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ORIGINAL RESEARCH

Bacterial Profiling of Fish in Kathmandu Valley Market

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ABSTRACT

Objectives: The purpose of the study was to identify the bacterial species in fishes as well as to enumerate the total viable count and total coliform count in the fish sample from Kathmandu valley market.

Methods: A descriptive study was conducted where two species of fish i.e., Rohu and Bachua were collected from several shops within the Kathmandu valley. Each fish was separated into gills, gut, and skin portions, constituting a total of 18 samples and the samples were then processed according to standard laboratory methods for the isolation and identification of bacteriological species.

Results: In a total sample size of 18 samples, 44 isolates were isolated where 20.46% of the isolates were found to be gram-positive bacteria and 79.54% of the isolates were found to be gram-negative bacteria. *Escherichia coli* accounted for 38.64%, resulting in being the dominant organism. The sample collected from Kalimati in Bachuwa showed the highest bacterial count (1.01×10^7cfu/gm) while the sample from Lalitpur in Rohu showed the lowest bacterial load (9.68×10^4cfu/gm). In Rohu samples, the highest coliform load and lowest coliform load was collected from Kalimati (4.25×10^5cfu/gm) and Lalitpur (3.78×10^3cfu/gm), respectively.

Conclusion: The highest bacterial load, coliform load and isolated pathogens in fishes available in the market of Kathmandu valley from this study concluded that the fishes are highly vulnerable to bacterial contamination, and suggest the potential risk for public's health issues.

Keywords: Fish, Rohu, Bachuwa, Bacterial load, Coliform

INTRODUCTION

Fish and fish products only supply about 34 calories per person per day on average worldwide. Fish does, however, contribute significantly to the diet in terms of high-quality, readily digested animal proteins and particularly in the prevention of micronutrient defi ciencies, in addition to serving as an energy source (FAO, 2018).

Consuming oily fish that is high in long-chain omega-3 fatty acids can help lessen the risk of cardiovascular disease and minimize systemic inflammation(Bowen et al., 2016).In Nepal, aquaculture is one of the agricultural subsectors with the quickest rate of growth. The most popular species cultivated are rainbow trout, pangas catfish, and both native and exotic carp. Although aquaculture's institutional development in Nepal began about seven decades ago, the industry developed at a very modest pace. All the same, this industry has made tremendous strides in the last ten years. In Nepal, people consume comparatively less fish than they do chicken, hog, beef, and mutton. People's growing health consciousness has increased demand for aquaculture sectors and resulted in a surge in fish consumption.

Fish is an essential part of the human diet, and in Nepal, the number of fish consumed per person is rising. However, the rapid expansion of industry and agriculture may contaminate both naturally occurring and artificially created aquatic ecosystems, which could

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have an impact on fish health and raise questions about the safety of fish intended for human consumption (Novoslavskij et al., 2016).

Fish illnesses caused by bacteria are among the most prevalent and challenging medical issues to treat. Fish fingerlings, fry, and eggs have all been known to contract bacterial infections that result in high mortality rates. These microbes are essentially opportunistic pathogens that enter the tissues of fish hosts that have been exposed to stressors that can cause infection (Guzman et al., 1988). Columnariosis, farunuclosis, tail rot/fin rot, bacterial gill diseases, aeromoniasis, edwardsiellosis, vibriosis, eye disease, pseudomonasis, and enteric red mouth disease are among the bacterial infections that are frequently observed. The parasite condition alone results in an 8–12% reduction in productivity when compared to other illnesses. Under favourable circumstances, fish parasites proliferate quickly, impacting fish health and frequently resulting in high mortality. Parasites affect host nutrition by interfering with the metabolism and secretary functions of the alimentary canal and damaging the nervous system (Shrestha et al., 2019).

Fish from the natural environment are known to harbour various species. Regular exposure to contaminated water causes bacterial colonization on fish skin and gills, and contaminated feed or water can harm the digestive tract. When immunological resistance is weakened, fish muscles may also become contaminated (de Cuesta et al., 2011). A study from UK reported that total bacterial count (TBC) on the skin of salmon (*Salmo salar*) varied from 10^2 to 10^3 cfu/cm^3. Meanwhile,a similar study carried out in Turkey revealed a higher number of 10^1 to 10^7 cfu/cm^3 on salmon skin and aerobic microorganisms was detected more often than anaerobic (Minniti et al., 2019). It is commonly known that the bacteria that exist in water that is contaminated are similar to those found on fish skin These bacteria include *Aeromonas* spp, *Flexibacter* spp, *Proteus* spp, *Providencia* spp, *Psychrobacter* spp, *Moraxella* spp, *Pseudomonas fluorescens, Acinetobacter johnsonii, Alcaligenes piechaudii, Enterobacter aerogenes, Escherichia coli, Micrococcus luteus,* and *Vibrio fl uvialis* (Novoslavskij et al., 2016).

METHODS

Research design, duration, and laboratory setting

This study was a descriptive study conducted at KIST College of Management, Kamalpokhari, Kathmandu, Nepal from December, 2023 to May, 2024. A total of six fishes of 2 different species were collected from several shops in Kathmandu Valley. Each fish was separated into gills, gut, and skin portions, constituting a total of 18 samples. These fishes were transported in sterile zip-lock bags along with ice and brought to the laboratory and the samples were then processed according to standard laboratory methods (Cheesbrough, 2006).

Study sites

Sites were selected in various locations: Kathmandu (Kalimati), Bhaktapur (Sukuldhoka), and Lalitpur (Mangalbazar). Parameters such as cleanliness, store location, and general hygiene around the fish shops were recorded. The samples' properties, including temperature, location, date, and time, were appropriately labelled.

Sample collection and Transportation of sample

The fish was placed into a zip-lock, hygienic plastic container with ice box. Surgical gloves and sterile hands cleaned with chloroxylenol were used during the transfer. Each time a sample was to be taken; this process was repeated. With caution to avoid overfilling the container, each sample was moved to a different container. It was sent straight to the lab, where it was handled with care to preserve the sample's integrity by creating an atmosphere that would not change the microbial flora in the fish sample in any manner.

The sample was brought to the lab in minutes or hours in a sterile container. The temperature, amount of sunshine, and other environmental elements was carefully controlled to avoid changing the sample's pre-existing microflora (Cheesebrough, 2006).

Sample processing

Homogenization

Using a sterile blade, the fish sample was cut into tiny pieces. 25 grams of each gill, gut, and skin from the fish samples were weighed and homogenized separately using a sterile mortar and pestle. Then, each homogenized sample was mixed with 250ml of diluent for the microbiota enumeration process (Cheesebrough, 2006).

Enumeration of bacteria and coliform

For enumeration, 1 ml of diluent of the homogenized sample was serially diluted from 10^{-1} to 10^{-6} dilutions. 1 ml of dilution was taken and poured onto sterile petri plates for each dilution. Then, about 25ml of molten Plate count agar (PCA) and Violet red bile agar (VRBA) were poured onto these dilutions for Total Plate Count and Total Coliform Count respectively. Finally, Petri plates

were incubated at 37°C for 24 hours. After 24 hours, the number of colonies on each plate was enumerated.

Enrichment of the Sample

For the isolation of pathogenic bacteria like *Salmonella* and *Vibrio*, the homogenized sample was enriched in their respective enrichment media such as Selenite F broth and Alkaline Peptone water. About 10 ml of each homogenized sample was transferred to 50 ml of Selenite F broth for *Salmonella* as well as Alkaline Peptone water for *Vibrio*. Then, it was incubated at 37°C for 24 hours (Cheesebrough, 2006).

Isolation of pathogenic bacteria

To isolate pathogenic bacteria from the fish samples, different selective and differential media were prepared. For example: Mannitol Salt Agar (MSA), MacConkey Agar (MA), Xylose Lysine Deoxycholate (XLD) Agar, and Thiosulfate Citrate Bile salts Sucrose (TCBS) Agar. MSA is a selective media for the isolation of gram-positive *Staphylococcus* spp MA is commonly used for isolating the Enterobacteriaceae family as well as differentiating lactose fermenters and non-lactose fermenters. XLD agar is used for the isolation of *Salmonella* whereas TCBS agar is used for *Vibrio* spp. A loopful of suspension of diluent was taken in a sterile loop and streaked over the media. This process was repeated for every media prepared. Then, these plates were incubated at 37°C for 24 hours. After 24 hours, the colony characteristics were studied, sub-cultured on the Nutrient Agar (NA), and again incubated at 37°C for 24 hours. The colonies from NA plates were subjected to gram staining and biochemical tests (Cheesebrough, 2006).

Identification

Staining

The gram staining was performed from the isolated pure culture in NA.

Biochemical Tests

Biochemical tests such as Catalase test, Oxidase test, Indole test, Methyl red test, Voges-Proskauer test, Citric acid utilization test, Triple sugar iron agar test, Urease test, Motility test, Sulphide production test, Gas production test, etc. were performed by standard method (Cheesebrough, 2006).

RESULTS

Percentage-wise distribution of bacteria in fish samples

In a total sample size of 18 samples, 44 isolates were isolated where 20.46% of the isolates were found to be gram-positive bacteria and 79.54% of the isolates were found to be gram-negative bacteria. *Escherichia coli* (*E. coli*) accounted for 38.64%, resulting in being the dominant organism. Likewise, *Staphylococcus aureus* accounted for 13.64% of total organisms isolated, while the percentage of *Salmonella* was found to be 11.36%. *Pseudomonas* spp and *Vibrio* spp were both found to be 9.09% and also both *Staphylococcus epidermidis* and *Proteus* were found to be 6.82%. *Klebsiella* was also isolated and found to be 4.55% of the total bacteria isolated from the sample (Figure 1).

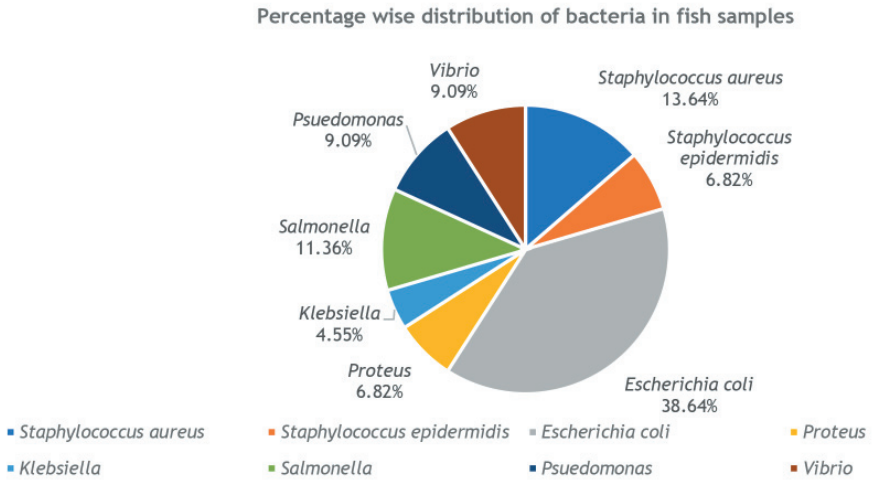

Figure 1: Percentage-wise distribution of bacteria in fish samples

Site-wise distribution of bacterial species in different fish samples

Two species of fish (Rohu and Bachuwa) were collected from above mentioned locations, totalling six fishes. Each fish was separated into gills, gut, and skin portions, constituting a total of 18 samples.

Site-wise distribution of total bacterial load (cfu/gm) in fish sample

The sample collected from Bachuwa in Kalimati showed the highest bacterial count (1.01×10^7cfu/gm) followed by Bachuwa in Lalitpur (2.66×10^6cfu/gm) (Table 1).

Site-wise distribution of total coliform load (cfu/gm) in fish samples

The highest coliform load was found to be in Rohu collected from Kalimati (4.25×10^5cfu/gm) followed by the sample Rohu collected from Bhaktapur (3.65×10^4cfu/gm) (Table 1).

Site-wise distribution of bacterial species in different fish samples

In a total of 18 samples, *Staphylococcus aureus* was present in 6 samples. Among these 6 samples, 3 were from Kalimati, 1 was from Bhaktapur and 2 were from Lalitpur. For *Staphylococcus epidermidis*, 2 samples were from in Kalimati sample and 1 sample from the Lalitpur. From a total of 17 samples, *E. coli* was isolated which was the highest among all the organisms. Likewise, *Proteus* was found in 3 samples, one from Kalimati and two from Lalitpur. *Klebsiella* was seen in each sample from Bhaktapur and Lalitpur. *Salmonella* was present in a total of 5 samples, i.e., 2 samples in Kalimati, 1 sample in Bhaktapur, and 2 samples in Lalitpur. *Pseudomonas* and *Vibrio* were present in 4 samples. Similarly, *Vibrio* was found in 1 sample of both Kalimati and Lalitpur. Kalimati had the highest number of bacterial loads (17 samples) followed by Lalitpur (15 samples) and Bhaktapur (12 samples) (Table 2).

Bacterial species distribution in different parts of fish samples

The skin accounted for the highest number of organisms isolated which was 17 isolates while the gill accounted for the lowest number of organisms which was 12 isolates. *E. coli* was the most dominant bacteria in all three parts of the fish samples (Table 3).

Comparing bacterial species isolated in two different fish samples i.e. Bachuwa and Rohu

Among the 2 species of fish, Rohu accounted for the highest number of isolates which was 23 and Bachuwa accounted for 21 isolates (Table 3). The result provided a statistical basis to conclude that there was no association of bacterial species isolated in two different fish samples i.e., Bachuwa and Rohu ($p>0.05$) (Table 3)

Table 1: Site-wise distribution of total bacterial load (cfu/gm) and total coliform load (cfu/gm) in fish samples

Site	Fish	Parts	Total bacterial load (cfu/gm)	Average bacterial load (cfu/gm)	Total Coliform Load	Average Coliform Load
Kalimati	Rohu	Gill	1.62×10^7		2.42×10^5	
		Gut	3.25×10^6	1.29×10^6	1.57×10^5	4.25×10^5
		Skin	1.93×10^7		8.78×10^5	
	Bachuwa	Gill	6.85×10^5		8×10^3	
		Gut	1.21×10^4	1.01×10^7	4.11×10^3	6.05×10^3
		Skin	2.96×10^7		0	
Bhaktapur	Rohu	Gill	4.02×10^6		8.85×10^5	
		Gut	6.22×10^5	1.4×10^6	1.42×10^5	3.65×10^4
		Skin	8.09×10^4		6.94×10^4	
	Bachuwa	Gill	3.02×10^5		6.7×10^2	
		Gut	1.18×10^5	1.51×10^5	2.58×10^4	1.32×10^4
		Skin	3.45×10^4		0	
Lalitpur	Rohu	Gill	1.94×10^4		1.81×10^3	
		Gut	2.68×10^5	9.68×10^4	9.53×10^3	3.78×10^3
		Skin	2.91×10^3		10	
	Bachuwa	Gill	1.85×10^5		6.7×10^3	
		Gut	7.79×10^6	2.66×10^6	2.16×10^3	4.43×10^3
		Skin	9.4×10^2		0	

Table 2: Site-wise distribution of bacterial species in different fish samples

	No. of samples from				
Microorganism	**Kalimati**	**Bhaktapur**	**Lalitpur**	**Total**	**Total %**
Staphylococcus aureus	3 (17.64%)	1 (8.33%)	2 (13.33%)	6	13.64
Staphylococcus epidermidis	2 (11.76%)	0	1 (6.66%)	3	6.82
Escherichia coli	6 (35.29%)	6 (50.0%)	5 933.33%)	17	38.64
Proteus	1 (5.88%)	0	2 (13.33%)	3	6.82

Microorganism	No. of samples from				
	Kalimati	Bhaktapur	Lalitpur	Total	Total %
Klebsiella	0	1 (8.33%)	1 (6.66%)	2	4.53
Salmonella	2 (11.76%)	1 (8.33%)	2 (13.33%)	5	11.36
Pseudomonas	2 (11.76%)	1 (8.33%)	1 (6.66%)	4	9.09
Vibrio	1 (5.88%)	2 (16.66%)	1 (6.66%)	4	9.09
Total	**17 (38.63%)**	**12 (27.27%)**	**15 (34.1%)**	**44**	**100**

Table 3: Bacterial species distribution in different parts of fish samples

Microorganisms	Bachuwa			Rohu			Total
	Skin	Gill	Gut	Skin	Gill	Gut	
Staphylococcus aureus	1 (11.11%)	1 (16.66%)	0	3 (37.50%)	1 (16.66%)	0	6 (13.63%)
Staphylococcus epidermidis	1 (11.11%)	0	0	1 (12.50%)	1 (16.66%)	0	3 (6.82%)
Escherichia coli	2 (22.22%)	3 (50.0%)	3 (50.0%)	3 (37.50%)	3 (50.0%)	3 (33.33%)	17 (38.63%)
Proteus spp	1 (11.11%)	0	1 (16.66%)	0	0	1 (11.11%)	3 (6.82%)
Klebsiella spp	1 (11.11%)	0	0	0	0	1 (11.11%)	2 (4.54%)
Salmonella spp	1 (11.11%)	1 (16.66%)	1 (16.66%)	0	1 (16.66%)	1 (11.11%)	5 (11.36%)
Pseudomonas spp	0	1 (16.66%)	0	1 (12.50%)	0	2 (22.22%)	4 (9.10%)
Vibrio spp	2 (22.22%)	0	1 (16.66%)	0	0	1 (11.11%)	4 (9.10%)
	9 (20.45%)	6 (13.64%)	6 (13.64%)	8 (18.18%)	6 (13.64%)	9 (20.45%)	44 (100%)

DISCUSSION

A total of 44 isolates were isolated from these 18 samples. Among these 44 isolates, 9 isolates were found to be gram-positive bacteria (20.46%) while 35 isolates were found to be gram-negative bacteria (79.54%). *Staphylococcus aureus* and *Staphylococcus epidermidis* were among the gram-positive bacteria while gram-negative bacteria included *E. coli, Proteus, Klebsiella, Salmonella, Pseudomonas,* and *Vibrio.* Out of 9 gram-positive bacteria, 6 of the isolates were *Staphylococcus aureus* (13.64%) and 3 of the isolates were *Staphylococcus epidermidis* (6.82%). Out of 35 gram-negative bacteria, 17 of the isolates were *E. coli* (38.64%), 3 *Proteus* spp 6.82%), 2 *Klebsiella* spp (4.53%), 5 *Salmonella* spp (11.36%), 4 *Pseudomonas* spp (9.09%) and 4 *Vibrio* spp (9.09%).

Comparing the two species of fish, Rohu had a higher number *of E. coli* than Bachuwa and the bacteria was most frequently isolated from the gut region of the fish. *E. coli* can cause serious complications related to the gastrointestinal tract such as dysentery, urinary tract infections, diarrhoea, meningitis and even pneumonia (Johnson et al., 2009). However, *E. coli* is not a common microflora of gut in fish, which could indicate that the organism came into existence from the faecal contaminated water (Lovell & Barkate, 1969).

After *E. coli, Staphylococcus aureus* and *Salmonella* were the frequently observed bacteria. *Staphylococcus aureus* was present in both skin and gill but absent in the gut. *Staphylococcus aureus* commonly found on human skin and environment can cross-contaminate with fish. They are present in the environment including water and on surfaces where fishes are processed or stored. Poor sanitation practices can contribute to the presence of these bacteria in the fish handling and processing environment (Taylor & Unakal, 2023).

Infections caused by *S. aureus* range from mild to life-threatening producing skin infections, often causing abscesses including bacteraemia, endocarditis, and osteomyelitis. Some strains of *S. aureus* produce toxins that cause staphylococcal food poisoning or toxic syndrome (Taylor & Unakal. 2023).

In the case of *Salmonella*, it was mainly isolated from the gut region of the fish. *Salmonella* is considered unfit for humans to be consumed contaminated with food. *Salmonella* in freshwater fishes has been usually related to the faecal contamination of water from where fish were harvested. *Salmonella* is the causative agent of salmonellosis, a severe form of gastroenteritis which is still a major prevalent considered one of highly common food-borne illness and a major public health problem. It is obvious that consumption of *Salmonella*-infected fishes can increase public health problems. The contamination of fish through aquatic environment, contaminated by humans and poultry itself may create a secondary food reservoir (Bibi et al., 2015).

About 4 isolates of *Vibrio* was found mostly in Bachuwa. This organism was most common in the gut and skin. *Vibrio* spp is key pathogen in many aquacultures system which are part of normal flora of intestine of many aquatic species. Therefore, vibriosis

is a major fish disease among many species of cultured fish (Manchanayake et al., 2023).

Fish hold an important position as a food component for a large section of world population, including Nepal. In Nepal, the consumption of fish and fish products has contributed a remarkable position in the market for many years. So, maintenance of appropriate quality of the products is regarded as vital for achieving desired success in trade of the product and health of the consumer. However, there still exists a huge problem in the fish markets in terms of microbiological view point. As the fish markets lacks in sanitation criteria, it could result in huge economic losses. The main source of contamination in fish usually starts from the fisheries. There might possibility of contaminated water being used in the fisheries. Even when these fish are brought from fisheries to market, they could get contaminated due to a lack of proper transportation facilities. The local cold stores in our locality are not well managed, measuring devices/knives are not fully sanitized and the shopkeeper use barehand to pick the fish and pack it. There can be possibilities of flies flying around the fish if the fish products are not kept inside the glass cover.

CONCLUSION

The highest bacterial load, coliform load and isolated pathogens in fishes available in the market of Kathmandu valley from this study concluded that the fishes are highly vulnerable to bacterial contamination, and suggest the potential risk for public's health issues. The differences in bacteriological load between fish samples highlight how storage, handling, and environmental factors affect the degree of microbial infection. The study emphasized the need for future research to create better methods for preventing bacterial contamination in fish.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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