A Bacteriological Investigation of Selected Flounder, Crab and Lobster Collected from St. John's Harbour, June, 2001.

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A Bacteriological Investigation of Selected Fish and Crustacean Samples Collected From St. John's Harbour, NF - Summer 2001.

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ABSTRACT

Samples of flounder (*Pseudopleuronectes americanus*, N=12), crab (*Hyas arenias*, N=10) and lobster (*Homarus americanus*, N=1) were collected from St. John's Harbour, Newfoundland in May and June of 2001 and analysed for bacterial content. Total bacterial counts (cfu/gram) were determined, as well as growth on media selective for enteric bacteria. The crab samples showed the highest total bacterial counts when compared with the flounder and lobster. Several species of enteric bacteria were isolated and identified using the Analytical Profile Index (API). Species identified from crab samples included *Escherichia coli, Aeromonas hydrophila, Hafnia alvei, Yersinia enterocolitica, Plesiomonas shigelloides,* and *Enterobacter aerogenes*. Bacteria identified from flounder included *Escherichia coli, Aeromonas hydrophila, Klebsiella oxytoca, Enterobacter aerogenes, Yersinia enterocolitica,* and *Enterobacter agglomerans*. With the exception of *Yersinia enterocolitica* and some strains of *E. coli,* all of the bacterial species identified are opportunistic pathogens. *E. coli* and *Y. enterocolitica* are considered to be enteropathogenic to man. Other selective media produced colonies characteristic of other human pathogens, such as *Vibrio* (TCBS), *Listeria monocytogenes* (PALCAM), and *Staphylococcus* spp. (MSA). Further identification using API is required to confirm these findings.

Introduction

Many bacterial species of enteric origin can be isolated from harbours which are located around sites of human habitation, including *Bacillus cereus, Staphylococcus aureus, Vibrio parahaemolyticus, Salmonella spp., Escherichia coli, Shigella spp., Listeria monocytogenes, and Klebsiella spp.* These bacterial species are commonly isolated from waters which contain fecal materials (Badley *et al.*, 1990; Jones and Summer-Brason, 1998; Martinez-Manzanarez *et al.*, 1992). Pathogenic bacteria in seawater are most abundant in sediments (Martinez-Manzanarez *et al.*, 1992), but are also seen in increased concentrations in the surface film, as compared with the water column (Plusquellec *et al.*, 1991). As a result, shellfish and other benthic fish, such as flounder, show elevated levels of these bacteria, which can also cause disease in fish, as well as human hosts (Martinez-Manzanarez *et al.*, 1992; McVicar *et al.*, 1988). Untreated sewage can cause disease in humans as a result of eating contaminated shellfish and groundfish species. Also, antibiotic resistant strains of *Escherichia coli* have been isolated from environmental samples (Baldini and Cabezali, 1991) as well as pathogenic viruses (Goyal *et al*., 1984). Skin infections can also result from contact with contaminated water (Kueh and Brunton, 1992). Effects of sunlight, temperature, salinity and pH on survival of enteropathogenic bacteria have also been studied (Pommepuy *et al.*, 1996; Solic and Krstulovic, 1992).

St. John's Harbour has been receiving 120 million liters of untreated sewage and stormwater runoff per day for many years. The resulting buildup of organic material has led to eutrophication of the waters as bacteria multiply in the rich organic waste. Much of the organic material in the harbour is human in origin, and contains high levels of enteric bacteria (City of St. John's, unpublished data). Since some of these bacteria may be pathogenic, it is therefore important to monitor the harbour on a regular basis to discern the potential health hazard to people working in or on the harbour basin.

The objective of this study is to determine presence and number of bacterial species in fish and crustacean samples collected from St. John's Harbour using basic nutrient media and to select for and differentiate between species of enteric bacteria using the selective media technique. Particular emphasis is also placed on isolation and possible identification of enteric pathogens.

Materials and Methods

Samples of flounder *(Pseudopleuronectes americanus),* crab *(Hyas arenias)* and lobster (*Homarus americanus)* were collected from St. John's harbour in May and June of 2001. They were packed on ice until they reached the lab of Oceans Ltd., where dissections were carried out to remove liver and other organs for analysis. Approximately 100g samples were sent to MUN for bacterial analysis. Samples were dissected using equipment dipped in 95% ethanol to sanitize them. Samples were

placed in sterile bags on ice for transport to the lab at MUN, where they were stored at 5 °C until processed.

A 25g representative sample was taken from each specimen and placed in 250 mL of Butterfield's phosphate buffer. The 1:10 mixture was emulsified using a stomacher for 2 minutes. A dilution series was prepared from 10^{-1} to 10^{-7}. Each sample in the dilution series was spread plated on TSA (trypticase soy agar) using 0.1 mL inoculum per plate. All dilutions were plated in triplicate. Plates were incubated for 48 hours at 37 °C, then colony counts were taken. Colony numbers between 30 and 300 per plate were used to calculate cfu/gm (colony forming units per gram of sample).

After total bacterial counts were obtained, the samples were spread plated on selective media in duplicate, using the optimum dilution of sample from the initial counts (i.e. the dilution which gave colony numbers between 30-300 per plate). Samples were so prepared using MYP (Mannitol egg yolk agar), SS (*Salmonella/Shigella* agar), VRBA (Violet Red Bile agar), EMB (Eosin-Methylene blue agar), TCBS (Thiosulfate-Citrate-Bile-Sucrose agar), MacConkey agar, MSA (Mannitol Salt Agar), and *Listeria* enrichment broth (LEB). Selective media were incubated at 37 °C for 24-48 hours and the results were observed and recorded. LEB was incubated at 30 °C (optimum temperature for *Listeria*) and observed for turbidity. Those tubes showing growth were then streaked on PALCAM agar (also selective for *Listeria spp.*).

After obtaining colony counts from selective media, typical colony types from each media were described and isolated. An attempt was made to identify selected isolates using the API system for bacterial classification.

Results and Discussion

A total of 23 samples were analyzed in this study. Of these, twelve were flounder, ten were crab, and one was lobster. The results of the total bacterial counts are shown in Table 1. CFU/gram of the samples showed a higher bacterial load on average in the crab samples, as compared to the flounder and lobster. When preparing the samples, parts representative of the organism as a whole were included. For example, the crab samples included some of the mouthparts, shell, legs, and body flesh. The flounder was provided in the form of a sample of skin and muscle only. This difference in the type of material used to prepare the suspensions may account for the higher bacterial loads observed in the crab, as compared with the flounder.

The dilution series prepared for each sample was also used to inoculate selective media, as described in the Materials and Methods section. The results of growth on these media are summarized in Table 2. Growth was observed in all samples when plated on EMB, VRBA, MacConkey's agar, TCBS agar, *Salmonella/Shigella* agar (SS), and *Listeria* enrichment broth (LEB). Although the

Table 1. Bacterial counts (cfu/gm) of fish and crustaceans taken from St. John's Harbour, summer 2001.

Sample #	Dilution	Average count	cfu/gm	Sample #	dilution	Average count	cfu/gm
C1	1/100	87	$8.7x10^4$	F2	1:10	33.667	$3.367x10^3$
C21	1/1000	36.67	$3.66x10^5$	F2	1/100	119(2)	$1.19x10^5$
C22	1/1000	93.33	$9.33x10^5$	F3	1:10	98.67	$9.87x10^4$
C23	1/100	249.3	$2.493x10^5$	F4	1:10	30(1)	$3.0x10^3$
C24	1/10000	56	$5.6x10^6$	F5	1:10	45	$4.5x10^3$
C25	1/100	98.33	$9.833x10^4$	F6	1:10	63	$6.3x10^3$
C26	1/1000	45	$4.5x10^5$	F7	1:10	48	$4.8x10^3$
C27	1/1000	258.5 (2)	$2.585x10^6$	F8	1/100	121	$1.21x10^5$
C28	1/100	213.33	$2.133x10^5$	F9	1/1000	43.67	$4.367x10^5$
C29	1/10000	39.67	$3.97x10^6$	F10	1/100	177	$1.77x10^5$
				F11	1/100	171	$1.71x10^5$
				F12	1/1000	51.67	$5.167x10^5$
L31	1/100	110.33	$1.103x10^5$	F13	1/1000	107	$1.07x10^6$

Notes: 1. Average counts are of 3 duplicate plates of each dilution, unless otherwise specified (in brackets)

2. Cfu/gm = colony forming units per gram of sample.

3. C = crab, F = flounder, L = lobster.

Table 2. Colony counts on selective media for fish and crustacean samples from St. John's Harbour, Summer, 2001.

	EMB		VRBA		MAC		MYP	TCBS	
ID	metallic/black	colorless	pink/red	colorless	pink	colorless	yellow	yellow	
C1	8.95×10^4	1.56×10^5	6.0×10^4	6.75×10^4	2.85×10^4	2.34×10^5	TFTC	TNTC	
F2	1.81×10^6	TNTC	TFTC	TNTC	TFTC	TNTC	5.1×10^5	4.1×10^5	
F3	5.55×10^3	1.05×10^4	4.9×10^3	4.9×10^3	3.4×10^3 (1)	8.55×10^3	ND	TNTC	6.0 X 1
F4	3.60×10^5 (1)	TFTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	
F5	TNTC	TNTC	TNTC	TNTC	9.65×10^5	TNTC	TNTC	TNTC	
F6	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TFTC	TNTC	
F7	6.2×10^3	7.2×10^3	4.3×10^3	3.75×10^3	3.3×10^3 (1)	6.2×10^3	ND	TNTC	
F8	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	
F9	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	2.58×10^5	1.5
F10	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	9.0
F11	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	2.05×10^5	
F12	TNTC	TNTC	TNTC	TNTC	2.34×10^5	TNTC	TNTC	9.7×10^4	9.35
F13	TNTC	TFTC	1.47×10^6	2.53×10^6	9.35×10^5	2.4×10^6 (1)	1.76×10^6	2.37×10^6	
C21	1.44×10^6	TFTC	4.25×10^5	5.15×10^5	3.5×10^5	7.4×10^5	TNTC	3.0×10^5 (1)	
C22	6.4×10^5 (1)	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC	
C23	7.05×10^4	9.25×10^4	1.15×10^5	4.35×10^4	1.25×10^5	TNTC	TNTC	TFTC	
C24	1.64×10^7	0	TFTC	TFTC	TFTC	1.16×10^7	4.7×10^6	0	
C25	TNTC	TFTC	7.1×10^4	9.5×10^4	TNTC	TNTC	TNTC	6.35×10^4	
C26	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	4.3×10^5	
C27	1.44×10^6 (1)	0	TFTC	5.7×10^5	TFTC	1.23×10^6	1.02×10^6	TFTC	
C28	TNTC	TFTC	1.26×10^5	2.96×10^5	2.66×10^5 (1)	TNTC	TNTC	4.8×10^4	5.6
C29	2.35×10^6 (1)	4.8×10^5 (1)	5.35×10^5	TNTC	4.3×10^5	1.94×10^6	1.91×10^6	5.65×10^5	
L31	1.85×10^4	8.5×10^4	TNTC	TNTC	7.6×10^4	2.42×10^5	8.1×10^4	5.55×10^4	

Notes: 1. TNTC = Too Numerous to Count; TFTC = Too Few to Count; ND = Not Determined; N/A = Not Applicable

2. All counts are expressed as cfu/mL (cfu= colony forming units) using an average count of two plates ur

3. EMB = Eosin Methylene Blue agar; VRBA = Violet Red Bile Agar; MAC = MacConkey Agar; MYP = Mar

TCBS = Thiosulfate Citrate Bile Sucrose agar; SS = Salmonella/Shigella agar; MSA = Mannitol Salt Aga

same dilutions were used as those which provided the most reliable overall bacterial counts (i.e. colony numbers between 30-300), some of the selective plates produced colonies too numerous to count. The plates that produced quantifiable results are shown in Table 2. Some morphological characteristics of the colonies observed on selective media are presented in Table 3. The following is a discussion of results obtained for each medium type.

EMB (Eosin Methylene Blue agar)

This medium is selective for Gram negative enteric bacteria. It is "recommended for the detection and isolation of the Gram negative intestinal pathogenic bacteria" (Difco Manual, 1998). According to DIFCO Manual (1998), the following colony types are the most predominant:

Green metallic colonies with black centres– characteristic of *Escherichia coli*,

Pink, mucoid colonies – characteristic of *Enterobacter aerogenes*

Colorless colonies – characteristic of *Salmonella/Shigella*

The most predominant colony types observed in our samples were the green metallic colonies with black centres (characteristic of *Escherichia coli*), with bacterial counts ranging from 5.55×10^3 (sample F3) to 1.64×10^7 (sample C24). Colorless colonies were also observed, ranging in numbers from 7.2×10^3 (sample F7) to 4.8 x

Table 3. Morphological characteristics of selected microbial species from fish and crustacean samples taken from St. John's Harbor.

Sample ID	Source (Type of Media)	Colony Characteristics	Gram Reaction	Shape	Arrangement
F 2A	VRBA	tan/beige, circular, raised, entire, smooth, shiny, ≅ 1mm	-	rods	single, strings
F 2A	MAC	colorless, circular, raised, entire, smooth, shiny, ≅ 2 mm	-	rods	single, strings
F 2A	MYP	white/yellow with yellowing of the media, circular, raised, entire, smooth, shiny, ≅ 2 mm	-	rods	single
F 3A	SS	cream-colored center with pink perimeter, irregular, convex, entire, smooth, shiny, somewhat mixed, ≅ 2 mm	-	rods	single
F 3A	Mannitol Salt	colorless, media changed from red to a pale peach, circular, raised, entire, smooth, shiny with slight texture, ≅ 1 mm	+	cocci	grape clusters
F 5A	MAC	purple with a ring of lighter purple at edges, circular, raised, entire, smooth, shiny, ≅ 3 mm	-	rods	single
F 6A	VRBA	bright pink, circular, entire, smooth, shiny, ≅ 1 mm	-	rods	single
F 6A	MAC	purple/red, produce purple/red precipitate in media, circular, flat/raised, entire, shiny, pinpoint	-	rods	single, strings
F 7A	LEB	white, circular, raised, entire, smooth, shiny, ≅ 2 mm	+	rods	single
F 8A	Mannitol Salt	peach, media showed a slight tinge of not pink (from red), circular, raised, entire, smooth, shiny with slight texture, ≅ 3 mm	-	rods	single
F 8A	LEB	beige, circular, umbonate, entire, smooth, shiny, ≅ 3-4 mm	+	rods	single
F 9A	EMB	metallic green, circular, raised, entire, shiny with little texture, ≅ 2mm	-	rods	single
F 9A	TCBS	green, circular, raised, entire, shiny, slightly textured, ≅ 2 mm	-	rods	single, strings
F 9A	SS	colorless, circular, slightly convex, entire, smooth, shiny, ≅ 2 mm	-	rods	single
F 10A	EMB	dark purple center with lighter purple/pink margin, circular, umbilicate, entire, smooth, shiny, ≅ 3 mm	-	rods	single
F 11A	EMB	bright pink, circular, convex, entire, smooth, shiny, ≅ 1mm	-	rods	single

F 11A	MYP	white/pink with media not pink, circular, convex, entire, smooth, shiny, ≅ 1 mm	-	rods	single
C 21A	VRBA	purple/red (darker near the edges), circular, raised, entire, textured, shiny, ≅ 2-3 mm	-	rods	strings
C 21A	MAC	dark purple/red, circular, raised, undulate, rough, shiny, ≅ 2-3 mm	-	rods	single, strings
C 21B	VRBA	purple/grey, circular, raised, entire, smooth, shiny, ≅ 2-3 mm	-	rods	strings
C 21B	MAC	pink, circular, convex, entire, smooth, shiny, slightly mucoid, ≅ 3 mm	-	rods	strings
C 23A	EMB	pink, slightly convex , raised, entire, smooth, shiny, ≅ 3 mm	-	rods	very short
C 23A	TCBS	yellow, circular, raised, entire, shiny, slightly textured, ≅ 2 mm	-	rods	single
C 23A	Mannitol Salt	peach/pink, media showed pink tinge, circular, raised, entire, smooth, shiny, ≅ 1 mm	no growth		
C 24A	VRBA	clear, circular, raised, entire, smooth, ≅ 1mm	-	rods	single, strings
C 24A	MAC	purple/red, circular, umbonate, undulate, smooth, shiny, ≅ 1-2 mm	-	rods	single
C 24A	LEB	colorless, slightly white at center of colony, circular, convex, entire, smooth, shiny, ≅ 2-3 mm	-	rods	single
C 27A	MAC	dark purple in center with light purple raised ring, circular, umbilicate, entire, smooth, shiny, ≅ 3 mm	-	rods	single, strings
C 27A	Mannitol Salt	pink, media showed a slight pink tinge from red, circular, convex, entire, smooth, mucoid, ≅ 3 mm	no growth		
C 28A	EMB	dark purple, circular, raised, entire, smooth, shiny, ≅ 3 mm	-	rods	single
C 28A	SS	colorless, brighter red, circular, slightly umbilicate, entire, smooth, shiny, ≅ 2-3 mm	-	rods	single
C 28A	LEB	slightly beige, darker in the center, circular, slightly umbonate, entire, smooth, shiny, ≅ 3-4 mm	-		
C 28B	SS	Bright pink with a darker center, circular, convex, entire, smooth, shiny, mucoid, ≅ 2-3 mm	-	rods	single
L 31A	MAC	purple, circular, convex, entire, smooth, shiny, somewhat gummy in appearance, ≅ 3 mm	-	rods	single

10^5 (sample C29). All isolates were Gram negative when streaked on TSA from the selective media (i.e. single colony isolates).

VRBA (Violet Red Bile Agar)

This medium is also selective for Gram negative enteric bacteria. The predominant colony types are as follows:

Red colonies – characteristic of *E.* coli (red color due to bile precipitate)

Pink/Red colonies – characteristic of *Enterobacter*

Colorless colonies – characteristic of lactose non-fermenters, such as most *Salmonella/Shigella spp.*

Both types of colonies were observed in approximately equal numbers (Table 2). Representative samples isolated on TSA were all shown to be Gram negative (samples F2, F6, C21).

MacConkey's Agar

This medium is selective and differential for "use in detection and isolation of all types of dysentery, typhoid and paratyphoid bacteria from stool specimens, urine,

and other materials harbouring these organisms" (Difco, 1998). The representative colony types are as follows:

Pink colonies – characteristic of *E. coli*

Colorless colonies – characteristic of *Salmonella or Proteus spp.*

The colorless colonies were most numerous in the samples tested, with colony counts ranging from 6.2×10^3 to 1.16×10^7. Pink colonies, characteristic of *E. coli* ranged in number from 3.3×10^3 (sample F7) to 9.65×10^5 (sample F5) (Table 2). All isolated colonies were Gram negative, which is characteristic of the coliforms, typhoid, and paratyphoid organisms within this group.

MYP Agar (Mannitol egg yolk – Polymixin agar)

This medium is selective for *Bacillus cereus*. Growth was observed in all but two of the samples tested. Colony counts ranged from 8.1×10^4 (sample L31) to 1.91×10^6 (Table 2). Yellowing of the medium occurred in all plates that showed growth. This indicates the presence of *Bacillus* species other than *B. cereus*, which cannot ferment mannitol, and thus does not show yellow colonies on MYP (Sneath *et al.*, 1986) One half of all plates inoculated had colonies too numerous to count. Samples F2 and F11 showed Gram negative cells when isolated and stained. This was unexpected, since *Bacillus* species are Gram positive. This result can possibly be explained if the cultures were not in exponential phase when the smears were made.

TCBS Agar (Thiosulfate-Citrate-Bile-Sucrose Agar)

This medium is selective for *Vibrio cholerae* and other enteropathogenic vibrios. This medium is recommended for use in isolating *Vibrio spp.* from stool specimens (Difco, 1998). Growth was observed in all samples, with counts ranging from 4.8×10^4 (sample C28) to 2.37×10^6 (sample F13) (Table 2). Yellow colonies were produced, indicative of *Vibrio cholerae, V. alginolyticus, or V. harveyi*, all of which are pathogenic to man (Krieg and Holt, 1984). One third of the samples showed colonies too numerous to count. Colonies were isolated using TSA with added salts (as *Vibrio* spp. are marine organisms). Gram stains of isolates C1 and L31 showed Gram negative, rod shaped cells, which is characteristic of this group (Krieg and Holt, 1984).

SS (*Salmonella/Shigella* agar)

This medium is "a highly selective medium recommended for the isolation of *Shigella* and *Salmonella* from stools and other materials suspected of containing these organisms" (Difco, 1998). Growth was observed in all samples. Typical colony types include the following:

Pink colonies – characteristic of lactose fermenting organisms, such as *E. coli*

Colorless colonies – characteristic of *Shigella* spp.

The colorless colonies were the most numerous, with counts ranging from 5.4 x 10^4 (sample L31) to 1.19 x 10^6 (sample C29). Pink colonies, characteristic of *E. coli*, ranged in numbers from 6.0 x 10^3 (sample F3) to 1.5 x 10^5 (sample F9) (Table 2). Gram stains of samples F3, F9 and C28 showed Gram negative rods, characteristic of this group (Krieg and Holt, 1984).

LEB (*Listeria* enrichment broth)

This medium is used to encourage the growth of *Listeria* spp. from mixed culture. It is a broth medium, and therefore is used in conjunction with other solid media, such as PALCAM agar, to isolate *Listeria* spp. (Difco, 1998). Growth was observed in all samples when inoculated into LEB. The turbid broth was then inoculated onto PALCAM agar. Five out of twenty-three samples produced grey colonies on PALCAM agar, typical of *Listeria sp*. Colony counts were not recorded for this medium. *Listeria* is also of marine origin, so it was isolated using TSA with salts added (as in the TCBS cultures previously discussed). Also, 20/23 PALCAM plates showed darkening of the media after 24-48 hr incubation at 30 °C. This usually indicates the presence of *Listeria*, but 15/20 plates did not produce observable colonies.

LEB showed a mixture of Gram positive and Gram negative rods when stained. Isolates obtained from streaking onto PALCAM (i.e. grey colonies) were

Gram positive when stained (samples F7 and F8) which is characteristic of *Listeria spp.* (Sneath *et al.*, 1986). Further identification is required to confirm these findings.

MSA (Mannitol Salt Agar)

This is a selective medium for the isolation of pathogenic staphylococci. The pathogenic strains usually produce colonies with yellow zones around them. The yellowing of the agar is due to the fermentation of mannitol, which causes the pH to drop, thus producing the colour change. Nonpathogenic strains produce colonies with red or purple zones (Difco, 1998). Samples C1, C24, and F3 showed colonies with yellow haloes, characteristic of pathogenic staphylococci, such as *Staphylococcus aureus* (Table 2). Sample F3 was isolated using salt TSA and showed grape-like clusters of Staphylococci, which stained Gram positive, characteristic of this species (Sneath *et al*., 1986). Samples C23, C25, C26, C27 and C28 showed colonies with pink haloes, indicating growth of the nonpathogenic strains (Table 2). Further identification is required to confirm these findings.

API Identifications

Table 4 shows the results of API identification of selected colonies isolated from selective media. The species isolated and identified from Violet red Bile agar (VRBA) include *Aeromonas hydrophila,* which was found in samples F2

and C21, *Escherichia coli,* which was isolated from samples F6 and C21, and *Yersinia enterocolitica,* which was isolated from sample C24 (Table 4). The latter two species belong to the family Enterobacteriaceae, which is composed of facultatively anaerobic, Gram negative, rod-shaped, motile bacteria. *A. hydrophila* belongs to the family Vibrionaceae, which also includes Gram negative, rod-shaped motile bacteria.

Aeromonas hydrophila is a facultatively anaerobic, Gram negative, rod shaped organism that is commonly found in fresh water and sewage. It can be pathogenic to frogs, fish and mammals, including man (Krieg and Holt, 1984).

Escherichia coli is also a Gram negative, facultatatively anaerobic rod which is found in the large intestine of animals and man. It is a common component of sewage – contaminated waters. It acts as an opportunistic pathogen, and some strains can cause diarrhea and intestinal disorders, due to the production of enterotoxins. The degree of virulence is coded for by genes carried on a plasmid (extra DNA outside the main circular strand) (Krieg and Holt, 1984). Further tests, such as serology of O, K, and H antigens (corresponding to cell membrane, capsule and flagella, respectively) are required to determine whether the species isolated in this study are of the pathogenic variety. *Yersinia enterocolitica,* the third species isolated from VRBA, is also a facultatively anaerobic, Gram negative, motile bacterium which "is responsible for diarrhea, terminal ileitis, mesenteric lymphadenitis, arthritis and septicemia in man

Table 4. API Identifications of Some Bacterial Species Isolated from Selective Media					
Sample ID	Media Source	Species Name	Notes		
F2 (A1,2)	VRBA	Aeromonas hydrophila	very good identification		
F3 (A1)	SS	Klebsiella oxytoca	good identification		
F3 (A2)	SS	Enterobacter aerogenes	48%		
F6 (A1)	VRBA	Escherichia coli	acceptable identification		
F9 (A1,2)	EMB	Yersinia enterocolitica	excellent identification		
F9 (A1)	SS	Escherichia coli	low discrimination		
F10 (A1,2)	EMB	Enterobacter agglomerans	very good identification		
F11 (A1,2)	EMB	Hafnia alvei	excellent identification		
C21 (A1)	VRBA	Escherichia coli	acceptable identification		
C21 (B1,2)	VRBA	Aeromonas hydrophila	acceptable identification		
C23 (A1,2)	EMB	Hafnia alvei	excellent identification		
C24 (A1,2)	VRBA	Yersinia enterocolitica	very good identification		
C28 (A1,2)	EMB	Yersinia enterocolitica	excellent identification		
C28 (A1,2)	SS	Plesiomonas shigelloides	52.40%		
C28 (B1,2)	SS	Enterobacter aerogenes	48.00%		
Notes: 1. Sample ID: F=Flounder,C=Crab, A1, A2,B1,B2=different colony types taken from each plate					
2	Media types: EMB=Eosin Methylene Blue Agar, SS=Salmonella/Shigella agar,				
	VRBA=Violet Red Bile Agar				

and animals" (Krieg and Holt, 1984). It can be isolated from a wide variety of environmental sources, including healthy man and animals. This species was also isolated from EMB agar (samples F9 and C28, Table 4).

Other species isolated from Eosin Methylene Blue agar (EMB) include *Enterobacter agglomerans* (sample F10) and *Hafnia alvei* (samples F11 and C23) (Table 4). Both species belong to the family Enterobacteriaceae (described above), and both can act as opportunistic pathogens in immunocompromised individuals. *E. agglomerans* can be isolated from plants, water, soil, and foodstuffs, as well as man and animals. It is often isolated from blood culture in hospitals due to contamination of catheters and intubation equipment. It causes septicemia in susceptible individuals (Krieg and Holt, 1984).

Hafnia alvei is found in the feces of man and other animals, including birds. It is also isolated from sewage, soil, water and dairy products. It is often isolated from clinical specimens, such as blood, sputum, wounds, and abcesses, and has been implicated as a causative agent of intestinal disorders and opportunistic infections (Krieg and Holt, 1984).

Salmonella/Shigella agar isolates were identified as *Escherichia coli* (sample F9 -described under VRBA above), *Klebsiella oxytoca* (sample F3), *Enterobacter aerogenes* (samples F3 and C28), and *Plesiomonas shigelloides* (sampleC28) (Table 4). *Klebsiella* and *Enterobacter* are both members of the Family Enterobacteriaceae, while *Plesiomonas* belongs in the Family Vibrionaceae. All are facultatively anaerobic, Gram negative rods (Krieg and Holt, 1984).

Klebsiella oxytoca can be isolated from a variety of sources, such as intestinal contents of man and animals, soil, water and grains. This species is an opportunistic pathogen which can cause bacteremia and urinary tract infections in man. The outermost part of the organism consists of a polysaccharide capsule, which can protect the bacterium from host defences. Several strains are also capable of antibiotic resistance, making this species a common source of infections in hospitals, where immunocompromised individuals are especially at risk (Krieg and Holt, 1984).

Enterobacter aerogenes can be isolated from water, sewage, soil and feces of man and animals. Despite its presence in feces, it is not considered to be an enteric pathogen. However, it is an opportunistic pathogen, and can be isolated from clinical samples of blood, pus, spinal fluid, genitourinary tract and gastrointestinal tract of man (Kreig and Holt, 1984).

Plesiomonas shigelloides is a Gram negative, motile rod-shaped bacterium found in fish and other aquatic mammals. “It probably does not belong to the normal intestinal flora of man, but can cause diarrhea in man” (Krieg and Holt, 1984).

Conclusions

St. John's Harbour is contaminated with a wide variety of bacteria due to the influx of massive amounts of untreated organic waste, much of it of fecal origin. The species identified in this study are mostly opportunistic pathogens, such as *Enterobacter agglomerans, Hafnia alvei, Klebsiella oxytoca, Enterobacter aerogenes*, and *Plesiomonas shigelloides,* but some are enteropathogens, such as some strains of *Escherichia coli* and *Yersinia enterocolitica*. The other selective media, such as TCBS, *Listeria* selective media (PALCAM) and Mannitol salt agar also produced colonies characteristic of other human pathogens, such as *Vibrio* (TCBS), *Listeria monocytogenes* (PALCAM), and *Staphylococcus* spp. (MSA). Further identification using API is recommended for these media to confirm these results. Further sampling and monitoring of the harbour water and fish is also recommended to further elucidate the species of interest to health officials.

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