



The Bacterial Microflora in the Fish Organs-A Public Health Aspect

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ABSTRACT

Water sample from Kengerilake (polluted) and Kommaghatta lake (fresh water) was collected to know their water quality. Fishes (*Glossogobius giuris* and *Labeo rohita*) were collected from fresh water and polluted water resp. The bacterial load in various tissues like skin, gills, intestine were analysed. The bacterial load in polluted water fish tissues were comparatively higher. Maximum bacterial counts were seen in intestine followed by gills and skin in both the analysed fishes. The bacterial genera *Pseudomonas*, *Acinetobacter*, *Aeromonas*, *Enterobacteriaceae*, *Micrococcus*, *Bacillus*, and *Lactobacillus* species were isolated. The findings of this study confirm the moderate occurrence of organisms belonging to *Enterobacteriaceae* family in the fish; therefore it should be monitored in epidemiological studies of human gastroenteritis causes considering the public health significance. The antibiogram of the isolated bacteria was done, multiple antibiotic resistances were observed in some isolated organisms.

Key words: Fish, antibiotic resistance, health, Kengerilake, Kommaghatta lake

1. INTRODUCTION

Fish are the primary sources of protein for humans in many parts of the World; this is especially true in most of the developing countries. Fish industry has declined due to series of factors which include over fishing, loss of fish habitats and environmental pollution. Disease outbreaks (infectious and non-infectious) resulting in high mortalities occurs more often when fish were held under relatively crowded and confined conditions. Mass mortality of healthy fish occurs even under good environmental conditions when an infectious agent is accidentally introduced in the culture system. The presence of microorganisms in internal fish organs could indicate the breakdown of immunological defense mechanisms. Although there is no certainty about the existence of strictly aquatic bacteria, the most widespread opinion among the various authors is that the majority of the bacteria found in aquatic environments is of soil origin and carried into the water due to rain or to accidental introduction of natural or a direct consequence of human activity. However, every water mass has its bacterial community, although these communities may vary greatly in both the present groups and the number of cells [1]. Microorganisms are widely distributed in nature and are found mostly in natural water. In urban and densely populated rural areas, the microbiological quality of fresh water is frequently threatened by contamination with untreated domestic wastewater [2]. Antibiotics resistant microorganisms may be associated with reduced penetration of the antibiotic into the cell, or can result from active processes such as changes in the

transport of those compounds into or from bacterial cells [3].

2. MATERIALS AND METHODS

The fish *Glossogobius giuris* and *Labeo rohita* (HAM) were randomly collected for the period of three years i.e. January 2005 to December 2007 using gill nets (10mm) and immediately transported to the laboratory where they were sacrificed. The organs gills, intestine and skin were aseptically removed. About 250mg of the above tissue was homogenized in 5ml sterile distilled water and serial dilution of the sample was made with distilled water to the level of 10^{-5} . 0.1ml of aliquots ranging from 10^{-3} to 10^{-5} dilution were plated onto plate count agar and incubated for 48-72 hours at 22 °C. After incubation, the total number of CFU/ml as well as the CFU/ml corresponding to each plate were determined and representative colonies of different morphological types present in each plate were isolated and subjected to biochemical tests. Isolated bacteria were subjected to antibiotic sensitivity test by Kirby-Bauer disc diffusion method on Muller Hinton Agar [4].

2.1 Concentration of Antibiotic disc

Ampicillin (10 µg), Carbenicillin (100 µg), Nalidixic acid (30 µg), Erythromycin (15 µg), Tetracycline (10 µg) and Penicillin (2 µg).

3. RESULTS AND DISCUSSION

At the time of sampling the water temperature ranged from 25-28.5 °C, pH and DO ranged 7.5-8.0 and 5.0-6.2mg/L resp. in Kommaghatta lake while

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Table 1: Showing the bacterial counts in various tissues of fresh water and polluted water fish *Glossogobius giuris* and *Labeo rohita* resp.

Tissue	Name of Fish	10 ⁻³	10 ⁻⁴	10 ⁻⁵
Skin	<i>Glossogobius giuris</i>	21.5*10 ²	10.2*10 ²	8.3*10 ²
	<i>Labeo rohita</i>	32.2*10 ²	27*10 ²	17.8*10 ²
Gills	<i>Glossogobius giuris</i>	23.5*10 ²	15.8*10 ²	14.1*10 ²
	<i>Labeo rohita</i>	28.1*10 ²	21*10 ²	12.6*10 ²
Intestine	<i>Glossogobius giuris</i>	59.5*10 ²	38.6*10 ²	23.2*10 ²
	<i>Labeo rohita</i>	32*10 ³	27*10 ³	10.4*10 ³

in Kengeri (Doddakere) lake the water temperature ranged from 32-35.5 °C, pH and DO ranged 8.0-8.5 and 3.2- 4.0mg/L resp. Quantitative composition of the viable bacteria in the various organs of the freshwater fish *Glossogobius giuris* and polluted water fish *Labeo rohita* is summarized in table1. Observation of the *Glossogobius giuris* revealed the population count was maximum in the intestine 59.5*10² colonies/ g wet weight of the tissue, gill showed 23.5*10² colonies/ g wet weight of the tissue while the skin showed 21.5* 10² colonies/ g wet weight of the tissue in 10⁻³ dilution. Minimum bacterial colonies count were recorded in 10⁻⁵ dilution, showing 8.3*10² colonies/ g wet weight of the tissue in skin, 14.1*10² colonies / g wet weight of the tissue in gill and 23.2*10² colonies/ g wet weight of the tissue in intestine. While observation of the *Labeo rohita* revealed maximum population count in the intestine 32.2*10³ colonies/ g wet weight of the tissue, the skin showed 32.2* 10² colonies/ g wet weight of the tissue while gill showed 28.1*10² colonies/ g wet weight of the tissue in 10⁻³ dilution. Minimum bacterial colonies count were recorded in 10⁻⁵ dilution, showing 12.6*10² colonies/ g wet weight of the tissue in gill, 17.8*10² colonies / g wet weight of the tissue in skin and 10.4*10³ colonies/ g wet weight of the tissue in intestine. The qualitative composition of bacteria genera isolated from the skin, gill and intestine of fish *Glossogobius giuris* and *Labeo rohita* is indicated in Fig. (1-2). The Antibiotic susceptibility pattern of the isolated bacteria to six antimicrobial substances are shown in Fig. (4). *Pseudomonas* showed high resistance followed by Enterobacteriaceae and *Micrococcus*. Low resistance was shown by *Lactobacillus*. The resistance of bacteria to particular drugs is represented in Fig. (6). Penicillin was highly resistant followed by Nalidixic acid while Erythromycin was highly susceptible to the isolated bacteria. The bacterial infection in fishes can lead to poor fish health and high mortality rate [5]. Bacterial infection of fish and fish products may influence human health directly by inducing diseases or indirectly through residues of antimicrobial growth agents used to treat such

infections in fish. Human infections that may be caused by bacteria in fish include food poisoning and gastroenteritis. Depression of humoral immunity may lead to increased susceptibility of fish to infection [6]. Further food products prepared from such fish's results in spoilage pointed out, the occurrence of an infection in a fish could not be necessarily an abnormal event nor would it lead to a disease situation. Moreover, under natural conditions, most infectious agents coexist with their host without causing significant disease. However stress factors such as overcrowding found in aquaculture facilities are frequently blamed for the dissemination of infections and also for the occurrence of many diseases outbreaks [6]. From the organs of the fish included in this study bacterial strains belonging to *Pseudomonas*, *Acinetobacter*, *Aeromonas*, Enterobacteriaceae, *Bacillus*, *Lactobacillus*, and *Micrococcus* were isolated. The presence of bacteria in the internal organs has been often observed by [7]. The population density of fishes usually varies quantitatively and qualitatively in different organs such as intestine, skin and gill. Highest bacterial load is seen in intestine followed gill and skin of the fish *Glossogobius giuris* while in the fish *Labeo rohita* highest bacterial load is seen in intestine followed skin and gill (figure 1&2). The composition of the intestinal flora is related in varying degree to the level of contamination of water and food in the environment. Therefore, the higher yield in respect of intestine might be due to bacterial replication Our result is similar to the findings of [8]. The composition and biomass of the intestinal microflora vary depending on specific features of water bodies, the intensity of feeding and some other factors [9]. The variation in the density on the intestine can be attributed to motility factor. The growth promoting and inhabiting substance of the digestive system in fish determine the dominance of particular group of bacteria as also suggested by [10]. The bacteria load is comparatively low in the gill than other organ of freshwater fish *G giuris*. The low number suggests that under normal circumstances this microflora is

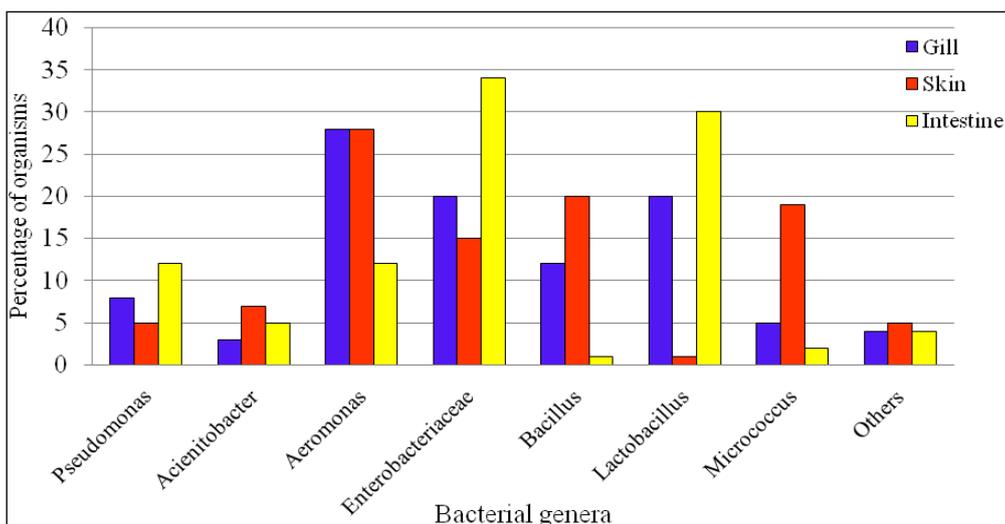


Figure 1. Qualitative composition of bacteria from various tissues of the fish *Glossogobius giuris*.

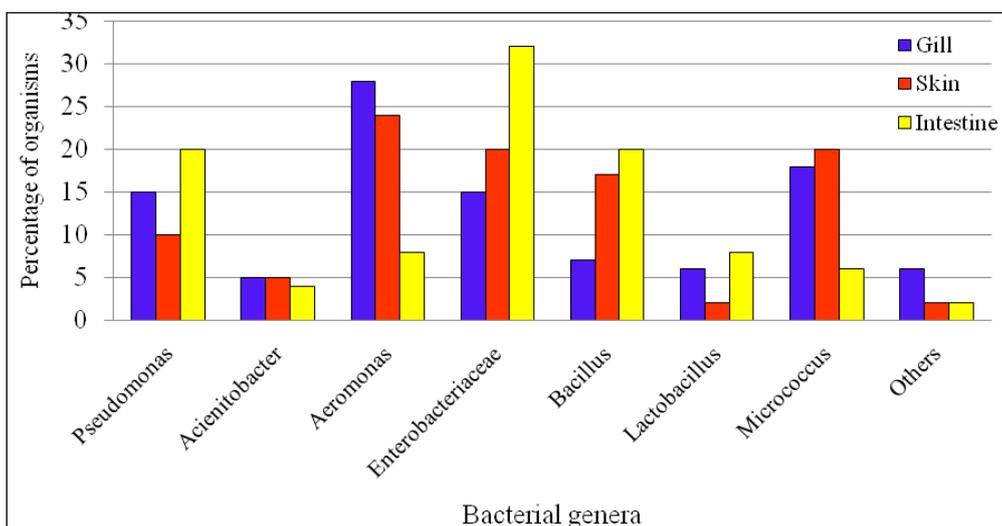


Figure 2. Qualitative composition of bacteria from various tissues of the fish *Labeo rohita*.

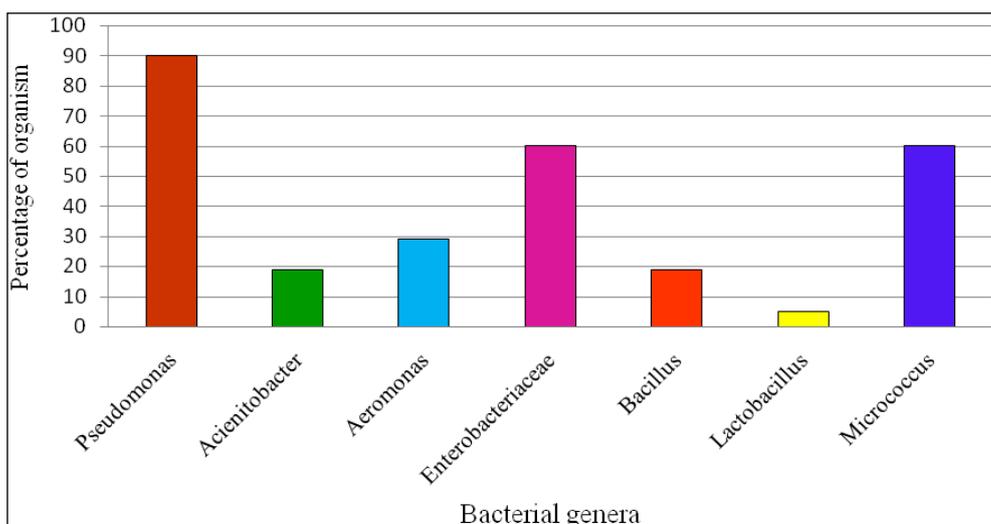


Figure 3. Various bacterial genera isolated from fish (in%).

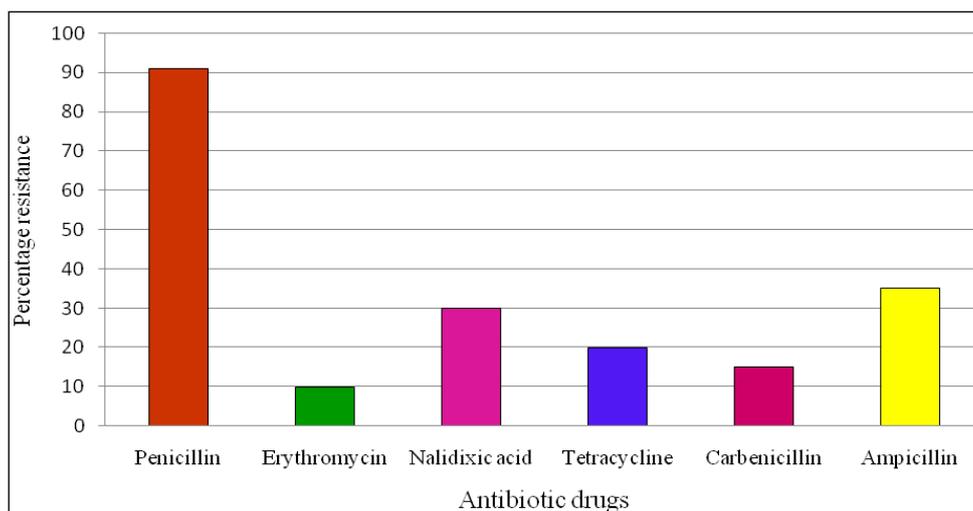


Figure 4. Antibiotic resistance of the actinial genera to antibiotic drugs (in%).

unlikely to alter significantly the physical and chemical environment of the gill.

As [11], has suggested that, chemical environment is important for these bacteria be located in the functional surface area of the gills (Gill lamellar). The means by which the fish is able to keep the bacterial number on the skin low are probably tissue integrity or tissue or secretory bactericidins. Another nonspecific mechanism is likely to be production of mucus. This latter mechanism has an inimical effect on the fish since it is likely to increase the diffusion distance for ions, water and gases. Circulating antibodies and complement activity are probably important protective mechanisms [12]. The polluted water fish showed higher count in skin than the freshwater fish this may be due to the higher load of microorganisms in the polluted waterbody.

Bacteria inhabiting the fishes are characterized by multiple antibiotic resistance (figure 3). Excessive use of antibiotic substance in aquaculture has led to increase of resistance rates [13]. Several different kinds of antibiotics are used in fish farms to control bacterial and fungal diseases by incorporation into the feed [14], resistance can also be associated with the production of enzymes that modify and inactivate antibiotics [15].

The present study has displayed that bacteria isolated from fish have a high degree of resistance to most of the antibiotic under investigation (figure 4). Such a high level of resistance might result from the fact that about 50% of the bacteria have plasmids where antibiotic fighting genes are assembled, which protect the microorganisms from being affected by antibiotics [16]. The R plasmid can be transferred between various strains of

bacteria through conjugation and transformation processes [14]. There are four classic mechanism of resistance specified by plasmids: inactivation impermeability, by passes and altered target site, all occur in aquatic environments. Also, intracellular binding seems to be a valid mechanism for immobilizing an inhibitor [17].

According to [18] the antibiotic resistance of bacteria depends on their taxonomic position rather than their origin because particular taxonomic groups of bacteria have similar genetic information with codes for resistance. According to [14] multiple resistances may be coded on plasmids, mutational events or on even smaller and mobile genetic elements called transposons. Transposons are able to move between plasmids and bacterial chromosomes. According to [19] chemical treatment, grain based artificial diet and accumulation of organic wastes in the form of excess feed, excreta and dead plankton have residual influence on the antibiotic resistance of aquatic bacteria.

4. CONCLUSION

The results presented in the study prove that antibiotics are significant selection factor and probably are important in regulating the composition of bacterial communities in fishes. The present investigation disagrees with prophylactic uses of antibiotic in the aquaculture systems because of the development of resistant bacteria. It would be impossible to treat diseases of aquatic organism caused by resistant bacteria and also human diseases with antibiotic-resistance bacteria of animal origin. Hence, the present study the use of antibiotic to treat diseases, but not as prophylactic use.

5. REFERENCES

- Sousa, J. A.; Eiras, J. C.; Alexandrino, A. C.; Ishikawa, C. M.; Ranzani-Paiva, M. J. T. and Eiras, A. C. (1996) a. Preliminary bacteriological evaluation of semi-intensively farmed fish (*Piaractus mesopotamicus*, *Prochilodus scrofa* and *Colossoma macropomum*) in São Paulo State, Brazil. *Revista UNIMAR*, **18**, 299-309.
- Griesel, M. and Jagals, P. 2002. Faecal indicator organisms in the Renoster Spruit system of the Modder-Riet River catchment and implication for human users of the water. *Water SA*, **28**, 227-234.
- Hermansson, M., Ones, G. W. and Kjelleberg, S. (1987) Frequency of antibiotics and heavy metal resistance, pigmentation and plasmids in bacteria of marine air-water interface. *Applied and Environmental Microbiology*, **53**, 2338.
- WHO. (1992). World health organization. Geneva.
- Ter Hofte., Barrom B. and Arend, P. (1997). Frick hingers: Gesund wie der Fisch im Wasser ? . 192.
- Madhu, P. Saxena., Paviter Kaur, H.M. Saxena and Kapur-Ghai, J. (2006) Resistant bacteria isolated from Fish died on exposure to chromium. *Journal of Fisheries and Aquatic Science*, **1**, 209-212.
- Mc Vicar, A.H. (1997) Interactions of pathogens in aquaculture with wild fish population. *Bulletin of European Association of Fish Pathology*, **17**, 197-200.
- Toranzo, A.E., Novoa, B., Romalde, J.L., Nunez, S., Devesa, S., Marino, E., Silva, R., Martinez, E., Figueras, A. and Barja, J.L. (1993) Microflora associated with healthy and diseased turbot (*Scophthalmus maximus*) from three farms in Northwest Spain. *Aquaculture*, **114**, 189-202.
- Venugopalan, U.K., R. Nandakumar and Ramesh, A. (1985) Microflora of pond cultured milk fish, *Chanos chanos* (Forsk.). *Proceedings of the symposium on Harvest Technology*, 680-688.
- Austin, B. (2002). The Bacterial Microflora of Fish, *Science World Journal*. **2**,558-572.
- Mercy Abraham. (1989) Studies on the nutritional grouping of bacteria in the gut of culturable freshwater fish, *Labeo rohita*. M.Phil. dissertation Kerela University.
- Brock, T.D. (1966) Microbial ecosystems. In principles of microbial ecology. Englewood cliffs: Prentice-Hall, Inc.
- Snieszko, S. F. (1970) Bacterial gill disease of freshwater fishes. Fish Disease Leaflet 19. Washington: United States Department of the Interior, *Bureau of Sport Fisheries and Wildlife*, 464.
- Aoki, T. (1992). Present and future problems concerning the development of resistance in aquaculture- Chemotherapy in Aquaculture: from theory to reality. Office international des Epizooties. Michel, C., Alderman, D.J., editors. Paris, 254-262.
- Herwig, R.P., Gray, J.P and Weston, D.P. (1997) Antibacterial resistant bacteria in surficial sediments near salmon net cage farms in Puget sound Washington. *Aquaculture* 149-263.
- Koch, A.L. (1981) Evolution of antibiotic gene function. *Microbiological Reviews*. **54**, 355.
- Kobori, H., Sullivan, C.W., Shizuya, H. (1984) Bacterial plasmids in Antarctic natural microbial assemblages. *Applied and Environmental Microbiology*, **48**, 515.
- Foster, T.J. (1983) Plasmid- Determined resistance to antimicrobial drugs and toxic metal ions in Bacteria. *Microbiological Reviews*, **47**. 361.
- Nemi, M., Sibakov, M., Niemela, S. (1983) Antibiotic resistance among different species of Fecal Coliforms isolated from water samples *Applied and Environmental Microbiology*, **45**, 79.