MICROBIAL SAFETY AND QUALITY ANALYSIS OF FISH MEAT AT RETAILERS IN GREATER NIODA, INDIA.

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Abstract: The purpose of present study was to evaluate the microbiological quality of raw fish received from retail market of India. Overall, 180 sample from 6 fish spp of Indian *oil Sardins (Sardinella longiceps) Tilapia (Oreochromis niloticus), Indian Macierels (Rastrelliger kanagurta), Common carp (Cyprinus carpio), Pabda (Ompok pabda) and Mrigal carp (Cirrhinus cirrhosis)* were collected at some stage in March 2019. This samples had been enumerated, such as aerobic mesophilic bacteria, total and faecal coliforms and *Salmonella prevalence. Salmonella* became identified in 5 of 180 fish samples, means that, five samples had been positive for *Salmonella.* Total bacterial count in raw fish ranged from 2.4 to 5.85 cfu/ml.Total bacterial count in common carp (5.85 cfu/ml) were significantly higher ($p<0.05$) than in Tilapia ((3.6 cfu/ml), this indicates that high total bacterial count numbers were determined in raw fish samples, as well as raw fish at retail level..

Key words: raw fish, microbiological quality, *Salmonella*.

Introduction

Fish are a source of excessive satisfactory animal protein, holding good sized quantities of treasured lipids, minerals and nutrients. It's far supposed that freshwater fish merchandise are healthful meals for human vitamins (Steffens W. and Wirth M. 2005). On the other side, fish nutrient composition and excessive moisture content material let the growth of a massive variety of microorganisms, which have an effect on fish exceptional and protection rendering fish unacceptable for human intake (Gram L. and Huss H.H. 2000). Microbiological exceptional of raw fish effects from microbiological load of aquatic habitat, strategies of capture, transportation, chilling and garage situations. (Eizenberga et al., 2015).

Total bacterial count, *Enterobacteriaceae* and *Escherichia coli* are regularly used as standards to measure the first-class and safety of meals. Total bacterial matter is used to evaluate the overall microbiological excellent of fish and may be a beneficial indicator to expect the shelf life of uncooked fish .*Enterobacteriaceae* and *E. coli* are used as pointers of the capacity existence of pathogens and showing sanitary standing of fish. (Length, F. 2017).

Numerous pathogenic bacteria can additionally either be gift in the atmosphere or pollute fish throughout managing .*Salmonella* and *Listeria monocytogenes* are said to motive foodborne toxic in human beings through the absorption of uncooked or ineffectively treated polluted fish or fish merchandise Pathogenic bacteria may be conveyed to purchaser by means of uncooked fish at retail stage, which increase the risk of meals-borne contaminations. Consequently detection of microbiological exceptional and pathogens in raw products is critical for the identity and prevention of problems associated with public health and protection(Eizenberga et al., 2015).

Fish spoilage is resulting from bacterial enzymes. Microorganisms settling the pores and membrane, guts and gills are often inoffensive for stay fish, and started to duplicate hastily afterwards it's died (Shamsuzzaman *et al.*, 2011).Bacterial weight on the pores and membrane, gills and guts of fish residing in pure waters is commonly excessive, and muscle tissues are expected to stay hygienic(Pamuk *et al.*, 2011). The fish can be infected afterward fishing or all through conveyance to marketing. After infection and reproduction of bacteria, deterioration takes place and the intake will become risky. Fish fine is motivated by means of several reasons because the cause, chilling strategies, handling, container, packing. The pleasant and cleanness of fish are swiftly decayed thru bacteriological and biochemical mechanisms. Bacterial activity effects in ugly odour due to conversion of amino acids into biogenic amines, sulfides, natural acids etc. (Stratev, et al, 2015).

Several pathogenic and decomposition microbes are capable of adher on foodstuff sides, and continue to be feasible even when washing and sterilization .The nice of fish could be tainted thru a difficult system, wherein the physical, chemical and microbiological kinds of worsening are complicated (Pal, et al, 2016). The enzymatic and biochemical reactions are normally accountable for the primary lack of cleanness however bacterial interest is answerable for the clear spoilage, and thus starts product shelf life .Sensory strategies are the maximum excellent for measuring the damage and brightness of fish .A main intention for the food processing manufacturing is to afford secure, healthful, and proper food to the patron.. Regulator of microbes applied thru high stage of sanitation and effective washing, and decontamination applies at some point of the treating and renovation processes are vital to satisfy this goal. (Pal, et al, 2016).

Human and microorganisms have a long past collectively. The common bacterial plants includes organisms that create their homebased on or in some part of the frame. In a healthful individual, such organisms infrequently motive disorder. Microbes of the regular flora might be in interdependent dating, in which together microorganism and mass gain. The enteric micro-

organism that arrangement the ordinary flowers of the intestine help in the mixture of diet ok and some of the vitamins of the B complex. In commensalism, microorganisms are neither useful nor dangerous to their host as within the case of the huge collection of bacterial plant life that stay at the skin, and the mucous membranes of the top breathing tract and intestines.(Pal et al., 2016).

Fish collected from infected waters, which have stayed inappropriately conserved afterward collecting, are acknowledged to show an crucial role in infections by means of Vibrio spp.Intake of raw fish, specially shellfish, infected with V. parahaemolyticus might also cause growth of acute gastroenteritis characterized by means of diarrhea, headache, vomiting, nausea, belly cramps, and coffee fever (Johnston 2006).

Research show that there are numerous microorganism which have been remoted from 3 foremost elements of fresh fish: mucus cover, gills and the intestinal tract. The bacteriological variety of the sparkling fish muscle determined at the fish landing and ecological elements about it. Seafood from warm waters ordinarily port mesophilic gram-negative micro-organism though cold waters port commonly psychrophilic, gram-effective bacteria. Those microbial separates are categorized into 2 groups: native and post-harvest bacteria. Earlier researches have additionally verified the existence of indicator microbes of faecal contamination, resourceful and pathogenic micro-organism to human beings in fish. (Length, 2017)

The starting place of maximum microbes in fish and fish merchandise may not be exactly recognized, some people have associated bacterial contaminations or pollution of fish to quite a number of causes consisting of detrimental situations inside the fish subculture gadget(Okpokwasili and Ogbulie, 1993), contamination and periodic adjustments (Moore 2002), fish managing and treating together with personnel and processing equipment (Length, 2017). Fish consists of protein and vitamins good for bacterial assault even after treating. This frequently leads to fish decay and dietary deformation.

2. RESEARCH METHODOLOGY

2.1 Study design and period

A cross-sectional study was performed among January 2019 and April 2019, to evaluate the bacteriological quality of fish product which can be promoting round Greater Noida. The investigation become centered on fish retailers of Jagat market positioned at Greater Noida metropolis; the specified fish samples had been collected from the observe places throughout 4 months.

2.3. Fish samples collection

The row fish samples were gathered from fish retailer market in Jagat Greater Noida, state of Uttara Pradesh in India. Sample from 6 fish species of Indian oil Sardins (Sardinella longiceps) Tilapia (Oreochromis niloticus), Indian Macierels (Rastrelliger kanagurta), Common carp (Cyprinus carpio), Pabda (Ompok pabda) and Mrigal carp (Cirrhinus cirrhosis) were collected in plastic bags,chilled below 4°C,transported to the laboratory and tested within a short paired of time.

2.4. Fish Preparation

Six types of fish species were brought to laboratory of Sharda College on ice and below hygienic situations. The fish sample washed with disinfected distilled water to take away any out of doors dust, dried with disposable cloth. The fish flit samples have been gutted and mad filleted fish, washed over again in smooth distilled water and left to empty. Disposable towel paper become used to facilitate the draining gadget. After that samples have been measured and weighted one after the other every fish samples further organized for bacterial isolation (Fawole and Oso, 2001)

2.5. Bacteriological analysis of fish samples

2.5.1. Preparation of samples

Twenty five gram of fish samples had been aseptically weighted individually from 150g of every gathered six samples and homogenized in 225ml of sterile zero.1% (w/v) buffered peptone water (Oxoid, England) for (30–60) seconds in a sterilized mixer machine.

Ten-fold serial dilutions (10-2 to 10-4) were prepared from homogenized samples the usage of 9ml sterile saline solution clean as diluents. The homogenate became used to enumerate, isolate and describe microorganism groups from fish samples (Ray, 2004).

2.5.2. Enumeration of Aerobic mesophilic bacteria

One ml of homogenized serial diluted sample from 10-2, 10-3 and 10-4 had been pour plated without delay to plate count agar (Oxoid, England) in triplicates, effectively leveled Petri dishes. The plate had been allowed to solidify and incubated at 37°C for 24 hours. After incubation the Petri dishes holding 30 to 3 hundred colonies have been decided on and counted the usage of colony counter and explicit as colony forming units in keeping with gram (Tortorello, e tal.2003)

2.5.3. Enumeration of total and faecal coliforms

Total coliforms

Three tube most-probable-number method the use of lauryl tryptose broth changed into used for inventory of total coliforms .One ml aliquots from each dilution (102,103 and 10-4) have been aseptically relocated in to triplicate tubes containing sterile lauryl tryptose broth (Oxoid Ltd, Basingstoke, Hampshire, England) with inverted Durham's tubes. Incubation modified into completed at 37°C for 24 hours. Tubes displaying gas and growth had been considered as presumptive positive for total coliforms. Then, a loop complete of inoculum from all presumptive-positive lauryl tryptose broth tubes was inoculated into tubes which contained 10ml of Brilliant Green Lactose Bile broth and incubated at 37 °C for twenty-four hours. Following incubation period, Brilliant Bile Green broth tubes had been found for gas formation inside the Durham tubes.

All positive Brilliant Green Bile broth tubes were taken into consideration positive for coliform affirmation. The huge form of coliforms changed into expected from MPN table. For records analysis functions, overall coliform counts that were a great deal much less than the finding restrict (<3.0 MPN/g) were assigned 1.5 MPN/g, that is centrally among absence of colonies and the finding restriction (Vrints,,2007) positive.

Faecal coliforms

The identical manner of total coliforms was done for fecal coliforms, triplicate tubes holding Lauryl Tryptose broth (Blulux Laboratorie Ltd, India) with inverted Durham tubes raised at 44.5°C or a maximum of 24 hours. Then, confirmatory check for fecal coliforms turned into done the usage of MacConkey broth with inverted Durham tubes and incubated at 44.5°C for a most of 24 hours. Confirmation turned into acquired by means of gas production. The result become suggested as the maximum in all probability variety consistent with gram of meals (Yousef, e.tal 2003)

2.6. Isolation of Salmonella species

Enrichment of the sample: in the fish samples, Salmonella and Shigella species can be determined in small numbers with different microorganisms or they may be damaged. To lessen the danger of acquiring improper results pre-enrichment via peptone water and selective enhancement with the useful resource of Selenite Cystin broth have been used. The homogenized interruption emerge as incubated at 37°C for 24 hours for the metabolic healing and propagation of cells. From this, 1ml of manner of life changed into transferred into separate tubes every containing 10 ml of Selenite Cystein Broth (Oxoid). Selenite Cystein broth was incubated at 37°C for 24 hours.

Presumptive detection: After selective enrichment, means from the enrichment broth turned into one after the other streaked on plates of Xylose Lysine Desoxycholate medium and MacConkey Agar (each from Oxoid) and incubated below aerobic ecosystem at 37°C for 24 hrs (Renatus et al., 2012). Pink colonies with/without black middle on Xylose Lysine Desoxycholate agar were taken into consideration as Salmonella species red or purple colonies on Xylose Lysine Desoxycholate agar and dense or obvious colonies on MacConkey Agar were careful as Shigella species. Such characteristics of colonies have been picked and streaked into Nutrient agar (Oxoid, England) for purification motive. After 24 hrs of incubation at 37°C under cardio environment, a single colony of microorganism become taken from the nutrient agar and inoculated into Tryptic Soy agar slant. The slant turned into incubated at 37°C underneath cardio surroundings for twenty-four hrs. Sooner or later, after 24 hrs of incubation the slant was preserved in 5°C for the cause of biochemical description, (Abila, e.tal, 2003).

2.7. Statistical analysis

Statistical analyses had been achieved on log-10 converted statistics. The prospect dgree at which statistical analyses had been time-honored as significant became < 0.05. Data were analyzed (means, standard deviations) the usage of the software program ANNOVA.

3. RESULTS AND DISCUSSION

3.1. Aerobic mesophilic counts

Total viable counts and Salmonella species in fish meats are provided in Tables 1 and a connect of the microbial loads in fish products have been found in common carp (5.85cfu/ml), Indian Macierels (5.78 cfu/ml) Pabda (5.56 cfu ml), Indian oil Sardins (4.46 cfu/ml) and Mirgal carp (4cfu /ml) followed by means of Tilapia (3.6 cfu/ml) Maximum polluted with Salmonellosis species. were Indian Macierels and Pabda. Salmonella species were no longer attached in Tilapia and Indian oil sardines counted. According to (Mol and Tosun 2011) in Istanbul. Studied horse mackerel samples showed great entire feasible bacterial counts, as much as (7.04 log cfu/ml) we have sited out in our observe that Indian mackerel samples were the less contaminated with microorganism in comparison to the earlier studied at (5.78 cfu/ml) mentioned that studied Pabda, Tilapia, Indian oil sardine, Indian mackerel, Mrigal and Common carp samples from retailers have been of good satisfactory, In Indian Macierels, the level of total bacterial count and salmonella species turned into the highest (5.78 cfu/ml respectively), at the same time as the lowest total bacterial count turned into detected in Tilapia (3.6 cfu/ml) .The mean values for total bacterial count, remember for fish samples are given in table 1.

Table 1: Aerobic mesophilic bacteria of Fish from a retailers in Greater Noida city, India, 2019.

Fish type	No.of samples	Mean±SD	Minimum	Maximum	P-valu
Pabda	30	4.28±1.09	2.78	5.56	
Tilapia	30	1.26±1.75	0	3.6	
Common carp	30	4.28±1.31	2.63	5.85	0.01
Indian oil Sardins	30	2.16±2.06	0	4.46	
Indian Macierels	30	4.25±1.25	2.66	5.78	

Figure 1: aerobic mesophilic bacteria of Fish from a retailers in Greater Noida city, India, 2019.
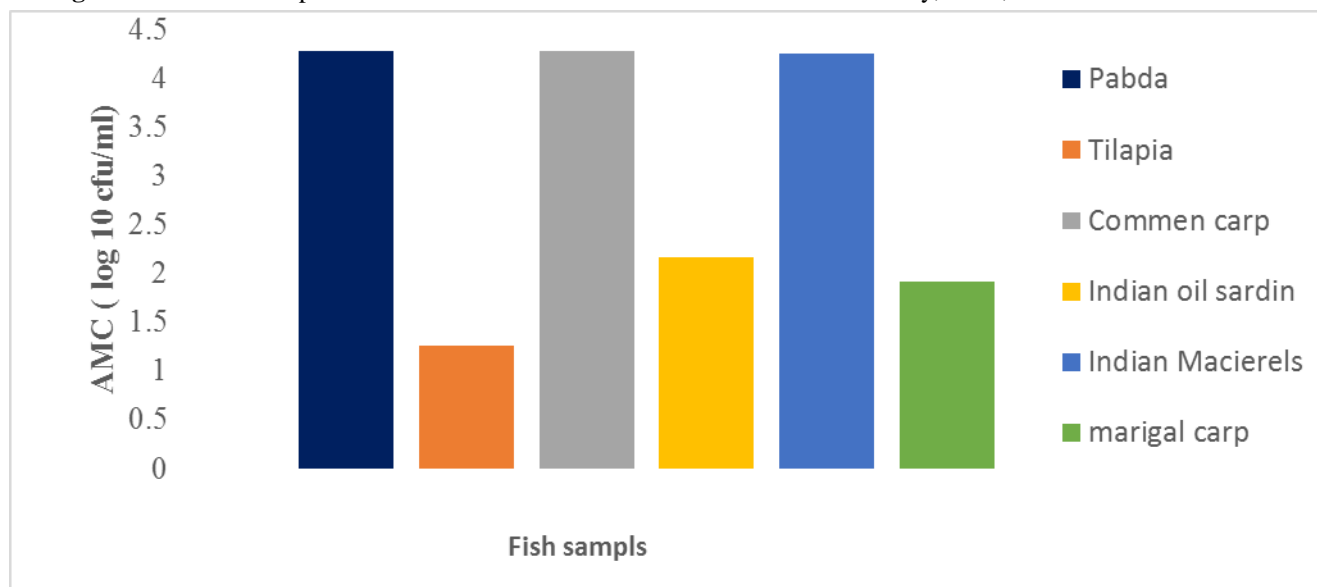
Result of this study directed that total bacterial count of Indian mackerel (5.78 cfu/ml) and common carp sample effects had been lower than the result of (Stratev,et al.2015) who reported that common carp (6.81cfu/ml) and Hors mackerel (5.9 cfu/ml) of samples from Republic of Bulgaria .According to Wenjiao et al. (2013), the carp is one of the economically crucial freshwater fish species for eastern nations because of its widespread growth, easy culturing, good feed adaptation and high dietary value. besides, the carp is an without problems spoiled food because of excessive water interest, autolytic enzymes content material and comparatively excessive quantities of risky basic nitrogen and ftree amino acids.

3.2. Total and faecal coliforms
Total coliforms

Table 2: Mean and range of total coliforms (MPN/100ml) of Fish at a retailers in Greater Noida city, India, 2019.

Fish samples	Mean count ±SD		Range MPN/100ml	p-value
Indian Macierels	475.00	±288.05	150 - 800	0.00
Mrigal carp	315.67	±260.05	80 - 550	

The present study in total coliform counts revealed that, 10 (33.3%) in Indian Macierels and 4 (13.3%) of the samples in Mrigal carp were recorded (Table 2). A study revealed in South Africa Vincent et al. (2012) revealed that, total coliform counts with a range of 1.9×10^2MPN/100ml to 3.8×10^7MPN/100ml.In another study total coliform level recorded was with a range of 4×10^3 to 8×10^4MPN/100ml in Kumasi, Ghana (Nana et al., 2012). If large number of coliforms is found in fish meat there is a probability that pathogenic bacteria exist as a result contaminate produce.

Faecal coliforms
Faecal coliform bacteria are Gram-negative bacteria, also known as thermotolerant coliforms or presumptive E. coli. E. coli are specifically of faecal origin from birds, humans and other warm blooded animals. Faecal coliform bacteria are therefore considered to be a more specific indicator of the presence of faces. In this study, the mean faecal coliform counts of row fish at retailers was a range of 110-800MPN/100ml with a mean of 689.67MPN/100ml and 90-500MPN/100ml with a mean of 390.00MPN/100ml in Indian Macierels and Mrigal carp fish samples.

Table 3: Mean and range of faecal coliforms (MPN/100ml) of Fish at a retailers in Greater Noida city, India, 2019.

Farming site	No of samples	Mean ±SD		Range MPN/100ml	p-value
Indian Macierels	30	689.67±	572.04	110- 800	0.01
Mrigal carp	30	390 ±	226.04	90- 500	

The presence of faecal indicator bacteria in the row fish sample suggests faecal pollution raising the possibility of the presence of pathogenic microorganisms in raw fish and a threat to public health.

3.3. Prevalence of Salmonella

The present study documented on isolation of Salmonella species these organisms are known food borne pathogens that have been implicated in food borne disease outbreaks (Mudgil et al., 2004). In this study, 30 samples of each fish spp. were used to detect Salmonella. Mirgal carp fish and Indian Mackerels fish samples were contaminated with Salmonella spp according to Table 4 indicated below. The prevalence of Salmonella in raw fish is associated with water pollution or poor disinfected situations throughout the fish handling.

Table. 4. Occurrence of Salmonella spp Fish from a retailers in Greater Noida city, India, 2019.

Fish spp	No.of samples	No.of *salmonella* positive samples	Percent (%)
Pabda	30	0	0
Tilapia	30	0	0
Indian oil Sardins	30	0	0
Common carp	30	0	0
Indian Macierels	30	3	10
Mrigal carp	30	2	7.6
Total	**180**	**5**	**2.8**

Information is important in prevalence of Salmonella isolates on raw fish related to the potential health hazards that poses to humans. Salmonella occurs mostly in the intestinal tract of animals, but occasionally is found elsewhere in the body. In this study, Salmonella was isolated in 3(10%) and 2 (7.6%) of Indian Macierels and Mrigal carp samples, respectively (Table 4). However, none of the tested fish samples contained Salmonella. Consequences for fish species and pathogens are placed in table 4. It has been advised that Salmonella is not always an indigenous bacterial flora of fish, but occasional instances on Salmonella presence in fish have been documented (Cloete T.E, et al. 1984) .Salmonella can be announced inside the aquatic surroundings to animal and human fecal dropping or manure contamination (Amagliani G 2012), Listeria species. and Salmonella may infect fish in aquatic surroundings, throughout conveyance to fish markets
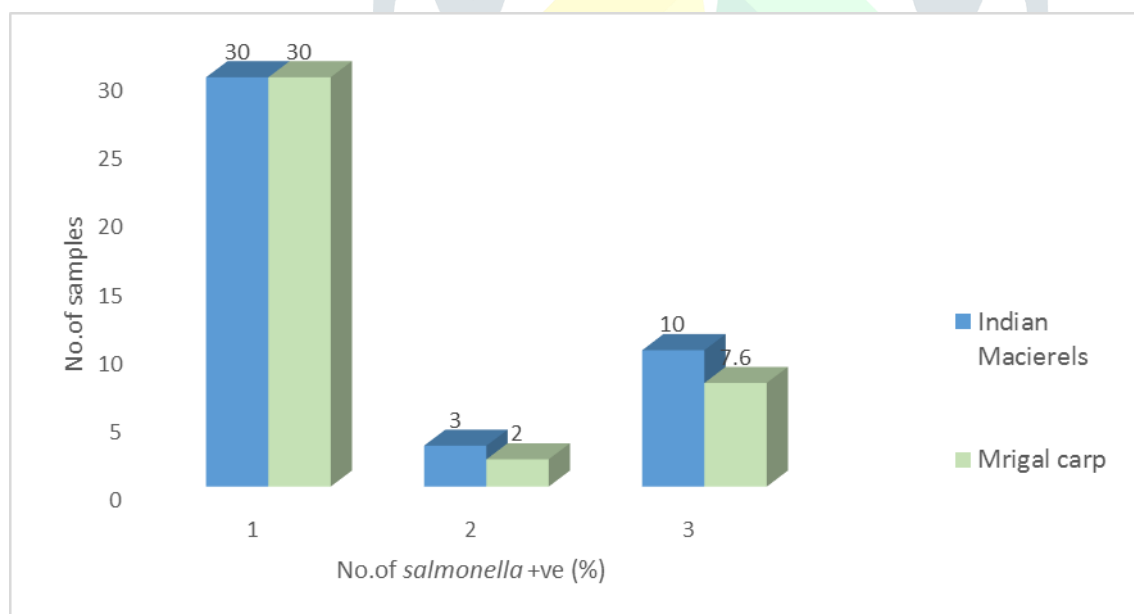

Figure 2: Isolation of salmonella from fish samples at a retailers in Greater Noida city, India, 2019.

The presence of Salmonella in 25 g of a sample examined is regarded as potentially hazardous to consumers, and is unacceptable for consumption (Cheung et al., 2007).

Fish look like passive vendors of salmonella exhibit no clinical sickness and might defecate Salmonella species without obvious hassle. The pollution of the organism originates from native assets and fish can used as a vector for Salmonella spp (Chattopadhyaya, 2000). The occurrence of Salmonella species is of public health distresses, and shows feasible infection attributable to water resources (Pal, 2010). The serious trouble associated with fish safty is the infection with bacterial pathogens. Moreover, fish merchandise are sensitive to spoilage due to their high water content, impartial pH, excessive quantity of amino acids and naturally existing autolytic enzymes (Jeyasekaran et al., 2006).

4. CONCLUSION

Raw fish at retail market in India incorporate averege counts of total bacterial count and Enterobacteriaceae between all samples that means fish species. Salmonela were obtained in five trials. Even though this have a look that indicates the existing of Salmonella, but uncooked fish at retail level was observed to be especially infected with extraordinary pathogenic bacterias, consequently consumers should ignore cross-contamination of meals in the course of fish coaching and keep away from the intake of uncooked fish to save you foodborne contaminations. in order to verify the deficiencies of fish microbiological pleasant, there may be a necessity of similarly studies between whole fish supply sequence, inclusive of marine surrounding, transportation, chilling and storage situations of fish, quality of water used, fishing equipment's, personal hygiene of the fish handlers and sanitary conditions of the landing site. This observe additionally have same remaining works they are not included.

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