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BACTERIAL FISH DISEASES IN THE ESTUARIES OF GOA

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Diseases in fish, of bacterial origin were observed in the estuaries of Goa. High fish mortality rate was seen in the months of July to October, of year 1993, and the disease persisted at a reduced intensity upto January 1994. It was also observed that the disease was severe in the water bodies, that were near the agricultural fields. The disease affected the estuarine fish, like Catfish (*Macrones oculatus*), Mullet (*Mugil cephalus*), Karchani (*Gerres.* sp), Pearlspot (*Etroplus suratentsis*), etc. The morbid fish showed skin ulceration and infection all over the body. Pathogens were isolated also from internal organs such as, kidney, liver and spleen, besides isolating them from infected skin and gills.

Diseases in fish occur often due to proliferation of bacteria, fungi, protozoa or viruses in the skin, blood or the organs such as gills, kidney, air bladder, gonad, liver, etc. (1-13). Some time parasites such as crustaceans, nematodes, trematodes etc., are also responsible for the fish disease (13).

The unplanned industrial growth and other developmental activities, result often in the release of effluents into the water bodies, leading conditions of stress and strain on aquatic flora and fauna which includes fish. Large scale discharges from refineries, chemicals, fertilizers, pesticides, metal extraction, food fibre and pharmaceutical units have adversely affected the development of fisheries (7).

In the last few years, in Goa, fish mortality and morbidity is being observed in the brackish waters of the Mandovi-Zuari Basin, and rivers such as Chapora and Terakhol specially, during the monsoon period from July to October and extending upto January of the following year. The affected fish showed the redening of the skin surface. The skin peeled off extensively wherever the intensity of infection was acute. These symptoms were distinct at the regions of mouth, gills, fins and the mid region of the fish body. Often the diseased fish observed had their tails completely degraded, or their lower jaw portion showed signs of necrosis.

Disease in fish, resulted due to the lowering of resistance in fish in polluted environment. Thus the bacteria proliferated and indicated the different organs of the fish such as skin, gills, fins, kidney, liver, etc. The opportunistic bacteria invade the different organs of fish and thus cause disease. It was our endeavour to isolate and study the potential pathogens infecting the fish.

EXPERIMENTAL RESULTS AND DISCUSSION

During the present investigation samples of live and dead fishes with symptoms of the disease were collected from five affected regions in Goa . Help of the local fishermen was taken to obtain fresh and live fish by casting nets into the estuarine waters and catching them for our investigation.

Table 1a Microbial pathogens on fish

Isolate No.	Identity	Isol. form	Features of Micro-organisms
Culture I	Bacillus sephaericus	Infected surface and kidney	Gram +ve bacilli,not fermenting any Carbohydrates source capsule present.
Culture II	Micrococcus species	-do-	Gram +ve cocci in tetrads B-haemolytic
Culture III	Klebsiella species	-do-	Gram -ve bacilli, ferments most carbohydrate source with gas production capsule present.
Culture IV	Klebsiella species	-do-	Gram -ve bacilli, ferments most carbohydrate source with gas production capsule present.
Culture V	Acinethobacter species	-do-	Gram -ve bacilli in chains utilizes NO_3 as Nitrogen source.
Culture VI	Acinethobacter species	-do-	Gram -ve coccobacillli capsule present.
Culture VII	Klebsiella species.	-do-	Gram -ve bacilli

During our field visits fishes were caught from waters where high mortality of fish was reported to us by the local fishermen. These samples were directly brought to the laboratory for detailed examination.

The fishes were identified, their weight and size determined, and then subjected to a detailed microbiological study and analysis. The affected regions were then examined under the stereo microscope. The Gram staining of the infected tissues were carried out. Under sterile conditions the tissue samples from skin, heart, liver, spleen and kidney were plated out on microbiological media. Among the media used for isolation of bacteria were, nutrient agar, sea water nutrient agar, tryptone soya agar and the TCBS agar. The bacterial pathogens isolated were identified as per Bergey's Manual (VIII edition). The ability of the isolates to degrade nitrates, urea and phosphates was determined.

The details of the infected fish and the microorganisms isolated and identified are presented in Tables 1 (a & b). Our results clearly indicate that the infection was predominantly of bacterial origin. The bacterial species, commonly isolated were, *Bacillus sphaericus, Micrococcus sp., Acinetobacter sp., and Klebsiella* sp. *Bacillus sphaericus* which occurred in chain produced capsules and spores was commonly found in most diseased fish samples from Curtorim. Other bacterial species like *Micrococcus* sp., *Acinetobacter* sp.

Table 1b Ecological studies and symptoms of diseased fish

Sample No.	Place	Region	Name of fish Size (cm) Weight (gms)	Symptoms	Bacteria isolated
1	2	3	4	5	6
1.	Curtrim	Agrl. fields	Cat fish 13.5 cms: 22.5 gms.	Infection at the upper jaw	*Bacillus Micrcoccus*
2.	Curtrim	Agrl. fields	Cat fish 15 cms. 34.5 gms.	Infection between the two gills	*Bacillus Micrcoccus*
3.	Curtrim	Agrl. fields	Karsani 14 cms. 20.5 gms.	Infection near the doorsal fin at the side	*Bacillus Klebsiella* (No. III)
4.	Curtrim	Agrl. fields	Cat fish 12 cms. 12.8 gms.	Infection at the lower jaw	*Bacillus Micrcoccus*
5.	Curtrim	Agrl. fields	Mullet 12.5 cms. 13 gms.	Infection near the ventral fin and the gills	*Bacillus Klebsiella* (No. IV)
6.	Curtrim	Agrl. fields	Cat fish 10 cms. 9 gms.	Infection near the sides of the fish	*Bacillus Micrococcus Klebsiella* (No. IV)
7.	Curtrim	Agrl. fields	Mullet 13 cms. 21 gms.	Infection near the tail &sides of the fish	*Bacillus Klebsiella* (No. IV)
8.	Curtrim	Agrl. fields	Khoru 6.5 cms. 2.5 gms.	Infection near the tail andsides of the fish	*Bacillus Micrococcus Klebsiella* (No. IV)
9.	Curtrim	Aqua. culture farm	Cat fish 19 cms. 48 gms.	Redening all over the body	*Klebsiella* (No. VII)
10.	Cuorao	Aqua. culture farm	Cat fish 16.2 cms. 38 gms.	Redening all over the body	*Klebsiella* (No. VII)
11.	Curtrim	Aqua. culture farm	Cat fish 12 cms. 25 gms.	Redening all over the body	*Klebsiella* (No. VII)
12.	Santan	Agrl. fields	Cat fish 20 cms. 38 gms.	The mouth was infected and the fish was bleeding from nostrils	*Acineto-bacter* (No. V)

- continued-

1	2	3	4	5	6
13.	Santan	Agrl. fields	Mullet 18 cms. 35 gms.	Infection near the tail region	*Acineto-bacter* (No. V)
14.	Santan	Agrl. fields	Cat fish 18 cms. 38 gms.	The muth was infe-cted and the fish was bleeding from noostrils.	*Acineto-bacter* (No. V)
15.	Santan	Agrl. fields	Mullet 12 cms. 26 gms.	Infection near the tail region and the caudal fin regioon	*Acineto-bacter* (No. V)
16.	Terakhol	Agrl. fields	Mullet 13.4 cms. 28 gms.	Infection near the tail region	*Acineto-bacter* (No. VI)
17.	Keri	Agrl. fields	Mullet 13.4 cms. 29 gms.	Infection near the tail region and mouth reion	*Acineto-bacter* (No. VI)

Table 2 Biochemical tests to confirm the ability of bacteria to utilize chemical fertilizers.

Sr. No.	Biochemical test	C-I	C-II	C-III	C-IV	C-V	C-VI	C-VI
1.	PO_4 test	+	+	+	+	+	+	+
2.	Urea test	+	-	+	+	+	+	+
3.	No_3 test	-	+	+	+	-	+	+

and *Klebsiella* sp. were isolated from kidney of the diseased Catfish and Mullet sampled from Curtorim, Satan, Terekhol, Keri, and Chorao. All the bacteria isolated, when injected into healthy fish, were able to reinfect the fish, causing similar symptoms of the disease.

It was observed that the intensity of the disease was very high in estuarine regions which were close to agricultural fields. The water bodies in Curtorim, Santan, Britton, Chorao and Siridao, are regions where the intensity of the infection was acute. It was observed that these regions were close to the agricultural land (Table 1b). It is pertinent to note that with the onset of South west monsoon, when the rainfall is heavy, the agricultural activity is intense and fertilizer use is evident. Our observation showed that the disease was at its peak in the months

of July to October of 1993, as the rainfall starts and the fertilizer drains off into the water bodies. The washing off of excessive chemical fertilizers in estuarine bodies could be one factor responsible for the growth of bacteria and their subsequent pathogenicity (12). The ability of isolated bacteria to grow on Urea, Nitrates and organic phosphate suggests the strong possibility of these bacteria to survive on fertilizer run off from agricultural fields (Table 2).

Among the antibiotics tested it was observed that tetracyclin in 500 ug concentration was most effective in controlling all the test isolates, followed by streptomycin and chloramphenicol, indicating that in aquaculture conditions. Tetracyclins could be effectively used to control that disease.

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