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Presence of *Salmonella* spp. and *Campylobacter* spp. in shellfish

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SUMMARY

Bivalve molluscs, (cockles, mussels, scallops and oysters) were examined according to EC shellfish bed classification regulations for faecal coliforms, *Escherichia coli* and salmonella, and for coliforms and campylobacter which are not specified by these regulations. Salmonella serotypes were detected in 8% of 433 molluscs. Seven salmonella isolations (2%) were made from category A beds, nominally suitable for immediate consumption according to *E. coli* counts. A higher percentage of salmonella isolates (6%) was detected in shellfish which require relaying or depuration prior to eating. In another survey, thermophilic *Campylobacter* spp. were found in 42% of 380 shellfish. These findings show that shellfish bed classification on the basis of indicator organisms alone is not sufficient to assure the absence of bacterial, and no doubt viral, pathogens. Depuration and end product specifications which require the absence of salmonellae are an essential part of these regulations. Microbiologists may wish to consider whether tests for pathogens such as salmonella and campylobacter should be included when determining the suitability of shellfish for human consumption.

INTRODUCTION

It is well known that estuarine filter-feeders are prone to contamination by faecal pathogens from sewage polluting the waters in which they grow [1–3]. Viral agents such as small round virus (SRV), small round structured virus (SRSV), hepatitis A virus, poliovirus and rotavirus are responsible for more cases of illness than are bacterial pathogens [1, 4]. Salmonella have been shown to survive for over a month in aqueous-sediment microcosms [5, 6] and to be detected in a high proportion of dried oysters which had not been depurated [7]. Depuration in clean water may remove bacterial contaminants [4], but is recognized as unreliable for the elimination of viral contamination [8].

In the absence of straightforward and reliable tests for viruses, the EC Directive on shellfish hygiene EC 91/492/EEC [9, 10] employs bacterial indicator organisms for the classification of shellfish harvesting beds and requires relaying or depuration or approved heat treatment for those not meeting the specification for immediate consumption (Table 1). Shellfish harvested from beds which are considered suitable must also meet end product criteria for visual inspection, salmonella, chemical, radionuclide and marine biotoxin levels. Molluscs harvested from category A beds which do not meet the end product criteria are not suitable for immediate human consumption.

United States standards likewise rely heavily on bed classification using faecal coliforms/*E. coli* indicators

Table 1. *Shellfish classification categories and their criteria (EC 91/492/EEC)*

Category A	< 230 *E. coli*/100 g	May go for direct consumption if end product standard met.
Category B	< 4600 *E. coli*/100 g in 90% of samples	Must be depurated, heat treated or relaid to meet category A.
Category C	< 60000 faecal coliforms/100 g	Must be relaid for a long period (at least 2 months) to meet category A or B, or heat treated.
Prohibited	> 60000 faecal coliforms/100 g	Harvesting prohibited.

[11] in addition to temperature control, HACCP and education. It is acknowledged in this document that faecal coliforms/*E. coli* do not correlate with viral contamination or other bacterial pathogens.

Risk assessment for viral contamination has been performed [12] but considerable uncertainty remains. Even depurated oysters from class A beds have been responsible for viral outbreaks [13]. Investigation of viral gastroenteritis outbreaks associated with oysters has shown that monitoring waters for faecal coliforms is insufficient to indicate the presence of viruses [14, 15]. These comments have been validated by the finding [16] that rotavirus and hepatitis A virus were present in around half a category A (Table 1) mussels sampled, and that the risk of infection could be dramatically reduced by cleansing in ozonated marine water and by steaming. Mussels and cockles are generally steamed before consumption in any case. Oysters are eaten raw, but sometimes are lightly cooked when incorporated in value-added products.

The clinical and economic consequences of eating contaminated shellfish may be serious, and inadequate microbiological quality assurance of end product shellfish has led to charges of manslaughter [17], albeit unsuccessful [18].

The aim of the present study was to investigate whether shellfish bed classification and current end product specifications are adequate to protect individuals eating shellfish from bacterial pathogens.

MATERIALS AND METHODS

Marine bivalve molluscs (cockles, *Cardium edule*; mussels, *Mytilus edulis*; scallops, *Pecten maximus*; and oysters, *Crassostrea gigas*) were collected from authorized harvesting beds by hand fishing, dredging and diving between January and December 1994, and examined within 12 h of collection.

Shucked meat was examined for vibrio, salmonella, *Clostridium perfringens*, coliforms, faecal coliforms, and *E. coli*, generally by statutory methods [2, 10].

Examination for *Cl. perfringens* took place between March and December 1994.

Campylobacter spp. were sought by enrichment culture [19] between October 1993 and August 1994, and presumptive colonies tested essentially as described by Abeyta and colleagues [3] with the exception that Exeter medium was used instead of Campylobacter Blood Free Selective Charcoal Agar Base. Latex agglutination was not used. Presumptive colonies from Exeter agar plates were examined by light microscopy and the hanging drop technique. Typical isolates exhibiting a curved (gull) shape and rapid, darting, corkscrew-like motility were tested biochemically for genus confirmation and species identification as described by Abeyta and colleagues [3].

RESULTS

Between January and December 1994, 433 shellfish samples were examined. Of these, 36 (8%) contained salmonellas of which 7 (2%) were in shellfish from beds classified as Category A (Table 2). Most isolations were made in the cooler months between October and January (Table 2). Also, 131 shellfish (30%) contained *Clostridium* spp., mainly *Cl. perfringens*. In all cases, the numbers of clostridia present were low ($\leqslant 10^3$ cfu/g). *Vibrio* spp. were not isolated from any samples.

Examination for *Campylobacter* spp. was carried out on both shellfish which had been recently harvested directly from the seabed and on those which had been depurated. Of 380 shellfish tested, 331 were cockles, mussels or scallops examined shortly after harvesting and 47% contained *Campylobacter* spp. The remaining 49 samples were oysters which had been depurated and were ready to eat. Three (6%) of the depurated oysters contained *Campylobacter* spp. These three isolates were *C. lari* biotype 0075, UPTC biotype 2030 and an atypical *Campylobacter* sp. biotype 0531. None of these isolates was phage-typeable. Overall, 159 (42%) of 380 shellfish were positive. The seasonality of campylobacter isolations

Table 2. *Indicator organisms, shellfish bed category and presence of* salmonella

Month	Indicator organisms cfu/g				Salmonella	Category
	*Cl. perfringens**	Coliforms	Faecal coliforms	*E. coli*		
Jan.	nt	$2{\cdot}2 \times 10^3$	110	110	*S. stanley*	A†
Jan.	nt	$4{\cdot}3 \times 10^3$	320	320	*S. agona*	B
Jan.	nt	160	160	70	*S. derby*	A†
Jan.	nt	$9{\cdot}1 \times 10^4$	$3{\cdot}5 \times 10^4$	$3{\cdot}5 \times 10^4$	*S. newport*	C
Jan.	nt	$1{\cdot}6 \times 10^5$	$5{\cdot}4 \times 10^4$	$3{\cdot}5 \times 10^4$	*S. newport*	C
Jan.	nt	$9{\cdot}1 \times 10^4$	$9{\cdot}1 \times 10^4$	$9{\cdot}1 \times 10^4$	*S. newport*	C
Jan.	nt	$9{\cdot}1 \times 10^4$	$3{\cdot}5 \times 10^4$	$3{\cdot}5 \times 10^4$	*S. newport*	C
Jan.	nt	$3{\cdot}5 \times 10^4$	$3{\cdot}5 \times 10^3$	$3{\cdot}5 \times 10^3$	*S. san-diego*	B
Jan.	nt	$2{\cdot}2 \times 10^4$	$5{\cdot}4 \times 10^3$	$5{\cdot}4 \times 10^3$	*S. heidelberg*	C
Jan.	nt	$5{\cdot}4 \times 10^4$	$1{\cdot}1 \times 10^4$	$3{\cdot}5 \times 10^3$	*S. typhimurium*	B
Jan.	nt	$2{\cdot}4 \times 10^4$	$3{\cdot}5 \times 10^3$	$2{\cdot}4 \times 10^3$	*S. kimuenza*	B
Jan.	nt	$3{\cdot}5 \times 10^4$	$3{\cdot}5 \times 10^3$	$3{\cdot}5 \times 10^3$	*S. kimuenza*	B
Jan.	nt	$5{\cdot}4 \times 10^4$	$3{\cdot}5 \times 10^3$	$2{\cdot}4 \times 10^3$	*S. wien*	B
Jan.	nt	$1{\cdot}6 \times 10^4$	750	750	*S. ohio*	B
Jan.	nt	$5{\cdot}4 \times 10^4$	230	230	*S. ohio*	B
Feb.	nt	$5{\cdot}4 \times 10^4$	$2{\cdot}2 \times 10^3$	$2{\cdot}2 \times 10^3$	*S. bredeney*	B
Feb.	nt	$> 1{\cdot}8 \times 10^5$	$4{\cdot}3 \times 10^3$	$4{\cdot}3 \times 10^3$	*S. newport*	B
Mar.	< 100	$3{\cdot}5 \times 10^3$	320	250	*S. agona*	B
Apr.	100	$2{\cdot}4 \times 10^3$	$2{\cdot}4 \times 10^3$	$2{\cdot}4 \times 10^3$	*S. newport*	B
Apr.	400	$5{\cdot}4 \times 10^3$	$5{\cdot}4 \times 10^3$	$5{\cdot}4 \times 10^3$	*S. heidelberg*	C
May	< 100	$1{\cdot}1 \times 10^3$	90	50	*S. virchow*	A†
May	< 100	$1{\cdot}1 \times 10^3$	90	50	*S. virchow*	A†
Aug.	100	$1{\cdot}1 \times 10^4$	$1{\cdot}7 \times 10^3$	$1{\cdot}1 \times 10^3$	*S. typhimurium*	B
Aug.	400	$9{\cdot}1 \times 10^3$	$1{\cdot}4 \times 10^3$	$1{\cdot}1 \times 10^3$	*S. typhimurium*	B
Sept.	< 100	$2{\cdot}4 \times 10^3$	50	50	*S. typhimurium* PT104	A†
Sept.	< 100	$2{\cdot}4 \times 10^3$	50	50	*S. typhimurium* PT104	A†
Oct.	< 100	310	50	50	*S. typhimurium*	A†
Oct.	700	$5{\cdot}4 \times 10^4$	$2{\cdot}4 \times 10^4$	$2{\cdot}4 \times 10^4$	*S. typhimurium*	C
Oct.	< 100	$9{\cdot}1 \times 10^3$	$2{\cdot}2 \times 10^3$	$2{\cdot}2 \times 10^3$	*S. enteritidis*	B
Oct.	200	$9{\cdot}1 \times 10^3$	$5{\cdot}4 \times 10^3$	$5{\cdot}4 \times 10^3$	*S. bredeney*	C
Oct.	100	$2{\cdot}4 \times 10^3$	500	500	*S. istanbul*	B
Dec.	1000	$> 1{\cdot}8 \times 10^5$	$1{\cdot}6 \times 10^5$	$1{\cdot}6 \times 10^5$	*S. newport*	D
Dec.	< 100	$1{\cdot}1 \times 10^3$	750	750	*S. newport*	B
Dec.	< 100	$2{\cdot}4 \times 10^3$	$1{\cdot}1 \times 10^3$	$1{\cdot}1 \times 10^3$	*S. newport*	B
Dec.	400	$1{\cdot}7 \times 10^4$	$1{\cdot}6 \times 10^4$	$1{\cdot}6 \times 10^4$	*S. newport*	C
Dec.	400	$5{\cdot}4 \times 10^3$	$3{\cdot}5 \times 10^3$	$3{\cdot}5 \times 10^3$	*S. newport*	B

* nt, not tested; † nominally suitable for immediate consumption.

Table 3. *Seasonal variation in* campylobacter *isolations from shellfish*

Sampling period	Number tested	Number positive	% positive
11 Oct. 1993–31 Jan. 1994	103	83	81
1 Feb. 1994–31 Apr. 1994	115	67	58
1 May 1994–31 Aug. 1994	162	9	6
Total	380	159	42

was marked (Table 3) with few recovered between May and August, and most in the cooler winter months (November–March).

The percentages of *Campylobacter* spp. among these isolates were: *C. jejuni*, 2%; *C. coli*, 8%; *C. lari*, 24%; *Campylobacter* spp., 9%; urease-positive thermophilic campylobacters (UPTC) 57% (20).

Table 4 shows the relationship between the indicator organisms used for shellfish bed classification and the detection of campylobacters. Campylobacters were isolated from 14% of shellfish where faecal

Table 4. *Relationship between indicator organisms and campylobacter isolation from shellfish*

Indicator organism	Counts (cfu/g)	Number* containing campylobacter (%)
Faecal coliforms	0	12 (14)
	1–1000	66 (75)
	> 1000	10 (11)
E. coli	0	18 (21)
	1–1000	61 (69)
	> 1000	9 (10)

* $n = 88$.

coliforms were not detected, and in 21% of those where no *E. coli* were detected.

DISCUSSION

It is noteworthy that salmonella were found in shellfish less often in the warmer summer months when the incidence of human salmonellosis is highest. In waters off Morocco, from beds which broadly corresponded to category B/C, no salmonella were found in mussels despite their much greater ability to concentrate bacteria from seawater than other shellfish [21]. This absence in warm waters and marked seasonality in temperature seawater suggests that like campylobacter [22, 23], salmonella may survive better at low temperatures. It is known that *Campylobacter* spp. die off after a few days in seawater, but survive in freshwater and shellfish meats [22].

Shellfish serve as efficient filtering devices, sampling and concentrating their surrounding aqueous environment [21]. If lower than expected numbers are isolated it suggests that salmonellae may be dying or entering a non-recoverable state [6]. Faecal contamination of marine waters with salmonella could be expected to be greater during the months of highest infection (July–September). However, the isolation of salmonella from human cases in Northern Ireland during the last quarter of 1994 was the highest ever recorded [24]. Of 276 reported salmonella isolations, 108 were made during October–December. The shellfish isolations may therefore indicate both the increased presence of salmonellae in sewage and increased survival in cooler weather. Of these 276 isolations, 129 were *S. enteritidis* (101 of these PT4), 58 *S. typhimurium*, 31 *S. bredeney*, and 11 *S. virchow*. Most of the *S. bredeney* isolates were associated with a community outbreak in the south of the Province; the single isolate of *S. bredeney* from shellfish was from the northern coast and most likely unconnected. The serotypes isolated from shellfish (Table 2) were not representative of those found in human infections.

Likewise, campylobacter isolation was lowest during the summer months. Human campylobacteriosis peaks in May and shows a smaller secondary park in the early autumn. It might be expected that human sewage, wild birds and farm run-off would lead to higher numbers of these organisms in the marine environment during the summer. In fact the opposite was found. Less than 6% of shellfish examined between May and August ($n = 162$) contained campylobacters. It is likely that this reflects the short survival of campylobacters in the marine ecosystem rather than their numbers entering it. The sharp rise in the number of cases in May must give rise to much larger numbers of these organisms entering the sewage system, although these may be greatly reduced by effective sewage treatment [25]. Higher temperatures and the photo-toxic effects of UV light in shallow tidal waters appear to prejudice their survival [23, 26, 27].

Published studies on *Campylobacter* spp. in sewage and river water, which may provide input into shellfish-growing beds, often do not agree. It was found [28] that most campylobacters in sewage effluent originated from animal slaughter and processing plants. A seasonal peak was noted in May and June when campylobacter counts rose from 10^3 cfu/100 ml to 10^5 cfu/100 ml. This was judged to be due mainly to zoonotic infections which also peak during this period. An opposite seasonal trend was noted in a study of river water [26]. In this study the highest counts of campylobacters were found in late autumn and winter. Raised counts were found downstream of sewage treatment plants with the highest numbers being 10^2 cfu/100 ml. An Italian study of sewage [29] found the greatest frequency of isolation and highest counts during May, June and July, coinciding with the peaks of human and animal infections. *C. jejuni* predominated in incoming sewage, but *C. coli* was more prevalent in activated sludge effluent due to its greater oxygen tolerance. These workers attributed the differences in seasonal isolations between studies to the closed, protected nature of the sewage system relative to surface and river waters. Seasonal variation and influences could not be ascertained in a study of a sewage system in Germany [30], but counts generally lay between 10^2 and 10^4 cfu/100 ml. Another study [31] found that sewage treatment plants reduced the campylobacter counts by 99·5%, and that *C. coli* was

found more frequently than *C. jejuni*. However, the unreliability of enumerating *Campylobacter* spp. in sewage was highlighted [32] with minimum and maximum values lying at least two orders of magnitude apart. The effects of differential survival of *Campylobacter* spp., weather, sewage, agricultural effluent and rivers feeding into seawater shellfish beds are therefore complex and much work is required to be done in elucidating the relationships of these factors to the routes of infection.

Thermophilic *Campylobacter* spp. were detected in 42% of the shellfish. The greatest percentage of these (57%) were UPTC [33, 34], atypical types which do not appear to be closely associated with domestic or farm animals, or man. Their significance in foodborne human disease is probably minimal. These campylobacters may be native to the aquatic environment [20], but have been associated with clinical conditions such as diarrhoea [35] and urinary tract infection [36]. These atypical campylobacters have not been commonly reported elsewhere. This may be due to their misidentification as *C. coli* if the urease test is not included in phenotypic characterization tests [37]. The urease test should be included in the characterization of campylobacters from marine environments and faecal specimens.

C. lari represented almost a quarter of the campylobacters isolated and has been associated with the colonization of seagulls and the infection of humans via contaminated water [38]. As judged by the species present, most of the campylobacters found in shellfish probably originate from agricultural run-offs and wild birds. The low infectious dose of campylobacters [39], and their prevalence in shellfish, indicate that great care must be taken to avoid cross-contamination of ready-to-eat foods. Depuration has been shown by these results to be not fully effective in the elimination of potential bacterial pathogens and this indicates that some thermal processing of shellfish is generally advisable. Cockles and mussels are usually steamed or boiled before consumption, but oysters generally are not.

Vibrios, which are responsible for large numbers of cases of shellfish-associated illness [1] in warm climates, were not detected in the cooler waters around Northern Ireland.

Cl. perfringens counts broadly reflected the bed classification and *E. coli* counts. Category A beds often had counts of < 100 cfu/g while counts > 400 cfu/g were associated with category C or prohibited beds. Nevertheless, salmonellae were found in category A shellfish which did not contain *Cl. perfringens*. All salmonella-containing shellfish from category A beds contained < 100 cfu/g *Cl. perfringens*. This shows that neither *E. coli* nor *Cl. perfringens* [40] is a suitable indicator of hygienic quality. It has been stated [40] that *Cl. perfringens* has a number of advantages over *E. coli* as an indicator of shellfish hygiene: it is present in greater numbers, isolated more frequently, more persistent, and in seawater indicates fresh input of spores. These workers proposed *Cl. perfringens* as an indicator based on sampling of 24 batches of shellfish from a limited geographical area (Strangford Lough). In the present study, sites within Strangford Lough were sampled but none was found to contain salmonella. The present work therefore cannot be compared directly with the earlier study. Nevertheless, it appears that outside the complex currents of this Lough, *Cl. perfringens* is not an adequate indicator to assure the safety of shellfish for immediate human consumption. Likewise, conflicting studies on the usefulness of faecal coliforms as indicators of campylobacter contamination of water have been reported [41]. In the present study, faecal coliforms and *E. coli* were absent in 14 and 21% respectively of samples which contained campylobacters (Table 4). Like *Cl. perfringens*, these are also inadequate indicators of safety from campylobacters. A period of depuration is necessary for shellfish harvested from beds of any category to ensure bacteriological safety [16]. In Northern Ireland, this is usually carried out on all shellfish, including those harvested from category A beds. However, one producer has recently begun to market category A shellfish without depuration. Bacteriological safety, which may be achieved largely by depuration, does not imply safety from pathogenic viruses which are more persistent because of their intracellular location in shellfish tissues. Where possible, thermal processing may be advisable to provide additional assurance against such contamination.

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