Fishery Technology
2005, Vol. 42(2) pp : 209 - 216

Level of *Escherichia coli* in Seafood in Domestic Trade and their Antibiotic Resistance Pattern

A. Surendraraj, Nirmala Thampuran* and P.K. Surendran
Central Institute of Fisheries Technology,
Cochin - 682 029, India

Bacteriological quality of fish/shellfish sold in retail outlets in and around Cochin, India was studied. The TPC for the different samples varied from 5.5 to 8.5 log cfu/g. The total *Enterobacteriaceae* count varied from 2.5 to 6.5 log cfu/g. The lactose fermenters count and total coliforms count varied from 2 to 6 log cfu/g. Seafood samples had *Escherichia coli* count ranging from 2 to 5.5 log cfu/g. The faecal streptococci count was between 2 to 5 log cfu/g. The majority of the *E.coli* isolates showed a Multiple Antibiotic Resistance (MAR) index of more than 0.5, suggesting that they may be from high risk sources. Only a low percentage of samples were acceptable for human consumption as per Indian Standards for market sold fresh fishes. Higher count of microbial indicator organisms confirms possible presence of bacteria of public health significance and demands stringent improvement in handling and sanitary practices in the markets.

Keywords : Bacteriological quality, fish/shellfish, retail markets, *E.coli*, MAR index.

Microbial safety is one of the public health issues associated with seafood consumption. Contamination due to unhygienic handling entails the risk of spreading the bacterial, viral or pathogenic agents of communicable enteric diseases. It has been established that quality of fish sold in domestic market is poor compared to that of export trade and they were mostly contaminated with pathogenic organisms (Nambiar & Iyer, 1990). The bacteriological quality of freshly landed and retail level seafood sold in different parts of the country has been reported by previous workers (Comar *et al.*, 1979; Lakshmanan *et al.*, 1984; Iyer *et al.*, 1986; Pradeep & Lakshmanaperumalsamy 1986; Varma *et al.*, 1988; Nambiar & Iyer 1990). And these studies have established that a sizable portion of the seafood available in the market for consumption is not meeting the quality criteria prescribed by Indian Standards.

The occurrence of Multiple Antibiotic Resistance (MAR) among the enteric bacterial species could be a problem associated with transfer of resistance to other organisms of human/veterinary significance (Toranzo *et al.*, 1983). Parveen *et al.* (1997) proposed MAR index for differentiating the sources of pollution.

This study is intended to estimate whether global impacts of recent years have improved the quality of seafood of retail level and to assess the risk of prevalence of antibiotic resistance among the *E.coli* isolates from retail sold seafood samples.

Materials and Methods

Seafood viz. finfishes, Indian oil sardine (*Sardinella longiceps*), Indian mackerel (*Rastrelliger kanagurta*), Pink perch (*Nemipterus japonicus*) and Pearlspot (*Etroplus suratensis*), Shrimp (*Metapenaeus dobsonii*), and Black clam both fresh and boiled (*Villorita cyprinoides*) from various markets in and around Cochin, were collected. Five market places were selected for study over a period

* Corresponding author.

of 3 months. As far as possible the same type of samples were collected from each market. A total number of 104 samples were analysed during the period of study. Samples were brought to the laboratory in sterile polythene bag without any time lag. 10 gm of each sample was aseptically weighed and homogenized with 90 ml normal saline (0.85%). Serial decimal dilutions were prepared to enumerate total plate count, total *Enterobacteriaceae* count, total lactose fermenters count, *E.coli* count, and faecal streptococci count.

The total plate count was enumerated by plating the samples on Tryptone Glucose Agar (TGA), Total *Enterobacteriaceae* count on Violet Red Bile Glucose Agar (VRBGA, Oxoid) and total coliforms as the count of lactose fermenters on Violet Red Bile Lactose Agar (VRBLA, Oxoid). *Escherichia coli* was estimated on Tergitol-7 and faecal streptococci in KF streptococcus Agar (KF). In general the methodologies outlined in USFDA (2001) and APHA (1976) were used.

The *E.coli* isolates from T_7 plates were confirmed by streaking on Eosine Methylene Blue Agar (EMB). Characteristic colonies were purified and confirmed biochemically as *E.coli* using IMViC test. These isolates were tested for resistance to eight individual antibiotics using the standard single disc diffusion method (Bauer *et al.* 1966). Antibiotic discs used were Bacitracin (8 µg), Methicillin (5 µg), Neomycin (30 µg), Nitrofurantoin (300 µg), Novobiocin (30 µg), Rifampicin (5 mg), Tetracycline (30 µg), Trimethoprim (5 µg). Organisms were classified as resistant or intermediate or sensitive to the antibiotics under tests according to the criteria of Bauer *et al.* (1966) and Anon (1993). The MAR index, a ratio between the number of antibiotics to which the organism is resistant and the total number of antibiotics used, was also determined.

Results and Discussion

Results of the bacteriological analysis pertaining to quality are presented in Table 1 to 3. The TPC varied from 5.5 log cfu/g to as high as 8.5 log cfu/g. The minimum value was recorded in the case of fresh clam from market E. The maximum value (8.31 log cfu/g) was recorded in the case of shrimp samples from market C. The present study showed a slightly higher count than the earlier reports, where TPC of only 3 to 7 log cfu/g was reported, for sardine, mackerel, and pink perch (Nambiar & Iyer 1990; Lakshmanan *et al.* 1984). The present study showed 1.5 to 4.5 log cfu/g higher than the previous studies for samples from Bombay and Mangalore (Iyer *et al.* 1986; Jadhav & Magar 1970). The TPC of shrimp, pearl spot

Table 1. Total Plate Count (log cfu/g) in fish/shellfish samples from Cochin markets

Samples / Markets	Sardine (20)	Mackerel (20)	Pearl spot (20)	Pink perch (8)	Shrimp (20)	Fresh Clam (8)	Boiled Clam (8)
Market A	6.24	5.93	5.92	7.04	7.59	ND	ND
Market B	6.34	6.55	7.20	ND	7.73	ND	ND
Market C	6.93	6.20	8.19	8.29	8.31	ND	ND
Market D	7.44	6.67	6.21	ND	7.70	5.50	6.93
Market E	5.79	5.78	6.55	ND	8.30	5.91	7.63

ND - Not Done
No in parenthesis indicate total no of samples

Table 2. Total *Enterobacteriaceae* Count (log cfu/g) and total coliforms count (log cfu/g) in fish/shellfish Samples from Cochin markets

Samples / Markets	Sardine (20)*	Mackerel (20)*	Pearl spot (20)*	Pink perch (8)*	Shrimp (20)*	Fresh Clam (8)*	Boiled Clam (8)*
Total *Enterobactericeae*							
Market A	4.52	3.97	3.86	4.71	4.47	ND	ND
Market B	4.56	4.33	4.49	ND	5.27	ND	ND
Market C	4.25	5.26	6.09	6.18	6.15	ND	ND
Market D	4.92	4.53	5.24	ND	5.17	2.60	4.92
Market E	4.30	3.68	4.91	ND	5.70	3.00	5.12
Total coliforms							
Market A	3.14 (4.2)	2.97 (10)	2.90 (61.5)	4.43 (51.9)	3.71 (17.16)	ND	ND
Market B	4.00 (27.6)	3.84 (32.5)	3.41 (8.3)	ND	3.14 (0.74)	ND	ND
Market C	2.39 (1.39)	3.94 (4.74)	5.83 (54.03)	5.07 (7.63)	5.58 (26.78)	ND	ND
Market D	4.59 (46.4)	3.56 (10.7)	5.06 (65.43)	ND	5.10 (84.9)	2.41 (65)	4.79 (75.3)
Market E	3.42 (13.3)	2.54 (7.3)	4.35 (27.45)	ND	5.30 (39.4)	2.17 (14.9)	4.70 (38.3)

ND - Not Done
No in parenthesis indicate % total coliforms in *Enterobacteriaceae.*
* No of samples tested

and boiled clam of the present study were in tune with that of previous studies involving samples from Cochin and Bombay (Pradeep and Lakshmanaperumalsamy 1986; Iyer *et al.* 1986 and Varma *et al.* 1988).

The total *Enterobacteriaceae* count is not reported in any of the studies and hence there is dearth of this data. In the present study the total *Enterobacteriaceae* count ranged from 2.5 to 6.5 log cfu/g (Table 2). This agrees well with the studies conducted by Gorczyca *et al.* (1985) for Australian samples of fish, oyster, scallops and mussels. But the seafood samples from Cochin had higher count than that of retail raw shrimp from Saudi Arabia, where a count not exceeding 4 log cfu/g only was reported (Al-Dagal 1996). As *Enterobacteriaceae* count is related to both hygiene and safety of foods, high count observed in these samples implies prevalence of poor sanitary and hygienic practices in Cochin markets.

The count of lactose fermenters on VRBLA give an estimate of bacteria able to ferment lactose in presence of bile i.e. coliforms. The total coliforms count ranged from 2 to 6 log cfu/g (Table 2). The minimum value was again recorded in the case of fresh clam from market E. Pearl spot samples analysed from market C showed the highest value of 5.83 log cfu/g. Shrimp, pearl spot, pink perch and boiled clam invariably from different markets showed higher values for coliforms. The present study showed about 2 log cycle increase in count than the count for samples from landing centers and retail markets from India and Australia by different authors (Rao and Gupta 1978, Gorczyca *et al.* 1985; Iyer *et al.* 1986). In the case of clam samples Comar *et al.* (1979) and Garcia *et al.*

Table 3. *E.coli* (log cfu/g) and faecal streptococci count (log cfu/g) in fish/shellfish from Cochin markets

Samples / Markets	Sardine (20)	Mackerel (20)	Pearl spot (20)	Pink perch (8)	Shrimp (20)	Fresh Clam (8)	Boiled Clam (8)
E.coli							
Market A	3.60	3.60	3.77	3.60	4.01	ND	ND
Market B	3.80	3.60	3.30	ND	3.50	ND	ND
Market C	3.86	3.82	5.00	4.90	5.53	ND	ND
Market D	3.66	2.69	4.19	ND	4.30	2.04	3.95
Market E	2.00	2.30	3.77	ND	4.73	2.22	3.60
faecal streptococci							
Market A	3.31	3.09	3.38	3.96	4.69	ND	ND
Market B	3.65	4.47	3.84	ND	4.32	ND	ND
Market C	4.93	3.48	4.87	4.59	4.71	ND	ND
Market D	4.03	2.30	3.92	ND	4.48	2.25	3.92
Market E	2.14	2.14	3.39	ND	3.85	2.26	3.69

ND - Not Done
No in parenthesis indicate total no of samples analysed.

(1995) reported counts very identical with the present study.

The *E.coli* count for different samples varied from 2 log cfu/g to 5.5 log cfu/g (Table 3). All samples, whether shellfish or finfish, exceeded a count range of more than 2 log cfu/g. The present data is at par with the earlier studies on sardine from landing centers and retail markets of Cochin, Andhra Pradesh, Bombay (Rao and Gupta 1978; Lakshmanan *et al.* 1984; Iyer *et al.* 1986 and Nambiar and Iyer 1990). Pink perch and pearl spot samples analysed showed a count comparable to that reported by Nambiar and Iyer (1990). Clams had a high count as reported by Varma *et al.* (1988). Higher value of *E.coli* indicated the faecal contamination rate is also high.

The count of faecal streptococci varied from a range of 2 to 5 log cfu/g (Table 3). The present data showed a higher count of faecal streptococci than that of Iyer *et al.* (1986); Varma *et al.* (1988) and Nambiar and Iyer (1990) for retail and freshly landed fish

Table 4. Bacteriological quality of fish/shellfish sample from Cochin markets based on microbiological criteria of Indian Standards

Samples	No of Samples analysed	TPC Greater than 5 Lakh (%)	*E.coli* Greater than 20 (%)	Faecal *Strep.* Greater than 100 (%)	Faecal *Strep.* Greater than 1000 (%)	TPC Greater than 10^7 /g (%)	*E.coli* Graeter than 400 (%)
Sardine	20	20 (100)	20(100)	20 (100)	16 (80)	4 (20)	
Mackerel	20	20 (100)	20 (100)	20 (100)	12 (60)	0 (0)	
Pearl spot	20	20 (100)	20 (100)	20 (100)	20 (100)	8 (100)	
Pink perch	8	8 (100)	8 (100)	8 (100)	8 (100)	8 (100)	
Shrimp	20	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)
Boiled Clam	8	8 (100)	8 (100)	8 (100)	0 (0)	4 (50)	8 (100)
Fresh Clam	8	4 (50)	8 (100)	4 (50)	0 (0)	0 (0)	0 (0)

and shellfish samples from Cochin and Bombay. This higher count of faecal streptococci value confirms the persistence of high faecal pollution at different markets of Cochin in recent years, as they are indicators of faecal pollution that occurred a long time back.

In general, it is observed that shellfish samples showed comparatively higher count than finfish samples. Among the fish samples Pink perch showed higher count. Shewan (1977) reported that shellfishes were observed to have higher count over that of fin fishes. The catching method for shrimps from Cochin is primarily trawling. This may be the reason for high count. Fresh clam samples, which were depurated in water in order to maintain in live condition by retailer, showed lower count consistently. Market C samples are prone to probable cross contamination from nearby sewage and hence showing a higher count than other markets for all samples except mackerel. Boiled clam meat was observed to have a higher count than fresh one. This can be attributed to higher rate of recontamination during further handling and exposure to ambience. Present data on retail market samples showed higher bacteriological count than that of landing center samples (Lakshmanan *et al.* 1984). This may be because of the effect of transportation, distance between landing center and the market, the better cleanliness in the landing center and handling and subsequent contamination in markets (Nambiar and Iyer 1990).

As per Indian Standards (IS: 4780 & 4781, 1978) except two fresh clam samples all other samples in the present study exceeded the TPC of 5 lakhs/g limit (Table 4). When TPC level suggested by ICMSF (1974) for fresh fish is concerned, most of the samples in the present study exceeded a count limit of 10^7/g. All the mackerel and fresh clam samples were within the prescribed limit. Among the other samples sardine (20%), pearl spot (40%), boiled clam (50%) samples exceeded the limit of acceptability.

As per IS: 6032 (1978), limit for *E.coli* is 20 nos/g and all the samples analysed exceeded the limit of 20/g. According to ICMSF specification for fresh clam, limit of *E.coli* is 400 nos/g, and in the present study the clam samples were under acceptable range. All the shrimp and boiled clam samples exceeded this limit. In the case of faecal streptococci a count less than 100/g is the acceptable limit as per Indian Standards. All the samples analysed crossed the acceptability limit. Iyer *et al.* (1973, 1986) suggested a limit of 1000/g and based on this the clam samples both fresh and boiled, 20% of sardine and 40% of mackerel were acceptable microbiologically. All other samples exceeded the limit. Acceptability percentage in the present study is less than those reported for retail and landing center samples from Cochin, Bombay and Melbourne (Lakshmanan *et al.* 1984; Gorczyca *et al.* 1985; Iyer *et al.* 1986 and Nambiar and Iyer 1990).

Table 5. Antibiotic resistance pattern of *E.coli* (50 nos.) isolate for different antibiotics

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Bacitracin B^8	0 (0)	6 (12)	44 (88)
Methicillin M^5	0 (0)	0 (0)	50 (100)
Neomysin N^{30}	38 (76)	12 (24)	0 (0)
Nitrofuranthin Nf^{300}	2 (4)	20 (40)	28 (56)
Novobiocin No^{30}	0 (0)	0 (0)	50 (100)
Rifampicin R^5	0 (0)	8 (16)	42 (84)
Tetracyclin T^{30}	0 (0)	12 (24)	38 (76)
Trimethoprim Tr^5	38 (76)	2 (4)	10 (20)

Presence of large number of antibiotic resistant *E.coli* in fish samples may lead to serious consequences. It can also spread of antibiotic resistance to human pathogens if the antibiotic resistance is of plasmid-mediated type (Toranzos *et al.* 1983). Table 5 shows sensitive, intermediate and resistance pattern of 50 *E.coli* isolates from different market samples for the 8 antibiotics tested. All the isolates showed resistance to Methicillin and Novobiocin. About 70 to 90 percentages of the *E.coli* isolates were seen to be resistant to Bacitracin, Rifampicin and Tetracycline. 38 isolates (76%) were sensitive to Neomycin and Trimethoprim.

The MAR index in the present study for *E.coli* isolates from various sample sources, was always higher than 0.5 (Table 6). It was reported that strains with MAR value higher than 0.2 may have originated from high-risk source of contamination (Toranzos *et al.* 1983). Resistant pattern for different antibiotics and MAR index of the *E.coli* isolates in present study are comparable with the earlier studies for isolates from river water, sediment and aquaculture pond samples (Harish *et al.* 2003; Hatha *et al.* 1999).

Table 6. MAR index of *E.coli* isolates from different samples

Sample source	No of isolates	No. of antibiotics tested	MAR index
Sardine	14	8	0.66
Mackerel	14	8	0.64
Shrimp	4	8	0.69
Pearl spot	12	8	0.625
Pink perch	4	8	0.75
Clam	2	8	0.625

Higher value for TPC and other microbial quality indicators for different samples in these markets show that the sanitary and hygienic practices followed have not improved in recent years. Other factors like improper icing of fish, exposure of samples to sun, quality of the water used for washing the fish etc might also have contributed to this higher count as well as variations in count among the samples and markets.

References

Al-Dagal, M.M. (1996) Microbial Safety and Quality and Protein integrity of shrimp sold in shops in Riyadh, Saudi Arabia. *J. Food Prot.*, **59**, pp 988-991.

APHA (1976) *Compendium of Methods for the Microbiological Examination of Foods.* (Speck, M.L., Ed), American Public Health Association. New York.

Anon (1993) Chapter 16. Antibiotics chemotherapy. In: *Microbiology*, 2nd Edn, (Prescott, L.M., Harley, J.P. and Klein, D.A.), Wm.C.Brown Publishers, Oxford, England, pp 325-343.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Jurk, M. (1966) Antibiotic susceptibility testing by a standardized single disk method. *Amer. J. Clin. Pathol.* **45**, pp 493-496.

Comar, P.C., Kanc, B.E., Jr., and Jeffreys, D.B. (1979) Sanitary significance of bacterial flora of brackish water clam, *Rangia cunneata* in Albermarle Sound, North Carolina. *Proc. Natl. Shellfish. Assoc. Md.*, **69**, pp 92-100.

Gorczyca, E., Chong, M.P., and Green, J. (1985) The hygiene status of seafoods in Melbourne. In: *Spoilage of tropical fish and product development- Proceedings of a symposium held in conjunction with the sixth session of the Indo Pacific Fishery Commission working party on Fish Technology and Marketing*, (Reilly, A., Ed.), pp 182-190, FAO Fisheries Technical Report no 317 supplement. Food and Agricultural Organization, Rome.

Harish, R., Sumitha, C.M., and Hatha, A.A.M. (2003) Prevalence and antibiotic sensitivity of *Escherichia coli* in extensive brackish water aquaculture ponds. *Fish. Technol.* **40,** pp 8-12.

Hatha, A.M.M., Dhanalakshmi, P., Smith Kuriakose, Lakshmi, P., and George, L. (1999) *Poll. Res.* **18,** p 519.

ICMSF (1974) *Microorganisms in foods.2.Sampling for microbiological analysis; Principles and Specification*, pp 92-104, International Commission on Microbiological Specifications for Foods, University of Toronto Press, Toronto, Canada.

IS: 6032 (1971) *Specification for Mackerel Fresh*, Indian Standards Institution, New Delhi.

IS: 4781 (1978) *Specification for Threadfin Fresh, First Revision*, Indian Standards Institution, New Delhi.

IS: 4780 (1978) *Specifications for Pomfret Fresh, First Revision*, Indian Standards Institution, New Delhi.

Iyer, T.S.G., Annamma Mathew, Joseph, A.C., Rao, K. and Pillai, V.K. (1973) Faecal *Streptococci* in frozen shrimp. *Fish. Technol.* **10,** pp 66-70.

Iyer, T.S.G., Damle, S.P., Garg, D.K., Nambiar, V.N., and Vasu, N.M. (1986) Quality of fish in retail markets of Bombay. *Fish Technol.*, **23**, pp 78-83.

Jadhav, M.G., and Magar, N.G., (1970) Preservation of fish by freezing and glazing. I. Bacteriology of fresh, frozen and glazed fish. *Fish. Technol.*, **7**, pp 86-90.

Lakshmanan, P.T., Mathen, C., Varma, P.R.G., and Iyer. T.S.G. (1984) Assessment of quality of fish landed at the Cochin fisheries harbour. *Fish. Technol.*, **21**, pp 98-105.

Nambiar, V.N., and Iyer, K.M. (1990) Microbial quality of fish in retail trade in Cochin. *Fish.Technol.*, **27,** pp 51-59.

Parveen, S., Murphree, R.L., Edmiston, L., Kaspar, C.W., Portier, K.M., and Tamplin, M.L. (1997) Association of multiple antibiotic resistance profiles with point and nonpoint sources of *E. coli* in Appalachicola Bay. *Appl. Environ. Microbiol.*, **63,** pp 2607-2612.

Pradeep, R., and Lakshmanaperumalsamy, P., (1986) A quantitative study of *Vibrio parahaemolyticus* (Sakazaki *et al*) in *Etroplus suratensis* (Bloch) and *Metapenaeus dobsoni* (Miers) from Cochin backwater. *Fish. Technol.*, **23**, pp 66-69.

Rao, C.C.P., and Gupta, S.S. (1978) Enteropathogenic *E.coli* and other coliforms in marine fish. *Fish. Technol.*, **15,** pp 45-47.

Shewan, J.M. (1977) The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. In: *Proceedings of the Conference on Handling, Processing and Marketing of Tropical Fish.*, pp 51-66, Tropical Products Institute, London.

Toranzos, A.E., Barja, J.L., Lolwell, R.R. and Hetrick, F. M. (1983) *Infect. Immunol.*, **39**, pp 184-189.

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

Varma, P.R.G., Iyer. T.S.G., and Mathen, C. (1988). Quality of commercial frozen boiled clam meat. *Fish Technol.*, **25**, pp 36-39.