Preliminary Screening of Pathogenic Bacteria in *Clarias batrachus* (Linn)

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**Abstract:** Human infections caused by pathogens transmitted from fish or the aquatic environment are quite common depending on the season, patients contacted with fish and related environment. Hence an attempt has been made to study the screening of pathogenic bacteria from *C. batrachus*. The study revealed that three genera of the pathogenic bacteria were identified belonging to the family *Enterobacteriaceae* namely *Aeromonas hydrophilia*, *Escherichia coli*, *Vibrio cholerae* and *Vibrio parahaemolyticus*.

**Key words:** Fish, *Aeromonas*, Ulcer, Pathogen, screening.

**Introduction:**
Aquaculture is the farming of animal in an aquatic environment. It includes production, processing and marketing of aquatic organisms. The main feature of culture fishery is in cultivation of fish for assured production of food from aquatic resources. Freshwater play a prominent role in fish production through aquaculture. A quaculture output of freshwater fishes is dominated by Asian countries particularly China, the highest producer. One of the important events of aquaculture is the disease management. Diseases are mainly caused by microbes. It includes viruses, bacteria, fungi, protozoan and some algae. The cause, symptoms and treatment of fish disease must be understood clearly by studying microorganisms present in the culture medium and on the body tissue of the fish for profitable aquaculture used for healthy food.

The fish and other food items are normally affected by many kinds of pathogens before they reach the kitchen. Definitely it may affect the health of the human beings. Therefore, it is essential to understand the health of fishes and other food organisms, cultured for human consumption. The
walking cat fish *Clarias batrachus* is a species of freshwater air breathing catfish native to South East Asia, but also introduced outside its native range, where it is considered an invasive species. It is named for its ability to walk across dry land, to find food or suitable environments. Candidates of the genus *Clarias* has been traveled to many continents, adapting itself successfully and found throughout Asia and Africa. In India, particularly in West Bengal and Tripura is considered as a medicinal fish and traditionally among the pregnant and lactating mothers, the elderly and children. Many times consumption of “Magur machh” (Local name of *C.batrachus* in Assam) is prescribed prophylactically to the anemic and malnourished individuals as well as for the convalescent of the patients due to the nutritional superiority. Intensive *C.batrachus* culture in several Indian states as in rural Bengal and Tripura have much potential towards livelihood development, employment generation and ensuring nutritional enrichment in the regular diet among the people. According to the data released by the Fisheries and Aquaculture department, Food and Agriculture Organization of the United Nations, *C.batrachus* has been propagated throughout the Asia from Thailand and Indonesia (Java). The species has been introduced to as far as Europe (United kingdom), USA and Australia (Papua New Guinea) from various pockets of South East Asia. FAO data also divulge the regular growth in the global production, processing and subsequent earning from different catfish varieties (global market value 80000 USD).

Establishment of *Clarias batrachus* in several continents and its popularity as a freshwater culturable fish species among consumers made the species suitable for meticulous reviews with respect to various parameters. Besides in order to protect the genetic resources of this species from unwanted hybridization, which is very much vulnerable, the fish geneticists and the government bodies should work together.

Habitat protection and sustainable consumption of this excellent fish species is the call of the day. *C.batrachus* has exceptionally well tolerance level in varied environment that suggests an advantageous evolutionary trait. Toxicity and stress studies can through light on the adaption strategy of a species according to the vibrant changes of the environment and the changed character of the wild habitat, much of which is altered by now due to increasing human intervention and exploitation.

Naqvi *et al* (1993) evaluated the severe hematotoxic effect of the commonly used farm fertilizer Diammonium-Phosphate compared to urea in *C.batrachus*. Effect of pollutants as pesticides, drugs, radio nuclides (Joy and Sathyansan 1981) and heavy metals (Panigrahi *et al* 1990) on factors like hematological indices, characters (Ray *et al* 1990) injuring the liver (Goel and Agarwal 1981), brain (Kirubagarn and Joy 1990), etc and general metabolism (Begum and Vijayaragavan 1995) are also well documented in *C.batrachus*. Reproductive cycles etc influenced by allogen are indicating towards the immediate need to preach and practice habitat protection and implement stringent regulation against tempering with the environment. Histopathological changes in liver and kidney caused by the bacterium in the fish. Mostofa *et al*., (2008) studied experimental pathogenesis of *A. hydrophila* bacteria in the same fish. Islam *et al*., (2008) studied histopathological changes in experimentally infected shing with the same bacteria. Lately the bacteria *A. hydrophila* was isolated from Thai pangus *Pangasianodon hypothalamus* (Siddik,2009) and from carps *Labeo rohita*, *Catla catla* and *Cirrhinus cirrhosus*, perch *Anabas*
testudineus, catfishes Heteropneustes fossilis and Clarias batrachus and eel Mastacembalus armatus (Ahmed, 2009).

Muthukrishnan et al., 2008. reported flor of the liver, gills, intestine and muscle of Epizootic Ulcerative syndrome (EUS) infected murrel, Clarias batrachus revealed total viable microbial count was measured as highest range in gills (5.9±0.5×10^7) CFU (Colony forming units) g-1 and lowest range in intestine (8.7±1.8×10^4) CFU g-1. In total 17 bacterial and fungal species were isolated and identified as Aeromonas hydrophila, Enterobacter sp, Vibrio sp., Pseudomonas sp, Escherichia coli, Aphanomyces invadens and Aspergillus sp.

Aeromonas hydrophila, is one of the most important fresh water, fish pathogen isolated from EUS affected fish pathogen isolated from EUS affected fish (Karunasagar et al., 1995). Bacterial flora of some freshwater fishes in tropical water showed that Aeromonas sp. was the most predominant microorganism isolated from the skin, gills and intestine of the fish (Karatas Dugenci and Candan., 2003). Rich data are also available regarding the pathogenic mechanism and virulence of A. hydrophila, which caused mass mortality in cultured Japanese catfish larvae (Kuge et al., 1992). Hence an attempt has been made to study the pathogenic bacteria associated with Clarius batrachus.

Materials and Methods
The infected fish showed ulceration and necrosis were aseptically collected from culture Pond, Needamangalam, Thiruvarur (Dist.) 28;10.47°N;79;100E, Tamil Nadu, India. Immediately the infected samples were brought to the laboratory. The infected area were scrapped and serially diluted and plated on Aeromonas agar, Eosin Methylene Blue agar (EMB, pH 7 ± 0.2 Hi Media, Mumbai), and Thiosulphate Citrate Bile salt sucrose Agar (TCBS, pH 8.2 ± 0.2 Himedia Mumbai). (Aneja, 1994). The plates were incubated at 37°C for 24 hrs. After 24 hrs the colony morphology were observed and the isolates were subjected to series of biochemical tests. The results were compared with Bergey’s manual of systematic bacteriology and the isolates were characterized.

Results and Discussion
In the present study, the isolation and screening of pathogenic bacteria from the infected Clarius batrachus revealed following observation. In the morphological observation the infected C. batrachus showed necrosis and ulceration (Table-1). Based the microbial analysis and the biochemical tests compared with Bergey’s Manual revealed the presence of four different bacterial isolates namely Aeromonas hydrophila, Escherichia coli, Vibrio cholera and Vibrio parahaemolyticus were isolated The bacterial composition may change with age, individuals, nutritional status, environmental conditions, and the complexity of the fish. (Table-2 & Table-3) Like all animals, fish suffers from environmental, nutritional and infectious diseases. Organisms such as bacteria and fungi are capable of causing disease under stressful conditions. Intensive and super intensive fish culture practices involve high rates of stocking and supplementary feeding which has substantially enhanced the incidence of disease in fishes in our country.
Table 1: Colony Morphology and color on different media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Colour</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeromonas Agar</td>
<td>Pale pink smooth, elevated Colony</td>
<td>Rod</td>
</tr>
<tr>
<td>TCBS Agar</td>
<td>Yellowish Green colour colonies</td>
<td>Rod</td>
</tr>
<tr>
<td>EMB Agar</td>
<td>Metallic Sheen colonies</td>
<td>Rod</td>
</tr>
</tbody>
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Table 2: Biochemical tests of different isolates

<table>
<thead>
<tr>
<th>Biochemical tests</th>
<th>Isolated bacterial colony*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IB1</td>
</tr>
<tr>
<td>Motility test</td>
<td>+</td>
</tr>
<tr>
<td>Indole test</td>
<td>+</td>
</tr>
<tr>
<td>M ethyl red test</td>
<td>_</td>
</tr>
<tr>
<td>Voges- proskauer test</td>
<td>_</td>
</tr>
<tr>
<td>Citrate utilization test</td>
<td>_</td>
</tr>
<tr>
<td>Triple sugar iron agar</td>
<td>_</td>
</tr>
<tr>
<td>Urease hydrolysis test</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase test</td>
<td>_</td>
</tr>
<tr>
<td>Catalase test</td>
<td>_</td>
</tr>
<tr>
<td>Carbohydrate Fermentation test:</td>
<td></td>
</tr>
<tr>
<td>a) Maltose test</td>
<td>+</td>
</tr>
<tr>
<td>b) Sucrose</td>
<td>+/G</td>
</tr>
<tr>
<td>c) Mannitol</td>
<td>+</td>
</tr>
<tr>
<td>d) Glucose</td>
<td>+</td>
</tr>
<tr>
<td>e) Adonitol</td>
<td>+</td>
</tr>
<tr>
<td>f) Arbanose</td>
<td>_</td>
</tr>
<tr>
<td>g) Lactose</td>
<td>+</td>
</tr>
<tr>
<td>h) Sorbitol</td>
<td>+</td>
</tr>
<tr>
<td>i) Rhamnose</td>
<td>+</td>
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</table>

*Based on the results of the colonies IB1, IB2, IB3, IB4
The identification test results of bacterial isolates from infected fishes are in agreement with previous studies. These bacteria are a part of the normal microbial flora of water (Lio-Po et al., 1998) and are a potential disease agent for fish (Cortran et al., 1999). Clinical signs were also similar to previous findings (Yadav et al., 1992, Prasad and Verma, 2003). Rodgers and Burke (1981) observed infectious prevalence in estuarine fish populations with seasonal aggregations of fish stressed by low or rapidly changing water temperatures and rapid or prolonged depressions of salinity. Lowered salinity due to rainfall events (Callinan et al., 1995; Virgona, 1992) and excess water discharges appear to play a role subsequent appearance of lesions in fish (Kane et al., 2000). Progression of the disease to ulcers is reported to occur after rainfall and High River flows which also caused rapid changes in various water quality parameters such as salinity, temperature, dissolved oxygen, pH and turbidity (Virgona, 1992). Mohan and Shankar (1994) reported that the dissolved oxygen of the water had close relationship with distribution, seasonal changes and zoosporic production of aquatic fungi.

Bodhe and Wadhai (2014) reported microbial infected *Clarias batrachus* from Wardha and Wainganga River during 2011-12 and 2012-13 in winter season. During 2011-2012 out of 69 samples collected from Wardha River, 43 were microbial affected, in 2012-2013, 53 samples out of 83 samples were found to be microbial affected counting to 63.86% infection respectively. However, from Wainganga River 54.22% and 59.09% infected sample were collected during 2011-2012 and 2012-13 respectively. Intensive aquaculture of *C. batrachus* in the rural water bodies with very little infrastructure development may bring about socioeconomic development in many parts of Bengal and Northeast India. Coordination between government bodies with respect to skill upgradation of the workers, market regulation etc together with the scientific community ensuring timely delivery of better quality seed stock will generate success stories in intensive *Clarias batrachus* culture. Since the species is a part of the natural fauna in this region therefore culture practices will be much easier to follow and therefore much more viable in economic point of view. Regional rural banks and agro finance bodies may be approached for capital requirement and the local governance may forward financial help to the rural entrepreneurs. (Surajith 2011).

Improper Storage of the fishes, usually at temperatures above 20°C appears to be the most important predisposing factor. The organisms most commonly involved are *Proteus spp.*, *Clostridium spp.*, *Escherichia spp.*, *Salmonella spp.*, *Shigella spp.*, *Morganella morganii*, followed by *V. parahaemolyticus* and *V. alginolyticus*.

The outbreak of a disease with more than 75% mortality due to *A. hydrophila* was reported by Lakshmanan et al., (1989). Similarly, Lio-po et al., (1992) reported that several species of bacteria
and fungi were found to be associated with EUS (Epizootic Ulcerative Syndrome) affected snake head \textit{C. striatus} and among that 89% of the total isolates were \textit{A. hydrophila}.

Katoch \textit{et al.}, 2003 identified 25 bacterial and fungal species from freshwater carp at Himachal Pradesh, India. 15 bacterial species are isolated in hybrid tilapia from Saudi Arabia. \textit{Aphanomyces invadans}, a highly invasive specific, slow growing fungus cause Epizootic Ulcerative Syndrome (Lilly and Roberts, 1997) Mycobiotic agent, \textit{Aspergillus flavus} and \textit{Aspergillus fumigatus} were the main fungi isolated from the Nigerian freshwater fish culture (Opkanasili \textit{et al.}, 1998).

\textbf{References:}


