

A STUDY ON ISOLATION AND
CHARACTERISATION OF BACTERIAL
PATHOGENS PRESENT IN GUT OF FISH *PUNTIUS*
TICTO, COLLECTED FROM SATAPADA AREA OF
CHILIKA LAKE

A

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MASTER OF SCIENCE IN ZOOLOGY

By

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CERTIFICATE-I

This is to certify that the thesis entitled “**A study on isolation and characterisation of bacterial pathogens present in gut of fish *Puntius ticto*, collected from Satapada area of Chilika lake**” submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science (Zoology)** to the Odisha University of Agriculture and Technology is a faithful record of bonafied and original research work carried out by **Miss Monalisa Sarkar** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by her from various source during the course of investigation has been duly acknowledged.

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CERTIFICATE- II

This is to certify that the thesis entitled “**A study on isolation and characterisation of bacterial pathogens present in gut of fish *Puntius ticto*, collected from Satapada area of Chilika lake**” submitted by **Miss Monalisa Sarkar** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of **Master of Science(Zoology)** has been approved by the student’s advisory committee and the external examiner.

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(Monalisa Sarkar)

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LIST OF ABBREVIATIONS

et al.	et alibi (and others)
%	Percentage
NB	Nutrient Broth
NA	Nutrient Agar
EMB	Eosin Methylene Blue
SS	Salmonella Shigella
KSA	Klebseilla Selective Agar
TCBS	Thiosulfate Citrate Bile Salts Sucrose
H ₂ O ₂	Hydrogen Peroxide
TSI	Triple Sugar Iron
S	Sensitive
R	Resistance
I	Intermediate
°C	degree Celsius
gm	gram
ml	millilitre
ABST	Antibiotic sensitivity test
TPC	Total plate count
MPN	Most probable number

EC	Electrical conductivity
TDS	Total Dissolve Solids
ppt	Parts per thousand
ppm	Parts per million
μS	micro Simen
cfu	Colony forming unit
AMC	Amoxyclav
GEN	Gentamycin
CIP	Ciprofloxacin
CL	Colistin
AZM	Azithromycin
AK	Amikacin
AMX	Amoxicillin
AMP	Ampicillin
PB	Polymyxin-B
B	Bacitracin
N	Neomycin
CTR	Ceftriaxone
PIT	Piperacillin/Tazol
CN	Cefalexin

ABSTRACT

Fishes are a good source of nutrients and are preferably consumed worldwide. Presence of pathogens in them can affect human health. A total of 5 live specimens of fish, *Puntius ticto*, collected from Satapada area of Chilika Lake were aseptically dissected and their gut contents were used for the study of isolation and characterisation of pathogenic bacteria. All the collected samples were streaked in different enriched media like EMB, TCBS, SS and KSA for the study of the type of bacteria. Gram staining and urease, catalase, oxidase, citrate, indole, mannitol, motility and TSI (Glucose, Lactose, Sucrose, H₂S, Gas) biochemical tests were performed for characterisation of the bacteria. This test noticed the prevalence of bacterial suspects like *Salmonella*, *Vibrio* and *E. coli*. The antibiogram of the isolated bacteria indicated higher sensitivity to Colistin, Ciprofloxacin, Piperacillin/Tazol, Amikacin and Neomycin and higher resistance to Bacitracin. Piperacillin/Tazol had wider inhibition zone, which indicated its highest effectiveness. Microbiological test of the fish inhabiting water indicated greater microbial load. It was inferred that the surrounding water may be a source of contamination of fishes. So, proper handling care should be taken from farm to fork.

CHAPTER-1

INTRODUCTION

Bacteria are found in every conceivable environment like the air we breathe, the food we eat, the water we drink and the dresses we wear. Fishes are excellent sources of nutrients in the terms of protein and calorie (Ravichandran *et al.*, 2012). However, the nutritional value is perishable due to susceptibility to microbial spoilage. Fishes harbour high microbial load on the surface of the skin, in the intestine and in the gills. A quantum number of opportunistic and pathogenic bacteria has been isolated and described by many workers.

Intestinal micro-biota plays a crucial role in the life of many organisms as it renders active role in nutrient assimilation, growth and health (Brugman *et al.*, 2010).

Besides this, gastrointestinal micro-biota takes an active part in several physiological functions of hosts viz. Digestion, development of mucosal system, angiogenesis and protection against diseases. Besides above, the total heterotrophic population also renders probiotic role and augments the digestive ability by producing microbial enzymes.

Fish gut houses about 10^7 to 10^{11} bacteria per gm of intestinal content (Nayak *et al.*, 2010).

The present study is designed to study bacterial population harbouring in the gastrointestinal tract of fish.

There are likely to have three fates of bacteria coming in contact with fish (Austin *et al.*, 2006):

- The microorganism become closely associated with and/or colonizes the external surface of the fish. Even they can get accumulated at the site of damage viz. missing scales or abrasions.
- The microbes may enter into the mouth with water or food and colonize the digestive tract.
- The microorganisms coming in contact with fish surfaces may be competed out by the resident micro-flora or by natural inhibitory compounds present on or in the fish.

The quality of fish inhabiting water influences its gut micro-flora. The quality of water can be determined by a number of indicators (viz. physical, chemical and biological). Coli-titre and coli-index are two quantitative biological indicators of faecal contamination of water. Presence of *Escherichia coli* is an indicator of faecal contamination of water, soil, food products and other objects of the external environment. Coli-titre is the smallest amount of material studied in millimetres in which one *E. coli* is found. Coli-index is the number of *E.*

coli found in one litre of the material under study. Though, *E. coli* are good gut bacteria but their prevalence often ensures presence of other pathogenic bacteria in the water.

The fish digestive tract confers a favourable culture environment for microorganisms. Hence, number of beneficial bacteria increases in the intestine dramatically. Low pH range in fish GI tract poses lethal effect on beneficial probiotics in the gut (Sullam *et al.*, 2012; Ringo *et al.*, 2016).

Gastrointestinal micro biota has maximal threat to the consumers owing to their zoonotic potential as they can successively surpass any processing method that the sellers usually adopt as precautionary measures.

Some of the dominant intestinal micro-biota in freshwater fishes are *Salmonella*, *Shigella*, *Yersenia*, *Aeromonas*, *Pseudomonas*, *Clostridium*, *Micrococcus* and *Acinetobacter* etc. (Austin *et al.*, 2006; Gómez *et al.*, 2008).

In the contrary, the intestinal micro-biota of marine fishes harbour dominantly facultative anaerobes like *Vibrio*, *Pseudomonas*, *Acinetobacter*, *Corynebacterium*, *Alteromonas*, *Flavobacterium* and *Micrococcus* (Onarheim *et al.*, 1994).

The present study is made on a particular fresh water fish that belongs to the genus *Puntius*. Live samples collected from Satapada fish Jetty located along the coast of Lake Chilika, Khordha, Odisha have been used for the study.

Puntius is a small fresh water fish of family Cyprinidae, which is native to South Asia and Mainland Southeast Asia. It is a delicacy in most parts of India and is known under several trivial names across different parts of the country such as “Karandi” in Odisha, “Pungti” in West Bengal etc. These fish reach to an utmost size of 5 to 15 cm and resemble the appearance of miniature carp. Chilika is the largest brackish water lagoon and harbours mostly 3 to 4 species of this genus viz. *P. chola*, *P. sophore*, *P. ticto*. *Puntius*, owing to its abundance, cheap price and high protein content, regarded as a delicacy in Odisha.

One of the cost effective post harvesting methods of preservation includes freezing at < 10° Celsius. This can retain the fish edible quality up to circa 40 hours. After that fish begins to spoil due to its autolytic activities and chemical changes. Then degradation of fish is geared up by the microorganisms. Cadaveric remains of fish are further spoilt by the microorganisms which harboured the surface, gills and gut, while it was alive. These

microorganisms begin to utilize the fish protein and food nutrients resulting in loss of nutritional value thereby imparting undesirable changes like off-flavours, texture and appearance.

Hence, microbial association with fish compromises with the safety and quality for human consumption, particularly critical is when microorganisms are of opportunistic and/or pathogenic.

The present study emphasises upon the following objectives:

- To study the physico-chemical characteristics and microbial load of fish inhabiting water.
- Collection of aseptically dissected fish guts contents.
- Isolation and culture of pathogenic bacteria by using different selective media.
- Morpho-physiological characterization of the bacteria through various biochemical tests and Gram staining technique.
- Antibigram profiling to check the susceptibility of bacteria to antibiotics.

CHAPTER-2

REVIEW OF LITERATURE

2.1 PHYSICO-CHEMICAL CHARACTERISTICS OF FISH INHABITING WATER AND ITS MICROBIAL LOAD

Ratto *et al.* (1989) carried out an experiment by taking twenty samples of drinking water from five different distribution line sources in Lima, Peru using the techniques like Presence/Absence (P/A) test, H₂S paper strip test and total coliform and faecal coliform MPN tests. They reported the incidence of coli phage in these water sources that further speculated the survivability of human pathogenic virus. (A. Ratto “portable water safety assessed by coli phage and bacterial test” water research vol.23 issue2 feb 1989 pg. 253-255)

Noble *et al.* (2003) studied the recreational quality of water in California beaches by using three bacterial indicator tests : TC (Total Coliform), FC (Faecal Coliform) and EC (Enterococci Coliform) in different weather conditions on a comparative analysis. They reported that the concentration of indicator bacteria in water was not necessarily pathogenic but ensured prevalence of many pathogenic organisms that could grow in similar prevailing conditions. They concluded that *Enterococcus* was the most suitable bacterial indicator regardless of various weather conditions. EC tests were conducted on a majority of samples following the protocol that included membrane filtration , multiple tube fermentation etc.

Rai *et al.* (2011) carried out a study on two severely polluted sites of river Ganga at Patna, Bihar by taking into account several physico-chemical parameters such as pH, Electrical conductivity (EC), Alkalinity, Total Solids (TS), Hardness, Chloride, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD) and Most probable number (MPN) during pre- and post-monsoon seasons in a year. They reported that one of the two selected sites was exceeding the safe limits, which indicated that river Ganga has been moderately polluted due to discharge of sewage effluents. (Arvind kumar Rai , Journal of advanced laboratory research in biology 2 (4), pg-136-140,2011)

2.1.1 EFFECT OF TEMPERATURE AND SALINITY ON THE ADHESION PROCESSES OF *VIBRIO SPECIES* TO FISH EPITHELIAL CELL

Bordas *et al.* (1996) reported that the capability of adhesion of pathogenic bacteria to the mucous layer of fish is considered as a bacterial virulence factor. Bacterial attachment to

external surfaces and tissues involving specialised structures “adhesions” is an essential step in the colonization of host tissues and subsequent occurrence of infection.

Westerdahl *et al.* (1999) showed antimicrobial property of the mucous layer of fish but later on, subsequent workers advocated that pathogenic bacteria used this external mucous layer as a nutrient medium also and facilitate the invasion of these pathogens into the host's body.

Laurencin *et al.* (2000) conducted experiment with *Vibrio anguillarum* infecting rainbow trout to demonstrate the effect of temperature and salinity on adhesion of fish pathogenic bacteria. He reported that minimal number of adhesions occurred at low temperatures of 4°C and 10% salinity while highest number of adhesions were observed at 22°C and 17% salinity.

Belas *et al.* (2002) supported this by illustrating the synthesis of lateral flagella of pathogens which was recorded to be maximal at temperature ranging from 15°C to 37°C and salinity ranging from 10 to 20 %.

2.1.2 EFFECT OF pH AND LOW TEMPERATURE ON GROWTH OF *SALMONELLA*

Due to varying composition of food or addition of preserving and flavouring agents, the pH of foods varies widely, which influences the growth of microbes. Dernby *et al.* (1921) reported growth of *S. typhosa* between pH 6.2 and 7.6, *S. Paratyphi* between pH 4.5 and 7.8 and *S. schottmuelleri* between pH 4.5 and 8.0.

Prescott *et al.* (1938) had earlier advocated no growth of *Salmonella* in food at temperature below 5°C. However, Matches *et al.* (1971) reported the growth of bacteria on fish fillets and 5 days old stale chicken at temperatures as low as 8°C. They stated that the composition of food serves as the medium for growth of bacteria and the growth is affected by a minimum temperature.

Matches *et al.* (1972) conducted experiments on combined effects of pH and temperature on growth of *Salmonella*. They reported growth of *Salmonella* over pH range 4.0 to 9.0 at low temperatures. Thereby, they concluded that food-poisoning *Salmonella* serotypes grow only over a narrow pH range at low temperature and food kept at

temperatures below 5°C may not support growth of *Salmonella* and hence, can be adopted as a method of preservation.

2.2 INSIGHTS INTO MICROFLORA IN FISH GASTROINTESTINAL TRACT

Most of the studies of GI micro-biota has been conducted on fishes like rainbow trout (*Oncorhynchus mykiss*) , common carp (*Cyprinus carpio*) , zebra fish (*Danio rerio*), Atlantic salmon (*Salmo salar*). However, literature on microbial aspects of *Puntius* is scanty.

2.2.1 COLONIZATION OF GI MICROBIOTA

Bell et al.(1971) showed major bacteria of healthy fish eggs includes *Cytophaga* , *Flavobacterium* and *Pseudomonas*.

Wang et al. (1993) have found that several sources contribute to the colonization of bacteria in the early development of fish such as eggs, larva rearing water and live feed.

Bergh et al. (1994) have conducted ELISA studies to reveal that the gut micro-biota of fish larva colonized after hatching during the early embryonic stages.

Eddy and Jones (2002) suggested that colonization of larval intestine follows a two step pattern; one during metamorphosis and the other during post larval stages.

Romero and Navarreta (2006) studied that stable colonies are formed after first feeding stages i.e. through the gills and its major components are acquired from environment while hatching.

Li et al. (2012); Navarrete et al. (2012); Bolnick et al. (2014) and Stephens et al. (2016) have discussed about the range of factors affecting micro-flora in the fish gut including host factors like genetic factor, gender, weight, age, immunity and intestinal motility. They also discussed about the impact of environmental factors like water, diet and antibiotics, and microbial factors like adhesion capacity, enzymes and metabolic activities.

Rawls et al. (2004) demonstrated in gnotobiotic zebra fish , the role of micro-biota in stimulating the rates of intestinal cell proliferation during normal development. The resident micro-organisms enhance the stability of β -catenin in intestinal epithelial cells and hence, promote cell proliferation.

Ray et al. (2012) overviewed that an extensive range of enzymes like amylase, cellulase, lipase, protease and phytase produced by intestinal micro-biota enhances the process of digestion.

Camp et al. (2012) demonstrated the effect of GI micro-biota on the fat storage mechanism with the zebra fish model. The expression of a circulating inhibitor of lipoprotein lipase called angiopoietin-like 4 (Angptl4) is suppressed in the intestinal epithelium upon colonization with micro-organisms.

2.2.2 INFLUENCE OF WATER

Yoshimizu et al. (1976) revealed that gut micro-biota of freshwater fishes are different from the marine fishes.

Navarrete et al. (2009) reported the prevalence of *Pseudomonas* in the gut of juvenile *Salmo salar* which was derived from the water influent.

Hagi et al. (2004) established the fact that water temperature and salinity are the two main factors affecting microorganisms in gut by comparing the intestinal lactic acid bacteria (LAB) in four different species of fishes.

Sullam et al. (2012) reviewed the work of Yoshimizu et al. (1976) by the intestinal micro-biota of fishes inhabiting estuarines appear to be similar to the freshwater fishes.

Wu et al. (2012) reported the resemblance of the intestinal micro-biota composition with that of in the culture water and sediment.

2.2.3 INFLUENCE OF DIET AND FEEDING HABIT

He et al. (2013) found out that bacterial population in herbivorous grass carp is more than the omnivorous gibel carp and black beam and carnivorous black carp under the same rearing environment.

Liu et al. (2016) reported the prevalence of cellulose-degrading bacteria *Clostridium* in the herbivores and protease –producing bacteria *Halomonas* in the carnivorous fishes.

Navarrete et al. (2009) reported that the bacterial composition of fish intestine is highest than other regions of gut. They came to this conclusion by analysing the bacterial composition of stomach, pyloric caeca and intestine from ten juvenile Atlantic salmon.

2.3 PREVALENCE AND PATHOGENESIS OF IMPORTANT BACTERIAL PATHOGENS IN FISHES

Ross *et al.* (1971) studied the prevalence of epizootic *Vibrio anguillarum* in a hatchery in Arizona in which rainbow trout were cultured in freshwater and fed on a pellet diet.

Rucker *et al.* (1971) studied another such outbreak in rainbow trout in freshwater hatchery in Washington where the infection persisted due to the use of marine fish in diet.

Rodgers *et al.* (1981) found that *V. alginolyticus* as a secondary invader of “red spot” disease caused by *V. anguillarum*. The source of secondary infection was found to be from surrounding waters when the temperature exceeded 16°C. *V. alginolyticus* can colonize human gut causing acute entero-colitis in individuals consuming contaminated fish. This pathogen is also known to cause skin infections like conjunctivitis and stump ulcer.

Knutton *et al.* (1987) investigated the presence of entero-pathogenic *E. coli* (EPEC) in the form of micro-colonies adhering to fish intestinal walls using Scanning electron microscope. They also stated that the entero-pathogenic *E. coli* causes alterations at the apical surface of infected enterocytes.

Hansen *et al.* (1990) studied on endocytosis of intact bacterial antigens and translocation of bacterial cells in the GI tract of fish larvae using microscopy. They reported that the endocytosis of intact bacterial antigens was restricted near the oesophagus of fish larvae. The uptake preference among bacterial species was in the order of *V. fischeri* > *V. salmonicida* > *Flavobacterium species*. The bacteria enter the digestive tract before the gut is developed and active feeding begins as the amount of the digestive enzymes is low during the developmental stage.

Magarinos *et al.* (1996) demonstrated the prevalence of strains of *Pasteurella piscidia* strongly adhered to the intestinal walls of sea bream, sea bass and turbot in numbers ranging from 10⁴ to 10⁵ bacteria per gram of intestine using Scanning electron microscope.

Venugopal *et al.* (2002) reported that fishery products are reservoirs of food borne pathogens like *Salmonella*, *Vibrio*, *Listeria*, *Escherichia coli* etc.

Ringo *et al.* (2003) conducted experiment with two fish pathogenic bacteria *V. salmonicida* and *V. anguillarum* showing their *in vivo* adherence to the intestinal epithelium of fish larvae and severe destruction of microvilli caused by them.

Aguirre *et al.* (2004) have reportedly found out the presence of *Vibrio anguillarum* in cultured and wild fishes, bivalves and crustaceans in salt and brackish water that can potentially cause haemorrhagic septicaemia, called vibriosis in human.

Senderovich *et al.* (2009) suggested the fishes as intermediate vectors of *Vibrio cholerae*, since they create a link in food chain between the copepods (crustaceans) and Chironomids (dipterans) that harbours *Vibrio* primarily and the migratory water birds. Migratory birds feeds on these infected copepods or carry them to other water bodies , thus spreading the pathogens across the continents

Selvaraj *et al.* (2010) advocated that presence of *Salmonella* in food stuffs as an indicator of their unsuitability for human consumption.

Prakash *et al.* (2011) found the prevalence of these pathogenic microbes in dried fishes and hence, inferred about its inedibility for human.

2.4 IMPACT OF ANTIBIOTIC TREATMENT ON HOST SUSCEPTIBILITY TO INFECTION

Barthel *et al.* (2003) demonstrated the effect of low doses of streptomycin, a broad-spectrum antibiotic and vancomycin, antibiotic targeting Gram-positive bacteria on mice. They have altered the intestinal micro-biota composition and increased the susceptibility to *S. typhimurium* infection. However, the overall number of intestinal micro-biota remained unchanged. The study on two selected populations of mice namely, 129S1/SvImJ mice and C57BL/6 mice revealed that 129S1/SvImJ mice were more resistant to *S. typhimurium* infection than C57BL/6 mice. It was also stated that the treatment with streptomycin and vancomycin increased both colonization of pathogen and intestinal inflammation, but treatment with metronidazole increased only the colonization without inducing colitis. Thus, they concluded that only specific members of micro-biota caused the infection.

Rosana *et al.* (2011) reported that different antibiotics have distinct effects on the composition of intestinal micro-biota. Any change in intestinal micro-biota causes a vast number of intestinal and extra-intestinal diseases.

Selvaraj *et al.* (2010) conducted an experiment to study the characterisation and antibiotic sensitivity pattern of the *Salmonella* found as contaminants in chicken carcass. They reported the overall incidence of *Salmonella* contamination of poultry carcass was found to be 4.90% with higher percentage of *Salmonella* being isolated from chicken meat, followed by liver and spleen. Biochemical profile and their sensitivity pattern to different antibiotics confirmed their prevalence. They also reported that Amikacin, Ciprofloxacin and Kanamycin were most effective against *Salmonella*.

CHAPTER-3

MATERIALS AND METHODS

The study entitled “A study on isolation and characterisation of bacterial pathogens present in gut of fish *Puntius ticto*, collected from Chilika, Odisha” was conducted in the Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Science and Animal Husbandry, Department of Microbiology and Department of Zoology, College of Basic Science and Humanities, Orissa University of Agriculture and Technology, Bhubaneswar during January, 2019 to April, 2019.

3.1 Collection of samples

Live *Puntius ticto* (Ticto barb) of size ranging from 5 to 7cm, (fig. 1) were collected from a fisherman of the Satapada fish jetty area of Chilika lake.

A total of 5 specimens were dissected aseptically and their gut contents were stored in sterilized vials in Microbiology Laboratory of the Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Science and Animal Husbandry for further study. Water sample from the same area was also collected in a sterilized bottle.

3.2 Isolation of bacterial pathogens

Gut contents were directly used for streaking on different Enriched media such as EMB , SS , TCBS and KSA (Himedia, India).

3.3 Preservation of Stock Culture

Bacterial cultures were streaked on the NA slants and preserved at 4°C for further use.

3.4 Morphological and Biochemical characterization

3.4.1 Gram staining

The bacteria were stained with the basic dye crystal violet followed by Gram’s iodine solution. Then washed by Gram’s decolourizer and counter stained by safranin. Gram positive bacteria were detected by appearance of blue to purple colour and gram negative bacteria by pink to red colour.

3.4.2 Oxidase test

The enzyme oxidase in the bacteria catalyses transport of electrons between bacteria and the redox dye e.g. dimethyl-p-phenylene diamine or methylene blue (oxidase reagents). It

gives a dark blue coloration when oxidised (OX+), and becomes colorless (OX-) when reduced. Discs were used for detection of oxidase production by microorganisms.

3.4.3 Catalase test

The enzyme catalase catalyses the breakdown of Hydrogen peroxide (1% of H_2O_2) into water and oxygen. It was studied on culture plates (NA) flooded with Hydrogen peroxide solution.

Effervescence of oxygen from the plate indicates positive reaction.

3.4.4 Urease test

The enzyme urease splits urea into ammonia and CO_2 . It was detected by inoculating bacterial cultures into the test tubes containing urease broth with filter sterilized urea at $35^\circ C$ for 24 hours.

Positive reaction was indicated by the light orange to magenta (pink) coloration whereas the negative reaction was indicated by the yellow coloration of the medium.

3.4.5 Indole Production test

This test is used to check the ability of the organisms to form Indole from tryptophan or to detect presence of the enzyme tryptophanase which converts tryptophan to indole. The test was performed by inoculating the bacterial cultures into the tubes containing tryptone broth incubated at $35^\circ C$ for 48 hours. Following incubation, few drops of Kovac's reagent were added to the culture broth. A positive result was indicated by the formation of a pink ring at the interface of the two solutions. Absence of pink ring indicated negative result.

3.4.6 Citrate Utilization Test

Bacteria were observed to grow in a medium containing citrate, which acts as a sole source of carbon and energy. Citrate utilization is monitored by the appearance of growth and increase in pH from 6.8, which is indicated by the change in colour of bromothymol blue indicator of the medium. This test was performed by inoculating the bacterial cultures in Simmon's citrate slants and incubated at $35^\circ C$ for 48 hours. Growth on the slants accompanied by change of colour of the slants to blue indicated positive results. No growth and yellowish green colour of the slants indicated negative result.

3.4.7 Mannitol Motility test

Mannitol Motility medium is a bacterial growth medium used to detect the ability of bacteria to ferment mannite and produce nitrogen gas and to indicate the motility of the organism. The fermentation of mannitol was indicated by acid production which changes the colour of the butt from red to yellow. Diffused growth along the stabbed line was considered as the positive result, while only stabbed line indicated a negative result for motility.

3.4.8 Triple Sugar Iron test

Lactose, Sucrose and Glucose are the fermentable carbohydrates. Sodium Thiosulphate and Ferric or ferrous ions make H₂S an indicator system. Phenol red is the pH indicator. Slants and butts were prepared in the test tubes containing TSI agar and microorganisms were first stabbed through the centre of the medium into butt and then streaked on the surface of the slants separately. The above inoculated tubes were incubated at 35°C for 48 hours.

3.5 Antibiotic Sensitivity test (ABST)

To determine the drug sensitivity pattern of the isolated bacteria and to interpret their disease potential, commercially available anti-microbial discs were used for the test.

1-2 ml of cultured bacteria was taken from NB and spread uniformly. Antibiotic discs were placed apart on the surface of inoculated plates aseptically with the help of a sterile forceps and incubated at 37°C for 24 hours.

After incubation, the plates were examined and the diameters of the zones of inhibition were measured. Depending on the area of the zone diameters for individual antibiotic was recorded as sensitive, intermediate and resistant.

3.6 Total Plate Count (TPC) method

The plate count method or spread plate technique is based on growing bacterial colony on a Nutrient Agar Plate. The number of colonies visible to the naked eye can be counted.

Serial dilution of the sample was made with sterile water and then known volume from the specific diluted sample was inoculated on a Nutrient agar plate at 22- 37° C for 24 hours.

The number of colonies were counted and the viable cell count was calculated.

3.7 Most Probable Number (MPN) technique

The presumptive test is used to detect and estimate coliform population of a water sample.

This test was carried out by inoculating a series of lactose broth medium or Mac Conkey broth with three different volumes (10 ml, 1 ml and 0.1 ml). The series consists of 3 groups , each composed of 5 tubes and each group is inoculated with the designated volume of water sample.

The greater the number of tubes per group, the greater is the sensitivity of the test.

Mac Conkey broth contains lactose, bile salts and pH indicator. Lactose and bile salts are selective for coli forms and lactose is only used by coli forms. Lactose is not used by other enteric bacteria and bile salts are depressant for enteric bacteria other than coli forms. A pH indicator such as neutral red or bromocresol purple is added to detect acid fermentation from lactose. Production of acid changes the colour of the medium to yellow. Gas production during fermentation is detected by inverted Durham tube in the medium. Positive test for coli forms is indicated by the production of acid and gas from lactose fermentation after 24-48 hours of incubation. Number of tubes showing positive test is counted and the result is expressed as most probable number (MPN) of coli forms by matching the results with those provided in a statistical table.

3.8 Measuring Physico-chemical properties of fish inhabiting water

The temperature of fish inhabiting water was measured using mercury thermometer at the site of collection. Besides, various physico-chemical properties such as salinity, electrical conductivity, total dissolve solid(TDS) and pH of the fish inhabiting water was measured using multiparameter (Labard, India) (fig.2)

CHAPTER – 4

RESULTS

4.1 Designation of gut content samples

The gut content samples collected from five *Puntius ticto* of size ranging from 6.2 cm , 6.6 cm , 5.5 cm , 5.9 cm and 5.7 cm. The samples were designated as F1, F2 , F3 , F4 and F5 respectively. The samples were cultured in EMB, SS, TCBS and KSA and were designated as E1 -E5, S1 – S5, V1- V5 and K1 – K5 respectively.

4.2 Physico-chemical characteristics of fish inhabiting water

Table 1. Physico-chemical characteristics of fish inhabiting water

Sample	Salinity (in ppt)	Electrical conductivity (EC) (in μ S)	Total Dissolve Solid (TDS) (in ppm)	pH	Temperature (in $^{\circ}$ C)
Fish inhabiting water	18.13	375	187.9	6.05	25

4.3 Total Plate Count (TPC)

The viable cell count in the sample was expressed in terms of colony forming unit (cfu) using the following formula with four-fold dilution of the sample.(fig. 15 and fig. 16)

$$\text{Colony Forming Unit (cfu)} = \frac{\text{no. of colonies}}{\text{Quantity taken} \times \text{Dilution factor}}$$

The viable cell count was calculated to be 35×10^6 cfu. Thus, the sample was found to be highly contaminated and supporting a great microbial load(Table.2).

Calculation:

Viable Cell count:

$$\text{cfu} = \frac{35}{0.1 \times 10^{-4}} = 35 \times 10^6 \text{ no. of cells}$$

Table 2. Enumeration of bacterial colonies with respect to dilution factor

Sl. No.	Dilution factor	No. Of Colonies
1	10^{-4}	35
2	10^{-4}	35
3	10^{-5}	28
4	10^{-5}	23

4.4 Most Probable Number (MPN)

Five samples of 10 ml, five samples of 1 ml and five samples of 0.1 ml were tested for calculation of MPN Value. Since, five, two and one samples respectively from above showed positive results, possible tube combination obtained was 5-2-1. (fig.17 – fig. 19) On matching it with the given table of MPN Index, the MPN value of the sample was found to be 70.(Table.3) This inferred that the sample is highly contaminated and unfit for drinking purpose.

Table 3. Estimation of MPN Index of sample

Sample	Strength	Volume inoculated (in ml)	Colour change	Gas productivity	Result MPN/100 ml
Inhabiting water of fish	Double Strength	10	5	5	70
	Single Strength	1	2	2	
	Single Strength	0.1	1	1	

4.5 Culture of bacteria in different selective media

Pure cultures from the samples were obtained through bacteriological media like EMB, SS and TCBS. However, no colony was found in KSA enriched plate (Table 4.)

After 24 hours of observations, the isolates S1, S2, S3 and S4 were found as positive for SS. (fig.3) The isolate S5 did not show proper characteristic colony growth hence, was not taken into account for the study.

Similarly, the isolates V1, V2 , V3 and V4 were found as positive for TCBS.(fig.4)

Then, the isolates E1 and E2 were found positive for EMB (fig.5).

The isolates V5, E3, E4 and E5 were also not taken into account for similar possible reasons. The isolates K1 – K5 did not show any growth of colony in it hence, were considered negative for KSA medium.(fig.6)

Table 4. Isolation of bacteria on different selective media

Sl. No.	Sample	EMB	SS	TCBS	KSA
1	S1	(-)	(+)	(-)	(-)
2	S2	(-)	(+)	(-)	(-)
3	S3	(-)	(+)	(-)	(-)
4	S4	(-)	(+)	(-)	(-)
5	V1	(-)	(-)	(+)	(-)
6	V2	(-)	(-)	(+)	(-)
7	V3	(-)	(-)	(+)	(-)
8	V4	(-)	(-)	(+)	(-)
9	E1	(+)	(-)	(-)	(-)
10	E2	(+)	(-)	(-)	(-)

4.6 Biochemical characterisation of isolated bacteria

Different biochemical tests like Urease , Catalase , Oxidase , Citrate, Indole , Mannitol , Motility , TSI (Glucose , Lactose ,Sucrose, H₂S, Gas) were performed and the results were analysed. (Table. 6)

4.6.1 Effect of Urease test on the cultured bacteria

The isolates S3, S4, V1, V2, V3, V4, E1 and E2 which indicated yellow coloration of the medium were identified as urease negative, where as S1 and S2 which changed colour from light orange to magenta (Pink) were identified as urease positive. (fig. 9)

4.6.2 Effect of Catalase Test on the cultured bacteria

All the isolates S1, S2, S3, S4, V1, V2, V3, V4, E1 and E2 produced effervescence of oxygen from the plate and indicated positive reaction. (fig.12)

4.6.3 Effect of Oxidase test on the cultured bacteria

The isolates E1 and E2 gave no coloration and were found to be oxidase negative while all other isolates S1, S2, S3, S4, V1, V2, V3 and V4 changed its colour to dark blue and found to be oxidase positive.

4.6.4 Effect of Citrate test on the cultured bacteria

The isolates V1 and V3 gave no change in colour and were found to be citrate negative. All other isolates S1, S2, S3, S4, V2, V4, E1 and E2 accompanied with change in colour from yellowish green to blue were found as citrate positive. (fig.8)

4.6.5 Effect of Indole test on the cultured bacteria

The isolates S1, S2, V4, E1 and E2 were found as indole positive as it formed a pink ring at the interface of two solutions whereas S3, S4, V1, V2 and V3 were found as indole negative due to the absence of pink ring at the rim of the medium. (fig. 7)

4.6.6 Effect of Mannitol test on the cultured bacteria

The isolates S1, S2, S3, S4, E1 and E2 changed the colour to yellow from red and were found to be positive for mannitol test. Other isolates V1, V2, V3 and V4 gave no change of colour and were found to be negative for mannitol test. (Fig.11)

4.6.7 Effect of Motility test on the cultured bacteria

The isolates S1, S2, S3, S4, E1 and E2 gave diffused hazy growth that spread throughout the medium rendering it slightly opaque were found to be positive whereas V1, V2, V3 and V4 gave growths that were confined to the stab-line having sharply defined margins, were negative for motility test.(fig. 11)

4.6.8 Effect of TSI test on the cultured bacteria

All the isolates S1, S2, S3, V1, V2, E1 and E2 were found to be glucose fermented as enough acid was produced to turn the butt yellow while the slants remain red. The isolates S1, S2, V1, V2, E1 and E2 were found to be lactose fermented as a large amount of acid is produced that turned the phenol red indicator yellow both in butt and in the slant. In isolate S3, lactose was not fermented but small amount of glucose was. Thus, the butt was yellow and the slant was red. The isolates S2, S3 and S4 produced H₂S as a result, the black colour of ferrous sulphide was seen while rest of the isolates were H₂S negative. All the isolates S1, S2, S3, S4, E1 and E2 produced gas in the medium while the other isolates V1, V2, V3 and V4 did not produce any gas.(fig. 10)

The results of the above tests indicated the presence of suspected bacteria namely, *Salmonella*, *Vibrio* and *E. coli* in the gut contents of *Puntius ticto* collected from Satapada, Chilika lake.

4.7 Effect of Gram staining technique on the cultured bacteria

All the isolates were found to be Gram negative with S1, S2, S3, E1, E2 and V3 having small rod shaped morphology while S4 shown normal rod morphology. Isolates V2 and V4 shown rod-comma shaped and V1 morphology shown very small rod shaped morphology. (fig. 13) (Table. 5)

Table 5. Morphology of isolated bacteria through Gram Staining

Sl. No.	Isolates	Results	Shape of the bacteria
1	S1	Gram negative	Small rod
2	S2	Gram negative	Small rod
3	S3	Gram negative	Small rod
4	S4	Gram negative	Rod
5	V1	Gram negative	Very Small Rod
6	V2	Gram negative	Rod-comma shaped
7	V3	Gram negative	Small rod
8	V4	Gram negative	Rod-comma shaped
9	E1	Gram negative	Small rod
10	E2	Gram negative	Small rod

4.8 Effect of antibiotic sensitivity test (ABST) on the cultured bacteria

Antibiotic sensitivity test (ABST) was performed by using disk diffusion method. Fourteen different antibiotic discs such as Colistin, Ciprofloxacin, Amoxyclav, Bacitracin, Gentamicin, Piperacilin/Tazobactam, Polymyxin-B, Ampicillin, Ceftriaxone, Cefalexin, Azithromycin, Amikacin, Amoxicillin and Neomycin were used. The isolates were classified as resistant, intermediate and sensitive, according to Clinical and Laboratory Standards Institute (CLSI) for most drugs. (fig.14). It was observed that the isolates showed 80% sensitivity to Colistin, Ciprofloxacin, Piperacillin/Tazol, Amikacin and Neomycin. Among them Piperacillin/Tazol had the most wider inhibition zone and thus, indicated highest effectiveness. The isolates showed 60% sensitivity to Gentamicin and Polymyxin-B and 70% sensitivity to Azithromycin. Most of the isolates were resistant to Bacitracin and intermediate for Ceftriaxone.(Table.7)

Table 6. Biochemical characterisation of final isolates

Sl. No.	Isolates	Urease	Catalase	Oxidase	Citrate	Indole	Mannitol	Motility	Glucose	Lactose	Sucrose	H ₂ S	Gas
1	S1	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(+)
2	S2	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
3	S3	(-)	(+)	(+)	(+)	(-)	(+)	(+)	(+)	(-)	(-)	(+)	(+)
4	S4	(-)	(+)	(+)	(+)	(-)	(+)	(+)	(-)	(-)	(-)	(+)	(+)
5	V1	(-)	(+)	(+)	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(-)	(-)
6	V2	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(+)	(+)	(+)	(-)	(-)
7	V3	(-)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
8	V4	(-)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
9	E1	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(+)
10	E2	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(+)

Table 7. Antibiotics sensitivity of the isolated bacteria

Sl. No.	Isolates	S=11 R=10 CL	S=21 R=15 CIP	S=20 R=19 AMC	S=13 R=8 B	S=15 R=12 GEN	S=21 R=17 PIT	S=12 R=11 PB	S=17 R=13 AMP	S=21 R=13 CTR	S=18 R=14 CN	S=18 R=13 AZM	S=17 R=14 AK	S=19 R=13 AMX	S=17 R=12 N
1	S1	11 S	23 S	- R	17 S	- R	19 I	16 S	- R	- R	17 I	19 S	19 S	17 I	18 S
2	S2	13 S	30 S	18 I	- R	19 S	23 S	- R	15 I	- R	- R	24 S	26 S	- R	17 S
3	S3	14 S	25 S	25 S	- R	- R	- R	15 S	19 S	19 I	- R	17 I	18 S	- R	20 S
4	S4	13 S	- R	- R	- R	- R	24 S	13 S	- R	17 I	- R	- R	- R	- R	- R
5	V1	17 S	32 S	27 S	- R	15 S	21 S	- R	14 I	28 S	21 S	22 S	23 S	20 S	19 S
6	V2	- R	27 S	18 I	- R	20 S	22 S	- R	- R	15 I	- R	30 S	16 I	19 S	- R
7	V3	- R	21 S	- R	- R	17 S	27 S	- R	- R	14 I	16 I	- R	24 S	- R	23 S
8	V4	13 S	23 S	31 S	- R	- R	25 S	18 S	- R	- R	24 S	26 S	22 S	27 S	- R
9	E1	15 S	35 S	- R	11 I	23 S	30 S	16 S	20 S	13 I	19 S	27 S	25 S	17 I	22 S
10	E2	16 S	18 I	26 S	- R	21 S	36 S	15 S	35 S	- R	20 S	24 S	18 S	23 S	19 S

R- Resistance

S -Sensitive

I- Intermediate

CHAPTER - 5

DISCUSSION

Fish is a preferable source of protein and calorie to mankind. However, a constant vigil should be kept on the microbiological profile of fishes to ensure the safety for consumption. According to Nayak *et al.* (2010), fish gut houses about 10^7 to 10^{11} bacteria per gm of intestinal content. The quality of fish inhabiting water influences its gut bacteria. The present study provides evidence that there is correlation between the gut bacteria of fish and its inhabiting water.

Rai *et al.* (2011) carried out a study on two severely polluted sites of river Ganga at Patna, Bihar by taking into account several physico-chemical parameters such as pH, Electrical conductivity (EC), Alkalinity, Total Solids (TS), Hardness, Chloride, Dissolved Oxygen (DO), Biological Oxygen Demand (BOD) and Most probable number (MPN) during pre- and post-monsoon seasons in a year. Laurencin *et al.* (2000) conducted experiment with *Vibrio anguillarum* infecting rainbow trout to demonstrate the effect of temperature and salinity on adhesion of fish pathogenic bacteria. He reported that minimal number of adhesions occurred at low temperatures of 4°C and 10% salinity while highest number of adhesions were observed at 22°C and 17% salinity. Matches *et al.* (1972) conducted experiments on combined effects of pH and temperature on growth of *Salmonella*. They reported growth of *Salmonella* over pH range 4.0 to 9.0 at low temperatures. Thereby, they concluded that food-poisoning *Salmonella* serotypes grow only over a narrow pH range at low temperature and food kept at temperatures below 5°C may not support growth of *Salmonella*. Li *et al.* (2012); Navarrete *et al.* (2012); Bolnick *et al.* (2014) and Stephens *et al.* (2016) have discussed about an array of factors affecting the micro-flora in fish gut. Physical factors like weight, gender age; environmental factors like salinity, pH of water and administration of antimicrobial agents affect the fish gut micro-biota. In the present study, several physico-chemical properties of the sample water were measured. The salinity was found to be 18.3 ppt, pH was measured to be 6.05 and temperature at the time of collection was recorded to be 25°C. Previous studies have already confirmed that favourable pH, temperature, salinity, TDS, EC etc. facilitate the adherence of bacteria to the intestinal walls of fish and such adherence may render virulence. Hence, one must be concerned about the cleaning, proper sanitation and processing of the fishes before consumption. Since, some of the bacteria are pathogenic and they possess zoonotic potential, safety disposal of waste parts is also important. Since fishes live in water, its qualities and microbial load have profound influence on it. So, utmost care of fish should be taken from farm to fork so that its nutritional value remains intact.

MPN is a statistical approximate measure on the test bacteria in the given sample and not the actual number. MPN method is used to detect the coli-form bacteria in water or food Surendran *et al.* (2006). Prakash *et al.* (2011) conducted an experiment to measure the total coli-forms (TC) of dried fish during different weather conditions. They reported TC of dried fish to be 30-65 MPN / 100g, 45-115 MPN / 100g and 65-150 MPN /100g in summer, monsoon and post-monsoon respectively. Total coli-form group can be sub-grouped as faecal and non-faecal coli forms. The presence of faecal coli-form organisms indicates possibly hazardous faecal pollution due to direct discharge of human, livestock and poultry excreta. In the present study, both TPC and MPN methods were envisaged to analyse the microbiological quality of fish inhabiting water. The total viable cell count was estimated to be 35×10^6 cfu/ml which denoted that the sample support a profuse microbial load. Most probable number test of the same sample was conducted which estimated the total coli-forms to be 70 MPN/100ml. The result suggested that the sample water was unsuitable for direct consumption by human beings.

Several workers like Ross *et al.* (1971), Rucker *et al.* (1971) and Rodgers *et al.* (1981) have reported the prevalence of *Vibrio* species in freshwater bodies, while workers like Ringo *et al.* (2003), Aguirre *et al.* (2004) and Senderovich *et al.* (2009) have reported the incidence of *Vibrio* species in marine and brackish water bodies. Other workers like Venugopal *et al.* (2002), Selvaraj *et al.* (2010) and Prakash *et al.* (2011) have reported the presence of enteric bacteria like *Salmonella*, *E.coli* etc. in fishery products. Selvaraj *et al.* (2010) conducted an experiment to study the characterisation and antibiotic sensitivity pattern of the *Salmonella* found as contaminants in chicken carcass. They reported the overall incidence of *Salmonella* contamination of poultry carcass to be 4.90% with higher percentage of *Salmonella* being isolated from meat, followed by liver and spleen of chicken. According to them the biochemical profile and their sensitivity pattern to different antibiotics also confirmed their prevalence. They reported that Amikacin, Ciprofloxacin and Kanamycin were most effective against *Salmonella*. However, little literature is available on the microbiological load on *Puntius*. In the present study, selective culture media like EMB, SS, TCBS and KSA were used for identification of bacterial flora. It was found that 40% each of the total isolates were positive for SS and TCBS. A total of 20% of the isolates were found positive for EMB while no characteristic colony growth was observed in KSA medium. Similarly, 70% and 60% of the isolates were tested positive for glucose fermentation and lactose fermentation respectively. Through the study, it was noticed that the prime bacterial

suspects in the *Puntius* gut content were *Salmonella*, *Vibrio* and *E.coli*. Subsequently, morpho-physiological characterisation of the cultured bacteria was done through biochemical tests and Gram staining and the dominance of the suspected bacteria was recorded. Moreover, all the cultures were found positive for the catalase test and most of them were also found positive for oxidase test, which indicated the presence of facultative anaerobic or aerobic organisms in the sample.

Selvaraj *et al.* (2010) in his work had found the effectiveness of two antibiotics Amikacin and Ciprofloxacin against the cultures. In the present study, antibiotic sensitivity of the isolates was also conducted using fourteen different antibiotic discs such as Colistin, Ciprofloxacin, Amoxyclav, Bacitracin, Gentamicin, Piperacilin/Tazobactam, Polymyxin-B, Ampicillin, Ceftriaxone, Cefalexin, Azithromycin, Amikacin, Amoxicillin and Neomycin. The isolates showed maximum sensitivity to Colistin, Ciprofloxacin, Piperacillin/Tazol, Amikacin and Neomycin and maximum resistance to Bacitracin. Among them Piperacillin/Tazol had the wider inhibition zone and thus, indicated highest effectiveness. The isolate suspected to be *Salmonella* were found to be highly resistant to the above antibiotics, while isolates suspected to be *Vibrio* and *E. coli* were highly sensitive to these antibiotics. All the isolates were found Gram negative. One of the established facts, is, Ciprofloxacin and Piperacillin/Tazol targets mostly Gram negative bacteria. This coincides with the findings of our study and thus, indicates their prevalence.

CHAPTER – 6

SUMMARY AND CONCLUSION

Many workers have advocated about the influence of gut micro-biota on the nutritional value of fish. The state Odisha has a vast coastline and pisciculture is one of the prime occupations practised. Fish being a rich source of protein and calorie, is cultured in abundance to quench the nutrient requirements of humans across the globe. So in order to ensure that fishes are not reservoirs of pathogenic micro-organisms which can possibly enter into the food-chain, some key measures should be considered:

- Maintaining the microbiological water quality of local capture.
- Post-harvest care
- Adequate hygiene conditions in the handling processes
- In case of fishery processed products, measures should be taken to ensure the bacteriological safety during all the processes.

Besides, to avoid human acquiring direct contamination with epizootic pathogens of fish, it is recommended not to consume raw or undercooked fish delicacies. The present study was designed with the objectives like collection of the fish gut contents, isolation and culture of pathogenic bacteria by using different selective media, their morpho-physiological characterization, antibiogram profiling and estimating the microbiological quality of surrounding waters.

The gut content samples from *Puntius ticto*, a freshwater fish, collected from Satapada, Chilika, were stored by aseptically dissecting the specimens. The samples were streaked onto different selective enriched media like EMB, SS, TCBS and KSA to obtain pure cultures. About 40 % of the isolates were found to be positive for both SS and TCBS, followed by 20% of them showing positive results for EMB. However, no colony growth was obtained on KSA media. Thus, the bacteria cultured were primarily suspected to be species of *Salmonella*, *Vibrio* and *E. coli*. For further confirmation, morpho-physiological characterization of the bacteria was performed through different biochemical tests and Gram staining, where all the isolates were found to be Gram negative bacteria with varied morphology. While 60% of the bacteria possessed small rod morphology, 20% of them showed rod-comma shaped morphology and only 10% of the total isolates exhibited each of the normal rod and very small rod morphology. The antibiogram profiling of the isolates was executed using different types of antibiotic discs in which Colistin, Ciprofloxacin, Piperacillin/Tazol, Amikacin and Neomycin showed maximum sensitivity. Among them Piperacillin/Tazol had the most wider inhibition zone and thus, indicated highest

effectiveness. The samples of fish inhabiting water supported a large amount of viable cell count and its MPN / 100 ml values depicted its unsuitability for drinking purpose. Various other physico-chemical parameters of the water samples were recorded that provided these pathogenic micro-organisms a viable environment to survive. It is inferred that the surrounding waters of fish can be a source of pathogens harbouring fish bodies. Thus, local municipalities and state legislatures should take optimum measures to deplete the level of pollution in water bodies mainly housing fish crop.

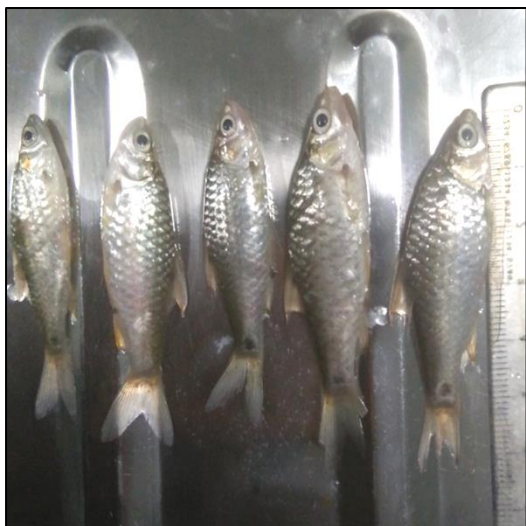


Fig. 1. Specimens of *Puntius ticto*



Fig. 2. Multiparameter (Labard, India)



Fig. 3. Growth on SS

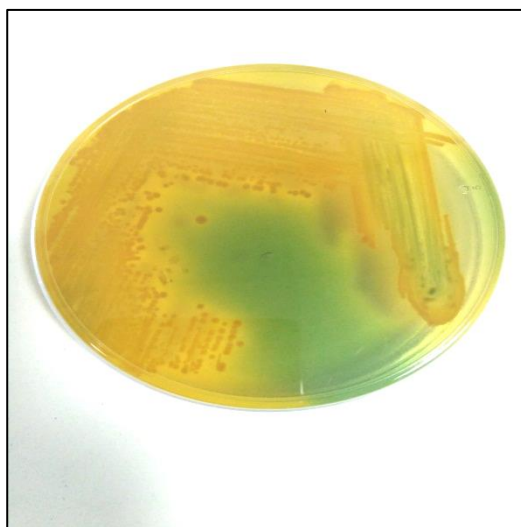


Fig. 4. Growth on TCBS



Fig. 5 Growth on EMB

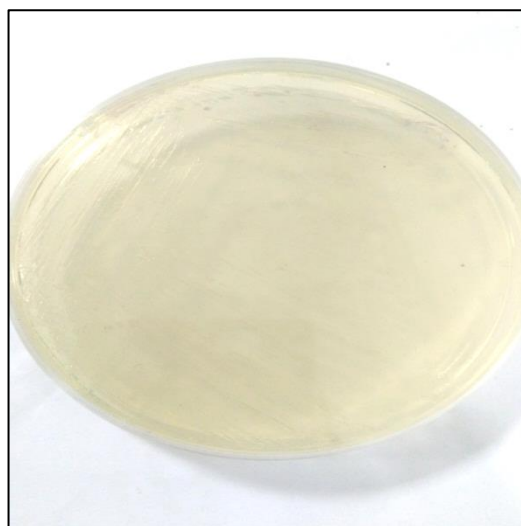


Fig. 6. Growth on KSA



Fig.7. Indole test



Fig.9. Urease test



Fig.8. Citrate test



Fig.10. TSI test



Fig.11. Mannitol Mortality

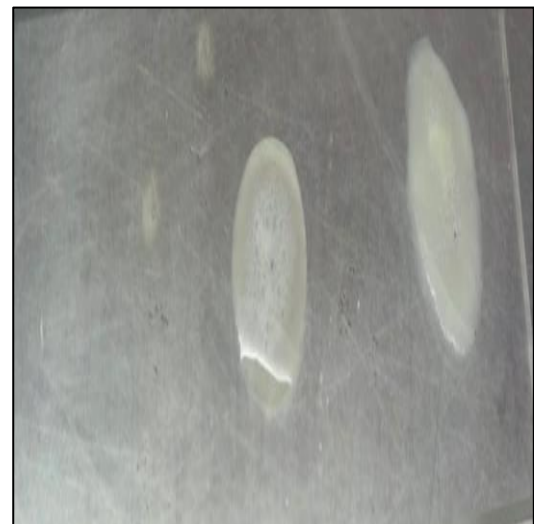


Fig.12. Catalase test

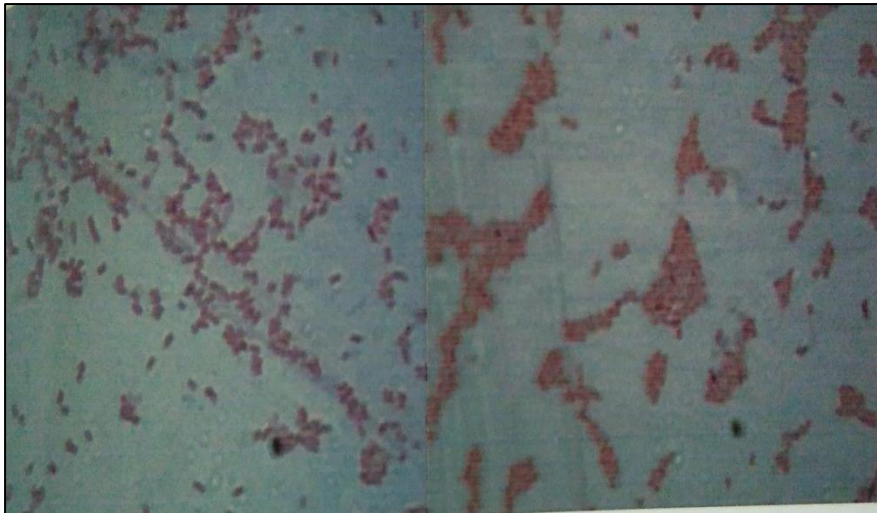


Fig.13. Rod-shaped Gram negative

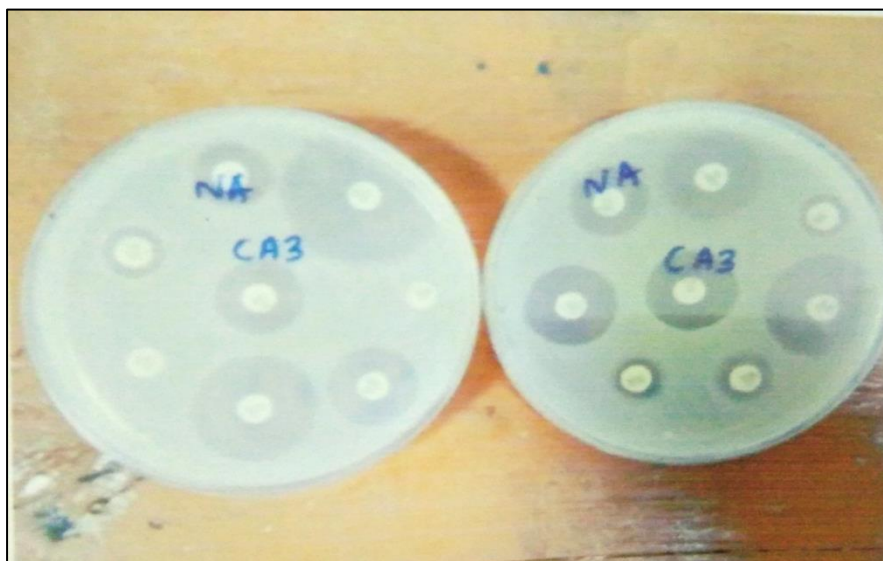


Fig.14. Antibiotic Sensitivity Test



Fig.15. Growth of Bacterial colonies on four-fold dilution of sample

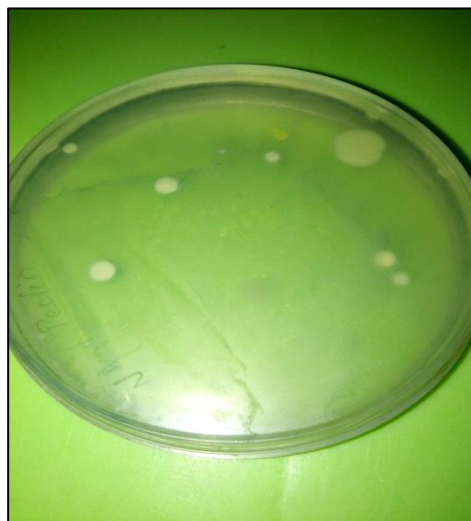


Fig.16. Growth of Bacterial colonies on five-fold dilution of sample



Fig.17. Double strength media inoculated with 10 ml sample

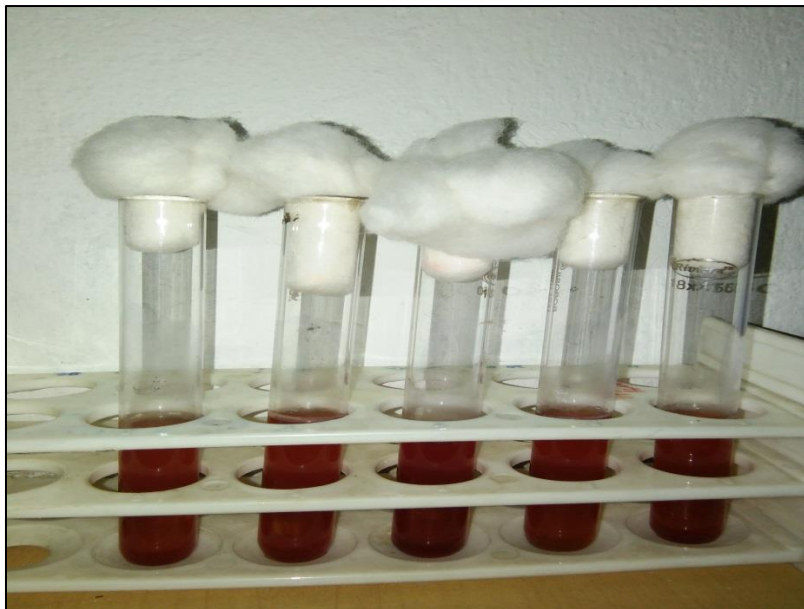
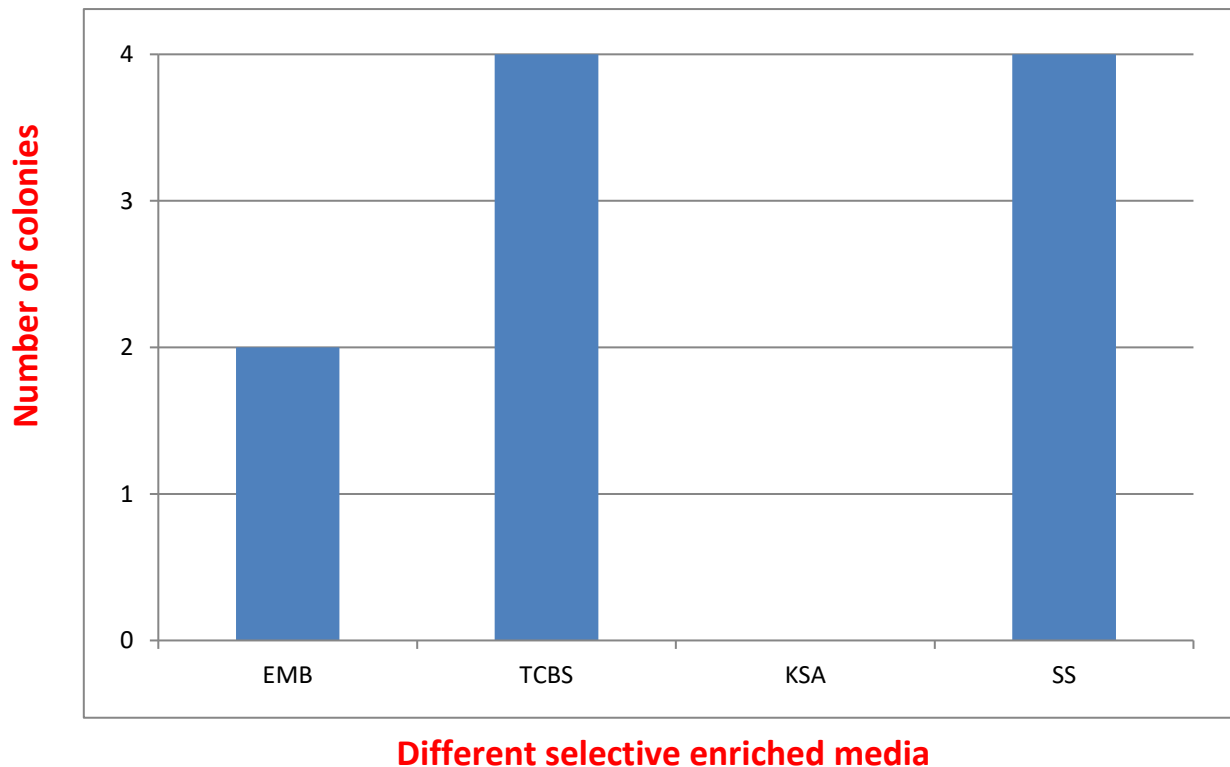


Fig.18. Single strength media inoculated with 1ml sample

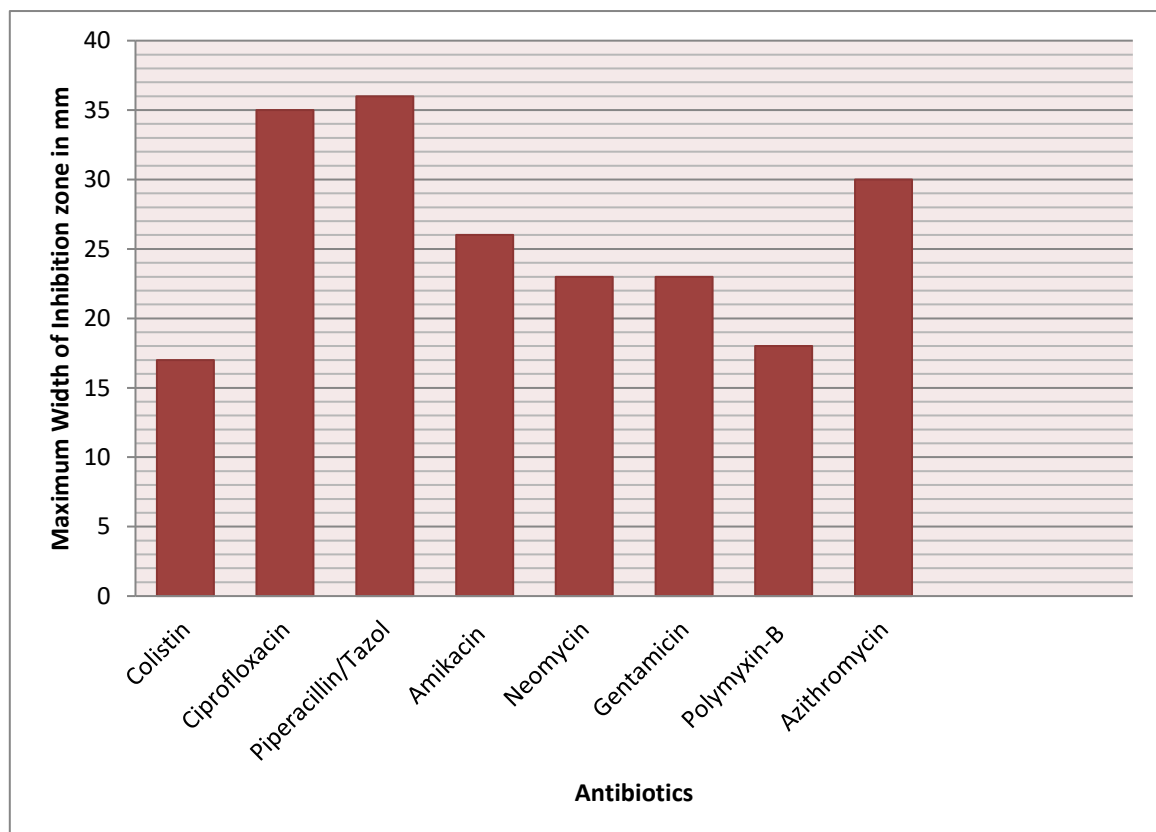


Fig.19. Single strength media inoculated with 0.1ml sample

Graph.1 Growth of bacteria on different selective enriched media



Graph 2. Maximum width of inhibition zone of sensitive antibiotics



CHAPTER- 7

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