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Incidence of Enteric Pathogens and Coliforms in Fish from Domestic Markets of Cochin

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The incidence of human enteric pathogens and other coliforms of dominance in the fish and shellfish from retail markets of Cochin were investigated. Total *Enterobacteriaceae* count in these samples varied from 2.5 to 6.5 log cfu/g. The generic composition of 248 *Enterobacteriaceae* isolates showed a dominance of genera *Enterobacter, Citrobacter* and *Escherichia.* A small fraction (13.8%) of the *E. coli* isolates were β haemolytic, but none of them could produce labile toxin when tested by reverse passive latex agglutination (RPLA) method. Other enteric pathogens detected in these samples were *Klebsiella, Shigella, Edwardsiella* and *Arizona.* Though they constituted only a minor fraction (14.1%), knowing the established pathogenic potentials of these bacteria in food borne illness, strict adherence to good hygienic practices in retail markets of Cochin is warranted. In the Most Probable Number (MPN) procedure for the detection of total coliforms, faecal coliforms and *Escherichia coli* along with enteric non-coliform bacteria, oxidase positive *Aeromonas* spp. interfered by causing positive reaction. The temperature of incubation, pH and bile salt level had a profound effect on lactose fermentation in Brilliant Green Bile Lactose Broth (BGLB) and Elevated Coliform (EC) broth in the MPN procedure. It is found that in the presence of divergent mesophilic flora of tropical conditions, the Ortho Nitro Phenyl b-D-Galactopyranosidase (ONPG) test proved to be more reliable test than lactose fermentation for coliform detection as it alleviated the effect of pH, temperature, etc. on the lactose fermentation.

Keywords : Enteric pathogens; coliforms; Seafood; Interference;

Fish and fishery products are in the forefront of food safety because of their indispensable role as cheap protein supplement and their significance as one of the most internationally traded foodstuff. In recent times the number of food borne disease outbreak (including seafood borne outbreaks) are rising. The microbiological safety of seafood is an important concern of consumer as the major share of the outbreaks related to consumption of fish are caused by bacteria such as *Clostridium botulinum, E. coli, Salmonella, Staphylococcus, Vibrio spp., Bacillus cereus* (Fleming *et al.*, 2000). The mesophilic *Enterobacteriaceae* population in seafood is of vital importance as they include potential pathogens such as *Salmonella, Shigella* and Enteropathogenic *E. coli.* Previous studies on the *Enterobacteriaceae* family (*Salmonella, Shigella, Escherichia, Yersinia*) from fish and fishery products established them as a potential agent of causing illness and intoxication (Nedoluha and Westhoff, 1997; Fleming *et al.*, 2000). *Klebsiella* spp., *Enterobacter* spp., and some strains of *E. coli* were reported to cause severe neonatal infection (Tullus *et al.*, 1988). Reports on the occurrence of pathogenic strains of *E. coli* in fresh fish and other retail seafoods, tuna paste, salmon roe etc., and outbreaks of illness due to their consumption are being reported from different parts of the world viz. India, Belgium, Japan, Brazil, and US (Ayulo *et al.*, 1994; Mitsuda *et al.*, 1998; Asai *et al.*, 1999; Kumar *et al.*, 2001; Teophilo *et al.*, 2002). Out of 217 seafood consignments

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exported from developing countries to European Union, 6 were rejected due to the presence of enteric pathogens during the year 2002 (Huss *et al.*, 2003). An insight into the incidence of enteric bacteria have become mandatory as some of them require only a very low infective dose to cause illness (1-100 cells for some *Shigella, Salmonella* serotypes and *E. coli* O157:H7).

The family *Enterobacteriaceae* as group or individual members such as total coliform, faecal coliform and *E. coli*, is used as an indicator of the hygiene and safety status of food (Cox *et al.*, 1988). The species-wise composition of the total and faecal coliforms in water (Evans *et al.*, 1981; Ramteke *et al.*, 1992; Dancer *et al.*, 1997; Niemi *et al.*, 2001) and fish samples (Souter *et al.*, 1976; Sangjindavong and Cjerde, 1977; Ogbondeminu, 1993; Apun *et al.*, 1999) is quite diverse. The existence of such closely related members of *Enterobacteriaceae* may lead to mis-interpretation of the valid data on quality and sanitation. Moreover recent observations with water samples established that the classical *E. coli* is a thermotroph and is the only reliable indicator (Leclerc *et al.*, 2001). Previous study from India confirmed that only 30% faecal coliform colonies were *E. coli* (Jeyasekaran *et al.*, 1990). These observations along with concerns raised by other authors (Geldreich, 1978; Jeyasekaran *et al.*, 1990; Ramteke *et al.*, 1992; Charriere *et al.*, 1994) regarding the reliability of the indicator counts has led to a need for evaluating the method of their detection in presence of the diverse flora of tropics.

Based on the above facts and to derive useful data for public health risk assessment, the present study was carried out 1) to determine the enteric pathogens in fish by studying the species composition of enteric bacteria in retail sold seafood from tropics, and 2) to establish the level of reliability of the existing procedure for quality control.

Materials and methods

Fish samples from five selected markets in and around Cochin, India were collected within a period of three months from September to December 2002. A total number of 104 samples including Indian oil sardine - *Sardinella longiceps* (20), Indian mackerel - *Rastrelliger kanagurta* (20), thread fin bream - *Nemipterus japonicus* (8), prawn - *Metapenaeus dobsoni* (20), pearlspot - *Etroplus suratensis* (20), black clam - *Villorita cyprinoides* (8) and boiled clam meat - *Villorita cyprinoides* (8) were analysed during the study.

Quantitative estimation of total *Enterobacteriaceae* was carried out by direct plating on Violet Red Bile Glucose Agar (VRBGA, Oxoid, UK). 10 g of a flesh portion of fish or meat without shell of shellfish was homogenised with 90 ml saline (0.85 % (w/v) NaCl) in a Stomacher 400 Lab Blender (Seward, London, UK) for 30 seconds. Further serial dilutions were pour plated with VRBGA. All plates were incubated at 37°C for 18-24 hours. For identification, 2-5 well-separated typical colonies were selected using Harrison's disc method (Harrigan and McCance, 1976). These cultures were purified by streaking on nutrient agar plates and stored for further study in nutrient agar slants. Altogether 248 pure culture isolates from sardine (44), mackerel (52), prawn (52), pearlspot (50), thread fin bream (20), fresh clam meat (10) and boiled clam meat (20) were obtained and identified up to the genus level with the help of an identification scheme (web http// www.vet.uga.edu/WEBFILES/) in consultation with Edwards and Ewings (1972) and Macfaddin (1980). About 5% of the isolates were crosschecked for identification using Analytical Profile Index 20 E (API 20 E, bioMerieux). Representative isolates of different genera (168 numbers) from seafood samples were investigated in detail for their physiological properties such as lactose fermentation, ONPG reaction, growth on BGLB, EC and

Indole production at 37°C and 44.5°C in serological water bath (Beston Instruments, India).

A total number of 36 *E. coli* isolates obtained in this study were tested using the VET (*Vibrio cholerae* Entero Toxin) RPLA kit (Oxoid TD 0920) for heat-labile enterotoxin (LT) production as per the procedure outlined by the manufacturers. Isolates that were sorbitol and methylumbelliferyl-b-glucuronide (MUG) negative were grown on BHI (Brain Heart Infusion; Oxoid, UK) slant overnight and were subjected to latex agglutination with *E. coli* serotype O157 specific antisera (Oxoid DR 620M, UK). *E. coli* O157:H7 (ATCC 43895) was used as positive control. All the strains were also tested for lysine and ornithine decarboxylase test (Macfaddin, 1980) and β-hemolytic activity was studied in nutrient agar with 5% human erythrocytes received from State Health Authorities, Cochin.

Results and discussion

The total *Enterobacteriaceae* count in fish and shellfish of domestic markets varied from 2.5 to 6.5 log cfu/g. The generic composition of 248 numbers of *Enterobacteriaceae* isolates from different fish and shellfish of the Cochin are shown in Fig. 1. The present scheme employed allows the identification of 16 genera in the family, *Enterobacteriaceae* and eleven genera were detected in this study. Though the relative proportion varied, the major genera recovered were comparable to earlier studies involving carps, white sucker, frozen shrimp and other freshwater fishes and fishery environment (Souter *et al.*, 1976; Ogbondeminu, 1993; Apun *et al.*, 1999).

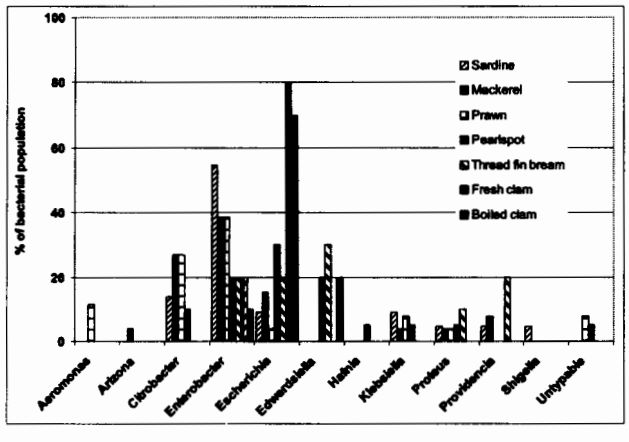

Fig. 1. Sample wise composition of enteric bacteria from retail seafoods

Among *Enterobacteriaceae*, *Enterobacter* spp., *Citrobacter* spp., and *Escherichia* spp., were the major genera found in all seafood samples, but their relative proportion varied with samples. *Enterobacter* spp. dominated in three [(mackerel (38.5%), prawn (38.5%), and sardine (54.5%))] of the seven samples (Fig. 1). *Enterobacter* sp along with other enteric bacteria *Klebsiella* spp. and *Citrobater* spp. from different food sources were reported to be responsible for the severe outbreaks of diarrhea lasting months or years (Lindsay, 1997). *Escherichia* spp. were detected as major genera in pearlspot, fresh and boiled clam samples (32, 80 and 70% respectively) and other groups in the order of dominance were *Citrobacter* spp, *Klebsiella* spp., *Proteus* spp., and *Providencia* spp. All the *E. coli* isolates obtained were studied for their disease causing potential and results were discussed.

Occasional food poisoning events were reported due to the consumption of shellfishes contaminated with *Edwardsiella tarda* (Fang *et al.*, 1991). In this study *Edwardsiella* spp. (19 out of 248 isolates) were detected only from Market V from the samples thread fin bream, pearlspot and boiled clam and *Hafnia* spp. (2 nos) could be obtained only from pearlspot samples. *Shigella dysenteriae* recovery and out break of shigellosis due to the consumption of a multitude of food products, including shrimp, clams and other molluscans were reported (Rheinstein and Klontz, 1993; Lipp and Rose, 1997). Recovery of 2 numbers *Shigella* spp., which is reported to have very low infective doses of 10-100

Table 1. Phenotypic traits of *E. coli* tested for pathogenicity from seafood

Properties (% positive)	Number of positives	
	Sorbitol Positive (%) n=30 (83.3)	Sorbitol Negative (%) n=6 (16.6)
EMB reaction (100)	30 (100)	6 (100)
IMViC TypeBiotype I (+ + - -) (86.1)	28 (93.3)	3 (50)
Biotype II (- + - -) (2.8)	0 (0)	1 (16.6)
Fecal type (+ - - -) (11.1)	2 (6.6)	2 (33.3)
Labile Toxin positive (0)	0 (0)	0 (0)
Latex Agglutination for O157 antiserum (0)	0 (0)	0 (0)
MUG positive (77.7)	25 (83.3)	3 (50)
Lysine Decarboxylase positive (63.8)	22 (73.3)	1 (16.6)
Ornithine Decarboxylase positive (72.2)	24 (80)	2 (33.3)
b-Hemolytic (13.8 %)	2 (6.6)	3 (50)

n - Numbers tested

cells (Lipp and Rose, 1997; Nedoluha and Westhoff, 1997) from sardine samples and *Arizona* spp., (2 nos) from mackerel samples warrant for strict adherence to Good Hygienic Practices in retail markets of Cochin. Present study failed to recover *Salmonella* spp. and *Serratia* spp., which was recovered by Souter *et al.*, (1976) involving Carp and white sucker samples. Psychrotrophic member of the *Enterobacteriaceae, Yersinia* spp., was not detected in the present study. Interference of *Aeromonas* spp. a non-enteric bacteria was observed in prawn samples and contributed to 11.5% of the total *Enterobacteriaceae*. This finding is line with the report of Evans *et al.* (1981) and Hazen *et al.* (1978) that *Aeromonas* interfere in the media used for *Enterobacteriaceae* detection.

All the 36 *E. coli* isolates were analysed for their phenotypic traits for toxigenicity and the results were given in table 1. All the

Table 2. Comparison of lactose, galactose and glucose fermentation with ONPG reaction among various *Enterobacteriaceae* isolates from seafood at 37°C

Genera	No. of cultures tested	Lactose		Galactose		Glucose		ONPG	
		+	-	+	-	+	-	+	-
Aeromonas	6	4	2	4	2	6	0	4	2
Arizona	2	2	0	2	0	2	0	0	2
Citrobacter	34	12	22	34	0	34	0	34	0
Enterobacter	56	30	26	56	0	56	0	56	0
Escherichia	36	34	2	36	0	36	0	36	0
Hafnia	2	2	0	2	0	2	0	2	0
Klebsiella	14	10	4	14	0	14	0	14	0
Proteus	8	4	4	8	0	8	0	0	8
Providencia	6	0	6	2	4	6	0	0	6
Shigella	2	0	2	0	2	2	0	0	2
Unidentified	2	2	0	2	0	2	0	2	0
Total	168	100	68	160	8	168	0	148	20

Table 3. Effect of incubation temperature on major identification traits of *Enterobacteriaceae* isolates from seafood

Genera	No. of cultures tested	Lactose positive		BGLB positive		EC positive		Indole positive	
		37°C	44.5°C	37°C	44.5°C	37°C	44.5°C	37°C	44.5°C
Aeromonas	6	4 (66.6)	4 (66.6)	4 (66.6)	2 (33.3)	4 (66.6)	4 (66.6)	6 (100)	2 (33.3)
Arizona	2	2 (100)	0 (0)	2 (100)	0 (0)	2 (100)	2 (100)	0 (0)	0 (0)
Citrobacter	34	12 (35.3)	2 (5.9)	12(35.3)	0 (0)	10 (29.4)	8 (23.5)	24 (70.6)	8 (23.5)
Enterobacter	56	30 (53.6)	16 (28.6)	28(50)	2 (3.6)	28 (50)	26 (44.8)	0 (0)	0 (0)
Escherichia	36	34 (94.4)	30 (83.3)	36(100)	18 (50)	36 (100)	32 (88.8)	36 (100)	36 (100)
Hafnia	2	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
Klebsiella	14	10 (71.4)	10 (71.4)	4 (28.6)	0 (0)	8 (57.14)	6 (42.9)	14 (100)	8 (57)
Proteus	8	4 (50)	2 (25)	2 (25)	0 (0)	4 (50)	4 (50)	8 (100)	8 (100)
Providencia	6	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (66.6)	0 (0)
Shigella	2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)
Unidentified	2	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
Total	168 (100)	100 (59.5)	68 (40.5)	92 (54.8)	26 (15.5)	96 (57.1)	88 (52.4)	97 (57.7)	66 (39.3)

Note: Number in parenthesis indicates % positive reaction

isolates in this study were Eosine Methylene Blue (EMB) positive and 77.7% of the *E. coli* isolates were MUG positive. March and Ratnam (1986) observed that typical verotoxigenic *E. coli* were sorbitol and MUG negative. It was observed in the present investigation that 83.3 % of isolates were sorbitol positive. Among clinical isolates, over 95% are reported to be sorbitol positive (Wells *et al.* 1991). 16.7% of the isolates were sorbitol negative. MUG negatives were more prevalent among sorbitol negative isolates (50%) than sorbitol positive isolates (16.7%). There are reports that more than 94-96% of *E. coli* from clinical sources were positive for glucuronidase activity (Edberg and Kontnick, 1986). However Chang *et al.* (1989) reported that only 65% of human faecal *E. coli* isolates were β-D glucuronidase positive.

Majority of the *E. coli* isolates (86.1%) were found to be Biotype I (+ + - -) (table 1) on the basis of IMVIC reaction (Geldreich, 1978). Fecal type is more prevalent in sorbitol negative isolates (33.3%). Haldane *et al.* (1986) have proposed supplementary tests such as lysine and ornithine decarboxylase as biochemical markers to improve the specificity of sorbitol screening procedure. On the contrary, there is small fraction of *E. coli* with positive lysine and ornithine decarboxylase activity among sorbitol negative *E. coli* and this point to the limitation of biochemical traits for identification of verotoxigenic *E. coli*. A rapid screening procedure based on hemolytic activity is also reported for differentiating pathogenic hemolytic strains of *E. coli* (Bettelheim, 1995). In the present study, 13.8 % of the total isolates showed the β-hemolysing activity (table 1). The hemolytic activity was more prevalent among the sorbitol negative (50%).

The rapid latex test proposed by Oxoid (U.K) is a reliable test to detect *E. coli* serotype O157 as supported by previous data (Haldane *et al.*, 1986; Fernandes *et al.*, 1997). There are reports on the Enterotoxigenic/shiga-like toxin producing *E. coli* from seafoods (Ayulo *et al.*, 1994; Mitsuda *et al.*, 1998; Asai *et al.*, 1999; Kumar *et al.*, 2001; Teophilo *et al.*, 2002). None of the isolates, whether of typical or atypical phenotype, gave a positive latex agglutination (table 1). All the isolates were negative for labile toxin production by VET-RPLA

method. Earlier attempts by Adesiyun (1993), Fernandes *et al.* (1997) and Surendran *et al.* (2000) investigating different fish sources in Trinidad, USA and Cochin respectively, have reported similar results.

The MPN analysis is the routinely employed procedure for estimating total coliforms, fecal coliforms and *E. coli*. As per APHA, AWWA and WPCF (1998), total coliforms include all the aerobic, facultatively anaerobic, gram negative, non-spore forming rod shaped bacteria that ferment lactose with gas production within 48hr at 35°C (APHA, AWWA and WPCF, 1998). But recent definition includes all ONPG positive *Enterobacteriaceae* members as coliform bacteria (Leclerc *et al.*, 2001). These coliform groups of bacteria are distributed among 20 genera and 80 species (Leclerc *et al.*, 2001). The coliform genera are as follows, *Arsenophorus, Budvicia, Buttiauxella, Cedecea, Citrobacter, Enterobacter, Erwinia, Escherichia, Ewingella, Hafnia, Klebsiella, Kluyvera, Leclercia, Moellerella, Pantoea, Rahnella, Serratia, Trabulsiella, Yersinia, Yokenella.* To evaluate the dependability of lactose fermentation as identification criteria for total coliforms, representative isolates (168 cultures) from different genera of *Enterobacteriaceae* recovered from fish and shellfish were checked for their ability to ferment lactose. The result of the study is shown in table 2. While all the isolates of typical coliforms such as *Escherichia, Enterobacter,* and *Citrobacter* were positive for glucose and galactose fermentation, only a lesser fraction was lactose positive. This may be due reason that, some members of the *Enterobacteriaceae* do not express lactose dehydrogenase enzymes while others non-coliforms express the enzymes under restricted conditions as it is affected by external factors (Leclerc *et al.*, 2001). A comparison of lactose fermentation with ONPG reaction (table 2) showed that except for *Aeromonas* spp., only those genera listed as coliforms by Leclerc *et al.* (2001), gave a positive reaction for ONPG and *Proteus, Providencia* and *Shigella* spp., were ONPG negative. This confirmed that ONPG reaction is more reliable in defining coliform as endorsed by Leclerc *et al.*, (2001).

As reported earlier (Hazen *et al.*, 1978; Hazen, 1988), in the present study also *Aeromonas* spp., (66.6%), a non *Enterobacteriaceae,* cause false positive reaction by fermenting both lactose and galactose and to a lesser extent in the ONPG (50%) reaction. However a positive oxidase test may differentiate coliforms from *Aeromonas* spp. It is revealed in the study that apart from *Escherichia, Enterobacter, Citrobacter* and *Klebsiella* spp., which are members of the coliform group (APHA, AWWA and WPCF, 1998), non coliform *Proteus* spp., *Arizona* spp., and non enteric *Aeromonas* spp., also gave positive reaction in BGLB at 37°C.

The effect of incubation temperatures of 37°C and 44.5°C on BGLB reaction showed that temperature exerted a profound effect on lactose fermentation reaction by decreasing the percentage of positives (table 3). Further it was seen that compared to EC broth (0.15% bile salt), BGLB (2% bile salt) was more inhibitory to *E. coli* isolates at 44.5°C (table 3). Only 50% of *E. coli* fermented lactose in BGLB at 44.5°C probably due to the increased presence of bile salt or pH range. To find out whether the inhibitory action was due to bile salt or pH on coliforms, trials were carried out with BGLB formulated at two pH values (7.0 and 7.4) and bile salt levels (0.15% and 2.0%). The result showed that at pH 7.4 with 2% bile salt, all the *E. coli* isolates failed to grow. At a bile salt level of 0.15% adjusted to pH 7.0 and 7.4, the percentage of recovery was 33 and 77 confirming the pH related inhibitory effect of bile salt on *E. coli.*

Elevated incubation temperature has been recommended to differentiate coliform bacteria of faecal habitats from those of

non-faecal sources (USFDA, 2001). In this study, it was noted in the faecal coliform test i.e. production of gas from lactose in EC broth at 44.5°C, that the percentage of positive were slightly lower at 44.5°C than at 37°C incubation. Out of 36 isolates of *E. coli*, only 32 strains could ferment lactose in EC broth at 44.5°C in 24 hours. This showed that while all of *E. coli* isolates are thermotolerant as shown by 100% growth at 44.5°C in tryptone broth, a small fraction (11%) may be missed on elevated coliform test. Apart from faecal coliforms *Escherichia, Klebsiella, Citrobacter, Enterobacter,* non coliforms *Arizona, Proteus,* and non enteric *Aeromonas* also gave positive results in EC test. While Ramteke *et al.,* (1992) reported recovery of *Proteus* spp., *Enterobacter* spp., *E. coli* and *Klebsiella* spp. as thermo tolerant coliform in water samples, others recovered only *E. coli* and *Klebsiella pneumonae* (Sangjindavong and Cjerde, 1977; Paille *et al.,* 1987; Bitton, 1994) in different seafood and water samples.

Production of indole at 44.5°C is considered as confirmed *E. coli* identification test and is used in the third level MPN. While 100% of the *E. coli* are indole positive at 44.5°C incubation, other genera *Proteus* spp., *Citrobacter* spp., *Aeromonas* spp., *Hafnia* spp., and *Klebsiella* spp. also produced indole at 44.5°C there by interfering with *E. coli* detection. A compulsory *E. coli* confirmation in EMB Agar plates may avoid any confusion in the final step of MPN. A parallel study also indicated that the EC positive and Indole negative strains that were encountered in the seafood isolates were *Citrobacter* spp.

Enterobacter spp., *Citrobacter* spp., and *Escherichia* spp., were the major enteric bacterial genera found in all seafood samples. Hemolytic types of *E. coli* with many of the accessory biochemical traits excepting latex agglutination with O157 antisera, attributable to verotoxigenic *E. coli* O157:H7 types were noticed. This suggests the presence of atypical strains that need further study at molecular level. Recovery of established enteric pathogenic genera *Shigella* spp. and *Edwardsiella* Spp. warrant for strict adherence to Good Hygienic Practices in retail markets of Cochin. The data from the study clearly show that a large portion (5.6 to 64.7%) of the isolates, which were reported as coliforms and faecal coliforms, failed to exhibit the typical traits of coliforms and faecal coliforms in the MPN procedure. Similarly some of the non-coliforms or faecal coliforms gave reactions of typical coliforms and thus interfere with their detection and estimation.

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References

Adesiyun, A.A. (1993) Prevalence of *Listeria* spp., *Campylobacter* spp., *Salmonella* spp., Yersinia spp. and toxigenic *Escherichia coli* on meat and seafoods in Trinidad. *Food Microbiol.* **10**, pp 395-403.

APHA, AWWA and WPCF (1998) *Standard methods for the examination of water and wastewater, 20th edn.* American Public Health Association, USA.

Apun, K., Yusof A.M. and Jugang, K. (1999) Distribution of bacteria in tropical freshwater fish and ponds. *Int. J. Environ. Health Res.* **9**, pp 285-292.

Asai, Y., Murase, T., Osawa, R., Okitsu, T., Suzuki, R., Sata, S., Yamai, S., Terajima, J., Izumiya, H., Tamura, K. and Watanabe, H. (1999) Isolation of Shiga toxin-producing *Escherichia coli* O157:H7 from processed salmon roe associated with the outbreaks in Japan, 1998, and a molecular typing of the isolates by pulsed-field gel electrophoresis. *Kansenshogaku Zasshi.* **73**, pp 20-24.

Ayulo, A.M.R., Machado, R. & Scussel, V.M. (1994) Enterotoxigenic E coli and Stapylococcus aureus in fish and seafood from the southern region of Brazil. *Int. J. Food Microbiol.*, **24**, pp 171-178.

Bettelheim, K.A. (1995) Identification of enterohemolytic *E.coli* by production of their enterohemolysin. *J. Appl. Bacteriol.* **79**, pp 178-180.

Bitton, G. (1994) *Waste Water Microbiology*, pp 101-113, Wiley-Liss, New York, USA.

Chang, G.W., Brill, J. and Lum, R. (1989) Proportion of b-D-glucuronidase negative *Escherichia coli* in human

Ayulo, A.M.R., Machado, R. & Scussel, V.M. (1994) Enterotoxigenic E coli and Stapylococcus aureus in fish and seafood from the southern region of Brazil. *Int. J. Food Microbiol.*, **24**, pp 171-178.

Bettelheim, K.A. (1995) Identification of enterohemolytic *E.coli* by production of their enterohemolysin. *J. Appl. Bacteriol.* **79**, pp 178-180.

Bitton, G. (1994) *Waste Water Microbiology*, pp 101-113, Wiley-Liss, New York, USA.

Chang, G.W., Brill, J. and Lum, R. (1989) Proportion of b-D-glucuronidase negative *Escherichia coli* in human faecal samples. *Appl. Environ. Microbiol.* **55**, pp 335-339.

Charriere, G., Mossel, D.A.A., Beaudeau, P. and Leclerc, H. (1994) Assessment of the marker value of various components of the coli-aerogenes group of *Enterobacteriaceae* and of a selection of *Enterococcus* spp. for the official monitoring of drinking water supplies. *J. Appl. Bacteriol.* **76**, pp 336-344.

Cox, I.J., Keller, N. and Schothorst, M.V. (1988) The use and misuse of quantitative determinations of Enterobacteriaceae in food microbiology. In *Society for applied bacteriology symposium series no 17. Enterobacteriaceae in the environment and as pathogens.* (Lund, B.M., Sussman, M., Jines, D. and Stringer, M.F., Eds), pp 237s-250s, Society of Applied Bacteriology, London.

Dancer, S.J., Shears, P. and Platt, D.J. (1997) Isolation and characterization of coliforms from glacial ice and water in Canada's High Arctic. *J. Appl. Microbiol.* **82**, pp 597-609.

Edberg, S.E. and Kontnick, C.M. (1986) Comparision of b-glucuronidase-based substrate systems for identification of *Escherichia coli. J. Clin. Microbiol.* **24**, pp 368-371.

Edwards, P.R. and Ewing, W.H. (1972) *Identification of Enterobacteriaceae*, 3rd Edn. 362p, Burges Publishing co., Minneapolis, USA.

Evans, T.M., LeChevallier, M.W., Warvick, C.E. and Seidler, R.J. (1981) Coliform species recovered from untreated surface water and drinking water by the Membrane filter, Standard, and modified Most Probable Number techniques. *Appl. Environ. Microbiol.* **41**, pp 657-663.

Fang, G. Araujo, V., and Guerrant, R. L. (1991) Enteric infections associated with exposure to animals and animal products. *Inf. Dis. Clin. North Am.*, **5**, pp 681-701.

Fernandes, C.F., Flick Jr. G.J., Silva, J.I. and McCaskey, T.A. (1997) Comparison of quality in aqua cultured fresh catfish fillets II. Pathogens *E.coli* O157:H7, *Campylobacter, Vibrio, Plesiomonas,* and *Klebsiella. J. Food Prot.* **60**, pp 1182-1188.

Fleming, L.E., Bean, J.A., Katz, D. and Hammond, R. (2000) The Epidemiology of Seafood Poisoning. In *Foodborne Disease Handbook,* Vol. 4 (Hui, Y.H., Kitts, D. & Stanfield, P.S. Eds.) pp 297-310, Marcel Dekker, Inc., New York, USA.

Geldreich, E.E. (1978) Bacterial population and indicator concepts in faeces, sewage, storm water and solid wastes. In *Indicators of viruses in water and food* (Berg, G. Ed.)

Haldane, D.J.M., Damm, M.A.S. and Anderson, J.D. (1986) Improved biochemical screening procedure for small clinical laboratories for Vero (Shiga-like)-toxin producing strains of *Escherichia coli* O157:H7. *J. Clin. Microbiol.* **24**, pp 652-653.

Harrigan, W.F. and McCance, M.E. (1976) *Laboratory methods in food and dairy microbiology*, pp 47-49, Academic Press, London, UK.

Hazen, T.C. (1988) Faecal coliforms as indicators in trophical waters-a review. *J. Toxic. Assess.* **3**, pp 461-477.

Hazen, T.C., Fliermans, C.B., Hirsch, R.P. and Esch, G.W. (1978) Prevalence and distribution of *Aeromonas hydrophila* in the United States. *Appl. Environ. Microbiol.* **36**, pp 731-738.

http//www.vet.uga.edu/WEBFILES/

Huss, H.H., Ababouch, L. and Gram, L. (2003) *Assessment and management of seafood safety and quality.* FAO Fisheries Technical Paper. No. 444. 230p, FAO, Rome, Italy.

Jeyasekaran, G. Karunasagar, I. and Karunasagar, I. (1990) Validity of faecal coliform test in tropical fishery products. In *Second Indian Fisheries Forum Proceedings*, May 27-31, 1990, pp 273-275, Mangalore, India.

Kumar, H.S. Otta, S.K. Karunasagar, I. and Karunasagar, I. (2001) Detection of shiga-toxigenic *Eschirichia coli* (STEC) in fresh seafood and meat marketed in Mangalore, India by PCR. *Lett. Appl. Microbiol.* **33**, pp 631-635.

Leclerc, H., Mossel, D.A.A., Edberg, S.C. and Struijk, C.B. (2001) Advances in the bacteriology of the coliform group: Their suitability as markers of microbial water safety. *Annu. Rev. Microbiol.* **55**, pp 201-234.

Lindsay J.A. (1997) Chronic Sequelae of Foodborne Disease *Emerging Infectious Diseases*, **3**, from web http://www.cdc.gov/ncidod/eid/vol3no4/lindsay.htm.

Lipp, E.K. and Rose, J.B. (1997) The role of seafood in foodborne diseases in the United States of America. *Rev. Sci. Tech.* **16**, pp 620-640.

MacFaddin, J.F. (1980) *Biochemical tests for identification of medical Bacteria*, II edn., pp. 439-464, Williams and Wilkins, Baltimore.

March, S.B. and Ratman, S. (1986) Sorbitol MacConkey medium for detection of *Escherichia coli* O157: H7 associated with hemorrhagic colitis. *J. Clin. Microbiol.* **23**, pp 869-872.

Mitsuda, T. Muto, T. Yamada, M. Kobayashi, N. Toba, M. Aihara, Y. Ito, A. and Yokota, S. (1998) Epidemiological study of a food-borne outbreak of enterotoxigenic Escherichia coli O25:NM by pulsed-field gel electrophoresis and randomly amplified polymorphic DNA analysis. *J. Clin. Microbiol.* **36**, pp 652-656.

Niemi, R.M., Heikkila, M.P., Lahti, K., Kalso, S. and Niemela, S.I. (2001) Comparison of methods for determining the number and species distribution of coliform bacteria in well water samples. *J. Appl. Bacteriol.* **90**, pp 850-858.

Nedoluha, P.C. and Westhoff, D.A.D. (1997) Microbiology of striped bass grown in three aquaculture systems. *Food Microbiol.* **14**, pp 255-264.

Ogbondeminu, F.S. (1993) The occurrence and distribution of enteric bacteria in fish and water of tropical bacyeria in fish and water of tropical aquaculture ponds in Nigeria. *J. Aqua. Trop.*, **8**, pp 61-66.

Paille, D.C., Hackney, L., Reily, M.C. and Kilgen, M. (1987) Seasonal variation in faecal coliform populations of Louisiana oysters and its relationship to microbiological quality. *J. Food Prot.* **50**, pp 545-549.

Ramteke, P.W., Bhattacharjee, J.W., Pathak, S.P. and Kalra, N. (1992) Evaluation of coliforms as indicators of water quality in India. *J. Appl. Bacteriol.* **72**, pp 352-356.

Rheinstein, P.H. and Klontz, K.C. (1993) Shellfish borne illnesses. *Am. Fam. Phys.*, **47**, pp 1837-1840.

Sangjindavong, M. and Gjerde, J. (1977) Classification of coliform bacteria isolated from marine environment and marine fish products. *Fiskeridir. Skr. Ser. Ernaering.* **1**, pp 67-74.

Souter, B.W., Sonstegard, R.A. and Mcdermett, L.A. (1976) Enteric bacteria in carp (*Cyprinus carpio*) and white suckers (*Catostomus commersoni*) *J. Fish. Res. Board. Can.* **33**, pp 1401-1403.

Surendran, P.K., Thampuran, N. and Nambiar, V.N. (2000) Comparative microbial ecology of freshwater and brackish water prawn farms. *Fish. Technol.* **37**, pp 25-30.

Teophilo, G.N.D., Dos, R.H.S., Fernandes-Vierra, Dos, D., Prazeres-Rodrigues and Menezes, F.G.R. (2002) *Escherichia coli* isolated from seafood: toxicity and plasmid profiles. *Int. Microbiol.* **5**, pp 11-14.

Tullus, K. Berglund, B., Fryklund, B. Kuhn, I. and Burman, L.G. (1988) Epidemiology of faecal strains of the family Enterobacteriaceae in 22 neonatal wards and influence of antibiotic policy. *J. Clin. Microbiol.* **26**, pp 1166-1170.

USFDA. (2001) *Bacteriological Analytical Manual.* 8th Edn. (Revised). Association of Official Analytical Chemists. Washington DC, USA.

Wells, J.G., Shipman, L.D., Greene, K.D., Sowers, E.G., Green, J.H., Cameron, D.N., Downers, F.P., Martin, M.L., Griffin, P.M., Ostroff, S.M., Potter, M.E., Tauxe, R.E. and Wachsmuth, I.K. (1991) Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like toxin-producing *E.coli* from dairy cattle. *J. Clin. Microbiol.* **29**, pp 985-989.