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Bacteriological Analyses of Fish Mucus from Fish Market with Reference to Consumer's Health

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Authors' contributions

This work was carried out in collaboration among all authors. Author NPS helped in sample analyzed and while wrote the manuscript. Author NCU was involved as research guide and helped in manuscript reviewed and editing whereas. Author YHM is involved in fish samples collection. All authors read and approved the final manuscript.

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ABSTRACT

A study was conducted to evaluate the bacteria load present in fish mucus collected from Nanpura wholesale fish market during the period from 30th December 2022 to 20th February 2023. Total 20 fish mucus samples were determined by Total Plate Count (TPC) and identification of bacteria. High bacterial load with pathogens such as Escherichia coli (Gram negative bacteria) and Staphylococcus aureus (Gram positive bacteria) were found in all fresh and marine fish samples from study area. These pathogens can survive and multiply in fish, transferred into consumer by way of food and main source of fish poisoning, diarrhoea, meningitis and septicemia which cause health risk to consumer. The fishes were contaminated with pathogenic bacteria indicated poor handling of fishes, lack of preservation facility and unhygienic condition of

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study area. Thus, it is strongly recommended to improve the quality control and quality assurance systems of the study area to minimize the prevalence of pathogenic bacteria.

Keywords: Wholesale fish market; total plate count; Escherichia coli, Staphylococcus aureus; Septicemia.

1. INTRODUCTION

"Aquatic animals had been a major source of food for many not only domestically but also globally because of its rich contents of nutrients that are essential to health and well-being of the human race" [1].

"The fisheries sector is acknowledged as a significant source of revenue since it both promotes the expansion of numerous subsidiary companies and supplies low-cost, nutritious food. It has a significant impact on the socio economic lives of thousands of people involved directly or indirectly because it gives millions of rural farmers, especially women, a source of job and money. The growth of fish production as well as development of fishery sector in terms of economy and infrastructure is highly dependent on an efficient fish marketing system" [2].

"Fish is a significant part of peoples' diet all around the world represents a relatively low coast and accessible source of high quality protein for poorer households" [3]. "Due to the greater expense of meat and other sources of animal protein, fish are a rich source of protein that is widely consumed as an alternative source of protein" [4]. "Fish is an extra relishing and nutritive source than the plant food. Fish meat was easily digestible and it was often favoured over meat and eggs. Due to its high protein content and nutritional value, fish is a significant food source in developing nations" [5]. Fish and fishery products are not only nutritionally important but also important in global trade as foreign exchange earner for a number of countries in the world.

"The sale of commercially valuable fish and fish products takes place in a fish market. Fish marketing is the process of regulating fish production and consumption through sales" [6]. "Market infrastructure comprises wholesale markets, retail markets, and fish retail outlets. Large quantities of fish are collected from the surrounding areas and sold to other retailers and wholesalers in wholesale markets" [7]. Fish commodities can be transported using any mode of transportation, including taxis, jeeps, pick-up trucks, buses, trucks, and lorries, but the most crucial factor is to maintain the necessary temperature to prevent the growth of organisms that cause spoilage [8]. "Consumption of fish and shellfish may result in disease due to infection or intoxication. Some of these diseases are caused by microorganisms found on the fish's external surfaces such as slime, gills, and gut. When fishes are alive, their natural defence mechanism keeps these microorganisms from invading the sterile flesh. After death, the microorganisms or enzymes they secrete are free to invade or diffuse into the flesh, where they react with the complex mixture of natural substances present, resulting in a well-defined sequence of changes in odoriferous and flavors compounds" [9]. "The bacteria found on fish are typically associated with those found in their natural environment and are influenced by the season and harvesting conditions" [10]. "The outlined and discussed the risks and challenges associated with handling fish during farming and capture, as well as environmental contaminants in seafood that may pose a risk to human health" [11].

2. REVIEW LITERATURE

Food security is a complicated issue, and due to the presence of pathogens, natural poisons, and other potential pollutants, fish and fishery products are typically considered to be high risk commodities [12]. Zoonosis can directly or indirectly spread a variety of infectious diseases [13]. The bodies of fish merchants and operators are contaminated with various pollutants and have been found to be a source of numerous microorganisms crucial to public health [14]. When customers buy these fish, the accompanying germs are passed on to the people who carry those [15].

Skin mucus functions as a barrier against pathogen attacks and sloughs off continuously [16]. "Mucus plays many biological and ecological roles, including osmoregulation, protection against environmental toxins and heavy metal toxicity, parental feeding, protection

against pathogens" [17]. A variety of antimicrobial substances such as pore-forming glycoproteins, enzymes (such as chitinases with antifungal activity) and proteins (such as apoliporotein-1 and warm temperature acclimation protein WAP65) have been discovered in fish external mucus [18]. Fish mucus has also been found to contain antibacterial peptides (AMPs), which are one of the key compounds used to combat diseases [19].

"Microbial contamination can happen at any point along the upstream supply chain during collection and processing, distribution, storage, marketing and preparation if suitable sanitary handling procedures are not followed" [20]. "Microorganisms have been found to colonize the skin, gills, and the gastrointestinal tract of fresh fish with some being possibly pathogenic" [21]. *Escherichia coli*, a coliform group member is present in the intestinal tracts of humans, animals as well as in fish [22] and indicator of fish and water faecal contamination [23]. *Staphylococcus aureus* is one of the most common causes of human diseases [24]. In the entire world, staphylococcal food poisoning is a prevalent foodborne infection [25].

3. MATERIALS AND METHODS

The mucus sample were collected from the fishes available at Nanpura wholesale fish market, Surat (Gujarat) to find out the edible quality of fishes.

3.1 Sample Collection

Fish samples collected in sterile thermocol boxes with ice were transported instantly to the research laboratory of Aquatic Biology Department, VNSGU, Surat (Gujarat). The mucus sample (1 ml) was carefully scraped from dorsal surface of body by moving a sterile plastic spatula in anterio-posterior direction (head to tail) and shifted in 10 ml of sterile distilled water underneath aseptic environment. Collection of mucus from the ventral area was avoided to eliminate intestinal and urinogenital contamination [26].

3.2 Total Viable Count/Standard Plate Count (Quantitative Examination)

Fish samples were examined for hygienic condition using Total viable count technique as described by Surendran et al [27, 28]. The distilled water (sterile 9 ml) was transferred aseptically into six tubes each and 1 ml of the original fish mucus 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 (1:20 dilution) and mixed it. Procedure continued until the sixth test tube. Each sample was diluted from 10^{-1} to 10^{-6}. Diluted solution (0.2 ml) was inoculated (10^{-4} to 10^{-6}) on each Nutrient agar plates using spread plate technique. All plates were incubated at a temperature of 37°C for 24 hrs. After 24 hrs., the mean colony count on the nutrient agar plates of each dilution was used to evaluated the total viable count for the samples in colony forming units per milliliter (CFU/ml).

3.3 Identification of Bacteria (Qualitative Examination)

Fish samples were examined for hygienic condition using quantitative technique as described by Patel and Patel [29- 31].

3.3.1 Characterization of microorganism (Cultural)

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

3.3.2 Characterization of microorganisms (Morphological)

The colony of isolate was chosen and suspension was made in 1 ml of sterile distilled water. The smear was prepared, after drying fixed with heat. Smear was stained with primary stain crystal violet for one minute. After one minute, smear was covered with Gram's iodine for thirty seconds and washed with distilled water. Thereafter, smear was covered with 95% ethanol for fifteen seconds followed by washing in distilled water. Then smear was stained with counterstain Safranin for five minutes and again washed with diatilled water. Slides were examined under oil immersion and morphological characteristics of microorganisms were noted.

3.3.3 Characterization of microorganisms (Biochemical)

Different microorganisms require different biochemical component for their growth. Selected isolates were biochemically characterized by Indole production test, Voges Proskauer test, Methyl red test, Citrate utilization test, Gelatin liquification test, Nitrate reduction

test, Urea hydrolysis test, Hydrogen sulfide test, Coagulase tests, Triple sugar iron agar and Sugar fermentation test.

4. RESULTS

Twenty fish samples of five species from Nanpura wholesale fish market were analysed to check the quality of fishes.

4.1 Total Viable Count (TVC)

The results of total bacterial count in fish samples expressed in colony forming unit per mililiter (CFU/ml) are shown in Table 1. Colonies were observed in plates ranged from 10^{-4}-10^{-6} dilution factor.

Results showed highest bacterial count 49.4x10^6 CFU/ml (Min. 17.9x10^6 CFU/ml Max. 65x10^6 CFU/ml) and 45.7x10^6CFU/ml (Min. 22.8x10^6 CFU/ml Max. 79.2x10^6 CFU/ml) in fresh samples of *Clarius garipinus* and *Labeo rohita* of Nanpura wholesale fish market. Apart from this, minimum bacterial counts 36.6x10^6 CFU/ml (Min. 20.1x10^6 CFU/ml Max. 57.3x10^6 CFU/ml) from *Pangasianodon hypophthalmus* was noted from same market. Simultaneously, results showed higher bacterial count 47.2x10^6 CFU/ml (Min. 31.2x10^6 CFU/ml Max. 61.7.3x10^6 CFU/ml) from marine fish *Sphyraena jello* too followed by 41.6x10^6 CFU/ml (Min. 23.9x10^6 CFU/ml Max. 58.3x10^6 CFU/ml) from marine fish *Solea solea.*

4.2 Gram Staining and Biochemical Test

The results of qualitative analysis are shown in Table 1. *Escherichia coli* and *Stayphylococcus aureus* were found in all twenty fish samples collected from Nanpura wholesale fish market.

Large circular, slightly raised, translucent white colonies of *Escherichia coli* with entire edge was seen on Nutrient agar (Fig.1) while lactose fermenting pink spreading colonies was on MacConkey's agar (Fig.2) and greenish metallic sheen was seen on selective media such as Eosine methylene blue (EMB) agar (Fig.3). *Escherichia coli* are motile and gram negative bacteria according to gram staining technique (Fig.4). *Escherichia coli* was found to be Indole and methyl red positive while Voges Proskauer and Citrate utilization tests were negative. Organisms fermented glucose, sucrose, lactose, maltose, xylose and mannitol. Triple sugar iron test (TSI) showed a yellow color, acidic slant and acidic butt. Results of various agar plates, gram staining and biochemical tests indicated confirmed presence of *Escherichia coli* in the fish samples.

Small circular, low convex, smooth, opaque white colonies of *Stayphylococcus aureus* with entire edge was seen on Nutrient agar (Fig.5) while golden yellow pigmented colonies were seen on selective media such as Mannitol salt agar (Fig.6). *Stayphylococcus aureus* are non-motile and gram positive bacteria according to gram staining technique. (Fig.7). *Stayphylococcus aureus* was found to be Coagulase, Methyl red, Voges Proskauer, Citrate utilization, Gelatin liquification, Nitrate reduction and Urea hydrolysis positive while Indole and Hydrogen sulfide production tests were negative. Organisms fermented glucose, sucrose, lactose, maltose, xylose and mannitol. Triple sugar iron test (TSI) showed a yellow color, acidic slant and acidic butt. Results of various agar plates, gram staining and biochemical tests indicated confirmed presence of Stayphylococcus aureus in the fish samples.

Table 1. Bacteriological Examination (Quantitative and Qualitative)

Sr. No.	Fishes' Name	Total Viable Count (TVC) (CFU/ml)			Name of the Bacteria
		Minimum	Maximum	Average	
1.	*Clarius garipinus* (fresh)	17.9x10^6	65x10^6	49.4x10^6	*Escherichia coli* *Staphylococcus aureus*
2.	*Labeo rohita* (fresh)	22.8x10^6	79.2x10^6	45.7x10^6	*Escherichia coli* *Staphylococcus aureus*
3.	*Pangasianodon hypophthalmus* (fresh)	20.1x10^6	57.3x10^6	36.6x10^6	*Escherichia coli* *Staphylococcus aureus*
4.	*Sphyraena jello* (marine)	31.2x10^6	61.7x10^6	47.2x10^6	*Escherichia coli* *Staphylococcus aureus*
5.	*Solea solea* (marine)	23.9x10^6	58.3x10^6	41.6x10^6	*Escherichia coli* *Staphylococcus aureus*

Fig. 1. Isolation of *Escherichia coli* on Nutrient agar

Fig. 2. Isolation of *Escherichia coli* on MacConkey's agar

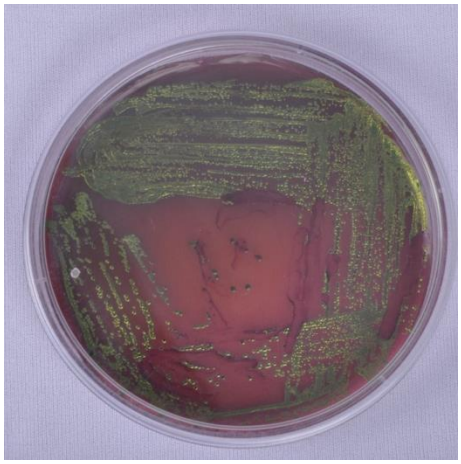

Fig. 3. Isolation of *Escherichia coli* on EMB agar

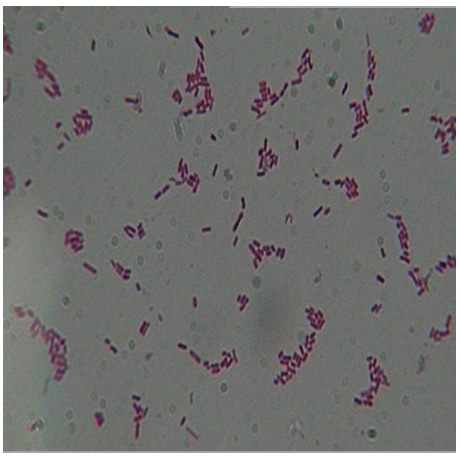

Fig. 4. Gram stain of *Escherichia colin*

Fig. 5. Isolation of *Staphylococcus aureus* on Nutrient agar

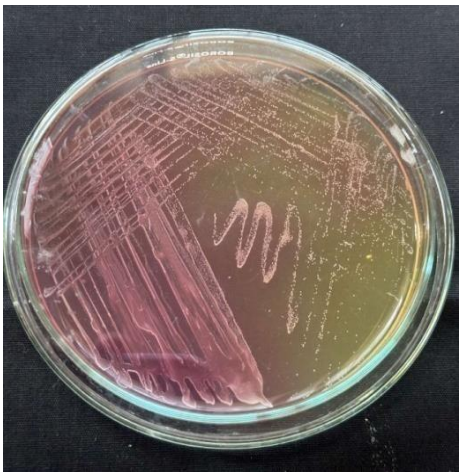

Fig. 6. Isolation of *Staphylococcus aureus* on Mannitol salt agar

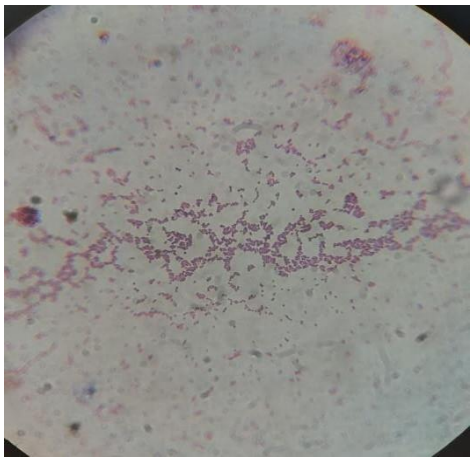

Fig. 7. Gram stain of *Staphylococcus aureus*

5. DISCUSSION

Fish is very important foodstuff in developing countries because of its high protein content, and nutritional value. Fish provides over 50% of the animal protein for the populations of 34 countries [32]. A change in fish or fish products known as spoilage renders them unfit for human eating or less acceptable, unsatisfactory, or harmful. Fish and shellfish can be involved in the spread of toxic substances and harmful microbes because the contamination typically originates from human and animal sources [33].

Microbiological analysis is a tool to assess the edible quality of fish and pathogenic bacteria harmful to humans. Type and total number of bacteria on fish skin, water, ice, mucus and contact surfaces usually indicate the quality of fishes. In present study, fish mucus of different fish samples was analyzed to evaluate the microbial quality of fishes.

In fish, the epithelial surfaces are covered by a slimy, slippery layer called the mucus. It can also be defined as a viscous colloid containing the antibacterial enzymes, proteins and water, etc known as mucins. It serves as an important component of innate immune mechanism in two ways. Firstly, by producing continuously and being sloughed off regularly, it prevents the adherence of pathogens and stable colonization of potential infectious microbes and invasion of parasites [34]. Secondly, it contains a number of factors of innate immunity like proteins and enzymes such as lysozyme, immuno-globulin, complement proteins, lectins, C-reactive protein (CRP), proteolytic enzymes, transferring, alkaline phosphatise (ALP) and various other antibacterial proteins and peptides etc. Arockiaraj et al [35, 36].

The aquatic environment is rich in pathogenic organisms and the aquatic animals including fish are obliviously prone to the invasion of these pathogens [37]. Therefore, the skin mucus in fish plays a significant role as it provides the first line of defence and is continuous with the linings of all body openings covering the fins also. The mucus has a wide range of functions including disease resistance, protection, as well as respiration, ionic and osmotic regulation, reproduction, excretion, communication, feeding and nest building [38].

But contrast to this research, high bacterial load (TPC) was found in mucus of all fresh (*Clarius garipinus*, *Labeo rohita* and *Pangasianodon hypophthalmus*) and marine (*Sphyraena jello* and *Solea solea*) fish samples from study area due to poor sanitary condition of the market and poor preservation facility. Preservation in low quality ice, handling with contaminated hands could also be liable for increased density of aerobic bacteria. Fish are very much prone to contamination with various bacteria because of their perishable protein content [39]. The prevalence of total bacterial counts in high ranges in various samples taken from retail market places raises concerns regarding the hygiene condition of the production and point-of-sale environment. The eating of raw or inadequately cooked fish and fish products, however, poses the biggest threat to human health [11]. Quantitative analysis of fish mucus was conducted first time in this particular area, so permissible limit of TPC for fish mucus was not described anywhere.

All fresh (*Clarius garipinus*, *Labeo rohita* and *Pangasianodon hypophthalmus*) and Marine (*Sphyraena jello* and *Solea solea*) fish sample from Nanpura wholesale fish market were contaminated with pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus.* It was due to unhygienic condition and inadequate use of ice. Temperature plays an important role in growth of bacteria. In support to current study, number of *Escherichia coli* had increased remarkably at the temperature between 31°C to 34°C in all fish samples collected from ponds of Nadia district of West Bengal as increase in temperature adversely affected the population of *Escherichia coli* reported by Dutta et al [40]. This warm temperature is extremely suitable for *Escherichia coli* development and proliferation. This organism is able to produce hazardous amounts of toxin histamine in a very short duration when the fishes are exposed to elevated temperature which cause food poisoning, diarrhoea, meningitis and septicaemea [41-43]. and a health hazard to human [44].

Staphylococcus aureus produces enterotoxins, which are important for public health because they may cause gastroenteritis when consumed with fish and related products [45]. *Staphylococcus aureus* is resistant to heat, drying, radiation, and it creates a toxin that is not eliminated by heat [46]. *Staphylococcus aureus* can create toxins and enzymes that can worsen several illnesses, including food poisoning, septic shock and toxic shock syndrome [47] which threatens consumer's health.

6. CONCLUSION

The findings of the present study indicated that fish mucus of all fresh and marine fish samples were contaminated with pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus.* Mucus secretion after exposure to bacterial challenges indicates its involvement in protection against pathogenic attacks. Higher mucus secretion could be an indicator of increased stress level in fishes. Above described pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* cause fish deterioration and food poisoning which make fishes unsafe for human consumption. Result also highlights the potential influence of local fish handling and processing on consumer health worldwide. The introduction of periodic training in fish handling from capture to marketing and hygiene for fish handlers as well as need to enhance an awareness in the public and in fish handlers about proper processing, preservation, storage and hygienic handling practices is essential for consumer's good health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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