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Research Article

Pseudomonas fluorescens associated with Bacterial Disease in Catla catla in Marathwada Region of Maharashtra

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ABSTRACT:

In the present study a detailed analysis was carried out to evaluate the association of various bacterial pathogens with *Catla catla* from Marathwada region of Maharashtra. The freshwater fishes were collected from different water bodies and fish culturing centre of eight districts of Marathwada region viz. Aurangabad, Jalna, Parbhani, Nanded, Hingoli, Latur, Beed and Osmanabad. The analysis could yield thirteen pathogenic bacteria from the fish samples that included *Micrococcus sp., Bacillus sp., Lactobacillus sp., Vibrio sp., Aeromonas sp., Streptococcussp., Flavobacterium sp., Vibrio sp., Proteus sp., Staphylococcus sp., Enterobacteria sp., E.coli , Pseudomonas sp.* The bacterial strains were identified based on colony morphology, cell morphology and biochemical chemical characters. The dominant bacterial pathogen was *Pseudomonas* sp. The *Pseudomonas* sp associated with *Catla catla* could survive on host as well as in water. *Pseudomonas fluorescens* was very sensitive to Kanamycin, Nalidixic acid, Gentamicin and Neomycin, less sensitive to tetracycline, amikacin and Chlorophenicol and very less sensitive to Oxytetracycline, Erythromycin and Penicillin.

Keywords: *Catla catla, Pseudomonas fluorescens, Marathwada.*

[I] INTRODUCTION

India is one of the best producers among the world freshwater fish producers with Indian major carp's viz. *catla, rohu, mrigal* and *pangas* is being the most preferred species [1]. *Catla catla* is one of the widely cultivated carp in all the states of India. It is preferred over other carp owing to the high consumer preference and good growth rate [2]. Bacteria are one of an important causative agent of fish diseases in both wild and cultured fish and are responsible for serious economic losses. Many pathogens are present as skin infections especially pseudomonads, aeromonads, vibrios etc. The bacteria of the genera *Pseudomonas* are ubiquitous facultative parasites. These genera belong to the normal bacterial flora of aquaria, the hatcheries, fish farms and bodies of water for domestic use. These bacteria show the appearance of the colonization of in the skin fins, gills and intestinal lumen of fish. These fish pathogenic bacteria can

survive in the environment or are associated with normal fish.

Pseudomonas fluorescens is a dominant component of freshwater ecosystem [3] *P. fluorescens* has been associated with septicemia and ulcerative conditions in wide range of fishes. It has been considered as a fish spoilage organism [4] as well as a primary, but poor pathogen [5]. *P. fluorescens* is normally found in water, soil and on the body of fishes.

These infections are often prominent due to stress conditions that alter the natural defenses. Infections in human caused by these bacterial pathogens transmitted through fish or the aquatic environment are common and associated with the various factors of fishes and human. Such infections are often by facultative bacteria that are pathogenic to both fish and human beings. These may be isolated from fish with or without apparent symptoms of the disease [6].

In Marathwada no systematic research on bacterial disease in fish has been carried out. Therefore the study of aquatic bacteria associated with fish is very limited in Marathwada region of Maharashtra. Our earlier work was a sincere attempt to assess the bacterial population in aquatic environment and their involvement in causing diseases in fish. Darak and Barde [7] reported *Aeromonas* sp. and *Pseudomonas* sp. are very common bacteria associated with major carp and live fishes. The aim of the present study is to identify the common bacterial pathogen associated with freshwater fish *Catla catla* and characterize the pathogen in detail with their susceptibility to antibiotics for developing a control measure in future.

[II] MATERIALS AND METHODS

2.1 Sample collection

The fish *Catla catla* were collected from various sources like fish farms, seed farms, cold storage and market. The fish samples were brought from all the districts of Marathwada, Maharashtra. The fish samples showed visible disease symptoms on the surface or other body parts. After collection fish samples were transported to the laboratory for further studies and were stored at low temperature for further studies.

2.2 Determination of total viable count and isolation of bacteria from infected fish

The fish samples with infected portions having visible symptoms were washed thoroughly with sterile distilled water. The infected fish samples were dissected and from the infected muscles, gills and liver, the pathogenic strains were isolated with help of sterile swab and swab was suspended in saline solution (0.1 % NaCl). Serial dilutions were made using saline solution, 10^{-5}, 10^{-6} and 10^{-7} were plated on nutrient agar (Hi media) and spread over the nutrient agar plates. The plates were incubated at 37°C for 24-48 hrs, after incubation. The total viable count as colony forming units (cfu) for each ml for each dilution was determined and average of number of colonies were recorded.

2.3 Identification of bacterial isolates based on morphological and biochemical characteristics

The morphologically different bacterial strains were identified based on their growth on solid medium in plates. The isolated colonies were streaked on nutrient agar slants, incubated overnight at 37°C. Biochemical tests were performed for identification of selected colonies. The morphological characteristics of bacteria isolated from diseased fish samples were done following the method of Collins *et al*., [8] Cappuccino and Sherman [9]. The cell as well as colony morphology were recorded. The colony characteristics size, shape, colour, margin, elevation, consistency and opacity were recorded. Biochemical characterization was carried out as per Bergey's manual and was identified according to Bergey's Manual of Systematic Bacteriology [10].

2.4 Antibiotic sensitivity Studies

The bacteria were tested for antibiotic sensitivity test by using Disc –Diffusions technique [11].

[III] RESULTS & DISCUSSION

Marathwada lies at an altitude of 300-650 m above mean Sea level gradually inclined from west to east and have adjoining region of Vidarbha to the North, Telangana to the East and South East, Karnataka to the South and Western Maharashtra on the West region with an area of 64,813 km. It comprises eight districts viz. Aurangabad, Jalna, Parbhani, Nanded, Hingoli, Latur, Beed and Osmanabad. The fishes are cultured and harevested throught the year and in all seasons but most in October while least is during rainy season. The increase in fish catch during the summer can be attributed to loss of water due evaporation. The stress induced due to human activities, water diversion, changes in method of water and land utilization had contributed to various impacts on fish population [12].

3.1 Total Viable Count from the samples collected:

The total viable count of bacteria (cfu/ml) was high from the fish samples and high viable count was attributed to swabs isolation from body surface of fish carrying significant population of pathogenic and non-pathogenic bacteria. The highest viable count of bacteria was observed from the Parbhani samples whereas lowest viable count was found in the sample from Aurangabad (Table 1). The high total viable count (cfu) of bacteria in Parbhani samples may be attributed to contamination of aquatic environment from domestic waste and sewage from city.

3.2 Identification and Characterization of the bacterial isolates

Bacterial colonies that were different in appearance were selected for further morphological and biochemical characterization. The different colonies were accounted to different thirteen types of colonies. These thirteen bacterial isolates were selected for characterization and identification. The cell and colony morphology, gram character, and biochemical characteristics of these thirteen were carried as per Bergey's Manual of Systematic bacteriology [10]. The isolates were Gram -ve rods, Gram +ve cocii and rods (Table 2). The biochemical characteristic leads to identification of isolates as *Micrococcus sp., Bacillus sp., Lactobacillus sp., Vibrio sp., Aeromonas sp., Streptococcussp., Flavobacterium sp., Vibrio sp., Proteus sp., Staphylococcus sp., Enterobacteria sp., E.coli , Pseudomonas sp.* The isolates in this study are commonly known fish and human pathogens that have been characterized up to species level [13, 14].

The most common and dominant bacterial pathogen was *Psuedomonas fluorescens*. *Psuedomonas fluorescens* was found to be associated with various freshwater and marine fishes. So this bacterial pathogen was further studied and described in detailed..

3.3 Characteristics of the Diseases

P. fluorescens is a ubiquitous bacterium found in soil, air and water. It is one of the most dominant components of the freshwater ecosystem [15]. *P. fluorescens* has been reported as a spoilage organism and a common contaminant or secondary pathogen of damaged fish tissues [16] . This organism is reported to be a primary but poor pathogen [17]. It has been reported to cause disease in a wide range of fish species and has been described [18]. *P. fluorescens* is associated with fin or tail rot in which the infected area is worn out. Elevated death rate up to 40 % of the total population was recorded with the infestation and infection of this pathogen. The visible symptoms included haemorrhagic skin lesions and at fins base. The typical generalised symptoms of bacterial septicaemia that included accumulation of Ascitic fluid in the peritoneal cavity and gills, kidney, liver with petechial haemorrhages [19]

3.4 Isolation of pathogen from infected tissue

P. fluorescens was isolated from gills, kidney, liver and most of the organs as pure culture growth on Pseudomonas F agar, blood agar, TSA

and nutrient agar with the incubation at 22–28 °C for 24–28 h.

3.5 Characteristics of the Pathogen Disease

The culture was further subjected to various determinative and diagnostic biochemical test. The result showed that the bacteria is Gram-negative, oxidative, catalase, gelatinase and oxidase producing rods. It is motile by polar flagella capable of growing in the range of 15°C but not at 40°C. The bacteria produced Fluorescent pigment (fluorescein) and but did not produced ß-galactosidase, H_2S, indole, amylase or urease. The Voges Proskauer reaction is negative. *P. fluorescens* possess the ability to utilise a wide range of compounds including D-alanine. Citrate is utilized, and acid is produced from arabinose, inositol, maltose, mannitol, sorbitol, sucrose, trehalose and xylose [20].

3.6 Control by Antimicrobial Compounds

The antibiotic namely, Kanamycin, Nalidixic acid, Tetracycline, Gentamycin, Neomycin, Amikacin, Oxytetracycline, Chloramphenicol, Erythromycin and Penicillin were tested *in vitro* against *P. fluorescens*. It was observed that the bacteria are very sensitive to Kanamycin, Nalidixic acid, Gentamicin and Neomycin, less sensitive to tetracycline, amikacin and Chlorophenicol and very less sensitive to Oxytetracycline, Erythromycin and Penicillin (Table 3)

P. fluorescens showed susceptibility to kanamycin, nalidixic acid gentamicin, neomycin kanamycin and tetracycline [21]. In another study *P. fluorescens* was susceptibile to gentamicin, kanamycin and neomycin, less to amikacin and oxytetracycline, and total resistance to chloramphenicol, erythromycin, penicillin and potentiated sulphonamides [22].

[V] CONCLUSION

P. fluorescens is ubiquitous facultative parasites and is potential pathogen posing a serious threat to freshwater aquaculture and fish industry. The visible symptoms included haemorrhagic skin lesions and at fins base. The typical generalized symptoms of bacterial septicaemia included accumulation of Ascitic fluid in the peritoneal cavity and gills, kidney, liver with petechial haemorrhages. *P. fluorescens* are very sensitive to Kanamycin, Nalidixic acid, Gentamicin and Neomycin, less sensitive to tetracycline, amikacin and Chlorophenicol and very less sensitive to Oxytetracycline, Erythromycin and Penicillin.

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Table 1 Total viable count of bacteria from infected fish samples.

Samples	Colony forming units/ ml		
	cfu x 10^{-5}	cfu x10^{-6}	cfu x10^{-7}
Aurangabad	60.3	57.6	42.3
Jalna	134	116	97.3
Parbhani	124	105	65.3
Hingoli	75.3	59	47.6
Nanded	68	42	27.6
Nanded2 (Kinwat)	66.7	47.3	25.3
Latur	98.3	67.6	28.3
Beed	73.6	53.3	33.3
Osmanabad	103.3	73.3	46.6

Table 2 Colony morphology, gram characters and Biochemical characteristics of selected bacterial isolates.

	1	2	3	4	5	6	7	8	9	10	11	12	13
Gram	+ve	+ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
Shape	cocci	rods	rods	rods	rods	cocci	rods	rods	rods	cocci	rods	rods	rods
Size (mm)	1	2	2	1-2	2	1	1-2	2-4	1-2	1-2	1-2	1-2	1-2
Color	white	white	white	pale	yellow	white	yellow	pale	pale	pale	pale	pale	pale
margin	irregular	irregular	irregular	circular	circular	cicular	circular	circular	circular	circular	circular	circular	circular
Elevation	raised	dull	dull	raised	flat	convex	raised	raised	raised	raised	raised	raised	raised
opacity	opaque	opaque	opaque	opaque	transparent	opaque	opaque	opaque	transparent	opaque	opaque	opaque	opaque
lustre	shiny	umbonate	umbonate	shiny	shiny	smooth	shiny	shiny	shiny	shiny	dull	dull	smooth
Edge	entire	rhizoidal	rhizoidal	entire	entire	entire	entire	entire	entire	entire	entire	entire	entire
Consistency	viscous	butyrous	butyrous	viscous	butyrous	viscous	viscous	viscous	butyrous	viscous	butyrous	butyrous	butyrous
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	-	-	-	+	-	+	+	+
Ammonia	-	+	-	+	-	-	-	+	-	-	-	-	+
M.R.	+	-	+	+	-	+	-	-	-	-	-	-	+
V.P.	-	-	-	-	+	-	+	+	+	+	+	+	-
Indole	-	-	+	-	-	-	-	-	-	-	+	+	-
Urease	-	-	-	+	-	-	-	+	+	+	-	-	-
Citrate	+	+	+	+	-	-	+	+	+	+	+	+	-
Gelatinase	+	+	+	+	+	+	+	+	+	-	+	+	+
Deamination	D	-	-	D	+	-	-	+	+	-	-	-	-
Starch	-	+	+	+	+	-	-	-	-	-	-	-	-
Esculin	+	+	+	+	-	+	-	-	-	+	+	+	-
Casein	-	-	+	+	+	-	D	+	+	+	-	-	-
Lipase	+	+	+	+	+	+	+	+	+	+	-	-	-
Nitrate Red	+	+	+	+	-	-	-	+	+	-	-	-	-
H_2S	-	-	-	-	-	-	-	+	+	-	-	-	-
T S I	-	+	+	+	+	-	-	-	+	+	+	+	-
OF	-	-	+	-	-	+	-	+	+	-	+	+	+
TCBS Agar	++	+	+	+	+	+	-	-	-	+	-	-	-
MacConkey's Agar	+	-	+	+	+	+	-	+	+	-	-	-	-
SS Agar	+	-	-	-	-	-	-	-	-	-	-	-	-
Pigmentation	-	-	-	-	+	-	-	+	+	+	-	-	+
Genus identification	*Micro coccus sp.,*	*Bacillus sp.,*	*Lacto bacillus sp.,*	*Vibrio sp.,*	*Aeromonas sp.,*	*Strepto coccus sp.,*	*Flavo bacterium sp.,*	*Vibrio sp.,*	*Proteus sp.,*	*Staphylo coccus sp.,*	*Entero bacteria sp.,*	*E.coli ,*	*Pseudomonas sp,*

Table 3: Antibiogram test on *Pseudomonas fluorescens* isolated from diseased fishes

S. No.	Antibiotic	*Zone of inhibition(mm)*
1	Kanamycin(30µg)	25
2	Nalidixic acid(30µg)	21
3	Tetracycline(30µg)	18
4	Gentamycin(30µg)	26
5	Neomycin(30µg)	22
6	Amikacin(30µg)	11
7	Oxytetracycline(30µg)	10
8	Chloramphenicol(30µg)	15
9	Erythromycin(30µg)	09
10	Pencillin(30µg)	08