Study on Isolation and Characterization of Enterobacteriaceae in Teleost Fishes of Bhopal Lakes

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Abstract: The present study was carried out for the biochemical detection and identification of Enterobacteriaceae from four different teleost fish species collected from five different water bodies of Bhopal viz. Upper Lake, Lower Lake, Shahpura Lake, Sarangpani Lake & Kolar dam in Bhopal region. The four different fish species were selected for the investigation. *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala* and *Tilapia tilapia* represented all water bodies. *Enterobacteriaceae* were isolated from total 112 samples of intestine and liver from 180 diseased fishes. Morphological and biochemical characterizations in cultured samples were carried out and the bacterial isolates were recovered. Upper Lake had maximum revival from total bacterial isolates. Among the bacterial isolates revived, the most dominant isolates were *Citrobacter diversus* (40.7) and *Klebsiella pneumoniae* (32.1). Other species collected were *Citrobacter freundii* (6.2), *Citrobacter braakii* (1.2), *Klebsiella ornithinolytica* (7.4), *Klebsiella planticola* (2.5), *Enterobacter cloacae* (6.2), *Enterobacter gergoviae* (1.2), *Enterobacter cancerogenouss* (1.2), *Yersinia intermedia* (1.2). Results showed that freshwater fishes in Bhopal lakes have remarkable proportion of enterobacteriaceae and these bacteria constitute a potential risk for fish population and public health.

Key Words: Enterobacteriaceae, Bhopal Lake, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Yersinia.*

Introduction

Fisheries and aquaculture stay important sources of food, nutrition, income and livelihood for million of people around the world. World per capita fish supply reached a soaring high of 20 kg in 2014. Fish continues to be one of the most-traded food supplies worldwide with more than half of fish exports by value originating in developing countries (FAO, 2016). Aqua industry is in fact a union of earthern pond fertilized with animal fecal waste, hence, 50% of the microorganisms from both fish and water of eastern pond fertilized with animal fecal waste, hence, enterobacteriaceae infection in fish is required to assess (Wogu and Maduakol, 2010). Until now, microbiological studies on freshwater fish appear to be limited especially in India. Hence, a study was carried out for the biochemical detection and identification of Enterobacteriaceae in liver and intestine of four teleost fish species collected from five different freshwater bodies of Bhopal. The present study may be useful to control the microbial growth to reduce its hazard by applying improvised processing of fishes in water bodies and to evaluate the public health aspects associated with the consumption of these fishes.

Materials and Methods

Study Sites & Sample Collection: Fishes were collected from the sites chosen to give a good depiction of the...
distribution of preferred fishes at Bhopal region. 40 diseased fishes were collected each from Upper Lake, Lower Lake, Sarangpani Lake and Kolar Lake (Fig.1). However, 20 fishes from Shahpura Lake. Total 180 diseased fishes were collected from these 5 different water bodies.

Clinical Examination: The diseased fishes collected were clinically examined according to Noga (2010) and the points taken into consideration were external lesions, and changes in color to shows the infectious characteristics by Enterobacteriaceae group.

Sample preparation: Fishes collected from sampling sites, were brought to the laboratory were kept in iceboxes and dissected within 1 hour or as early as possible. Pieces of fish liver and intestine were collected separately under aseptic conditions and put into sterile petri dishes. Corresponding organs from the same fish species were pooled, weighted and homogenized with a sterile warring blender. 10% homogenized suspensions each of intestine and liver tissues were prepared separately in distilled water (Fig. 2).

Bacterial Isolation and Identification: Fresh stocks of samples were streaked for isolation on the selective media plates of Eosin Methylene Blue (EMB) Agar, pH 7.2 ± 0.2 and Xylose Lysine Deoxycholate (XLD) Agar, pH 7.5 ± 0.2.

Plates were kept in an incubator (Thermo Scientific, USA) overnight at 37°C for growth. After incubation, different colonies formed on the plates were recovered and streaked again on the same media for pure culture. The pure cultures were maintained on Luria Agar slants, keeping in deep freezer at -20°C for further morphological and biochemical identification.

Morphological Characterization: Bacterial film was prepared from each suspected purified isolate and stained with Gram’s stain then examined under the bright field microscope (Carl Zeiss, Germany) with the oil immersion lens.

Biochemical Characterization: The cultures were then subjected to bio-chemical characterization by applying various tests i.e. Catalase, Triple Sugar Iron Agar, Indole, Voges-Proskauer and Methyl Red, Urea utilization, Simmon’s Citrate Agar, Motility and Oxidase test were done according to the biochemical identification keys using the standardized procedures as described by Aneja (2003).

Result and Discussion

In the present investigation, total 180 fishes of four species were undertaken for elusidation and characterisation of group enterobacteiraceae. Over 112 tissue samples of fish intestine and liver were analyzed for the presence of Enterobacteriaceae. It was interesting to find that fishes from Upper Lake were predominantly infested with Enterobacteriaceae as compared to other water bodies. Among the four species of fishes analyzed the maximum bacterial load was found in Labeo rohita (Fig. 3). Intestine was observed to be heavily infected with Enterobacteriaceae in comparison to the liver in all the fish species (Fig. 4).

The result of present study was based on screening of Enterobacteriaceae using the tests Catalase, Triple Sugar Iron Agar, indole, Voges-Proskauer and Methyl Red, Urea utilization, Simmon’s Citrate Agar, Motility and Oxidase as conformatary tests. We found, in particular, ten different species of Enterobacteriaceae from four fish species Catla catla, Labeo rohita, Cirrhinus mrigala and Tilapia tilapia.

Ten species of Enterobacteriaceae were found in all isolates in the clinical setting. These were Klebsiella pneumoniae, Klebsiella ornithinolytica, Klebsiella planticola, Citrobacter diversus, Citrobacter freundii, Citrobacter braakii, Enterobacter cloacae, Enterobacter gergovie, Enterobacter cancerogenus, Yersinia intermedia (Fig.5). Further, it is evident from our study that among all ten species of Enterobacteriaceae, the occurrence of Citrobacter species was maximum with 40.7% of which Citrobacter diversus contributed the most. These findings were in agreement with results of Yagoub, (2009) who reported 8.7% occurrence of Citrobacter species in raw fishes. Our research also shows second highest occurrence of Klebsiella species in both liver and intestine tissue samples with 32.1% specifically Klebsiella pneumoniae. These findings were supported by Elsherief et al., (2014) who reported 8% Klebsiella pneumoniae and Yagoub, (2009) who reported Klebsiella species with incidence of 10.9%. The occurrence of Enterobacter species may vary in intestine and liver including relatively higher occurrence i.e. 8.6% of Enterobacter cloacae. These observations are in line with those of the study reported with 4.8% by Elsherief et al., (2014) and 11.1% by Yagoub (2009). Also we found the enterobacterial species Yersinia intermedia with 1.2%. Generally, bacterial diseases do not developed only because of infectious agents (Wedekind et al., 2010). Ample density of microbial growth in fishes mostly arises due to stressful situations such as malpractice fishing, poor water quality and inadequate sanitation (Sheen et al., 2012). These environmental stresses decrease the ability of fishes to resist the pathogenic organism (Small et al., 2005). Klebsiella species have been considered to be the most important histamine-producing bacteria (HPB) isolated from fish which is a mild illness; however, in individuals with pre existing conditions severe complications such as cardiac and respiratory manifestations may occur (Lehane and Olley, 2000).

Likewise, Sekar et al., (2008), studied the Enterobacter cloacae from Mugil capito and reported it as the causative agent of selective mortality. The bacterial infectivity in fishes can be rigorous and proves to be devastating leading to severe mortality (Ter et al., 1997). One of the major factors of fish mortality due to bacterial infections is...
reduction in immune response. Human infections as food poisoning and gastroenteritis may be caused by bacteria in fish. Further, it is not necessary that any bacterial infection would cause a disease condition. In fact, many enterobacteriaceae members are opportunistic pathogens. However, stressful situations such as high organic content, increasing temperature and overcrowding in aquaculture amenities are responsible for disastrous outbreaks (Saxena et al., 2006). The quantitative and qualitative aspects of population density of bacteria usually vary in different organs of fish such as intestine, liver, muscle, skin and gill. Highest enterobacterial load is seen in liver in the fish Labeo rohita, Tilapia tilapia and Cirrhinus mrigala. While in the fish Catla catla highest enterobacterial load is seen in intestine. In concern to the composition of the intestinal flora, the level of contamination of water and food sources need to be monitored.
surrounding fish diversifies the flora (Latha & Mohan, 2013). Furthermore, the particular group of bacteria developed as the consequence of growth intensifying and inhabiting substance of the digestive system in fish (Austin, 2002). Thus, a wide interface between host species pathogens and its surrounding atmosphere is the principal occurrence of ailments in fish (Song et al., 2008).

Conclusion

This study revealed that the infectivity of fish through some enteropathogens has occupied the aqua industry of Bhopal. Further, the proportion of Enterobacteriaceae in fishes warns about bad sanitary conditions under which fish were open to the elements resulting in ailment of both public health and monetary losses.

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References


