

Microbial Populations in Deep Sea Water and Fish of South East Arabian Sea with Special Emphasis on *Escherichia coli* & Coliphages

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Deep sea fish, deep sea surface water (DSW) and deep sea water from 200m depth (DSW₂₀₀) and littoral sea water (LSW) of the Arabian Sea (8-13°N Latitude, 74-76°E Longitude) were analyzed for faecal indicators. *E.coli* could not be detected in DSW₂₀₀. However, two deep-sea fish, *Psenopsis cynea* and *Heterocarpus woodmasoni* had low levels of *E.coli*, 0.3 MPN/g and 0.7 MPN/g, respectively, at the time of harvest. *E.coli* strains isolated from deep sea environment have been found to be indistinguishable from terrestrial forms, either morphologically or biochemically. DSW had almost equal proportions of Gram -ve (22%) and Gram +ve (20%) bacteria whereas DSW₂₀₀ had a high percentage of Gram +ve (46%) bacteria. In deep-sea fish, Gram -ve were almost double of Gram +ve bacteria. *Pseudomonas* was the predominant bacterial genus in deep-sea water samples whereas *Vibrio* was preponderant in deep-sea fish. DSW₂₀₀ had significant proportions of *Arthrobacter* (24%) and filamentous bacteria (17%). Yeasts were detected only in deep-sea fish samples. LSW collected from a location 15 nautical miles off Quilon had high levels of faecal pollution. In LSW predominant Gram +ve bacterial genus was *Micrococcus* (16%) whereas *Pseudomonas* (13%) and *Enterobacteriaceae* (10%) were the prominent Gram -ve groups. *E.coli* and coliphages could not be detected in deep-sea waters. A few deep-sea fish at the time of harvest showed low levels of faecal pollution and were within the stipulated limits. The study showed that high count of *E.coli* and coliphages in deep-sea fish procured from landing centres is not due to polluted deep-sea fish or polluted deep-sea waters.

Key Words : Deep sea, *Escherichia coli*, coliphages, faecal pollution

Arabian sea has potentially rich unexploited deep sea fish resources. In the South East Arabian sea (8-13°N latitude, 73-76°E longitude) *Chlorophthalmus* formed the most dominant species. Other major exploitable species included *Cubiceps*, *Neopinnula orientalis*, *Psenopsis cynea*, *Chascanopsetta lugubris* and *Priacanthus hamrur* (Khan *et al.*, 1996). In the 200 - 500m depth region, the potential for deep sea fish resources is higher in South West Coast than in North West, Lower East and Upper East Coasts (Sudarsan *et al.*, 1990).

Monitoring the sanitation quality of water and fish is necessary for predicting

potential public health hazards. Coliform bacteria viz., faecal coliforms and *E.coli* are by far the most commonly used indicators of faecal contamination. Regulatory agencies had fixed the admissible level for these in water and food intended for human consumption (European Economic Communities, 1980; GOI, 1995). Salinity, light intensity, lack of adequate amounts of nitrogen, phosphorous, iron and low organic matter content of the sea water contribute significantly to the death of coliforms in marine environment (Borrego *et al.*, 1983, Lei *et al.*, 2000). As such very low counts of *E.coli* are expected in deep sea fish. However, when deep sea fish procured from fish landing

centers at Cochin were analyzed, we found that all the samples had very high numbers of faecal coliforms, *E.coli* and coliphages (Table 1). In order to ascertain whether *E.coli* and coliphages were present in the deep sea fish at the time of harvest and whether deep sea waters harbour these organisms, the present work was undertaken. Additionally, study on the microbial populations and generic distribution of bacteria in deep sea water and deep sea fish of the southwest coast was taken up, as the information available on this aspect was limited.

Materials and Methods

Deep sea fish and deep seawater samples were collected from the South East Arabian sea during Cruise 191 of FORV *Sagar Sampada*, in January, 2001, the area of operation being 8-13°N latitude, 74-76°E longitude and depths of 227m to 324m for deep sea samples and 51 to 70 m for littoral sea samples. The samples were analyzed in the on-board laboratory, immediately after collection.

Deep sea surface water (DSW) samples were collected from the surface (1-2m deep), at four locations, using presterilized bottles. Deep sea water samples from a depth of 200m (DSW₂₀₀) were collected at three locations using CTD system (Seabird Electronics, USA). Samples were dispensed immediately into presterilized bottles and analyzed. Littoral sea water (LSW) samples were collected from the surface (1-2m deep) at two locations, using presterilized bottles.

Five species of fish (*Psenopsis cynea*, *Chlorophthalmus agasizzi*, *Priacanthus hamrur*, *Trichurus lepturus*, *Neopinnula orientalis*), three species of prawn (*Plesionika ensis*, *Heterocarpus woodmasoni*, *Heterocarpus gibbosus*) and one species of lobster (*Panulirus sewelli*) were obtained from six locations from deep sea by

bottom trawling using high speed demersal trawl (fish version), high opening trawl and high speed demersal trawl. Immediately after hauling, the fish were aseptically picked from the trawl net, transferred into sterile plastic bags and analyzed microbiologically.

Two samples of fish (*Priacanthus hamrur*), four samples of shrimp (*Heterocarpus woodmasoni*, *Heterocarpus* sp. *Solenocera crassicornis*), two samples of lobster (*Panulirus sewelli*, *Panulirus polyphagus*), one sample of squid (*Loligo duvaucelli*) and one sample of oyster (*Crassostrea* sp.) were procured from fish landing centres in Cochin and were analyzed.

Aerobic Plate Count (APC) was done by pour plate method using sea water agar (peptone 0.5g, ferric phosphate 0.01g, agar 1.5g, aged seawater 100ml, pH 7.5±0.1). Aged sea water was autoclaved and used as diluent for homogenization. Plates were incubated aerobically at room temperature (26 – 28°C) for 10-12 days (ZoBell, 1990).

Original isolates from TPC plates were randomly picked using Harrison's disc (Harrigan and McCance, 1976b) and plated out to obtain a pure culture and put through a series of operations – microscopy (Gram's reaction, morphology) and biochemical reactions (Kovac's oxidase test, catalase test, Hugh & Leifsons glucose oxidation & fermentation reaction, sensitivity to 2.5 units of penicillin, growth at 0% NaCl) (Surendran & Gopakumar, 1981). The cultures were identified as per Surendran & Gopakumar, (1981); Bergey's Manual of Systematic Bacteriology Vol 1 & Vol 2, (1986a).

Total Coliforms, Faecal Coliforms and *E.coli* were determined by Most Probable Number (MPN) method (3step, 3tube MPN). MacConkey broth, brilliant green lactose bile broth 2%, EC broth and tryptone broth were

the media used. The incubation temperature for Step I and II was 37°C whereas for Step III it was $44.5 \pm 0.5^\circ\text{C}$ (Harrigan and McCance, 1976a).

Total Enterobacteriaceae Count was determined by employing VRBG Agar (Violet red bile glucose agar) using the pour plate method. Colonies that produced acid from glucose were counted as members of Enterobacteriaceae. Isolates which were Gram negative rods, oxidase test negative and H&L glucose fermentative were confirmed as members of Enterobacteriaceae (Bergey's Manual of Systematic Bacteriology Vol 1, 1986b).

From the positive MPN tubes, *E.coli* was purified by streaking on Levine eosin methylene blue agar (Difco, Becton Dickinson, USA) and confirmed using microscopy and biochemical tests. Isolates which were Gram -ve rods, oxidase -ve, catalase +ve, indole +ve, methyl red +ve, acetoin -ve, citrate -ve, H&L glucose fermentative with gas, gas from lactose at 44.5°C, ONPG +ve, utilised maltose, mannitol, sorbitol, trehalose and could not utilise adonitol and raffinose were

confirmed as typical *E.coli* (Bergey's Manual of Systematic Bacteriology Vol 1, 1986c)

Colonies from sea water agar APC plates were purified and identified as yeast based on their appearance in Gram's staining (blue to blue-black coloured yeast forms with budding and pseudo mycelium formation).

Coliphages were analyzed by single agar layer method (APHA, 1998), using *E.coli* (NCIM 2089) as host strain and nutrient agar medium (Rao & Surendran, 2001). Briefly, 1ml of host *E.coli* culture (24 h in nutrient broth), 5ml of water or 5ml of homogenized fish and 8ml of molten nutrient agar were mixed thoroughly and poured into petridishes and examined for the presence of plaques after 4-6 h.

Results and Discussion

Deep sea generally has none of the bacteria associated with terrestrial origin whereas those caught inshores may be heavily contaminated. Coliform bacteria especially *E.coli* indicates faecal pollution in water and fish. However, almost all samples of deep sea fish (one sample of *Priacanthus*

Table 1. Aerobic Plate Count and Faecal Indicators in Deep Sea Fish procured from Fish Landing Centres

Samples (Deep Sea Fish Species)	Aerobic Plate Count (cfu/g)	Faecal Coliforms (MPN/g)	<i>E.coli</i> (MPN/g)	Coliphages (pfu/g)
<i>Priacanthus hamrur</i>	7.7×10^5	0.7	0.7	NT*
<i>Priacanthus hamrur</i>	6.7×10^5	140+	110	NT*
<i>Heterocarpus woodmasoni</i>	1.3×10^6	110	110	75
<i>Heterocarpus sp.</i>	3.5×10^6	140+	140+	20
<i>Heterocarpus sp.</i>	6.0×10^6	140+	140+	4
<i>Solenocera crassicornis</i>	8.1×10^5	45	45	80
<i>Panulirus sewelli</i>	4.2×10^6	140+	140+	240
<i>Panulirus polyphagus</i>	4.3×10^7	140+	140+	14
<i>Loligo duvaucelli</i>	3.7×10^4	140+	45	4
<i>Crassostrea sp.</i>	1.1×10^8	140+	140+	6

* NT - Not Tested

Table 2. Aerobic Plate Count and Faecal Indicators in Deep Sea Water and Littoral Sea Water of South East Arabian Sea

Samples	Location Lat. - Long.	Water Depth (m)	Aerobic Plate Count (cfu/ml)	Total Coliforms (MPN/100ml)	Faecal Coliforms (MPN/100ml)	<i>E.coli</i> (MPN/100ml)	Coliphages (pfu/100ml)
DSW 1*	12°30'N 74°11'E	227	8	0	0	0	0
DSW 2	12°40'N 74°19'E	284	13	4	4	0	0
DSW 3	8°40'N 75°38'E	318	4	0	0	0	0
DSW 4	9°08'N 75°150E	324	4	3	0	0	0
DSW ²⁰⁰ 1*	9°22'N 75°47'E	295	1.6×10^3	0	0	0	0
DSW ²⁰⁰ 2	8°40'N 75°38'E	318	3×10^5	0	0	0	0
DSW ²⁰⁰ 3	9°08'N 75°50'E	324	4×10^2	0	0	0	0
LSW [↑] 1	8°52'N 76°18'E	51	2.9×10^4	250	250	45	30
LSW 2	8°39'N 76°20'E	70	2.3×10^4	0	0	0	0

* DSW - surface level deep sea water

* DSW²⁰⁰ - deep sea water collected from a depth of 200m

↑ LSW - surface level littoral sea water

hamrur, two samples of *Heterocarpus* sp., one sample each of *Heterocarpus woodmasoni*, *Solenocera crassicornis*, *Panulirus sewelli*, *Panulirus polyphagus*, *Loligo duvaucelli* and *Crassostrea* sp.) procured from fish landing centers at Cochin had very high levels of faecal coliforms, *E.coli* and coliphages (Table 1). Out of all the landing centre samples tested, only one sample of *Priacanthus hamrur* (0.7MPN/g) had acceptable levels of *E.coli*. The present study is undertaken to know the levels of faecal coliforms and *E.coli* in deep sea fish at the time of harvest and in deep sea waters.

Aerobic Plate Count values were very low in DSW (4 – 13cfu/ml) than in samples collected from 200m below the surface (4×10^2 to 3×10^5 cfu/ml) [Table 2]. This difference in abundance can be attributed to the concentration of easily assimilable organic matter. The raining down of organic matter, in the form of detritus/dead plankton from the euphotic zone, might have resulted in this high variability in counts. Higher counts of bacteria were reported at the 300m horizon followed by 100m, 25m and the 0m level (Labedeva *et al.*, 1963). Chandrika (1996) reported 10^8 cfu/ml in

Table 3. Aerobic Plate Count and Faecal Indicators in Deep Sea Fish, immediately after harvest

Fish Species	Aerobic Plate Count (cfu/g)	Total Coliforms (MPN/g)	Faecal Coliforms (MPN/g)	<i>E.coli</i> (MPN/g)	Coliphages (pfu/g)
<i>Psenopsis cynea</i>	2.1×10^2	0.3	0.3	0.3	0
<i>Chlorophthalmus agasizzi</i>	1.4×10^4	0	0	0	0
<i>Priacanthus hamrur</i>	3.7×10^6	0.9	0	0	0
<i>Trichurus lepturus</i>	2.5×10^6	0	0	0	0
<i>Neopinnula orientalis</i>	1.4×10^3	0.9	0	0	0
<i>Plesionika ensis</i>	5×10^4	0	0	0	0
<i>Heterocarpus woodmasoni</i>	6×10^4	1.5	0.7	0.7	0
<i>Heterocarpus gibbosus</i>	4×10^5	1.5	0.3	0.7	0
<i>Panulirus sewelli</i>	2.1×10^5	0	0	0	0
Range	2.1×10^2 to 3.7×10^6	0 – 1.5	0 – 0.7	0 – 0.7	0

surface and 50m deep waters around Lakshadweep islands but Karthiayani & Iyer (1975) obtained high aerobic plate counts in bottom mud (2.7×10^4 cfu/g) and lowest counts at surface waters (0-90/ml). In LSW samples total bacteria ranged between 2.3×10^4 and 2.9×10^4 cfu/ml (Table 2). Demersal deep sea fish at the time of harvest had APC values ranging between 2.1×10^2 and 3.7×10^6 cfu/g (Table 3). Bacterial load of ocean fresh prawn and fish was reported to be in the range of 10^3 to 10^5 cfu/g (Karthiayani & Iyer, 1975).

E.coli could not be detected in DSW as well as in DSW₂₀₀ samples. However, two of the DSW samples had total coliform levels of 3/100ml and 4/100ml, of which only one sample had faecal coliforms (4/100ml). None of the DSW₂₀₀ samples were positive either for total coliforms or faecal coliforms (Table 2). Five out of nine deep sea fish samples had total coliform levels ranging between 0.3MPN/g and 1.5MPN/g (Table 3). Three samples viz., *Psenopsis cynea* (0.3/g), *Heterocarpus woodmasoni* (0.7MPN/g) and *Hetrocarpus gibbosus* (0.3MPN/g) were positive for faecal Coliforms. *E.coli* was detected in *Psenopsis cynea* (0.3/g) and *H.gibbosus* (0.7/g). *E.coli*, the most commonly used indicator of faecal pollution, was detected in

two deep sea fish but was absent in deep sea surface waters and 200m deep waters. Till now *E.coli* was not reported from deep sea fish at the time of harvest. Although *E.coli* is regarded as unique to the gastrointestinal tract of warm blooded animals (APHA, 1995), studies showed that in natural waters certain cold blooded animals viz., fresh water turtles (Mitchell & McAvoy, 1990) and diamond back estuarine terrapins (Harwood *et al.*, 1999), harbour *E.coli* as part of commensal flora. The question then was, whether these isolates of *E.coli* were truly marine. The ability of the isolated *E.coli* strains to survive and grow rapidly at 0% salt concentration suggests that they might have been of terrestrial origin. *E.coli* strains isolated from marine environment were found to be indistinguishable from terrestrial forms. Coliforms and *E.coli* were found to be 100 to 1000 times more abundant in sediment samples than in surface samples and were detected upto 11 km for sediment samples and for sea water upto 5 km from the coastline (Yde *et al.*, 1980).

It can be said that *E.coli* was not present in marine environment either in deep sea fish or deep sea water and if at all present it might have been due to some human intervention. Deep sea fish did not harbour

Table 4. Qualitative distribution of microorganisms in sea water and fish

Littoral sea water (surface)		Deep Sea Water (Surface)		Deep Sea Water (at 200m)		Deep Sea Fish	
<i>Micrococcus</i>	16%	<i>Pseudomonas</i>	7%	<i>Pseudomonas</i>	27%	<i>Vibrio</i>	16%
<i>Pseudomonas</i>	13%	<i>Vibrio</i>	7%	<i>Arthrobacter</i>	24%	<i>Pseudomonas</i>	9%
<i>Filamentous bacteria</i>	13%	<i>Arthrobacter</i>	7%	<i>Filamentous Bacteria</i>	17%	<i>Filamentous Bacteria</i>	6%
<i>Enterobacteriaceae</i>	10%	<i>Filamentous Bacteria</i>	5%	<i>Acinetobacter</i>	2%	<i>Arthrobacter</i>	2%
<i>Arthrobacter</i>	10%	<i>Enterobacteriaceae</i>	2%	<i>Kurthia</i>	2%	<i>Acinetobacter</i>	1%
<i>Acinetobacter</i>	6%	<i>Photobacterium</i>	2%	Unidentified	2%	<i>Corynebacterium</i>	1%
		<i>Micrococcus</i>	2%			<i>Kurthia</i>	1%
		<i>Kurthia</i>	2%				
		<i>Corynebacterium</i>	%				
Yeast	16%					Yeast	17%
No Growth	16%	No Growth	59%	No Growth	24%	No Growth	44%
Total Isolates	31	Total Isolates	41	Total Isolates	41	Total Isolates	109

E.coli at the time of harvest and wherever present they were within the admissible limits. The high levels of *E.coli* encountered in the landing center samples might have been due to inadequate hygiene and sanitary practices. It is also possible that improper storage of fish might have helped the *E.coli* present in small numbers at the time of harvest, to multiply and made the fish unacceptable.

Total *Enterobacteriaceae* count is used as food safety indicator (Forsythe, 2000). Total *Enterobacteriaceae* were detected in DSW, DSW₂₀₀ and deep sea fish samples. Fish samples had relatively higher counts of total *Enterobacteriaceae*. However, on subsequent confirmatory tests it was found that majority were non-members of *Enterobacteriaceae*. Most of the colonies were either yeasts or filamentous bacteria. Only few were true members of *Enterobacteriaceae* belonging to the genera *Escherichia* and *Enterobacter*. Previous studies did not encounter *Enterobacteriaceae* in surface and 50m deep waters of Arabian Sea (Chandrika, 1996).

The group wise distribution of micro-organisms in deep sea fish and deep sea water samples is shown in Figure 1. The isolates were grouped as Gram -ve, Gram +ve, Yeast and No Growth based on their Gram's staining reaction, morphology and ability to grow on subsequent propagation. DSW had almost equal proportions of Gram -ve (22%) and Gram +ve (20%) bacteria whereas DSW₂₀₀ had high percentage of Gram +ve (46%) than Gram -ve bacteria (29%). In deep sea fish Gram -ve (26%) were almost double that of Gram +ve (14%) bacteria. 59% of DSW, 44% of deep sea fish and 24% of DSW₂₀₀ colonies from APC plates failed to grow on subsequent culture. Although the fact that, many marine bacteria do not undergo cell division in or on routinely employed bacteriological media (viable but non culturable state) is well known (Roszak and Colwell, 1987) but their ability to grow on initial isolation and failure to grow on subsequent culturing was seldom known. The generic distribution of bacteria in the commensal flora of deep sea fish and water was carried out and summarized in

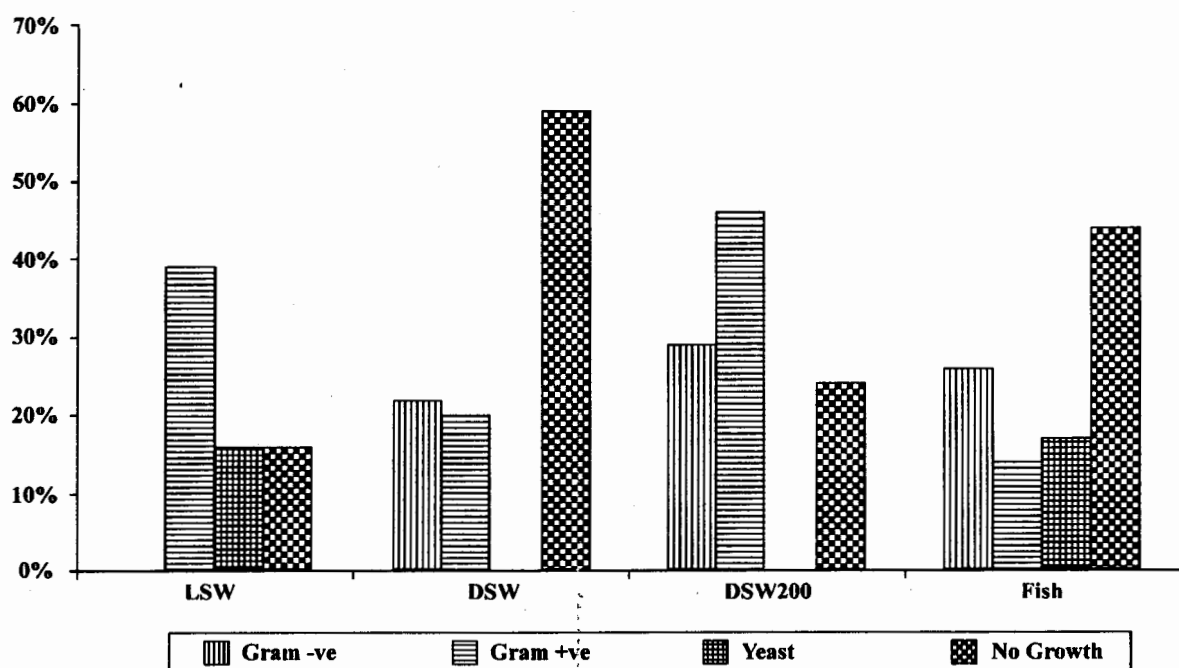


Fig. 1. Occurrence of Different Microbial Groups in Deep Sea Water and Fish

Table 4. *Pseudomonas* predominated in DSW₂₀₀ (27%) and DSW (7%). In deep sea fish, *Vibrio* (16%) was preponderant followed by *Pseudomonas* (9%). The dominance of *Pseudomonas* was expected as they were nutritionally non-exacting group, which utilize a wide spectrum of substrates. Upto the present, valuable data have accrued from numerous studies showed that the majority of forms cultivated from marine sources were Gram -ve asporogenous rods belonging to the *Vibrio* and *Pseudomonas* groups (Liston & Colwell, 1963; Skerman, 1963; Ohwada *et al.*, 1980; Chandrika, 1996). Other Gram -ve bacteria isolated belonged to *Acinetobacter* and *Enterobacteriaceae*. DSW₂₀₀ had significant proportions of *Arthrobacter* (24%) and filamentous bacteria (17%). The *Arthrobacter* isolates were all orange pigmented while the filamentous forms were highly coiled. *Arthrobacter* were found in soils where humus was the major ingredient (Schlegel, 1993). *Kurthia*, *Corynebacterium* and *Micrococcus* were the other Gram +ve bacteria observed. Previously, *Aeromonas* was reported to be the dominant genus in the surface waters of wedge bank region (Shankar, 1990).

Yeasts were noticed only in deep sea fish (17%) and LSW (16%). They could not be detected in DSW and DSW₂₀₀ samples. Yeast populations were reported to be most dense in the inshore waters and decrease progressively with distance from the shore. Yeast were also detected in the gut microflora of deep sea benthic microfauna (Ohwada *et al.*, 1980).

Littoral seawater had relatively higher Gram +ve (39%) than Gram -ve (29%) bacteria. The predominant Gram +ve bacterial genus was *Micrococcus* (16%) whereas *Pseudomonas* (13%) and *Enterobacteriaceae* (10%) were the prominent Gram -ve groups. Littoral sea water collected from a location

(8°52'99"N latitude, 76°18'273"E longitude, 51.2m deep water) which was 15 nautical miles off Quilon (Kerala Coast), a relatively bigger coastal town, had high levels of total coliforms (250/100ml), faecal coliforms (250/100ml), *E.coli* (45/100ml) and coliphages (30pfu/100ml) [Table 2]. The absence of all these indicators of faecal pollution in the littoral sea water collected from a location (8°36'340N, 76°23'408", 70m deep waters) 16 nautical miles off Neendakara (near Quilon), a small coastal town, suggests that littoral waters off densely populated coastal towns can pollute upto larger distances from the shore. Although our data was limited to arrive at a confirmed conclusion, it was in accordance with other studies which showed high *E.coli* levels in sea water samples from area with hinterlands of human populations and very low levels of *E.coli* in samples from areas with a hinterland of natural forest or very few dwellings (Owens, 1978). This assumes significance as the microbial populations in water can influence the microflora of fishes (Colwell & Liston, 1960). High total bacterial counts and *E.coli* counts were reported for different coastal waters (Owens, 1978; Yde *et al.*, 1980; Choi & Kim, 1988; Shin & Jung, 1996; Sunarya *et al.*, 1997; Vaidya *et al.*, 2001).

Coliphages were proposed as indicators of faecal pollution in marine waters (Borrego *et al.*, 1983; Legnani *et al.*, 1998). Coliphages were not detected in any deep sea water and deep sea fish samples. Only one littoral water sample yielded coliphages (30pfu/100ml). This sample had high levels of total coliforms, faecal coliforms and *E.coli* counts (Table 2).

E.coli and coliphages could not be detected in deep sea waters and most of the deep sea fish at the time of harvest or hauling. Few fish showed faecal pollution, although at very low levels and were within

the stipulated limits. The study showed that high count of *E.coli* and coliphages in landing centre samples is not due to the deep sea fish or sea water but could be only from terrestrial sources.

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References

- APHA (1995) *Standard methods for the examination of water and waste water*, 19th edn., American Public Health Association, Washington DC, USA.
- APHA (1998) *Standard methods for the examination of water and waste water*, 20th edn., American Public Health Association, Washington DC, USA.
- Bergey's Manual of Systematic Bacteriology* (1986a) Vol. 1 (Noel Krieg and John G Holt, Eds); Vol 2 (Sneath, P.H.A., Mair N.S. and Holt J.G., Eds), Williams & Wilkins, USA.
- Bergey's Manual of Systematic Bacteriology* (1986b) Vol. 1 (Noel Krieg and John G Holt, Eds), 414p, Williams & Wilkins, USA.
- Bergey's Manual of Systematic Bacteriology* (1986c) Vol. 1 (Noel Krieg and John G Holt, Eds), 408p, Williams & Wilkins, USA.
- Borrego, J.J., Arrabal F., Vicente A., Gomez, C.R. and Romero P. (1983) Preliminary study of microbial inactivation in the marine environments *J. Water Pollution Control Fed.* 55, pp 297-301
- Chandrika, V. (1996) Distribution of Heterotrophic Bacteria around Laccadive Islands. In: *Proceedings of 2nd Workshop of scientific results of FORV Sagar Sampada* (Pillai, V.K., Abidi, S.A.H., Ravindran, V., Balachandran, K.K. & Agadi, V.V., Eds), pp 97-102, Department of Ocean Development, India.
- Choi, Y.C. and Kim, J.K. (1988) Water quality of Tap-dong coastal area in Northern part of Cheju in winter season *Bull. Mar. Res. Inst. Cheju. Natl. Univ.* 12, pp 55-62
- Colwell, R.R. and Liston J. (1960) Microbiology of shellfish : bacteriological study of the natural flora of Pacific oyster (*Crassostrea gigas*) *Appl. Microbiol.* 8, pp 104-109
- European Economic Communities (1980) Council Directive of 15th July 1980, relating to the quality of water intended for human consumption (80/778/EEC) *Official Journal of European Communities*. No L 229, pp 11-28
- Forsythe J. Stephen (2000) *The microbiology of safe food*, 145p, Blackwell Science Ltd, London, UK.
- Government of India (1995) Ministry of Commerce. Order S.O. 729(E) dt. 21-8-1995.
- Harrigan, W.F. and McCance, M.E. (1976a) *Laboratory Methods in Food and Dairy Microbiology*, 141p, Academic Press, London, UK.
- Harrigan, W.F. and McCance, M.E. (1976b) *Laboratory Methods in Food and Dairy Microbiology*, 47p, Academic Press, London, UK.
- Harwood, V.J., Butler, J., Parrish, D. and Wagner, V. (1999) Isolation of faecal coliform bacteria from the diamond back terrapin (*Malaclemys terapin centrata*) *Appl. Environ. Microbiol.* 65, pp 865-867
- Karthiayani, T.C. and Iyer, K.M. (1975) The bacterial flora of certain marine fishes

- and prawns in Cochin waters in relation to their environs *J. Mar. Biol. Ass. India*. **17**, pp 96-100
- Khan, M.F., Zacharia, P.U., Nandakumaran, K., Mohan, S., Arputharaj, M.R., Nagaraja P. & Ramakrishna, P. (1996) Catch abundance and some aspects of biology of deep sea fish in the South East Arabian sea. In: *Proceedings of 2nd Workshop on scientific results of FORV Sagar Sampada*. (Pillai, VK, Abidi, S.A.H., Ravindran, V., Balachandran, K.K. and Agadi, V.V., Eds), pp 331-346, Department of Ocean Development, India.
- Labedeva, M.N., Anitchenko, E.J. and Gorbenko, J.A. (1963) Distribution of heterotrophic bacteria in some seas of Mediterranean basin. In: *Symposium of marine microbiology* (Carl Oppenheimer, Ed), 154p, Charles C Thomas Publishers, USA.
- Legnani, P., Leoni, E., Lev, D., Rossi, R., Villa, G.C. and Bisbin, P. (1998) Distribution of indicator bacteria and bacteriophage in shell fish and shellfish growing waters *J. Appl. Microbiol.* **85**, pp 790-798
- Lei Yang, Chang W. Shui and Lo Huang Mong N. (2000) Natural disinfection of waste water in marine outfall fields *Water Res.* **34**, pp 743-750
- Liston, J. and Colwell, R.R. (1963) Host and habitat relationships of marine commensal bacteria. In: *Symposium of marine microbiology* (Carl Oppenheimer, Ed), 611p, Charles C Thomas Publishers, USA.
- Mitchell, J.C. and McAvoy, B.V. (1990) Enteric bacteria in populations of fresh water turtles in Virginia *Va. J. Sci.* **41**, pp 233-242
- Ohwada, K., Tabor, P.S. and Colwell R.R. (1980) Species composition and barotolerance of gut microflora of deep sea benthic microfauna collected at various depths in the Atlantic ocean *Appl Environ. Microbiol.* **40**, pp 746-755
- Owens, J.D. (1978) Coliform and *E.coli* bacteria in sea water around Penang Island, Malaysia *Water Res.* **12**, pp 365-370
- Pramer, D., Carlucci, A.F. and Scarpino, P.V. (1963) The bactericidal action of seawater. In: *Symposium of marine microbiology* (Carl Oppenheimer Ed), 567p, Charles C Thomas Publishers, USA.
- Rao B. Madhusudana and Surendran P.K. (2001) Coliphages in fish and fishery environment *Applied Fisheries and Aquaculture* **1**, pp 87-89
- Roszak, D.B. and Colwell, R.R. (1987) Survival strategies of bacteria in the natural environment *Microbiol. Rev.* **51**, pp 365-367
- Schlegel, G.H. (1993) *General Microbiology*, 7th edn. 103p, Cambridge University Press, UK.
- Shankar, V.A. (1990) Heterotrophic bacteria in the surface layers of oceanic waters of the wedge bank region. In: *Proceedings of the first workshop of scientific results of FORV Sagar Sampada*. (Mathew, K.J. Ed), pp 33-35, Department of Ocean Development, India.
- Shin, S.U. and Jung, K.J. (1996) Bacterial flora of East China sea and Yosue coastal sea areas *J. Korean Fish. Soc.* **29**, pp 17-25
- Skerman, T.M. (1963) Nutritional patterns in marine bacterial populations. In: *Symposium of marine microbiology* (Carl Oppenheimer, Ed), 685p, Charles C Thomas Publishers, USA.
- Sudarsan, D., John, M.E. and Somvanshi (1990) Marine fishery resource potential in the Indian Exclusive Economic Zone: an update *Bull. Fish. Survey of India*, **20**

- Sunarya, A.K.S., Susilowati, B. and Pratiwi, T. (1997) Evaluation of microbiological quality of coastal waters from several landing sites along the North Java coastal area. In : *Asia Pacific Fishery Commission Summary Report* (James, D.J. Ed), pp 35-38, Working party of Fish Technology and Marketing, Colombo, Sri Lanka.
- Surendran, P.K. and Gopakumar, K. (1981) Selection of bacterial flora in the chlorotetracycline treated oil sardine (*Sardinella longiceps*), Indian mackerel (*Rastrelliger kanagurta*) and prawn (*Metapenaeus dobsoni*) during ice storage. *Fish. Technol.*, **18**, pp 133-141
- Yde, M., Maeyer S. de Cleempol and Dartevelle, Z. (1980) Faecal pollution in the North sea offshore Ostende (Belgium) *Rev. Int. Oceanogr. Med.* **59**, pp 47-54
- Vaidya, S.Y., Vala, A.K. and Dube, H.C (2001) Bacterial indicators of faecal pollution at Bhavnagar Coast *Indian J. Microbiol.* **41**, pp 37-39
- ZoBell, C.E. (1990) *Marine Microbiology - a monograph on hydrobacteriology*, 45p Bishen Singh Mahendra Pal Singh, Dehradun, India.