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Research Paper

Pathogenic Bacteria found in five Fish species sold in Bittan fish market of Bhopal

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Scope of Study:
The present study was conducted for microbiological assessment of five available fish species collected from Bittan markets of Bhopal during different seasons of the year For this, Total Bacterial Counts (TBC), Total Coliform (TC), Fecal Coliform (FC) and the occurrence of Salmonella and Vibrio spp., were determined by using serial dilution and spread plate technique. Bacterial densities of these fishes were higher than the acceptable limits. Findings of the present study suggest that these fishes may act as reservoirs of harmful pathogenic species which creates many dangerous diseases such as cholera, typhoid etc. and the consumers should be careful about the qualities of fishes.
Key words: *Pathogenic bacteria Human consumption ,Fish markets,*

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I. INTRODUCTION

Fish is one of the best sources of proteins, vitamins, minerals and essential nutrients required for supplementing both infant and adult diets (Abdullahi 2001). Fish are extremely susceptible to microbial contamination because of their soft tissues and aquatic environment. Contamination results mainly from rupturing of fish intestine during poor processing or unhealthy washing. Millions of bacteria, many of them potential spoilers, are present in the surface slime, on the gills and in the intestines of live fish, although the flesh itself is normally sterile. Bacterial growth and invasion on the fish are prevented by the body's natural defense system during life but after death the defense system breaks down and the bacteria multiply and invade the flesh (Abolagba and Uwagbai, 2011). Microbial action has been known to play a large part in the spoilage of fish (Eyo, 2001).

Fishes become contaminated in various ways. However, the type of microorganism associated with a particular fish depends on the waterbodies it was found (Thatcher and Clark, 1973; Clucas and Ward, 1996). Fishes which live in the polluted waterbodies can easily intake these bacteria while feeding along with contaminated aquatic foods. Phytoplankton such as Anabaena variabilis and zooplankton like copepode which are the reservoir of Vibrio and Salmonella as long term and short term, respectively and fish could easily feed on them and act as a reservoir or vector for the Vibrio and Salmonella. Thus, it is important to determine the relationship of occurrence of Vibrio and Salmonella with the feeding habits.

Pathogenic bacteria associated with fish and fishery products can be categorized into three general groups: (1) Bacteria (indigenous bacteria) that belong to the natural microflora of fish (Clostridium botulinum, pathogenic Vibrio spp., Aeromonas hydrophila), Enteric bacteria that are present due to fecal contamination (Salmonella spp., Shigella spp., pathogenic Escherichia coli, Staphylococcus aureus) Bacterial contamination during processing, storage or preparation for consumption (Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Clostridium perfringens, Salmonella spp.) (Lyhs, 2009).

Fish is also contaminated during post-harvest activities such as poor standards of hygiene and sanitation, inadequate processing, unhygienic condition of market etc. Most of persons associated with the culture and marketing of marine fishes in Bangladesh are not well educated and having no proper knowledge about hygiene and sanitation which lead to contamination of fishes by microbes. The present study was therefore aimed to estimate the microbial load in different organs of fishes.

II. MATERIALS AND METHODS

Bhopal, the city of lakes, is situated at 23˚16'N latitude and 77˚26'E longitude. It possesses a number of small and large water bodies, which in addition of promoting aquaculture activities also add to the scenic beauty of the city. However, these water bodies are under great environmental stress due to pollution from various sources. Since last few decades, private entrepreneurs have been using these water bodies for the production of fish. Generally the polyculture of Indian and exotic major carps is being practiced in these water bodies. Incidences of various health hazards have been observed in these fishes. Fishes, from these water bodies, with high microbial load reach the market where the prevailing improper handling and unhygienic conditions make them unfit for human consumption. Bittan fish market of Bhopal was selected for the present study.

Bittan fish market

It is situated about 12 km away from Bhopal railway station. There is no shelter for this market and the fishes are sold on road side under open sky. This fish market is run under the control of Bhopal Municipal Corporation. Besides freshwater fishes, considerable quantity of marine fishes, crabs, prawns, roasted fishes, sun dried fishes and salted fishes are marketed here. On the periphery of this market is situated chicken market. The condition of this market is also extremely unhygienic.

Bittan fish market

Fish sample : Five different fishes namely *Oreochromis mossambica, Rohtee cotio, Chanda nama Mastacembelus armatus. Hemirhamphus far* were selected for the present stud

Processing of samples:

At first the samples were washed with sterile Phosphate Buffer Saline (PBS) to remove sand, detritus as well as microorganisms attached to the surface of fish. Then the skin, gill and gut samples from each samples were collected aseptically and homogenized separately Each of the five tubes were filled with nine milliliters of PBS solution aseptically and 1 g of homogenized tissue of each sample was mixed with 9 mL PBS solution of first tube to prepare 10-1 dilution. The 1 mL was taken from the first tube and mixed to the second test tube to prepare 10-2 dilutions. The 10-3 to 10-5 dilutions were prepared by this subsequent serial dilution technique

Inoculation of plates for enumeration of bacterial load:

The l00 µL from diluted solution of each sample were transferred to culture media containing petri-dish and inoculated using spread plate method for bacteriological analysis. For enumeration of total bacteria in sample fishes, nutrient agar media was used and after inoculating incubated at 37°C for 18-24 h in the incubator. Salmonella spp., were counted on SS plate after 18-24 h of incubation and colorless, transparent, with a black center colonies were considered as Salmonella.

Observations

Fishery products which are of great importance for human nutrition worldwide and provide clear health benefits (Kromhout et al., 1985) can act as a source of food borne pathogens. If the bacterial loads of fishes are

greater than acceptable limit of bacterial pathogens in fishes, those fishes are unacceptable and pose a potential risk to public health.

Oreochromis mossambica

Table – 1: Showing bacterial flora in different tissues of *Oreochromis mossambica*				
Bacteria	skin	Gills	Muscles	Intestine
Aeromonas hydrophila	$8.0x10^3$CFU/g	$9.5x10^3$CFU/g	$2.0x10^3$CFU/g	$17.5x10^3$CFU/g
Pseudomonas fluorescens	$10.5x10^3$CFU/g	$10.0x10^3$CFU/g	$4.0x10^3$CFU/g	$10.4x10^3$CFU/g
Streptococcus iniae	$2.0x10^3$CFU/g	$1.0x10^3$CFU/g	$1.0x10^3$CFU/g	$3.0x10^3$CFU/g
Staphylococcus aureus	$4.0x10^3$CFU/g	$5.5x10^3$CFU/g	$5.0x10^3$CFU/g	$9.0x10^3$CFU/g
Salmonella sp.	$4.0x10^3$CFU/g	$10.0x10^3$CFU/g	$1.0x10^3$CFU/g	$7.0x10^3$CFU/g

Table – 2: Showing bacterial flora in different tissues of *Rohtee cotio*				
Bacteria	skin	Gills	Muscles	Intestine
Aeromonas hydrophila	$8.0x10^3$CFU/g	$7.5x10^3$CFU/g	$2.0x10^3$CFU/g	$10.5x10^3$CFU/g
Pseudomonas fluorescens	$10.5x10^3$CFU/g	$10.0x10^3$CFU/g	$4.0x10^3$CFU/g	$10.4x10^3$CFU/g
Streptococcus iniae	$4.0x10^3$CFU/g	$1.0x10^3$CFU/g	$2.0x10^3$CFU/g	$3.0x10^3$CFU/g
Staphylococcus aureus	$2.0x10^3$CFU/g	$5.5x10^3$CFU/g	$6.0x10^3$CFU/g	$1.0x10^3$CFU/g
Salmonella sp.	$2.0x10^3$CFU/g	$3.0x10^3$CFU/g	$1.0x10^3$CFU/g	$8.0x10^3$CFU/g

Table – 3: Showing bacterial flora in different tissues of *Chanda nama*				
Bacteria	skin	Gills	Muscles	Intestine
Aeromonas hydrophila	$4.0x10^3$CFU/g	$6.5x10^3$CFU/g	$1.0x10^3$CFU/g	$6.5x10^3$CFU/g
Pseudomonas fluorescens	$10.5x10^3$CFU/g	$10.0x10^3$CFU/g	$3.0x10^3$CFU/g	$10.4x10^3$CFU/g
Streptococcus iniae	$2.0x10^3$CFU/g	$1.0x10^3$CFU/g	$2.0x10^3$CFU/g	$2.0x10^3$CFU/g
Staphylococcus aureus	$1.0x10^3$CFU/g	$0.5x10^3$CFU/g	$1.0x10^3$CFU/g	$1.0x10^3$CFU/g
Salmonella sp.	$1.0x10^3$CFU/g	$4.0x10^3$CFU/g	$1.0x10^3$CFU/g	$6.0x10^3$CFU/g

Table – 4: Showing bacterial flora in different tissues of *Mastacembelus armatus*				
Bacteria	skin	Gills	Muscles	Intestine
Aeromonas hydrophila	$7.0x10^3$CFU/g	$6.5x10^3$CFU/g	$2.0x10^3$CFU/g	$6.5x10^3$CFU/g

Pseudomonas fluorescens	$10.5x10^3$CFU/g	$6.0x10^3$CFU/g	$1.0x10^3$CFU/g	$10.4x10^3$CFU/g
Streptococcus iniae	$1.0x10^3$CFU/g	$1.0x10^3$CFU/g	$1.0x10^3$CFU/g	$2.5x10^3$CFU/g
Staphylococcus aureus	$2.0x10^3$CFU/g	$1.5x10^3$CFU/g	$12.0x10^3$CFU/g	$1.0x10^3$CFU/g
Salmonella sp.	$10.0x10^3$CFU/g	$8.0x10^3$CFU/g	$3.0x10^3$CFU/g	$6.0x10^3$CFU/g

Table – 5: Showing bacterial flora in different tissues of *Hemirhamphus far*

Bacteria	skin	Gills	Muscles	Intestine
Aeromonas hydrophila	$4.0x10^3$CFU/g	$7.5x10^3$CFU/g	$2.0x10^3$CFU/g	$9.5x10^3$CFU/g
Pseudomonas fluorescens	$10.5x10^3$CFU/g	$10.0x10^3$CFU/g	$3.0x10^3$CFU/g	$10.4x10^3$CFU/g
Streptococcus iniae	$6.0x10^3$CFU/g	$5.0x10^3$CFU/g	$1.0x10^3$CFU/g	$10.5x10^3$CFU/g
Staphylococcus aureus	$1.0x10^3$CFU/g	$3.5x10^3$CFU/g	$1.0x10^3$CFU/g	$5.0x10^3$CFU/g
Salmonella sp.	$2.0x10^3$CFU/g	$1.0x10^3$CFU/g	$1.0x10^3$CFU/g	$4.0x10^3$CFU/g

III. CONCLUSION

Food borne pathogens are a growing concern for human illness and death (Losito et al., 2012). According to the guideline of ICMSF, acceptable limit of total bacterial counts for giant prawns and white fish are 106 and 5×105 CFU g-1, respectively. Therefore, the bacterial loads found in this study for different fishes were beyond the standard value suggested by ICMSF (1982) which indicate their unacceptability as food from public health point of view. Moreover, these fishes act as reservoirs of human pathogens which are a serious threat to the fish consuming community. Unhygienic fish handling practices of these infected fishes such as chances of cross contamination via kitchen utensils or by handling and inadequate cooking may further contribute to the spread of these pathogens. Hence, we are in urgent need to implement programmes such as HACCP as a part of Good Manufacturing Practices (GMP) and Sanitation Standard Operating Procedures (SSOP) to monitor the quality of the fishes (Mandal et al., 2011)

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Mastachembelus armatus

Hemirhamphus far

rohtee cotio

Chanda nama