Studies on *Aeromonas* infection in Indian major carp viz. Catla (*Catla catla*), Rohu (*Labeo rohita*) and Common carp (*Cyprinus carpio)*

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ABSTRACT

Aeromonas spp. were isolated and identified from a healthy and diseased Indian major carp viz. Catla (*Catla catla*), Rohu (*Labeo rohita*) and Common carp (*Cyprinus carpio)* collected from Latur city, India. *Aeromonas* spp. is Gram negative bacteria which are pathogenic to fish, amphibians and also humans basing on its biochemical properties, it was categorized into A1, A2 and A3 respectively. A1 showed positive reaction to lysine decarboxylase where as A2 showed negative to esculin hydrolysis. All the strain showed negative reaction to malonate where as other two showed positive reaction. The present study clearly indicates that this organism has a wide spread of spectrum to spread disease in Indian major and common carps

Keywords: *Aeromonas*, *Catla catla*, *Labeo rohita* and *Cyprinus carpio.*

I .INTRODUCTION

Fish represent the cheapest source of animal protein. It is one of the major food components of humans for many centuries and still constitutes an important part of the diet of many countries. The benefit of fish as a food resulted from its easy digestibility and high nutritional value. Fishes are found in different waters. Some are found in fresh water while some are found in salt water (sea and oceans). However, the type of microorganism found associated with a particular fish depends on the water it was found (26, 4). Freshly harvested aquaculture products, predominantly those from tropical regions may harbour pathogenic bacteria, which form part of natural micro-flora of fish ponds. Disease may occur systemically or be confined to external surfaces such as the skin or gills. In many instances, the pathogenic bacteria are ubiquitous in the environment, or may form part of the normal internal bacterial flora of an aquatic animal.

Kvenberg (13) and Rodricks (21) classified the bacteria pathogens associated with fish into two: the non-indigenous bacteria pathogen and the indigenous bacteria pathogens. The indigenous bacteria pathogens are those naturally living in the fish's habitat. They are the *Vibrio* species, *Aeromonas* species etc.

In India, *Aeromonas* spp. are common contaminants in fish, a variety of raw meat, milk and milk products, and other raw foods (25, 19) .*Aeromonas* is an opportunistic and zoonotically important bacteria belonging to the family *Aeromonadaceae*. Severalspecies, such as *Aeromonas hydrophila*, *A. bestiarum*, *A. sorbia*, *A.veronii*, *A. salmonicida*, *A. jandaei*, and *A. allosaccharophila*, have been known to be associated with several diseases, in both warm and cold blooded animals as a result of their virulence and pathogenicity (7). In humans they cause opportunistic infections and gastroenteritis, chronic diarrhea, wound infections, respiratory tract infections, peritonitis, urinary tract infections and septicemia (9, 1). The genus *Aeromonas* comprises of several species of gram-negative, rod shaped, motile and non-motile, oxidase and catalase positive, nitrate to nitrite reducing, glucose fermenting bacteria (3). In outlook of the various ways fishes could be contaminated with microorganisms, the present research was therefore aimed to study *Aeromonas* infection in Indian major carp Catla (*Catla catla*), Rohu (*Labeo rohita*) and Common carp (*Cyprinus carpio).*

II.MATERIAL AND METHODS

Sample preparation:-

With reference to the method described by Obi and Krakowiaka (18), 10 g of the fish sample was incise from the head, middle and tail regions with a sterile knife. The incise samples were crushed into small

pieces with about 10 ml sterile water. From the crushed sample, 1 ml aliquot volume was measured out and homogenized with 9 ml of distilled water giving a 1:10 dilution.

Medium preparation

The samples were cultured by streaking on three kinds of culture media: R-S agar (23), defibrinated sheep blood agar plates (Hi media, *Ltd*) and MacConkeys agar (Hi media, *Ltd*) plates. The cultured media were incubated at 25ºC and 37ºC for 24-48 hr aerobically. After incubation, the suspected colonies to be as motile aeromonads (i.e.: yellow and haemolytic colonies) were isolated. The identification of bacterial isolates as motile aeromonads was based on the colony morphology, Gram-staining, motility, produce of haemolysins, oxidase production, and glucose fermentation (Hi media, *Ltd*), as well as other biochemical tests (17,2).

Growth of bacteria if any was observed after 24-48 hours by noting the turbidity in the broth. Primary cultures were made from the turbid broth by streaking in nutrient agar plates. The colony characters such as shape, size, and color were for slant culture. The agar slants were incubated at 30ºC for 24-48 hours. Isolates in nutrient agar slants were maintained in the laboratory at 4ºC throughout the study period.

Preliminary identification was made on the basis of the colony characteristics. In addition to this, gram staining and cytochrome oxidase tests were done along with comparing the responses to various biochemical tests. On the basis of the various biochemical properties, different strains were demarked with different code numbers.

III. RESULTS

Gross Pathology:

Catla catla weighing about 250–350g and of length 20–30 cm showed severe ulceration with hemorrhagic spots around pelvic fin and snout. Scales appeared raised at the inflamed parts on the lateral side of the body. The entire region was disintegrated and severe ulcers were noticed exposing the muscles, which appeared lacerated. The ulcerative areas varied in sizes from 3 to 8 mm in diameter and 2 mm deep with grey slimy deposits.

Labeo rohita weighing around 350 – 400g and of length about 25 – 30 cm showed eye lesions characterized by periorbital oedema and corneal opacity. Clinically there was bilateral exophthamia with ground glass like appearance of the eyeballs. On necropsy on others lesions could be seen in any other organs.

Cyprinus carpio Weighing about 300 – 400g and of length 20 – 25 cm displayed scale-sac edema, ascites, exophthalmus and skin ulcers. Infected fish showed skin lesions, expanded scale sacs and an odematous dermis accompanied by haemorrhages and necrosis. Interestingly the underlined lateral line musculature became odematous and showed atrophied muscle fibres. Infected kidneys lost their texture. hemorrhagic spots at the base of the dorsal fin. There were whitish nodular cysts mainly confirmed to tail region. All the internal organs were highly hemorrhagic with marked congestion of the blood vessel.

Isolation and identification:

A. hydrophila was isolated from skin, body fluid, kidney and liver of *Catla catla*. Eye, heart and skin lesions of marginal showed presence of bacterial organisms (Table 1). Some other bacterial pathogens, which were isolated from the diseased fishes, have not been taken into account in the present study.

Table 1 . Species source of diseased fish and organs from which isolations were made

Sample collected	Strain No.	Fish	Organs
Kava Lake	A1	*Catla catla* *Labeo rohita* *Cyprinus carpio*	Skin ,Liver Eye Skin
Kava Lake	A2	*Catla catla* *Labeo rohita* *Cyprinus carpio*	Skin ,Body fluid Skin Skin
Kava Lake	A3	*Cyprinus carpio*	Skin

Morphology:

The colonies of *Aeromonas* A1 and A2, which were isolated appeared round convex and flattened in all solid media. The colonies were semitransluscent in nature . in *Aeromonas* isolation medium it appeared pinhead sized round and golden yellow in colour and were gram negative, coccobacilli, measuring about 2-2.5 times longer than width (0.3-0.9 µm in diameter and 1.0-3.2 µm in length) while the colony of *Aeromonas* A3 was gram negative in nature , small rods to cocco bacillus(1-2 mm). The bacterial isolates obtained from different organs of various affected species were subjected to identification using enterobacteriaceae kit, cytochrome oxidase test and biochemical characteristics. The detailed results are represented in Table 2

Table 2: Identification of *Aeromonas* using Biochemical test (Manually and Enterobacteriaceae Kit)

Biochemical test	A1	A2	A3
Morphology			
Flagella	+	+	+
Mortality	+	+	+
Capsule formation	+	+	+
Physiology			
Growth in Methyl Red	-	-	-
Growth in Voges Proskauer	+	+	+
Catalase Production	+	+	+
Growth in 2.5% NaCl	+	+	+
Growth in 6.5% NaCl	-	-	-
Carbohydrate Metabolism			
Malonate	+	+	-
Esculin Hydrolysis	+	-	+
Salicine	-	-	-
D-sorbitol	-	-	-
D-dulcitol	-	-	-
D -Mannitol	+	+	+
D-Sucrose	+	+	+

D-Maltose	+	+	+
Sorbose	+	+	+
D-Mannose	+	+	+
D-Lactose	-	-	-
L-Rhamnose	-	-	-
D-Xylose	-	-	-
L-arabinose	+	+	+
Glucose	+	+	+
Sucrose	+	+	+
Metabolism in Nitrogenous Compound			
Urease	-	-	-
Lysine decarboxylase	+	-	-
Ornithine decarboxylase	-	-	-
Hydrogen Sulphide	-	-	-
Starch Hydrolysis	+	+	+
Inositol	-	-	-
Adonitol	-	-	-
Nitrate Reduction	+	+	+
Citrate Utilization	+	+	+
0/129 vibriostat	**R**	**R**	**R**
Tween 80 esterase	+	+	+
ONPG	+	+	+
DNAse Hydrolysis	+	+	+
Gelatin	+	+	+
Indole	+	+	+
Gram Stain	-	-	-
Cytochrome oxidase	+	+	+

IV. DISCUSSION:

The determination of bacterial contamination of fresh fish (*Cyprinus carpio*) carried out in this study was necessary in safeguarding public health. This is because fresh fish which is contaminated with faecal material before or during harvest, may cause outbreak of intestinal infectious disease such as typhoid fever. The bacteria isolation assay result revealed the presence of commensal bacterial of viz: *Aeromonas*, *Flavobacterium* (Flexibacter), *Pseudomonas* . In view of the fact that these microorganisms could contaminate fish and therefore a source of food poisoning; harvesting, handling and cooking of fish especially (*Cyprinus carpio*) should be done properly so as to reduce the bacterial load. In this case, eating of *Cyprinus carpio* raw or half-boiled should be discouraged to eliminate zoonotic infections from fish . This study therefore is intended to provide basic information about these microorganisms likely to cause food-borne disease.

The isolation study revealed presence of *A. hydrophila* in skin, body fluid, heart, eye, kidney and liver of various species of fish collected from different geographical location. The high percentage incidence of *A. hydrophila* in diseased fishes strongly suggested the omnipresent nature of the bacterium. Similar observations were recorded by Llobrera and Gacuttan, (14) ; Sharif *et al*., (22); Kumar, (12); Karunasagar *et al*., (11); Mukherjee, (16); and Nayak *et al*. (10). Das (6) isolated the same bacteria from lesions of ulcers and other visceral organs of different species of fish examined in the Orissa state. The bacterium is frequently associated with infections as secondary invader as reported by Roberts (20) and Tonguthai (27) and has been branded as a facultative pathogen. It is thought that this organism invade only when the host resistance is lowered by environmental stress factors such as high organic load,

overcrowding and sub lethal oxygen levels in water (15, 24). Groberg *et al*., (8) demonstrated a positive correlation between water temperature and probability of mortality in steelhead trout (*Salmo gairdneri*) inoculated with low numbers of *A. hydrophila*. They concluded that higher temperature favored rapid proliferation of the bacteria and subsequent host mortality. In the present study the isolation of *A. hydrophila* was aptly done from the ulcerated skin lesion, kidney, liver, body fluid, eye and heart of catla, rohu and mrigal showing various clinical manifestations. However, no correlations could be made with the environmental temperature as the disease occurred throughout the year with varying atmospheric temperature.

Media employed for isolation of *A. hydrophila* were reviewed (5) and choice of Rimler-Shotts agar (23) was made in the initial phase of the study. The balance of ingredients in this medium provided a nutrient base and chemophysical stability. Shotts and Rimler (23) remarked that the yellow colonies indicated the presence of *A. hydrophila*. However it was also pointed out that all the yellow colonies having black centers should be subsequently tested for oxidase activities to eliminate the possibilities of *Citrobacter* sp. and / or other organisms. Similar methods were followed in the present study, which proved to be useful.

Until now the role of *A. hydrophila* in various fish diseases was not very clear. The present study has elucidated the role of this organism in dropsy, fin and tail rot and more elaborately in Epizootic Ulcerative Syndrome (EUS). It is beyond doubt that in the ulcerative disease of fish the organism may play an important role not only in necrotizing the muscle tissue but also damaging the internal organ like kidney, liver and spleen besides the peritoneum. It is on this basis that prophylactic measures could be formulated in order to contain various diseases caused by this organism.

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