



Status of retail fish markets of Choryasi taluka and Surat city (India) with reference to bacteriological examination of fishes

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Abstract

Quality of fish is an important criteria for human health. The pathogens like *Escherichia coli* and *Klebsiella pneumoniae* can survive and multiply in fish, transferred in to humans from fish used as food and cause food poisoning, diarrhoea, meningitis and septicaemia. Taking into consideration, bacteriological analyses were done to know the quality of fishes sold at retail fish markets of Choryasi Taluka and Surat city. Fish samples were collected from fish markets for enumeration of the total viable count (TVC) and identification of bacteria. Bacteriological analyses of fresh fish samples (*Mugil parsia* and *Stromateus cinereus*) showed higher bacterial load with pathogenic bacteria which crossed permissible limit (5×10^5 CFU/g) except the case of *Mugil parsia* from vendors of Gabheni village. The fishes were contaminated with *Escherichia coli* and *Klebsiella pneumoniae* indicating poor hygiene and sanitary condition of the retail fish markets. Apart from this, A innovative trend in live fish marketing was observed in retail fish market of Katargam to overcome from problems like contaminated and poor quality fishes. Fresh water fishes collected from this live fish market were completely free from bacteria indicating good quality fishes available in the market.

Keywords: *Escherichia coli*, *Klebsiella pneumoniae*, *Mugil parsia*, *Stromateus cinereus*, sanitary condition.

Introduction

Demand of fish and seafood products are ever increasing as becomes important food commodity in the international trade. Fish supplies about 60% of the world supply of protein and 60% of the world gets more than 30% of their animal protein from fish¹. Fish allows for protein improved nutrition in that it has a high biological value in term of high protein retention in the body, low cholesterol level and presence of essential amino acids. Fishes are very important due to health benefits, particularly against cardiovascular diseases². Fishes are also known for infant brain development due to their long-chain polyunsaturated fatty acids (PUFAs)³. Despite such nutritional benefits, infection of fishes by pathogenic microorganisms is a major concern for consumers. Fish contaminated with bacteria pathogens could cause severe food borne illness in human and reduce the health benefits of PUFAs⁴.

Pathogenic bacteria, viruses and parasites are mainly responsible for microbial contamination. Food born diseases in human are caused by pathogenic bacteria in fish. Bacterial load in fish species varies based on biological as well as environmental factors. Some fishes are naturally more prone to contamination depending on the fish, size, age, feeding, harvest season and geographical location⁵. The primary sources of pathogens in fish are human activities that create pollution in coastal waters⁶.

Intake of spoiled fish may cause diseases due to intoxication. Some of these diseases are caused by bacteria present on the external surfaces of fish. When fishes are alive, these microorganisms can't enter in the fish body due to normal defence mechanism of fish. On death, the microorganisms or the enzymes secrete to invade into the flesh where they react with natural substances resulting in a changes in odoriferous and flavourous compounds⁷. So, the fish quality could be damaged through the physical, chemical and microbiological deterioration of fish. The chemical and enzymatic reactions are usually main reasons for the initial loss of freshness while spoilage is due to microbial activity.

The quality of the raw fish (fresh) varies at different markets and need to check and to compare with quality standards. This kind of research work is almost not done in this particular area. Therefore, this study was carried out to to check the hygienic condition and bacteriological quality of fishes sold in retail fish markets of Choryasi taluka and Surat city.

Methodology

Bacteriological analyses was implemented to check the edible quality of fishes and pathogens harmful to consumers. Fish samples collected in zip-lock plastic bags were brought immediately to the laboratory. 1 gm of skin surface of fish was scraped with a sterile knife and transferred in 10ml of distilled water under aseptic conditions.

Total Viable count (Quantitative analysis): Fish samples are analyzed for hygienic condition using quantitative method⁸. 9 ml of sterile distilled water was poured aseptically into five tubes each and 1 ml of the original fish sample was added to the first test tube giving a 1:10 dilution. Again 1 ml was taken from the first tube and added to the second tube and mixed well. Procedure continued until the fifth test tube. Each sample was diluted from 10^{-1} to 10^{-5} . Nutrient agar plates were inoculated with 0.2ml of the diluted solution (10^{-2} to 10^{-4}) using spread plate technique. All plates were incubated at a temperature of 37°C for 24 hrs before colony enumeration and isolation. The mean colony count on the nutrient agar plates of each given dilution was used to estimate the total viable count for the samples in colony forming units per gram (CFU/g).

Identification of microorganisms (Qualitative analysis): Fish samples were analyzed for hygienic condition using qualitative method⁹⁻¹¹. Differential media (Nutrient agar and MacConkey's agar), selective media (Eosin methylene blue) and different biochemical media were used for identification. The organisms were recognized by cultural, morphological and biochemical characteristics.

Cultural Characterization of Microorganism: Cultural characteristics of the colony of selected isolate were examined from nutrient agar plate after 24 hrs of incubation. Different characteristics of colonies such as size, shape, edge, elevation, surface, texture, consistency, colour, transparency, pigmentation etc. were recorded.

Morphological Characterization of Microorganisms: The colony of isolate was selected and suspension was prepared in 1 ml of distilled water. The smear was prepared, allowed to dry and fixed with heat. Smear was stained with crystal violet stain for one minute. After one minute, smear was covered with Gram's iodine for thirty seconds and washed with running water. Thereafter, smear was covered with 95% ethanol for ten to fifteen seconds followed by washing in running water and was stained with Safranin for five minutes and again washed with running water. Slides were examined under oil immersion and morphological characteristics of microorganisms were recorded.

Biochemical Characterization of Microorganisms: Different microorganisms require different biochemical component for their growth. Selected isolates were biochemically characterized by different tests such as 'Sugar fermentation, Indole, Methyl red, Voges proskauer, Citrate utilization, Hydrogen sulphide production, Gelatin hydrolysis, Urea hydrolysis, Phenylalanine deamination and Triple sugar iron agar⁷.

Results and discussion

Sample collection was done thrice in a six months. Total twenty four fish samples from four fish markets were analyzed to check the quality of fishes. This table shows an average of bacterial count in the sample. Bio-oxidation reactions are important for bacteria which helps them to provide energy by fermentation. Based on the sebio-oxidation reactions, the bacteria were recognized and the results given in Table-1.

Results showed highest bacterial count (137.5×10^5 CFU/g and 95.3×10^5 CFU/g) in fresh samples of *Mugil parsia* and *Stromateus cinereus* of Dumas retail fish market followed by fishes from Hazira market because proper hygienic criteria were not maintained throughout catching, landing, transportation, handling and preservation. It was found that all fish samples crossed the permissible limit (5×10^5 CFU/g) with pathogenic bacteria *Escherichia coli* or *Klebsiella pneumoniae*. Quality depreciation of fresh fish occurs rapidly during storage and handling which reduces the shelf life of fish products as it is one of the most perishable food products¹². Fish spoilage in the dead fish is mainly due to enzymatic and bacterial action. When temperature is high, fish spoils within 15-20 hours depending on the capture method and fish species¹³. At the temperature between 31°C to 34°C, number of *Escherichia coli* had increased remarkably in all fish samples collected from ponds of Nadia district of West Bengal due to increase in temperature effected the population of *Escherichia coli*. This temperature is appropriate for *Escherichia coli* growth and production¹⁴. Fresh fish *Scomberomorus guttatus* sold in fish market of Madurai has high contamination, may be as a result of certain factors like temperature which supports growth of *Escherichia coli* and *Klebsiella pneumoniae* and handlers who were not keeping their own hygiene¹⁵. The contamination of fish with pathogenic bacteria indicates poor hygiene and sanitary condition of retail fish markets.

Table-1: Bacteriological Quantitative and Qualitative Analyses.

| Name of the Place | Name of the Fishes | Total Viable Count (TVC) (CFU/g) | Permissible limit of (CFU/g) in fish | Name of the Microorganisms |
|-------------------|------------------------------------|----------------------------------|--|------------------------------|
| Dumas | <i>Mugil parsia</i> (fresh) | 137.5×10^5 | Central Institute of Fisheries Technology, Cochin 5×10^5 | <i>Escherichia coli</i> |
| | <i>Stromateus cinereus</i> (fresh) | 95.3×10^5 | | <i>Escherichia coli</i> |
| Hazira | <i>Mugil parsia</i> (fresh) | 43.6×10^5 | | <i>Klebsiella pneumoniae</i> |
| | <i>Stromateus cinereus</i> (fresh) | 92.5×10^5 | | <i>Escherichia coli</i> |
| Gabheni | <i>Mugil parsia</i> (fresh) | No growth | | Absent |
| | <i>Stromateus cinereus</i> (fresh) | 28.5×10^5 | | <i>Escherichia coli</i> |
| Katargam | <i>Catlacatla</i> (fresh) | No growth | | Absent |
| | <i>Labeorohita</i> (fresh) | No growth | | Absent |

Fishes having Total viable count (TVC) above permissible limit with pathogenic bacteria were harmful to consumer. *Escherichia coli* and *Klebsiella pneumoniae* are pathogenic organisms of Enterobacteriaceae^{16,17}. Enterobacteriaceae count is considered as another index of fish quality because it is related to storage in ice, washing, and evisceration¹⁸. Monitoring of these microorganisms have been suggested as a measure of fish quality. Enterobacteriaceae may be present in the tissues of normal fish¹⁹. Whenever fish are exposed to environmental stressor injury, it causes disease with mortalities. Environmental stresses such as poor water quality, high temperature, high organic content contribute to the severity of Enterobacteriaceae infections in fish^{20,21}. These organisms are producing hazardous amounts of toxin histamine in a very short period of time when the fishes are kept at raised temperature and cause fish poisoning²²⁻²⁴. The most frequently occurring microorganism associated with fish histamine poisoning is *Klebsiella pneumoniae*²⁵. *Escherichia coli* and *Klebsiella pneumoniae* in fish and fish products cause a health hazard to human²⁶⁻²⁸. These two microorganisms cause food poisoning, diarrhoea, meningitis and septicemia²⁹. This point indicates poor sanitary conditions including fish handling and storage methods at market area. Poor hygiene practices and contamination from fish seller and equipment are mainly responsible for food borne diseases in human.

Fresh fish samples collected from vendors of Gabheni village showed less (28.5×10^5 CFU/g) or no bacterial count compared to other fish markets of study area. Transportation and selling of fresh fish is usually done on flaked ice, which keeps temperature slightly above 0°C³⁰. At a lower temperature, most spoilage processes are slowed down³¹. The results of present study claimed for proper handling and preservation in meagre amount of ice for bacterial load.

Apart from this, bacteriological analysis of fresh fish samples *Labeorohita* collected from Katargam fish markets were completely free from pathogenic bacteria due to proper and continuous aeration in oxygenated tank during fish selling. Retailers of Katargam fish market sold live fishes to local consumers. Dead fishes were sold at the rate of Rs. 100/Kg whereas live fishes cost was Rs. 200/Kg thus retailers fetched double price through selling of live fish. Elite class consumers preferred to pay higher price for live and quality fishes. Similar findings were observed except bacteriological analysis by Pathan, J.³¹. As bacteriological analysis is rarely done in this specific study area. Results of current study indicated that an innovative technology has enhanced fish quality up to higher standard which is a very important criteria for human health.

Conclusion

The study indicated that fresh fish samples collected from different markets and vendors showed high bacteria count with pathogenic bacteria which crossed permissible limit except in the case of *Mugil parsia* also collected from vendors of Gabheni

village. It indicates that proper hygienic and aseptic conditions were not maintained properly throughout catching, landing, transportation, processing, handling and preservation except Gabheni village. Findings of present work suggested that contaminated fishes were unsafe for human consumption due to presence of high bacterial load. Pathogenic bacteria *Escherichia coli* and *Klebsiella pneumoniae* in fishes cause a health hazard to human. So, the bacteriological quality of the fishes inspected is unacceptable and shows a potential risk to human health. To overcome of this problem, authority should monitor management practices to minimize the risk of degrading fish quality. On the other hand, fresh samples collected from Katargam live fish market were completely free from bacteria. Findings of present study showed that live fish marketing provides high quality of fishes due to use of continuous aeration during selling which were safe for human consumption.

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