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Prevalence of *Salmonella* spp. in Oysters in the United States

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Food-borne diseases such as salmonellosis can be attributed, in part, to the consumption of raw oysters. To determine the prevalence of *Salmonella* spp. in oysters, oysters harvested from 36 U.S. bays (12 each from the West, East, and Gulf coasts in the summer of 2002, and 12 bays, four per coast, in the winter of 2002–2003) were tested. *Salmonella* was isolated from oysters from each coast of the United States, and 7.4% of all oysters tested contained *Salmonella*. Isolation tended to be bay specific, with some bays having a high prevalence of *Salmonella*, while other bays had none. Differences in the percentage of oysters from which *Salmonella* was isolated were observed between the summer and winter months, with winter numbers much lower probably due to a variety of weather-related events. The vast majority (78/101) of *Salmonella* isolates from oysters were *Salmonella enterica* serovar Newport, a major human pathogen, confirming the human health hazard of raw oyster consumption. Contrary to previous findings, no relationship was found between the isolation of fecal coliforms and *Salmonella* from oysters, indicating a necessity for specific monitoring for *Salmonella* and other pathogens rather than the current reliance on fecal coliform testing.

Shellfish are known carriers of viral and bacterial pathogens (1, 4). In particular, the consumption of raw oysters has been linked to outbreaks of hepatitis A and viral gastroenteritis (1). The accumulation of pathogenic bacteria and viruses within the oysters make them a hazard for human consumption. Muniain-Mujika et al. (20) performed a study in which *Escherichia coli*, *Clostridium perfringens*, and somatic coliphages were isolated from 24% of shellfish, human adenovirus from 47% of shellfish, and enteroviruses from 19% of shellfish. In addition to these contaminants, oysters have also been responsible for disease outbreaks as a result of contamination with other infectious agents such as enterotoxigenic *E. coli*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Campylobacter jejuni*, noroviruses, *Staphylococcus aureus*, hepatitis A virus, and *Salmonella* (4, 7, 9, 11, 13, 15, 19–21, 24, 26, 28, 29). Seafood and shellfish accounted for 7.42% of all food poisoning related deaths from *Salmonella* infections between 1990 and 1998 (14).

The incidence of *Salmonella* infections has risen dramatically since the 1980s with a loss of productivity in billions of dollars annually (14), and many cases are linked to seafood (6), particularly to the consumption of shellfish (14). An estimated 1.4 million annual cases of salmonellosis in the United States result in approximately 500 fatalities yearly (www.cdc.gov/ncidod/dbmd/diseaseinfo/salmonellosis_t.htm). Salmonellosis is characterized by fever, abdominal cramps, and diarrhea.

Heinitz et al. (14) tested seafood and shellfish around the world for the presence of *Salmonella* spp. and found that U.S. shellfish, particularly oysters, had a 1.2% prevalence of *Salmonella* in domestic shellfish (14). Wilson and Moore (29) conducted a study that showed that 8% of 433 shellfish contained *Salmonella*. Harvesting areas have become more populated in recent years, with more human sewage discharged into coastal waters resulting in an increase in pathogens in these waters, and thus a higher incidence of food-borne disease from shellfish (18). Oysters are filter feeders; as water flows through them, they ingest and concentrate all particulate matter in the water, including pathogenic bacteria and viruses (18).

Since the 1970s, the Food and Drug Administration (FDA) has required the shellfish industry to use fecal coliforms as indicators of contamination within harvesting waters and oysters (14, 15, 19–21). Hood et al. (15) concluded that fecal coliforms were a sufficient indicator of other bacterial pathogens, as *Salmonella* spp. were not present in the absence of fecal coliforms. However, these results are inconsistent with the studies of Heinitz et al. (14), which indicated that *Salmonella* could be present in oysters that did not contain fecal coliforms. The FDA requires that each U.S. state test harvesting waters six times per year for the presence of fecal coliforms. If fecal coliforms are detected above the most probable number (MPN) of 230/g of oyster or 230/ml of water sample, then the waters are closed to harvesting (1, 5, 27). There are no current requirements for U.S. states to test harvesting waters for the presence of human pathogens, such as *Salmonella* spp.

To determine the prevalence of *Salmonella* in oysters and their relationship to oyster fecal coliforms, oysters harvested from 36 bays (12 per coast from the West, East, and Gulf coasts during the summer of 2002, and 12 bays, four per coast, in the winter of 2002-2003) were tested.

MATERIALS AND METHODS

Collection and shipment of oysters. Bays from which oysters were to be harvested were identified on the West, East, and Gulf coasts of the United States and were restricted to those from which licensed shippers, from the Interstate

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TABLE 1. Percentage of *Salmonella*- and fecal coliform-positive oysters per bay in summer 2002

West Coast bay (state)	% Oysters positive		East Coast bay (state)	% Oysters positive		Gulf Coast bay (state)	% Oysters positive	
	Sal.[a]	Coli.[b]		*Sal.*	Coli.		*Sal.*	Coli.
1W (OR)	19.4	20	1E (ME)	27.8	45	1G (FL)	0	100
2W (OR)	2.8	21	2E (ME)	0	72	2G (FL)	5.5	85
3W (WA)	11.1	0	3E (VA)	0	42	3G (FL)	16.7	0
4W (WA)	0	22	4E (NY)	0	38	4G (FL)	77.8	6
5W (WA)	16.7	0	5E (NY)	0	19	5G (LA)	0	97.5
6W (WA)	36.1	5	6E (ME)	41.7	12.8	6G (LA)	0	100
7W (CA)	0	5	7E (DE)	0	23	7G (LA)	0	100
8W (AK)	0	39	8E (DE)	0	93	8G (FL)	5.5	78
9W (AK)	0	39	9E (NY)	0	0	9G (FL)	0	0
10W (OR)	0	15	10E (SC)	0	95	10G (MS)	0	100
11W (AK)	0	19	11E (NY)	0	24.6	11G (LA)	0	75
12W (AK)	0	8	12E (NJ)	2.8	63	12G (LA)	0	100
Total	7.1	16.1	Total	6.0	44.0	Total	8.8	70.1

[a] *Sal.*, *Salmonella*. *Salmonella* is expressed as the percentage of oysters positive in the bay.
[b] Coli., fecal coliforms. Fecal coliforms are expressed as the percentage of oysters above the MPN.

Shellfish Sanitation Conference Shippers List, harvest oysters for consumer markets. Each bay was assigned a number and placed into an Excel spreadsheet where they were randomized, and 12 bays were chosen per coast. Each bay was assigned a specific code according to coast: West Coast, 1- to 12W; East Coast, 1- to 12E; and the Gulf Coast, 1- to 12G. Oysters were collected from all 36 bays in the summer of 2002 and from 12 bays, four per coast, in the winter of 2002–2003. The reduced winter sampling was a result of a lower availability of oysters due to lower harvesting rates. Summer samples were collected between May 2002 and September 2002, while the winter sampling was done between November 2002 and March 2003.

A laboratory member traveled to each bay and purchased 36 oysters. Chain of custody tags were examined to identify the actual location of harvest. Once purchased, the oysters were placed into sealed plastic bags and placed on ice in an ice cooler. The oysters were then shipped overnight to Tucson, Arizona. All oysters were processed within 48 h of purchase.

Processing of oysters for *Salmonella* spp. The oysters were washed individually in 70% ethanol to remove external dirt and debris. The oysters were then shucked aseptically with a sterile shucking knife, and the oyster meat was weighed and dissected with sterile scissors and forceps. One half of the oyster meat from each oyster was used for enrichment of *Salmonella* spp., according to FDA protocols in the Bacteriological Analytical Manual (1), with minor modifications.

The oyster meat was placed into 25 ml of sterile lactose broth supplemented with 1% Triton X-100 (EM Science, Gibbstown, N.J.) and blended (Oster blender) for 30 s on liquefy. Three milliliters of oyster homogenate from lactose broth was aliquoted for the testing of fecal coliforms. The remaining oyster homogenate was placed into conical tubes and incubated at room temperature for 1 h and then at 37°C for 24 h to enrich for *Salmonella* spp. One milliliter of each sample was placed into 10 ml of tetrathionate broth base (Difco, Detroit, Mich.), supplemented with iodine potassium iodide (0.6% iodine, 0.5% potassium iodide) and incubated at 37°C for 24 h. The vortexed tetrathionate broth suspension (100 μl) was plated onto freshly prepared bismuth-sulfite agar (Difco), and the plates were incubated at 37°C for 48 h. For each oyster sample, two putative *Salmonella* colonies (colonies that were black, black with metallic sheen, or brown) were subcultured onto Mueller-Hinton agar (Becton Dickson, Bedford, Mass.) plates. Putative *Salmonella* isolates were stored frozen at −80°C in Luria-Bertani broth supplemented with 20% glycerol.

Detection of fecal coliforms. Fecal coliforms were detected by using standard methodology (27). Ten-fold serial dilutions (1:10, 1:100, and 1:1,000) from the 3-ml aliquot of oyster homogenate were inoculated into five tubes of lauryl sulfate tryptose lactose broth (Difco) and incubated at 37°C for 48 h. Positive samples, determined by gas production in the tubes, were transferred to Brilliant Green lactose bile broth (Difco) for 48 h at 37°C to confirm the presence of coliforms. Positive samples were analyzed using the MPN method (27), providing an estimate of the number of fecal coliforms present per 100 g of oyster meat.

***Salmonella* confirmation by PCR.** Putative isolates were definitively identified as *Salmonella* spp. by PCR with the primers SHIMA-L (5′-CGTGCTCTGGAA AACGGTGAG-3′) and SHIMA-R (5′-CGTGCTGTAATAGGAATATCTTC A-3′), which amplify a 123-bp *Salmonella*-specific product from the *himA* gene (3).

PCR amplification was performed on whole cells in a Bio-Rad I-Cycler (Bio-Rad, Hercules, Calif.) by using a reaction mixture of 50 mM Tris-HCl, pH 8.9, 50 mM KCl, and 2.5 mM $MgCl_2$, containing 200 μM dNTPs (Sigma, St. Louis, Mo.), 0.5 μM each aforementioned primer (QIAGEN, Valencia, Calif.), and 2.5 U of *Taq* DNA polymerase (Biolase, Celmente, Calif.). PCR conditions were as previously published (3), with a 65°C annealing temperature to provide specificity for *Salmonella* spp. Amplified PCR products were separated in a 1.8% Low EEO agarose (Fisher, Pittsburgh, Pa.), 1× TBE (0.445 M Tris, 0.445 M boric acid, 0.1 M EDTA, pH 8.3) gel, containing 0.1 μg of ethidium bromide/ml to visualize DNA bands. A positive control strain, *Salmonella enterica* serovar Typhimurium, and a negative control strain, *E. coli* DH5α, were included in the PCRs.

Serotyping of *Salmonella* isolates. Isolates confirmed as *Salmonella* spp. by PCR were serotyped at the Animal and Plant Health Inspection Services in Ames, Iowa. Serotyping was performed by agglutination with specific antilipopolysaccharide antibodies.

RESULTS

Previous reports have suggested that oysters harvested and sold in the United States may be contaminated with potentially pathogenic *Salmonella* spp. (27). To assess the frequency of contamination, market oysters harvested from a total of 36 bays, 12 bays per coast (East, West, and Gulf coasts), were chosen randomly and 36 oysters from each bay were tested for the presence of *Salmonella* spp. by enrichment and culture on bismuth sulfite agar (Table 1). Ninety-three of 1,296 oysters examined were positive for *Salmonella* spp.

Salmonella spp. were isolated from oysters harvested on each coast; however, oyster contamination was not uniform. The prevalence of *Salmonella* isolation from the oysters was bay specific but not coast specific (Table 1). *Salmonella* spp. were detected in 5 of 12 West Coast bays: 2 of 3 in Oregon and 3 of 4 in Washington. *Salmonella* spp. were not recovered from oysters in any of the Alaskan bays. *Salmonella*-positive oysters were only obtained from 3 of 12 East Coast bays: 2 of 3 in Maine and the single New Jersey bay (12E), while none of 4 New York bays had detectable *Salmonella* contamination. The four Gulf Coast bays which contained *Salmonella*-positive oysters were located in Florida (4/6). *Salmonella* was not detected in oysters from the five Louisiana bays sampled. While oyster

TABLE 2. Percentage of *Salmonella*- and fecal coliform-positive oysters per bay in winter 2002-2003

West Coast bay (state)	% Positive oysters		East Coast bay (state)	% Positive oysters		Gulf Coast bay (state)	% Positive oysters	
	Sal.[a]	Coli.[b]		*Sal.*	Coli.		*Sal.*	Coli.
1W (OR)	8.3	61	1E (ME)	0	11	1G (FL)	0	86
2W (OR)	2.8	0	2E (ME)	2.8	83	2G (FL)	0	61
3W (WA)	0	86	3E (VA)	0	17	3G (FL)	0	55
4W (WA)	2.8	86	4E (NY)	0	0	4G (FL)	0	58
Total	3.5	58.3	Total	0.7	27.8	Total	0	65.0

[a] *Sal.*, *Salmonella*. *Salmonella* is expressed as the percentage of oysters positive in the bay.
[b] Coli., fecal coliforms. Fecal coliforms are expressed as the percentage of oysters above the MPN.

contamination with *Salmonella* was bay specific, the percentage of *Salmonella*-positive oysters within individual contaminated bays varied considerably from 2.8% in bay 2W (Oregon) and 12E (New Jersey) to 77.8% in bay 4G in Florida.

Twelve bays, four from each coastline, were randomly selected from those initially sampled during the summer of 2002, and a second set of oysters were examined from each of the bays approximately 6 months after the initial sampling. The average proportion of oysters harvested from the 12 bays during the summer that were contaminated with *Salmonella* spp. (13.4%) was higher than the average proportion of *Salmonella*-positive oysters harvested in the winter (1.6%) (Table 2). The difference between summer and winter samples was particularly marked in the four Gulf Coast bays, all of which were located in Florida. Three of the four bays showed *Salmonella*-contaminated oysters in summer, with an average percent *Salmonella*-positive oysters of 25% and a high of 77.8% of oysters in bay 4G. However, in winter, all four Florida bays were free of *Salmonella*-positive oysters. *Salmonella* spp. were detected in oysters harvested from a similar number of bays in the summer and winter months, on both the West and East coasts, but the proportion of positive oysters in individual samples was higher in samples harvested during the summer. These results suggest a decrease in *Salmonella* contamination in winter. However, this result is tempered by the detection of *Salmonella* in oysters from two bays, 4W and 2E, in the winter, where no *Salmonella* contamination was detected in the summer months.

***Salmonella enterica* serovar Newport was the predominant serotype isolated from oysters.** To determine whether the *Salmonella* spp. isolated from oysters were major human pathogens, all *Salmonella* spp. isolated during this study were serotyped by the National Veterinary Services Laboratories of the U.S. Department of Agriculture Animal and Plant Health Inspection Services. The serotype breakdown of the 101 isolates was as follows: 78 serovar Newport, 6 serovar Typhimurium (Copenhagen), 9 serovar Arizona (21:G,Z51), 2 serovar Agona, and one each of serovars Adelaide, Arizona (65: K-Z), Bardo, Hartford, Poona, and Reading. The majority of *Salmonella* isolates on all three coasts in both summer and winter were serovar Newport. A greater variety of *Salmonella* serotypes were isolated from oysters harvested on the West Coast, with five other strains isolated. However, with the exception of one serovar Bardo isolate, only serovar Newport was observed on the Gulf Coast.

Comparative presence of fecal coliforms and *Salmonella* spp. Fecal coliforms or total coliform assessments of water samples are currently used by shellfish sanitation agencies to determine the sanitary suitability of specific locations for shellfish harvesting (6, 8–11, 17, 23). For each oyster tested in this study for *Salmonella* spp., the MPN of fecal coliforms was determined and contrasted with the presence of *Salmonella* spp. (Table 1 and Table 2). The percentage of oysters within a bay that exceeded the MPN 230/g limit imposed by the FDA was compared to the percentage of *Salmonella*-positive oysters on all coasts, during either season, and no consistent trend could be found between the presence of fecal coliforms over the FDA imposed limit of MPN 230/g and that of *Salmonella* spp. Therefore, in this study, fecal coliform presence was not a viable predicator of *Salmonella* presence.

In the summer on the West Coast (Table 1), fecal coliforms were detected in oysters harvested from 10 bays, but *Salmonella* spp. were detected in oysters harvested in only five bays. Oysters harvested from two bays contained only *Salmonella* spp., while oysters harvested from seven bays contained only fecal coliforms. Only bay 1W (Oregon) had an equal occurrence of oysters contaminated with both fecal coliforms and *Salmonella* spp. On the East Coast (summer) (Table 1), fecal coliforms were detected in oysters harvested in 11 bays, some with a high percentage of *Salmonella*-positive oysters. Bays 8E (Delaware) and 10E (South Carolina) had over 90% of oysters coliform positive and 2E (Maine) and 12E (New Jersey) were over 50%. *Salmonella* was detected in oysters from three bays. Bay 6E (Maine) had 42% of oysters positive for *Salmonella* and only 12% positive for fecal coliforms, whereas the other two bays containing *Salmonella* had high fecal coliform occurrence. On the Gulf Coast (summer) (Table 1), fecal coliforms were identified in ≥75% of the oysters harvested from nine bays and 100% of the oysters harvested from five bays. However, oysters positive for *Salmonella* were harvested from only four bays. In one Florida bay, 8G, 5.5% of oysters examined had *Salmonella* spp., but no fecal coliforms were detected. In addition, *Salmonella* spp. were detected in 77.1% of oysters harvested in bay 4G (Florida) but only 5% of these oysters contained fecal coliforms at levels above the FDA limit. Oysters harvested from 10 of the 12 bays tested during winter had oysters with fecal coliform contamination (Table 2). However, *Salmonella* spp. were detected in oysters harvested from one of the two bays from which no fecal coliforms were detected.

DISCUSSION

Nontyphoidal *Salmonella* spp. are common food-associated pathogens, and *Salmonella* infections account for a large proportion of deaths associated with food-related illness (6, 18). Potential pathogenic serotypes of *Salmonella* were isolated from oysters harvested on all three U.S. coasts. The oysters sampled were harvested from waters approved for shellfish harvesting, intended for consumers, and sold by shellfish vendors. Although shellfish sanitation agencies work diligently to ensure oysters and other shellfish are harvested from sanitary waters, pathogens that pose a risk to human health are still being detected in oysters sold for human consumption.

The prevalence of *Salmonella* contamination in oysters appears to be bay specific but not coast specific. Despite the fact that there were bays on each coast that contained contaminated oysters, some with a higher prevalence of contamination, the majority of bays on each coast did not have contaminated oysters.

There was a difference between summer and winter contamination levels of fecal coliforms as well as *Salmonella* spp. When comparing the same bays from summer to winter, the summer percentage of oysters positive for *Salmonella* was 13.4%, while the winter proportion was 1.6%. Martinez-Urtaza et al. (18) also noted an increase in *Salmonella* isolation during summer, furthering the possibility that weather has an effect on the presence of *Salmonella* spp. Multiple weather-related factors may be responsible for the lower isolation of *Salmonella* during the winter. For example, in the Gulf Coast, where *Salmonella* prevalence was high in summer but no *Salmonella* was isolated in winter, several hurricanes occurred shortly before the summer harvesting. In addition, winter is the dry season in Florida, where less rainfall and runoff occurs which may potentially reduce the fecal coliform load of these bays. Differences in *Salmonella* isolation between summer and winter may be due to the temperature of the water, with colder waters reducing the presence of bacteria while warmer waters may allow increased bacterial survival (22). In addition, in the summer there is a significant amount of runoff from the mountain snow which contains contaminants and accumulates contaminants as it runs off into the bays (18). Animals are also more active during the summer months, and their increased defecation into streams that feed oyster bays may result in higher fecal, as well as *Salmonella*, contamination rates, since many animals harbor *Salmonella* in their intestines (8). A large number of *Salmonella* serotypes have been detected in cattle. Runoff from agricultural crops may also contribute to the increase of *Salmonella* contamination in summer, since most crops are irrigated with recycled water, which may contain fecal and *Salmonella* contaminants, as stated by the United States geological survey (http://water.usgs.gov/pubs/pp/pp1655.html). It has been documented that alfalfa sprouts also contain *Salmonella* spp., including serovar Newport (16, 26). If *Salmonella* is present when the crops are irrigated, the runoff goes into local streams. This would be a common occurrence on all coasts, crops such as alfalfa, wheat, grapes, and apples are abundant on the West Coast, tobacco on the East Coast, and citrus in the Gulf Coast.

All serotypes that were isolated from the oysters have been associated with human disease (www.cdc.gov/ncidod/dbmd/phlisdata/salmtab2001/SalmonellaAnnualSummary2001.pdf). Serovar Newport was the predominant *Salmonella* isolate from oysters, with 78 of 101 (77.2%) *Salmonella* isolates being of this serotype. Serovar Newport is emerging as an important human pathogen that has been extensively associated with cattle (8, 10). Serovar Newport also has a wide range of hosts such as cattle, swine, water fowl, and poultry, as well as marine life, which may contribute to the contamination of water sources in which oysters are harvested (8, 10, 14, 25).

Serovar Newport is an emerging *Salmonella* serotype associated with human salmonellosis. In 2001, serovar Newport was the third highest cause of *Salmonella*-associated human gastroenteritis, behind serovars Typhimurium and Enteritidis, and linked to nearly 10% of human cases of *Salmonella* infection (www.cdc.gov/ncidod/dbmd/phlisdata/salmtab/2001/SalmonellaAnnualSummary2001.pdf). The incidence of serovar Newport infections has increased probably as a result of multiple reservoirs for serovar Newport (8, 11, 16, 19, 22, 26) and the emergence of multidrug-resistant strains (12). The ability of serovar Newport to attach to cells from a number of different host species may be a function of the fimbriae produced by this organism (2, 7), allowing for many carriers.

One purpose of this study was to determine if the currently accepted method of fecal coliform testing was a sufficient way to examine the contamination rate of market oysters as previously proposed (15, 20). Our present study contradicts the previous work, which found no evidence of *Salmonella* spp. when fecal coliforms were not present (20). Our results may reflect an increasing incidence of *Salmonella* spp. in harvesting waters over the past decade, due to encroachment of human and animal habitation (18) on these waters. Consistent with this interpretation, our study showed a higher prevalence of *Salmonella*, with a national prevalence of 7.4% of oysters positive for the presence of *Salmonella*, than did Heinitz et al. (14), who reported just 1.2% of domestic U.S. oysters contaminated with *Salmonella*. While this increase seems significant, it is difficult to compare between studies because of differences in sampling strategies. However, in light of the apparent increase in prevalence of *Salmonella* in oysters, there is a need to update testing techniques for *Salmonella* in shellfish. The guidelines for oysters set forth in the Sanitation of Shellfish Growing Areas (National Shellfish Sanitation Program 1992) (5) state that the water should be tested for coliforms every three months and that the actual oyster meat be tested only every 10 years. This represents an unacceptable testing regimen for filter feeders such as oysters that concentrate all microorganisms ingested, and it ignores the apparently changing relationships between fecal coliforms and other pathogens, as indicated by Wilson and Moore (29) and our study. It is worth noting, that the waters from which the oysters in this study were harvested were not tested for coliforms or *Salmonella* spp., but it is likely that oyster concentrates are reflective of the contamination of the water.

The results of our study indicated that there was no correlation between fecal coliform numbers and *Salmonella* isolation. Some bays in which there was a high prevalence of *Salmonella*, as in 4G (Florida) with 77.8% of the oysters positive for *Salmonella*, there was a low prevalence of oysters with coliform MPN over the FDA limit. There were three bays, two in the summer and one in the winter, that only had *Salmonella*

present, and coliforms were not detected. There were many bays that had both present, sometimes with high coliform and low *Salmonella* contamination or vice versa. In addition, many bays with high numbers of coliform-positive oysters had no *Salmonella*, such as 1G in Florida. Thus, monitoring of bacterial contamination of oysters solely based on coliform testing or water sampling testing is not justified and in fact is likely to overlook *Salmonella*-contaminated oysters. The testing of the oyster meat specifically for *Salmonella* spp. on a regular basis throughout the year, in each bay open for harvesting, would appear to be the only mechanism to remedy this oversight.

In conclusion, both fecal coliforms and *Salmonella* spp. were isolated from oysters harvested and intended for human consumption. The current use of the MPN procedure to quantify the presence of fecal coliforms does not seem to be effectively eliminating the risk of infection with *Salmonella* when consuming oysters. A number of alternative indicators have been examined as potential replacements for fecal and total coliform monitoring. Isolation and characterization by DNA fingerprinting, using evolving molecular technology, is being increasingly examined as an adjunct for surface water monitoring. Reliance on a single indicator may be overly optimistic and the combination of a profile of indicators may prove more useful for surface water monitoring and the classification of shellfish growing areas as either open or closed for shellfish harvesting.

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REFERENCES

1. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella*, p. 5.01–5.20. *In* FDA Bacteriological Analytical Manual, 8th ed. (revision A). Food and Drug Administration, Washington, D.C.
2. **Baumler, A. J., R. M. Tsolis, F. A. Bowe, J. G. Kusters, S. Hoffmann, and F. Heffron.** 1996. The *pef* fimbrial operon of *Salmonella typhimurium* mediates adhesion to murine small intestine and is necessary for fluid accumulation in the infant mouse. Infect. Immun. **64:**61–68.
3. **Bej, A. K., M. H. Mahbubani, M. J. Boyce, and R. M. Atlas.** 1994. Detection of *Salmonella* spp. in oysters by PCR. Appl. Environ. Microbiol. **60:**368–373.
4. **Beuret, C., A. Baumgartner, and J. Schluep.** 2003. Virus-contaminated oysters: a three-month monitoring of oysters imported to Switzerland. Appl. Environ. Microbiol. **69:**2292–2297.
5. **Center for Food Safety and Applied Nutrition Shellfish Sanitation Branch.** 1993. National Shellfish Sanitation Program manual of operations, appendix K 1–4, p. C1–C4. C. K. Smoley, Boca Raton, Fla.
6. **Centers for Disease Control and Prevention.** 2000. Surveillance for foodborne disease outbreaks—United States, 1993–1997. Morb. Mortal. Wkly. Rep. Surveill. Summ. **49:**1–51.
7. **Chen, L. M., K. Kaniga, and J. E. Galan.** 1996. *Salmonella* spp. are cytotoxic for cultured macrophages. Mol. Microbiol. **21:**1101–1115.
8. **Clegg, F. G., S. N. Chiejina, A. L. Duncan, R. N. Kay, and C. Wray.** 1983. Outbreaks of *Salmonella* Newport infection in dairy herds and their relationship to management and contamination of the environment. Vet. Rec. **112:**580–584.
9. **Fair, G. M., J. C. Geyer, and D. A. Okun.** 1996. Water and wastewater engineering. Water Supp. Wastewater Rem. **8:**1–8.
10. **Fedorka-Cray, P. J., D. A. Dargatz, L. A. Thomas, and J. T. Gray.** 1998. Survey of *Salmonella* serotypes in feedlot cattle. J. Food Prot. **61:**525–530.
11. **Fiandrino, A., Y. Martin, P. Got, J. L. Bonnefont, and M. Troussellier.** 2003. Bacterial contamination of mediterranean coastal seawater as affected by riverine inputs: simulation approach applied to a shellfish breeding area. Water Res. **37:**1711–1722.
12. **Fontana, J., A. Stout, B. Bolstorff, and R. Timperi.** 2003. Automated ribotyping and pulsed-field gel electrophoresis for rapid identification of multidrug-resistant *Salmonella* serotype Newport. Emerg. Infect. Dis. **9:**496–499.
13. **Gerba, C. P., and J. S. McLeod.** 1976. Effect of sediments on the survival of *Escherichia coli* in marine waters. Appl. Environ. Microbiol. **32:**114–120.
14. **Heinitz, M. L., R. D. Ruble, D. E. Wagner, and S. R. Tatini.** 2000. Incidence of *Salmonella* in fish and seafood. J. Food Prot. **63:**579–592.
15. **Hood, M. A., G. E. Ness, and N. J. Blake.** 1983. Relationship among fecal coliforms, *Escherichia coli*, and *Salmonella* spp. in shellfish. Appl. Environ. Microbiol. **45:**122–126.
16. **Inami, G. B., and S. E. Moler.** 1999. Detection and isolation of *Salmonella* from naturally contaminated alfalfa seeds following an outbreak investigation. J. Food Prot. **62:**662–664.
17. **Lee, C. Y., G. Panicker, and A. K. Bej.** 2003. Detection of pathogenic bacteria in shellfish using multiplex PCR followed by CovaLink NH microwell plate sandwich hybridization. J. Microbiol. Methods **53:**199–209.
18. **Martinez-Urtaza, J., M. Saco, G. Hernandez-Cordova, A. Lozano, O. Garcia-Martin, and J. Espinosa.** 2003. Identification of *Salmonella* serovars isolated from live molluscan shellfish and their significance in the marine environment. J. Food Prot. **66:**226–232.
19. **Mead, P. S., L. Slutsker, V. Dietz, L. F. McCraig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe.** 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. **5:**607–625.
20. **Muniain-Mujika, I., M. Calvo, F. Lucena, and R. Girones.** 2003. Comparative analysis of viral pathogens and potential indicators in shellfish. Int. J. Food Microbiol. **83:**75–85.
21. **Nakano, T., K. Nakanishi, H. Ohashi, M. Araki, T. Ihara, H. Kamiya, Y. Iwade, A. Yamauchi, and A. Sugiyama.** 2002. Invasive food poisoning caused by *Salmonella oranienburg*. Pediatr. Int. **44:**106–108.
22. **Rhodes, M. W., and H. Kator.** 1988. Survival of *Eschericia coli* and *Salmonella* spp. in estuarine environments. Appl. Environ. Microbiol. **54:**2902–2907.
23. **Rose, J. B., and M. D. Sobsey.** 1993. Quantitative risk assessment for viral contamination of shellfish and coastal waters. J. Food Prot. **56:**1043–1050.
24. **Tenuis, P., A. Havelaar, J. Vliegenhart, and G. Roessink.** 1997. Risk assessment of *Campylobacter* species is shellfish: identifying the unknown. Water Sci. Tech. **35:**29–34.
25. **Thorton, V., W. Hazell, and G. Simmons.** 2002. Acute gastroenteritis associated with seafood privately imported from the Pacific Islands. N. Z. Med. J. **115:**234–236.
26. **Van Beneden, C. A., W. E. Keene, R. A. Strang, D. H. Werker, A. S. King, B. Mahon, K. Hedberg, A. Bell, M. T. Kelly, V. K. Balan, W. R. MacKenzie, and D. Fleming.** 1999. Multinational outbreak of *Salmonella enterica* serotype Newport infections due to alfalfa sprouts. JAMA **281:**158–162.
27. **Vanderzant, C., and D. Splittstoesser.** 1992. Molluscan shellfish: oysters, mussels, and clams, p. 60–62, 511. *In* C. A. D. S. Vanderzant (ed.), Compendium of methods for the microbiological examination of foods. Culinary and Hospitality Publishing, Weimer, Tex.
28. **Waage, A. S., T. Vardund, V. Lund, and G. Kapperud.** 1999. Detection of small numbers of *Campylobacter jejuni* and *Campylobacter coli* cells in environmental water, sewage, and food samples by a seminested PCR assay. Appl. Environ. Microbiol. **65:**1636–1643.
29. **Wilson, I. G., and J. E. Moore.** 1996. Presence of *Salmonella* spp. and *Campylobacter* spp. in shellfish. Epidemiol. Infect. **116:**147–153.