Asian J. Exp. Sci., Vol. 31, No. 1, 2017; 25-29

Online available on : www.ajesjournal.com, ISSSN : 0971-5444

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 pneumonie* (32.1). Other species collected were *Citrobacter fraundii* (6.2), *Citrobacter braakii* (1.2), *Klebsiella ornithinolytica* (7.4), *Klebsiella planticola* (2.5), *Enterobacter clocae* (6.2), *Enterobacter gergovie* (1.2), *Enterobacter cancerogenous* (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 aqua systems that culture different species of aquatic animals (Pillay, 1990). Fish had long been regarded as a desirable and nutritional source of high quality proteins and generous supply of minerals and vitamins constituting the major part of human diet (Hastein *et al.*, 2006).

Fishes are getting contaminated with number of micro-organisms and fishery products act as carriers of numerous microbial and other health hazards, therefore, preservence of quality is of supreme importance (Ponnerassery, 2012). Until now, microbial flora from cultured freshwater fishes has been least studied especially in India (Souter *et al.*, 1976; Sakata *et al.*, 1980; Surendran and Gopakumar, 1991; Surendraraj *et al.*, 2009). Evidently, a number of temperate and tropical fishes and water samples have been evaluated for the presence of wide variety of microorganisms and Enterobacteriaceae from skin, intestine, gills, kidney and muscle (Buras *et al*., 1987; Ogbendeminu, 1993; Saraswathi, 2015). Further, more attention should be given to the diversity of microflora and its composition as well as its effect on fish (Zmyslowska *et al.*, 2001). Enterobacteriaceae is a family of small Gram-negative, non-spore- forming straight rods. Some genera are motile by means of peritrichous flagella.

Their pathogenesis has been reported since 1950 (Austin, 2011). Various authors (Roberts *et al.*, 2009; Yagoub, 2009; Elsherief *et al.*, 2014; Hassan *et al.*, 2012; Austin and Austin, 2016;) accounted for the enterobacteriaceae distribution in number of fishes. Factors affecting physical condition of fish such as sewage pollution (Rajasekaran, 2008), environmental stress (Sekar *et al.*, 2008) or injury (Pal and Gupta, 1992) also significantly enhance the rate of fish mortality caused by enterobacteriaceae and has been reported as opportunistic pathogen in fish. Likewise fecal pollution in the surrounding lakes depicts the possibility of isolation of probable enterobacterial pathogenic organisms as *Klebsiella* spp., *Citrobacter* sp., *Salmonella* spp., *E. coli* and *Proteus* spp. from fish. Ogbondeminu and Okoeme (1989) reported the recovery of family Enterobacteriaceae of 50% of the microorganisms from both fish and water of earthern 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, weighed 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 37^0C 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 -20^0C 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

ın the present investigation, total 180 fishes of four species were undertaken for elusidation and characterisation of group enterobacteriaceae. 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 Labio 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 conformatory 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 pneumonie, Klebsiella ornithinolytica, Klebsiella planticola, Citrobacter diversus, Citrobacter fraundii, Citrobacter braakii, Enterobacter cloacae, Enterobacter gergovie, Enterobacter cancerogenous, 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 pneumonie*. These findings were supported by Elsherief et al., (2014) who reported 8% *Klebsiella pneumonie* 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

Fig. 1: Water bodies in Bhopal area
(Source: Google map)

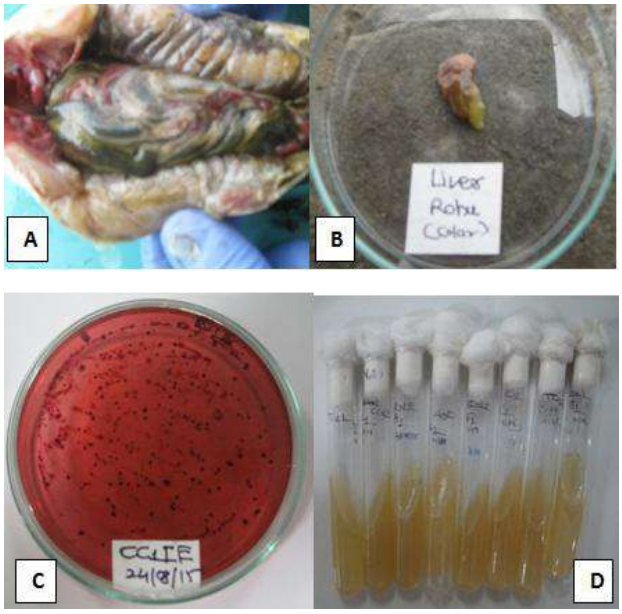

Fig. 2: Bacterial isolation and slant preparation: A- Intestine; B- Liver; C- Bacterial growth plate; D- Pure culture slants.

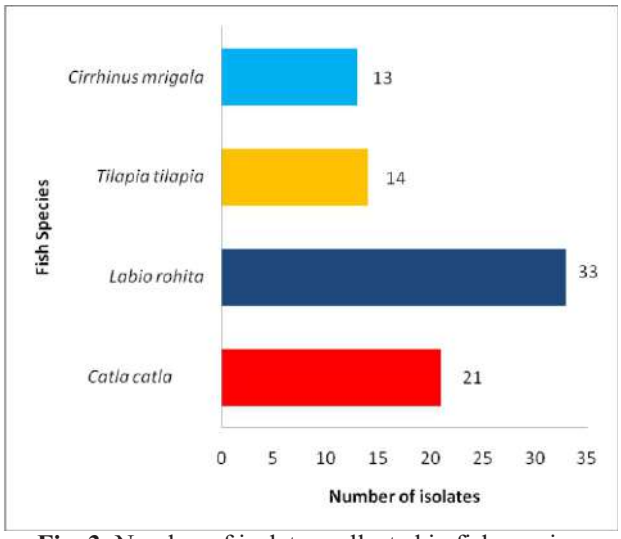

Fig. 3: Number of isolates collected in fish species.

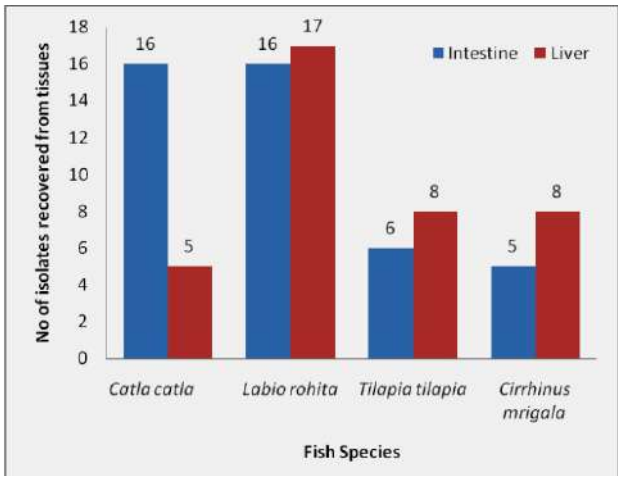

Fig. 4: Isolates recovered from different tissues of various fish species.

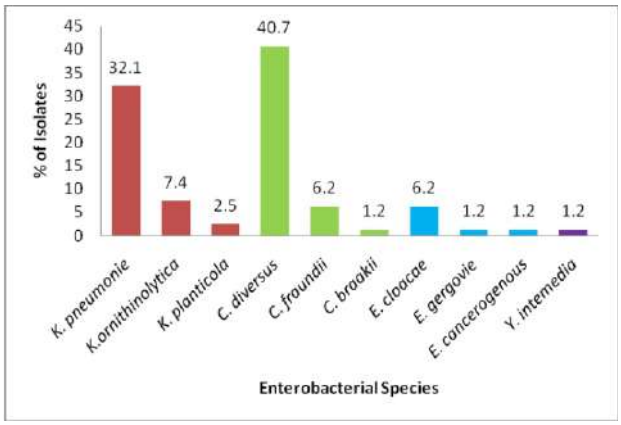

Fig. 5: Percentage of isolates recovered from various fish species.

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

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.

Acknowledgment

First author thankfully acknowledge help extended by Prof. P. K. Verma, former Director General MP Council of Science and Technology for the laboratory facilities during the research work.

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