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Isolation and Identification of Pathogenic Bacteria Associated with Diseased Fish *Cirrhinus mrigala* (Ham) in Glass Aquarium

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Abstract: Aquaculture products can anchorage several pathogenic bacterial species, which are known as part of the micro-flora of the environment. The pathogenic bacteria associated with fish can be transmitted to humans as a food source or by handling. The aim of this study was to isolate and identify pathogenic bacteria associated with diseased fish in aquarium. Sampling was carried out under aseptic conditions following standard protocol. Bacterial growth was obtained on different growth media. Total 26 bacterial species were isolated and identified on the basis of cultural, morphological, staining reactions and biochemical analysis. Of the 26 bacterial species, *Escherichia coli* was the most common bacterium accounting 34.62% (n=09) followed by *Aeromonas salmonicida* 30.77% (n=08), *Staphylococcus aureus* 23.07% (n=06) and *Salmonella typhi* 11.54% (n=03). The isolation of pathogenic bacterial species from fish samples may represent high risk for development of lethal infections in humans utilizing such contaminated/diseased fish as a food source. In summary, the present study has revealed the presence of pathogenic bacterial species in diseased aqua fish. It is therefore suggested to take authentic measures to limit the access of bacterial pathogens in fish acquirams.

Keywords: Pathogenic bacteria; Aquarium; Diseased Fish.

1. INTRODUCTION

Fish constitutes an essential part of the diet for human beings due to its easy digestibility and high nutritional value. It has also known as the most important source of high quality protein for a human and provides approximately 16% of the protein source consumed by the population in the world (FAO, 1997). Fishes can be found in different types of water, i.e., salt water (sea and oceans) and fresh water (Eze *et al*., 2011). Water serves as a major source indicating the type of microorganisms associated with particular fishes (Eze *et al*., 2011; Claucas and Ward, 1996). Aquaculture products may harbor pathogenic bacteria from fish ponds particularly in tropical regions (Claucas and Ward, 1996). Pathogenic microorganisms may also enter into sea foods due to inadequate processing, poor standards of hygiene and incorrect handling or storage (Eze *et al*., 2011). The bacterial pathogens associated with fish have been divided into two groups, i.e., indigenous and non-indigenous bacterial pathogens (Kvenberg, 1991; Rodrick, 1991). The indigenous bacterial pathogens are those that are naturally found in fish habitats, i.e., *Aeromonas* and *Vibrio* species while the non-indigenous bacterial pathogens can contaminate fish or fish habitats during handling and processing under unhygienic conditions. The non-indigenous bacterial pathogens include *Clostridium botulinum*, *Listeria monocytogens*, *Staphylococcus aureus*, *Salmonella* species, *Shigella* species and *Escherichia coli* (Kvenberg, 1991; Rodrick, 1991).

Besides being an important source of food with rich source of protein, Fish is also a ready source of income in farming sectors (Smith and Yoshida, 2000). However, fish diseases caused by pathogenic bacterial species are considered as the major factor for economic losses (Yiagnisis and Athanassopoulou, 2011). The bacterial infections in fish farms can lead to death or cause certain symptoms refer to deviation from normal function and structure of a fish (Hedrick, 1998). The production of clinical symptoms caused by pathogenic bacteria depend on the type of host, age and stage of disease, i.e., acute, chronic and sub-clinical form. Several sign and symptoms have been reported in fish infected by pathogenic bacteria, i.e., pale gills, lethargies, anorexia, darkening in color, abdominal retention, abdominal swelling, exophthalmos, external haemorrhages in eyes, head and skin gills (Yiagnisis and Athanassopoulou, 2011).

Globally fishes have impact of diseases in aquaculture systems (Plumb, 1997). The farmers of rural areas have been reported to face financial loss more than 15% in fish production due to fish diseases (Faruk *et al*., 2004). Moreover, in 1997, the World Bank had reported about 3 billion US dollars per anum loss of aquaculture. Commercial fish farms and ornamental fishes are in great threat due to most common infectious problem of bacterial diseases. A little number of pathogenic bacteria may be responsible for fish diseases in captivity and may cause of kidney disease, dropsy,

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enteric red-mouth, tuberculosis, vibriosis, motile Aeromonas septicemia, bacterial gill disease, mouth fungus, tail and fin rot, and columnaris diseases (Austin and Austin, 1999; Banu, 1996). The bacterial pathogen *Aeromonas* spp. is responsible for hemorrhagic septicemia, a disease affecting a wide variety of freshwater and marine fish (Paniagua, *et al*., 1990). The present study was thus focused on isolation and identification of pathogenic bacteria from a diseased fish in aquarium to control the spread of infections due to such diseased fishes.

2. MATERIALS AND METHODS

Sampling

Diseased fish was collected from the aquarium at the Institute of fresh water biology and fisheries. A live diseased fish with clear wound infection on the skin was aseptically transferred to the microbiology laboratory. Sample from the diseased fish was taken by rubbing the sterilized cotton swab over the infection on the skin of the fish. Inoculation was carried out using 10 fold serial dilutions in peptone water. Bacterial counts were enumerated using 0.1 ml inoculums in standard agar plate count method using Nutrient agar and MacConkey's agar as described by Slaby *et al*., (1981). The agar plates were incubated at 37ºC for 24-48hrs.

Preparation of serial dilutions

Sterile distilled water (9.0 ml) was poured aseptically into blank test tubes followed by adding 1.0 ml of the original sample mixture to the first test tube and from the first to second test tube and this whole process was continued until the fifth test tube. All the dilution tubes were mixed thoroughly. Finally, the original sample mixture was diluted from 10^{-1} to 10^{-5}.

Identification of bacterial isolates

Identification of the bacterial isolates was carried out using the phenotypic and biochemical characteristics (Cheesbrough, 1984). Primarily, pure cultures were obtained after repeated plating on selected nutrient media. Identification was based on morphological characteristics, motility, Gram-staining reactions, growth on various types of nutrient media and catalase as well as oxidase tests.

Sterilization of materials

All the glass wares were washed, air-dried and sterilized at a temperature of 180ºC for 1h in hot-air oven as described by Adibe and Eze, (2004). Culture media were prepared according to the manufacturer's instructions and were sterilized at a temperature of 121ºC for 20 min.

3. RESULTS AND DISCUSSION

A diseased fish with a clear wound infection on dorsal fin **(Fig.1)** collected from aquarium was processed for the isolation and identification of the microbial species responsible for wound infection. Total twenty six bacterial strains were isolated using ten-fold dilution method followed by standard plate count assay. Based on physiological, morphological, staining reactions and biochemical tests these bacterial strains were identified as *Aeromonas salmonicida*; *Salmonella typhi*; *Escherichia coli* and *Staphylococcus aureus* **(Table-1).** Out of the twenty six bacterial species; *Escherichia coli* were 34.62% followed by *Aeromonas salmonicida* 30.77%, *Staphylococcus aureus* 23.07% and *Salmonella typhi* 11.54% **(Table 2).** The association of the opportunistic and pathogenic microbial species with fish shows the critical condition compromising safety and the quality for the human being consumption (Sichew *et al*., 2014; Mhango *et al*., 2010). There is a potential risk of infections transmitted to humans from the diseased fish caused by pathogenic microbes.

Fig. 1 A fish *Cirrhinus mrigala* (Ham) with a clear wound-swelling from which sample was obtained.

Table 1. Biochemical reactions of the bacterial species isolated from diseased aquarium fish.

Bacterial isolate	Coagulase	Oxidsae	Catalase	Indole	Lactose	Manitol	Glucose	Sucrsoe	Fructose	Motility
Aeromonas salmonicida	–	+	+	+	+	+	+	+	+	+
Salmonella typhi	_	_	+	_	_	+	+	_	_	+
Escherichia coli	_	_	+	+	+	+	+	+/_	_	+
Staphylococcus aureus	+	_	+	_	+	+	+	+	+	-

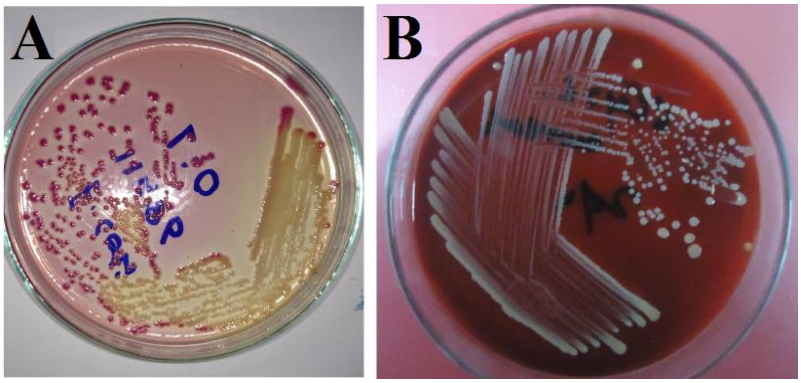

Fig. 2 Representative culture of the isolated bacterial strains on different nutrient media. "A" shows th pure culture of *E. coli* strain on MacConkey's agar and "B" indicates the pure culture of *S. aureus* strain on blood agar.

Table 2. Incidences of the bacterial species isolated from diseased aquarium fish

Name of Bacteria	Total bacterial number	Frequency (%)
Escherichia coli	09	34.62%
Aeromonas salmonicida	08	30.77%
Staphylococcus aureus	06	23.07%
Salmonella typhi	03	11.54%

The biochemical analysis of the isolated bacterial strains revealed that the *A. salmonicida* strain showed the positive reactions to oxidase, catalse, indole, lactose, manitol, glucose, sucrose and fructose test. While, coagulase test was recorded as negative (Table 1). The bacterial strains identified as *S. typhi* indicated the positive reactions to catalase, manitol, glucose; however, negative reactions were noted against oxidase, lactose, indole, lactose, sucrose, fructose and coagulase test. Most of the microbial species live normally in water; However, they cause infection due to injury, poor water quality, mishandling and contamination that leads to an increase in infections. The presence of *Aeromonas* and *Salmonella* species indicate a high risk factor to cause severe infections in fish. *Aeromonas* species have been shown to cause severe septicemia infections in fresh water fish worldwide (Austin, 2011). The biochemical analysis of the bacterial strains identified as *E. coli* **(Fig. 2a)** showed the positive reactions to catalase, indole, lactose, manitol, glucose and sucrose; while, negative reactions were noted against coagulase, oxidase and fructose. The bacterial strains identified as *S. aureus* isolated from diseased fish indicated the positive reactions to coagulase, catalase, lactose, mannitol, glucose, sucrose and fructose. The presence of enteric bacterial species such as *E. coli* in the diseased aqua fish shows the fecal contamination or pollution of water. While the presence of *S. aureus* **(Fig. 2b)** shows the contamination of aquarium due to direct human contact.

The infections in humans by fish pathogens are frequently due to direct contact with fish, dietary habits and the immune system of the exposed individuals. A large numbers of pathogens have been observed to cause infections in fish; however, potential bacterial pathogenic species associated with fish include *Vibrio* spp., *Mycobacteria*, *Salmonella* spp. *Aeromonads* etc. (Chattopadhyay, 2000; Lipp & Rose, 1997). The transmission of fish pathogens may also be due to cleansing the aquarium without protective gloves, handling fish ponds in tropical regions, preparation of fish dishes and processing fish in food industries (Notermans and Hoornstra, 2000). Fish has been recorded as the carrier of *Salmonella* bacterial spp. without any apparent sign and symptoms of the infection (Novotny *et al*., 2004). The contamination of the *Salmonella* spp. may be carried out from the terrestrial environment and fish can serve as the vector for these bacterial species (Novotny *et al*., 2004; Chatopadhay, 2000). *S. aureus* spp. associated with fish produce enterotoxins that may cause serious gastroenteritis infections after consumption of fish (Novotny *et al*., 2004).

Generally, outbreaks of food poisoning linked with fish and related food products are caused by the consumption of uncooked or insufficiently heat-treated fish spp., which may be contaminated with pathogenic bacteria through water environment or terrestrial environmental source. The determination of the bacterial pathogens in the present study in aqua fish would be significant in overcoming the spread of bacterial contamination in fish farms and to take necessary measures to safeguard public health.

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REFERENCES:

Austin, B. (2011). Taxonomy of bacterial fish pathogens. *Veterinary research, 42*(1).

Austin, B., and D. A. Austin, (1999). Bacterial Fish Pathogens: Diseases of Farmed and Wild Fishes. Springer-Praxis, Chichester, UK.

Adibe, N. C., and E. I. Eze, (2004). General laboratory techniques for tertiary institutions. Mike Social Publishers, Enugu, Nigeria.

Banu, G. R. (1996). Studies on the bacteria *Aeromonas* spp. In farmed fish and water in Mymensingh region. Thesis, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Chattopadhyay, G. N. and G. Dev, (2000). Potassium for balanced fertilization in fish ponds. *Fertiliser News, 45*(3), 61-64.

Cheesbrough, M. (1984). Medical Laboratory for tropical countries, 1st edn. Green Britain of the University Press Cambridge, UK.

Claucas, I. J. and A. R. Ward, (1996). Post-harvest fisheries development: A guide to handling, preservation, processing and quality. Charthan Maritime, Kent ME44TB, United Kingdom.

Eze, E. I. B. C. Echezona, and E. C. Uzodinma, (2011). Isolation and identification of pathogenic bacteria associated with frozen mackerel fish (Scomber scombrus) in a humid tropical environment. *Afr J Agri Res* 6(8), 1947-1951

FAO (1997). Review of the state of world aqua culture. FAO Fisheries Circular No. 886, Rev. 1. Rome, Italy.

Faruk, M. A. R., M. M. R. Sarker, M. J. Alam, and M. B. Kabir, (2004). fish diseases on rural freshwater aquaculture of Bangladesh. *Pakistan Journal of Biological Sciences*, *7*(12), 2086-2091.

Hedrick, R. P. (1998). Relationships of the host, pathogen and environment: Implications for diseases of cultured and wild populations. *J Aqua Anim Health,* 10 (2), 107-111.

Kvenberg, E. J. (1991). Non-indigenous bacterial pathogens, In: Microbiology of marine food products. (Eds). R. W. Donn, and H. Cameron, Van Nostrand Reinhold, New York, 263-291.

Lipp, E. K. and J. B. Rose, (1997). The role of seafood in food borne diseases in the United States of America. *Rev Sci Tech OIE,* 16, 620 -640.

Mhango, M., S. F. Mpuchane, and B. A. Gashe, (2010). Incidence of indicator organisms, opportunistic and pathogenic bacteria in fish. *Afr J Food Agric Nutr Dev*, 10(10), 4202-4218.

Novotny, L., L. Dvorska, A. Lorencova, V. Beran, and I. Pavlik, (2004). Fish: a potential source of bacterial pathogens for human beings. A review. *Veterinarni Medicina-UZPI (Czech Republic)*.

Notermans, S. and E. Hoornstra, (2000). Risk assessment of *Listeria monocytogenes* in fish products: some general principles, mechanism of infection and the use of performance standards to control human exposure. *International journal of food microbiology*, *62*(3), 223-229.

Paniagua C., O. Revero, J. Anguita, and G. Naharro, (1990). Pathogenicity factors and virulence for rainbow trout (*Salmo garidneri*) of motile *Aeromonas* spp. isolated from a river. *J Clinic Microbiol*, 28, 350-355.

Plumb, J. A. (1997). Trends in Fresh water Fish Disease Research. In: T. W. Flegel and I. H. MacRae (eds). Diseases in Asian Aquaculture III. Fish Health Section, Asian Fisheries Society, Manila, 35-47.

Rodrick, G. E. (1991). Indigenous pathogens: Vibrionaceae. In *Microbiology of Marine Food Products,* 285-300).

Slaby, B. M., R. E. Martin, and G. R. Ramsdell, (1981). Reproducibility of microbiological counts on frozen cod: A collaborative study. *J Food Sci*, 46(3), 716 719.

Smith, G. A. and I. Yoshida, (2000). Sustainable integrated Kysei nature farming EM technology and food security in Africa.

Sichewo, P. R., R. K. Gono, J. Muzondiwa, and W. Mungwadzi, (2014). Isolation and identification of pathogenic bacteria in edible fish: A case study of rural aquaculture projects feeding livestock manure to fish in Zimbabwe. *Int J Curr Microbiol App Sci*,3(11), 897-904.

Yiagnisis, M. and F. Athanassopoulou, (2011). Bacteria isolated diseased wild and farmed marine fish in Greece. *INTECH*.