactose aerogenically (4, 5, 6, 8, 9, 10, 12). In the study reported here, Chesapeake Bay oysters and oyster beds were examined over a 2-year period. Two sites in Chesapeake Bay, Tolly Point and Eastern Bay, were sampled on a routine basis. These sites were selected because they are commercially important oyster harvest areas and, in addition, the water column of both areas is subject to very little fecal contamination (mean total coliform completed test MPN, 8/100 ml). At approximately 1-month intervals during 1977 and 1978, bottom water samples were collected at one meter above the sediment by means of the Niskin sampler (General Oceanics, Inc.). Sediment samples were collected by using a Petite Ponar grab (Wildlife Supply Co.), and oysters were harvested using a drag-type dredge. All samples were processed within 30 min of collection.

Six oysters, each of which weighed ca. 16 to 20 g, including meat and liquor, after shucking, were scrubbed, rinsed, and aseptically shucked. The oyster tissue was pooled and homogenized in a solution consisting of sterile 0.5% (wt/vol) peptone (Difco Laboratories) in a 1:2 dilution of oyster tissue. Sediment samples were suspended in an estuarine three salts solution (3). Salinity and temperature were measured at the time of collection of the bottom water samples.

A five-tube, total coliform MPN analysis of each of the water, sediment, and oyster samples was performed in duplicate, and the results were normalized for 100 ml (or 100 g) of sample, following procedures recommended by the American Public Health Association (1). Samples (10, 1.0, 0.1, and, for sediment suspension, 0.01 ml) were transferred to appropriate tubes. Lactose broth (Difco), brilliant green bile (2%) broth (Difco), and eosin methylene blue agar (Difco) were employed. Total viable counts (TVC) of aerobic heterotrophic bacteria were enumerated on 30% strength 2216E Marine agar (Difco) (7) plates prepared in triplicate. The TVC plates were incubated at 17 ± 2°C for ca. 15 days before counts were made.

Within each MPN test series, the number of positive results at each successive step (Table 1) was compared, and the proportion of positive presumptive tests which failed to be confirmed as total coliform-positive was defined as the false-positive percent (FP%). The calculation was done using the formula: $FP\% = [(P - C)/P] \times 100$, where P is positive results and C is confirmed (or completed) positive results. This comparison was made for the presumptive-confirmed and also the confirmed-completed test steps. For each sample, the false-positive percentages were, in turn, compared with data for total coliform MPN, TVC, salinity, and temperature. Correlation coefficients were obtained using the Biomedical Computer Programs (BMDP) statistical package on the University of Maryland UNIVAC 1108 computer, and compared with values for critical r (15).

Bottom water salinities ranged from 7.4 to 15.0 ‰. TVC and temperature values are re-

TABLE 1. *Numbers of positive reactions obtained for presumptive, confirmed, and completed coliform MPN tests*

Date	Tolly Point			Eastern Bay			
	Temp (°C)[a]	No. of positive reactions[b]		Temp (°C)[a]	No. of positive reactions		
		Oyster	Bottom water		Oyster	Bottom water	Sediment
24 October 1977	15	21, 20, 20 (—)	12, 8, 8 (1.2E5)	13	10, 1, 1 (1.1E5)	10, 0, 0 (—)	21, 1, 1 (4.4E5)
18 November 1977	11	18, 15, 15 (—)	8, 5, 5 (—)	13	10, 7, 6 (—)	4, 3, 3 (—)	—, —, — (—)
20 December 1977	5	7, 4, 2 (2.0E3)	15, 9, 8 (—)	5	6, 3, 3 (—)	8, 4, 3 (—)	7, 4, 1 (—)
18 January 1978	1	2, 0, 0 (1.7E4)	20, 19, 10 (2.8E4)	1	7, 1, 0 (3.9E4)	9, 4, 1 (9.0E3)	22, 5, 1 (1.3E5)
28 March 1978	6	10, 9, — (5.0E3)	18, 17, — (—)	8	1, 0, — (—)	14, 4, — (—)	23, 0, — (—)
18 April 1978	10	13, 13, 13 (4.0E3)	7, 0, 0 (—)	9	1, 1, 1 (4.0E3)	7, 4, 4 (5.0E3)	23, 10, 3 (4.3E6)
19 May 1978	9	14, 14, 13 (4.7E4)	18, 10, 10 (7.4E4)	9	11, 4, 3 (3.6E5)	12, 3, 0 (6.0E4)	23, 13, 0 (—)
21 July 1978	26	5, 3, 3 (—)	3, 2, 2 (—)	24	5, 1, 1 (—)	1, 0, 0 (—)	4, 0, 0 (—)
6 September 1978	26	9, 7, 7 (9.3E4)	6, 5, 5 (2.0E3)	27	1, 1, 1 (2.7E4)	1, 1, 1 (5.0E3)	20, 1, — (2.3E7)
31 October 1978	14	11, 6, 6 (6.0E3)	9, 5, 5 (2.0E3)	15	17, 14, 14 (3.2E4)	0, 0, 0 (2.0E3)	16, 0, 0 (2.1E5)

[a] Temperature of water 1 to 2 m below surface.

[b] Positive results at each test level: first column is presumptive, second is confirmed, third is completed. Initial observations were recorded for 15 MPN tube series done in duplicate. Number within parentheses is the TVC for the corresponding sample. See text for procedures. —, No data.

ported in Table 1. Oyster total coliform MPN values were consistently low, averaging 81/100 g at Tolly Point and 34/100 g at Eastern Bay. Bottom water total coliform MPN values averaged ca. 12/100 ml and 3.2/100 ml, respectively. Sediment counts at Eastern Bay averaged 13/100 g.

The percent occurrence of false-positive presumptive and confirmed results are presented in Table 2 and Fig. 1, respectively. Overall, some parameters were not found to be correlated (probability, $P < 80\%$). For example, the percent occurrence of false-positive confirmed results did not correlate with: (i) percent occurrence of false-positive presumptive tests ($r = 0.068$); (ii) TVC ($r = 0.193$); or (iii) salinity ($r = -0.125$). Some equivocal correlations ($90\% < P < 95\%$) were noted, and these included total coliform MPN with false-positive percentages, both presumptive ($r = -0.269$) and confirmed ($r = -0.272$), and with salinity ($r = -0.113$).

The most important and definitive relationship detected was that of false-positive and confirmed results and sample temperature (Fig. 1). Although some variations were recorded between the stations as well as for each sample type, it was clear, particularly with regard to sediments (Table 1), that the number of false-positives detected in the confirmation tests increased significantly when the water temperature fell below 10°C. In fact, this relationship was statistically validated for each sample type and station. To cite composite data, water temperatures were found to have a strong negative correlation with percentage of false-positive confirmed tests (−0.593 correlation, significant at

TABLE 2. *Percentage of false-positive presumptive MPN results*

Date	% of false-positive results				
	Tolly Point		Eastern Bay		
	Oyster	Bottom water	Oyster	Bottom water	Sediment
24 October 1977	5	33	90	100	95
18 November 1977	17	38	30	25	—[a]
20 December 1977	43	40	50	50	43
18 January 1978	100	5	86	56	77
28 March 1978	10	6	100	71	100
18 April 1978	0	100	0	43	57
19 May 1978	0	44	64	75	43
21 July 1978	40	33	80	100	100
6 September 1978	22	17	0	0	95
31 October 1978	45	44	17	—	100

[a] —, No data.

the ≥99.9% confidence level; critical $r = 0.372$; $n - 3 = 45$) (Table 3). The temperature at which significant false-positive results begin to be observed may be related to changes in the composition of the bacterial population (14).

Based on the results of this study, it is concluded that, in the past, total coliform MPN (confirmed test) results for cold, estuarine water samples (i.e., <15°C) were subject to error, and reported values may have been higher than was, in fact, the case. It is recommended that the total coliform MPN evaluation of estuarine water, shellfish, and sediment samples include the completed test whenever the temperature of the water falls below 15°C.

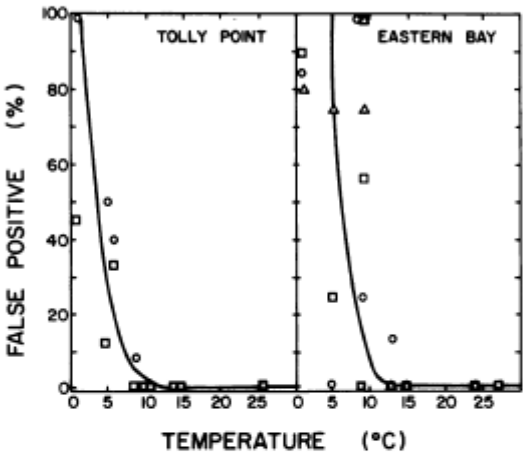

FIG. 1. *Effect of temperature on occurrence of false-positive confirmed MPN tests. Oysters (○), bottom water (□), and sediment (△) were sampled and processed as described in the text. Sample dates are listed in Table 1.*

TABLE 3. *Composite correlation matrix of salinity, temperature, and microbiological parameters*[a]

Parameter	Salinity (‰)	Temp (°C)	Log TVC	Presumptive FP%[b]	Confirmed FP%
Salinity	1.000				
Temperature	0.224	1.000			
Log TVC	0.376[c]	0.206	1.000		
Presumptive FP%	−0.014	−0.104	0.106	1.000	
Confirmed FP%	−0.125	−0.593[d]	0.193	0.068	1.000

[a] Results recorded for each sample, station, and date were combined, and the combined results were correlated.

[b] FP%, False-positive result, percentage of occurrence.

[c] Significant at the 0.05 level, critical $r = 0.367$.

[d] Significant at the 0.01 level, critical $r = 0.372$.

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Closing the Door on the Fecal Coliform Assay

The fecal coliform assay, the results of which have led to numerous misinterpretations over the years, may have outlived its usefulness

Michael P. Doyle and Marilyn C. Erickson

Since its inception in 1904, the fecal coliform assay has been used to assess the presence of fecal contamination in water and foods. Assays to detect *Escherichia coli*, a more specific indicator of fecal contamination, were previously not as popular due to the longer time period for detection required (five days) and their complexity. Recent advances in the detection of *E. coli*, however, have eliminated these impediments and detection occurs within 24 hours or less. Many limitations and complications have been associated with the fecal coliform assay, thereby raising questions about its continued appropriateness and usefulness in food and water testing. The microbiology literature is replete with reports of studies that correlate results of fecal coliform levels with the presence of *E. coli* including several recent examples that advocate the fecal coliform test as an acceptable indicator in manure composts and foods. However, the value of the fecal coliform assay as an indicator of fecal contamination is negated when bacteria of nonfecal origin are the principal microbes detected by the assay.

Historically, the definition of fecal coliforms has been based on methods used for their detection. Specifically, fecal coliforms are gram-negative bacilli, not sporulated, oxidase-negative, optional aerobic or anaerobic, able to multiply in the presence of bile salts or other surface agents that have equivalent properties, and are able to ferment lactose with acid and gas production in 48 h at the temperature of 44 ± 0.5°C. Several genera of bacteria that are common contaminants of nonfecal sources (e.g., plant materials and pulp or paper mill effluents) meet this definition. Examples include *Klebsiella*, *Enterobacter*, and *Citrobacter* species. Moreover, these bacteria which are false-positive indicators of fecal contamination can grow under appropriate conditions in nonfecal niches such as water, food, and waste. The International Commission on Microbiological Specifications for Foods in its evaluation of this issue reported the term fecal coliforms has arisen from attempts to find rapid, dependable methods for establishing the presence of *E. coli* and closely related variants without the need to purify cultures. Species of *Enterobacteriaceae* other than *E. coli* are associated with plants and do not indicate fecal contamination, yet they are identified as fecal coliforms by the fecal coliform assay. Hence, *E. coli* is the only valid index organism for the monitoring of foods containing fresh vegetables.

To reduce the possibility of false-positive results, a confirmatory test for *E. coli* is recommended. In spite of this precaution, there have been several instances where fecal coliform results have been incorrectly interpreted. One of the most sensational situations occurred in 1995 when the U.S. news media reported that high populations of fecal coliforms in restaurant-brewed tea indicated the presence of feces in tea. The dominant fecal coliforms identified were *Klebsiella pneumoniae* and some *Enterobacter* spp., but no *E. coli*. Although there was ample evidence of fecal coliform contamination of iced tea served in restaurants (e.g., 64% of samples at fecal coliform of >1,100 MPN/ml), there had been no history of outbreaks of illnesses resulting from consumption of iced tea.

Another instance where fecal coliform data have been inappropriately interpreted involved two Canadian recalls of sprouts where high levels of fecal coliforms were later identified to be *K. pneumoniae*. In the health hazard alert accompanying these recalls, a warning was issued that this organism could cause gastrointestinal illness in humans. While this bacterial strain is an opportunistic pathogen outside the intestinal tract causing respiratory and urinary tract infections, gastrointestinal illness rarely occurs. Hence, the overly cautious warning was likely due to the association of this bacterium with the fecal organism group.

A quick perusal of the Internet including both

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governmental and academic sites revealed information is being provided that fails to address the possibility that bacteria testing positive in the fecal coliform assay may originate from nonfecal sources. For example, a U.S. Environmental Protection Agency (EPA) page listing drinking water contaminants and their maximum contaminant levels stipulates that "fecal coliforms and *E. coli* only come from human and animal fecal waste." To the contrary, as noted above, there is a preponderance of data indicating that fecal coliforms do not only originate from fecal waste. Similarly, the Kentucky Division of Water site indicates that fecal coliform bacteria "are associated only with the fecal material of warm-blooded animals" and the Food Safety Authority of Ireland site reports that "faecal coliforms found in water are a direct indication that the water has been contaminated with animal or human effluent." Collegiate and K-12 academic sites also provide similar misleading information. Unfortunately, these generalizations can lead to misinterpretation of results by those who do not have a complete understanding of the fecal coliform assay and the subtleties associated with interpreting the results of such assays.

Concerns regarding the inappropriate interpretation of results of the fecal coliform assay and its limited usefulness as an indicator of fecal contamination are not new. They have surfaced several times over the past decade. When the issue of fecal coliforms in tea made media headlines, it was suggested that the fecal coliform assay be reevaluated for its usefulness in food testing. The following year, two commentaries published in *ASM News* opined that the fecal coliform term should be excluded from microbiology. This was further supported by investigators of a study comparing *E. coli*, total coliform, and fecal coliform populations as indicators of wastewater treatment efficiency, who concluded that *E. coli*-based effluent and stream standards (not fecal coliform standards) should be developed to protect public health. A subsequent review of the suitability of the coliform group as an indicator of microbial water safety led other investigators to recommend elimination of the fecal coliform assay. This proposal was further corroborated by studies revealing that only 50% of fecal coliform colonies enumerated as fecal coliforms in foods were identified as *E. coli*.

In the past few years, several changes in monitoring protocols have already been initiated by national and international regulatory agencies. In the European community as well as in Australia and New Zealand, the "fecal coliforms" term has been replaced by what is considered a more appropriate descriptor of this class of microorganisms, "thermotolerant coliforms". Both WHO's Guidelines for Drinking Water Quality and the Australian Drinking Water Guidelines, however, continue to advocate that thermotolerant coliform measurements are an acceptable alternative to *E. coli* measurements. While this change in terminology reduces the likelihood that positive results may be interpreted as meaning the presence of fecal contamination, it does not eliminate the possibility that nonfecal coliforms may be present and give positive results.

In 1986, the U.S. EPA published a document that encouraged states to use *E. coli* or enterococci as the basis of their water quality criteria to protect fresh recreational waters and to use enterococci as the basis for water quality criteria in marine waters. While these guidelines have been criticized, a systematic review and meta-analysis of data reaffirmed these recommendations. More specifically, this analysis revealed that *E. coli* was a more consistent predictor of gastrointestinal illness than other bacterial indicators in fresh water. Despite these recommendations, state and local authorities have been slow to respond in adopting these guidelines. To address some of the advantages and impediments to implementation of these guidelines, costs for the three bacterial indicators were surveyed in the Tacoma/Seattle region and were found to be fairly comparable and thus not a limiting factor. In contrast, an inherent weakness cited by the Washington State Department of Ecology was that using enterococci as an indicator organism in marine waters would complicate efforts to model data obtained from freshwater sources in which *E. coli* was monitored. Another weakness is the continuing requirement by the Food and Drug Administration to use fecal coliforms as an indicator microorganism in shellfish marketed across state borders. Despite this requirement, no significant relationship has been observed between levels of *E. coli* and enterococci and non-*E. coli* fecal coliforms in oysters. Consequently, the continued use of fecal coliforms as an indicator in shellfish would likely hinder widespread acceptance of more appropriate indicators. Moreover, in a National Academies of Science (NAS) report to evaluate candidate indicator organisms and/or indicator approaches, the committee was adverse to abandoning the current indicator microbes until new and better methods are developed and validated. While the NAS Committee foresaw the advent of increasingly sophisticated and powerful molecular biology techniques that would provide new opportunities for the development of improved assays for indicator microbes, we contend that immediate replacement of the fecal coliform assay with an *E. coli* assay would apply the best science available to providing public health protection.

In conclusion, physicians and public health officials have repeatedly misinterpreted results of the fecal coliform assay when applied to food, beverage, or water samples. To prevent future occurrences, the fecal coliform assay should at a minimum be redefined to specifically qualify that it is not a reliable indicator of either *E. coli* or the presence of fecal contamination. An even better alternative would be to eliminate the fecal coliform assay as an indicator of fecal contamination of foods, beverages, and water. The *E. coli* assay is a more reliable indicator of fecal contamination, although not absolute, and could serve as a replacement for the fecal coliform assay.

Literature citations and relevant references which provide the basis for this commentary can be found in the online version of this article.