Assessment of Bacterial flora in seven fish species sold in Govindpura market of Bhopal

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Scope of Study:

A study was carried out to investigate the bacterial load and the occurrence of potential pathogens from healthy seven fishes sold in Govindpura fish market. The skin, gills, muscles and intestine of the fish were examined. The samples were collected by swabbing aseptically over the areas of the fish and then processed for bacterial count, followed by isolation and identification of potential pathogens. Microbial load analysis revealed that the total viable count of bacteria ranged from 1.5 – 10.5 x 103 cfu/ g. The results show that the bacterial load of samples collected from Govindpura fish markets falls within the standard microbiological acceptable limit. The biochemical identification tests of the bacterial isolates confirm the presence of the following bacteria: Aeromonas hydrophilla, Pseudomonas fluorescens,Shigella sp.,Salmonella spp.,Vibrio parahaemolyticus, Straphylococcos,and Streptococos This study therefore reveals the bacterial load of these fishes with a view to provide information on the state of the environment and the poor hygienic conditions of the markets.

Key words:

Aeromonas hydrophilla, Pseudomonas fluorescens, Shigella sp., Salmonella spp., Vibrio parahaemolyticus, Straphylococcos, and Streptococos innie.

Introduction

Fish is a major food in prodigious demand throughout the world. Fish protein is healthier and safer than meat (animal protein) because it contains lower cholesterol (Leisner et al., 2001). As a result, there is a significant increase in the demand for fish being the cheapest source of animal protein (Ladipo et al., 1981; Roquia et al., 2014). The benefit of fish is its easy digestibility and high nutritional value. These important attributes make the commodity readily susceptible to microbial attack predominantly bacteria. Despite the enormous benefit of fish farming, fish diseases have been one of the threat and encompassing challenges to the aquaculture industry, of which bacterial fish diseases account for 55% among other fish pathogens. Bacterial fish diseases have become a significant concern to fish farming, especially with warm water temperature (Reddy et al., 1994). Different bacterial species have been reported pathogenic to fishes
including Aeromonas hydrophilla, Staphylococcus aureus, Vibrio parahaemolyticus and Streptococcus innie. regui et al., 2012; Zhang et al., 2018). The bacteria are transmitted by fish that have made contact with other diseased fish. Bacterial fish disease and infections are very common and one of the most challenging health problems to deal with. However, bacteria can enter the fish body through the gills or skin or it can stay on the surface of the body to cause infection when environmental conditions are not favorable (Roquia et al., 2014).

Fish have been reported to cause 24% of food borne outbreaks and 6% of all food poisoning/illness (CDC, 2013). Levels of bacterial pathogens in fish have been linked to environmental contamination and poor handling prior to their arrival (Ahmed, 2019) Bacteria associated with fishes could be transmitted to persons in contact and result in food borne illness (Ahmed, 2019). Therefore, monitoring of bacteria associated with both wild and farmed fish is essential to public health because they help to understand fish pathogens epidemiology and it as well demonstrate how they can transmit food borne infections from fish to humans. Disease outbreaks in fish rearing vessels can spread very rapidly and one has to first identify the type of disease before action can be taken. This study was undertaken to screen bacteria associated with fishes sold in Govindpura fish market and to determine their microbiological food safety of the fish.

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 to 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 the 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. Govind pura fish market of Bhopal was selected for the present study.

Govindpura fish market

This fish market is situated about 7 km away from Bhopal railway station. It also comes under the control of BHEL administration. This market also provided cemented platforms without any roofing. Both freshwater and marine fishes are marketed here. The condition of this market is not good and hygienic.

Bacterial analysis

Swabs were aseptically taken from the gills, muscles, skin and intestine of the sampled fish with a clean sterile swab stick. The swabs were immersed into a 100 ml conical flask containing buffered peptone water (0.1%) (Merck, Germany). After overnight incubation for 18 hours at 37°C, 1 ml was transferred for further analysis from the peptone water to Tryptone soy agar. Ten- fold serial dilutions (10-5) were carried out, and viable bacterial counts of the samples were obtained.

Bacterial enumeration

Enumeration of bacteria was carried out using a spread plate method. The plates containing Tryptone Soy agar (Himedia, India) were inoculated and incubated at 37°C for 24 hours after which the discrete colonies were observed and sub-cultured to get pure colonies of the isolates. Pure colonies of the isolates were further sub-cultured on Eosin methylene blue (EMB) agar, Salmonella-Shigella agar, Thiosulfate Citrate bile salts sucrose (TCBS) agar and Mannitol salt agar. The bacteria isolates were identified using some parameters such as gram staining reaction, cultural and morphological characteristics, and biochemical tests were also performed for presence of the suspected microorganism by their reactions to the tests according to Bergey's manual of determinative bacteriology, 8th Edition.

Observations

During present investigation carried out on seven species of fishes namely Sardinella sirm, Eutropiichthys vacha, Labeo gonius, Nemipterus japonicus, Notopterus notopterus, Hilsa toli and Mystus cavacius sold in Govindpura fish market of Bhopal, pathogenic bacteria viz. Aeromonas hydrophila, Pseudomonas fluorescens, Streptococcus iniae, Staphylococcus aureus, Shigella sp., Salmonella sp., Vibrio parahaemolyticus, have been isolated and identified

Hilsa toli

Nemipterus japonicus

Sardinella sirm

Mystus cavacius

Notopterus notopterus

Eutropiichthys vacha

Labeo gonius

Table – 1: Showing bacterial flora in different tissues of *Sardinella sirm*				
Bacteria	**Skin**	**Gills**	**Muscles**	**Intestine**
Aeromonas hydrophilla	$6.5x10^3$CFU/g	$4.5x10^3$CFU/g	$3.5x10^3$CFU/g	$6.0x10^3$CFU/g
Pseudomonas fluorescens	$3.5x10^3$CFU/g	$1.5x10^3$CFU/g	$2.5x10^3$CFU/g	$1.5x10^3$CFU/g
Streptococcus iniae,	$10.5x10^3$CFU/g	$1.5x10^3$CFU/g	$1.5x10^3$CFU/g	$6.5x10^3$CFU/g
Staphylococcus aureus	$3.5x10^3$CFU/g	$2.5x10^3$CFU/g	$6.5x10^3$CFU/g	$10.5x10^3$CFU/g
Shigella sp.,	$1.5x10^3$CFU/g	$1.5x10^3$CFU/g	$1.5x10^3$CFU/g	$4.5x10^3$CFU/g
Salmonella sp.,	$3.5x10^3$CFU/g	$1.5x10^3$CFU/g	$1.5x10^3$CFU/g	$4.5x10^3$CFU/g

Table – 2: Showing bacterial flora in different tissues of *Eutropiichthys vacha*				
Bacteria	**Skin**	**Gills**	**Muscles**	**Intestine**
Aeromonas hydrophilla	$4.5x10^3$CFU/g	$2.5x10^3$CFU/g	$1.5x10^3$CFU/g	$6.0x10^3$CFU/g
Pseudomonas fluorescens	$2.5x10^3$CFU/g	$1.5x10^3$CFU/g	$1.5x10^3$CFU/g	$4.5x10^3$CFU/g

Streptococcus iniae,	2.5x10^{3}CFU/g	1.5x10^{3}CFU/g	1.5x10^{3}CFU/g	3.5x10^{3}CFU/g
Staphylococcus aureus	1.5x10^{3}CFU/g	2.5x10^{3}CFU/g	2.5x10^{3}CFU/g	5.5x10^{3}CFU/g
Shigella sp.,	10.5x10^{3}CFU/g	1.5x10^{3}CFU/g	1.5x10^{3}CFU/g	4.5x10^{3}CFU/g

Salmonella sp., 5.5x10^{3}CFU/g 1.5x10^{3}CFU/g 1.5x10^{3}CFU/g 2.5x10^{3}CFU/g

Table – 3: Showing bacterial flora in different tissues of *Labeo gonius*

Bacteria	Skin	Gills	Muscles	Intestine
Aeromonas hydrophilla	2.5x10^{3}CFU/g	4.5x10^{3}CFU/g	1.5x10^{3}CFU/g	2.0x10^{3}CFU/g
Pseudomonas fluorescens	6.5x10^{3}CFU/g	4.5x10^{3}CFU/g	1.5x10^{3}CFU/g	3.5x10^{3}CFU/g
Streptococcus iniae,	3.5x10^{3}CFU/g	1.5x10^{3}CFU/g	1.5x10^{3}CFU/g	8.5x10^{3}CFU/g
Staphylococcus aureus	1.5x10^{3}CFU/g	2.5x10^{3}CFU/g	2.5x10^{3}CFU/g	5.5x10^{3}CFU/g
Shigella sp.,	10.5x10^{3}CFU/g	1.5x10^{3}CFU/g	1.5x10^{3}CFU/g	9.5x10^{3}CFU/g

Salmonella sp., 5.5x10^{3}CFU/g 1.5x10^{3}CFU/g 1.5x10^{3}CFU/g 4.5x10^{3}CFU/g

Table – 4: Showing bacterial flora in different tissues of *Nemipterus japonicus*

Bacteria	Skin	Gills	Muscles	Intestine
Aeromonas hydrophilla	3.5x10^{3}CFU/g	2.5x10^{3}CFU/g	1.5x10^{3}CFU/g	6.0x10^{3}CFU/g
Pseudomonas fluorescens	5.5x10^{3}U/g	1.5x10^{3}U/g	1.5x10^{3}U/g	4.5x10^{3}U/g

Streptococcus iniae,	9.5x10^{3}CFUg	1.5x10^{3}CFU/g	1.5x10^{3}CFU/g	5.5x10^{3}CFUg
Staphylococcus aureus	7.5x10^{3}CFU/g	2.5x10^{3}CFU/g	2.5x10^{3}CFU/g	5.5x10^{3}CFU/g
Shigella sp.,	10.5x10^{3}CFU/g	1.5x10^{3}CFU/g	1.5x10^{3}CFU/g	4.5x10^{3}CFU/g
Salmonella sp.,	10.5x10^{3}CFU/g	1.5x10^{3}CFU/g	1.5x10^{3}CFU/g	10.5x10^{3}CFU/g

Table – 5: Showing bacterial flora in different tissues of *Notopterus notopterus*

Bacteria	**Skin**	**Gills**	**Muscles**	**Intestine**
Aeromonas hydrophilla	5.5x10^{3}CFU/g	2.5x10^{3}CFU/g	2.5x10^{3}CFU/g	8.0x10^{3}CFU/g
Pseudomonas fluorescens	6.5x10^{3}CFU/g	1.5x10^{3}CFU/g	1.5x10^{3}CFU/g	4.5x10^{3}CFU/g
Streptococcus iniae,	4.5x10^{3}CFU/g	1.5x10^{3}CFU/g	1.5x10^{3}CFU/g	8.5x10^{3}CFU/g
Staphylococcus aureus	1.5x10^{3}CFU/g	2.5x10^{3}CFU/g	2.5x10^{3}CFU/g	10.5x10^{3}CFU/g
Shigella sp.,	10.5x10^{3}CFU/g	2.5x10^{3}CFU/g	1.5x10^{3}CFU/g	10.5x10^{3}CFU/g
Salmonella sp.,	5.5x10^{3}CFU/g	1.5x10^{3}CFU/g	3.5x10^{3}CFU/g	9.5x10^{3}CFU/g

Table – 6: Showing bacterial flora in different tissues of *Hilsa toli*				
Bacteria	**Skin**	**Gills**	**Muscles**	**Intestine**
Aeromonas hydrophilla	$2.5x10^3$CFU/g	$2.5x10^3$CFU/g	$1.5x10^3$CFU/g	$7.0x10^3$CFU/g
Pseudomonas fluorescens	$2.5x10^3$CFU/g	$1.5x10^3$CFU/g	$1.5x10^3$CFU/g	$5.5x10^3$CFU/g
Streptococcus iniae,	$4.5x10^3$CFU/g	$1.5x10^3$CFU/g	$1.5x10^3$CFU/g	$8.5x10^3$CFU/g
Staphylococcus aureus	$1.5x10^3$CFU/g	$2.5x10^3$CFU/g	$2.5x10^3$CFU/g	$5.5x10^3$CFU/g
Shigella sp.,	$10.5x10^3$CFU/g	$1.5x10^3$CFU/g	$1.5x10^3$CFU/g	$9.5x10^3$CFU/g
Salmonella sp.,	$5.5x10^3$CFU/g	$1.5x10^3$CFU/g	$1.5x10^3$CFU/g	$10.5x10^3$CFU/g

Table – 7: Showing bacterial flora in different tissues of *Mystus cavacius*				
Bacteria	**Skin**	**Gills**	**Muscles**	**Intestine**
Aeromonas hydrophilla	$5.5x10^3$CFU/g	$2.5x10^3$CFU/g	$15x10^3$CFU/g	$3.0x10^3$CFU/g
Pseudomonas fluorescens	$5.5x10^3$CFU/g	$1.5x10^3$CFU/g	$1.5x10^3$CFU/g	$6.5x10^3$CFU/g
Streptococcus iniae,	$7.5x10^3$CFU/g	$1.5x10^3$CFU/g	$1.5x10^3$CFU/g	$9.5x10^3$CFU/g
Staphylococcus aureus	$10.5x10^3$CFU/g	$2.5x10^3$CFU/g	$2.5x10^3$CFU/g	$9.5x10^3$CFU/g
Shigella sp.,	$10.5x10^3$CFU/g	$1.5x10^3$CFU/g	$1.5x10^3$CFU/g	$7.5x10^3$CFU/g
Salmonella sp.,	$10.5x10^3$CFU/g	$1.5x10^3$CFU/g	$1.5x10^3$CFU/g	$4.5x10^3$CFU/g

Discussion

The findings from this study shows that the skin and intestines of these fishes have higher bacterial load than the gills and muscles. These could be as a result of fish handling and environmental contamination. The result of lower bacterial load from the fish gills when compared to that of the skin is in total agreement with the study of Chessbrought (2006), who reported that the number of bacterial associated with gills are actively maintained at low level, thereby enabling the fish to keep the bacterial number low, and therefore afford it some degree of protection against bacterial invasion by the gills micro flora.

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