Nepal Journal of Biotechnology
Publisher: Biotechnology Society of Nepal ISSN (Online): 2467-9313
Journal Homepage: https://nepjb.com/index.php/NJB ISSN (Print): 2091-1130

Microbial Quality Assessment of Raw Freshwater Fish Sold in Local Markets of Kathmandu Valley

Shristi Prasai, Puja Shrestha, Sriniwas Pandey, Ishika Adhikari, Srijana Gurung, Kamil Prajapati
St. Xavier's College, Maitighar, Kathmandu, Nepal

Received: 14 May 2022; Revised: 8 Jul 2022; Accepted: 15 Jul 2022; Published online: 30 Jul 2022

Abstract

Microbial quality of *Labeo rohita, Cyprinus carpio* and *Clarias batrachus* collected from the markets of Kathmandu valley was evaluated. 9 freshwater fish (skin, gills, intestine) were sampled and were analyzed for Total Plate Count (TPC), Total Coliform Count (TCC) and Total Fecal Coliform Count (TFCC). The average TPC ranged from 4.1 x 10^7 to 1.02 x 10^8 cfu/gm, with the highest count in *C. batrachus* and the lowest in *C. carpio*, whereas the organ wise load was the highest in intestine with 1.3 x 10^8 cfu/gm and the lowest in skin with 1.02 x 10^7 cfu/gm. The highest TCC and TFCC was found in *C. carpio* and *C. batrachus* respectively, whereas organ wise distribution showed the highest count in intestine for both TCC and TFCC. The pathogens isolated from the samples were *Escherichia coli, Staphylococcus aureus*, Coagulase negative S*taphylococcus (CoNS), Vibrio cholerae, Salmonella* Typhi and *S.* Paratyphi. *E. coli* was isolated from 67% of *L. rohita*, 44.44% of *C. carpio* and 66.67% of *C. batrachus*. *S. aureus* was isolated from 44.44% of both *L. rohita* and *C. batrachus* whereas 55.55% of *C. carpio*. CoNS were isolated from 33.33% of *L. rohita*, 22.22% of *C. carpio* and 33.33% of *C. batrachus*. *S.* Typhi was isolated from 11.11% of *C. carpio* and 22.22% of *C. batrachus*. *S.* Paratyphi was isolated from 11.11% of both *L. rohita* and *C. batrachus*, *V. cholerae* was isolated from 11.11% of *L. rohita*, 33.33% of *C. carpio* and 22.22% of *C. batrachus*. The observation of this study showed higher bacterial load in all of the fishes above the acceptance level and presence of Total Coliform, Fecal Coliform and potential human pathogens suggests that the microbial quality of the fish available in the market is not satisfactory. Hence, the fishes possess a threat to public health safety and there is an urgent need to improve the Quality Control and Quality Assurance Systems for fish markets of Kathmandu valley.

Keywords: Raw fish, microbial quality, *E. coli*, *S. aureus*, *Vibrio cholerae*.

Corresponding author, email: kalkam2013@gmail.com

Introduction

Fish is one of the chief sources of protein and has remained an important part of consumption for many centuries[1]. The poikilothermic nature of fresh fish allows a wide variety of bacteria such as *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Shewanella*, *Flavobacterium*, and *Vibrio* among Gram negative and Gram-positive bacteria such as *Bacillus*, *Micrococcus*, *Clostridium*, *Lactobacillus*, and *Corynebacterium* [2]. The non-indigenous ones that contaminate the fish or the habitat include *Escherichia coli*, *Clostridium botulinum, Aeromonas*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Listeria monocytogens* and *Salmonella* spp. The indigenous bacterial pathogens that are found naturally in the fish habitat are *Vibrio* spp. and *Aeromonas* spp. [3].

Microbiological quality of raw fish results from microbiological load of aquatic habitat, methods of capture, transportation, chilling and storage conditions imposing a threat of food-borne infections as the pathogen can be conveyed to consumers at retail level through raw fish. Coliform, especially *Escherichia coli* are often used as criteria to assess the quality and safety of foods [1]. Extraneous bacteria, *Escherichia coli* is the fecal indicator capable of surviving in fish and found to be surviving and even multiplying in the digestive tract of rainbow trout (*Oncorhynchus mykiss*) [4].

Water being the habitat, fish is continually bathed in aqueous suspension of various microorganisms and their exterior surface, hence is in constant contact with these organisms. Some of the microorganisms may colonize the external parts of fish becoming the resident microflora. The presence of the microflora adds to the defense system of fish, thereby inhibiting the accession and consequent colonization by other potential pathogens. Obviously, the bacterial flora of fish depends on the fish's recent intake diet and the extent of contamination in the food [5].

A study performed on fish skin sampled from the lake Hawassa of Southern Ethiopia by [6], resulted in finding of the pathogenic strains of *E. coli* to be contaminating the fish with statistically significant results as defense to the fact that fish are contaminated enough to cause food-

borne illness. According to Sichewo et al. (2014) [7], inspection of various organs of fish such as skin, intestine, gills and mouth collected from different fish ponds of Zimbabwe showed presence of *Salmonella* Typhi from Nhengo, Imbayago and Nyamakwe. According to Kumari et al. (2001) [8], gill and intestine samples of *Labeo rohita* when processed revealed to be contaminated with coagulase negative *Staphylococcus* spp. and *Staphylococcus aureus*. According to Xu et al. (2019) [9], *V. cholerae* was detected from the intestines of ten freshwater fish species collected, including *Astatotilapia flaviijosephi*, *Barbus longiceps*, *C. idella*, *Cyprinus carpio*, *Mugil cephalus*, *Myripristis murdjan*, *Oreochromis aureus*, *Sarotherodon galilaeus*, and *Tilapia* spp. and *Tilapia zilli*.

Improper handling or consumption of undercooked or raw fish may contribute to the intake of pathogens which can potentially cause diseases. Diseases in human that can be caused by bacteria present in fish includes food poisoning and gastroenteritis, diarrhoea, superficial wound infections and ulcers, bacillary dysentery (shigellosis), clonorchiasis, dracunculiasis and paragonimiasis due to larvae and metacercariae ingested in fish and crustaceans, cholera, typhoid and paratyphoid, etc. [4]. *Salmonella*, *Staphylococcus* spp., *Escherichia* spp., *Vibrio parahaemolyticus*, *Clostridium perfringens*, *Clostridium botulinum* E and Enteroviruses are held responsible for majority of fish concerned with food borne diseases [2].

The safety concerns instigated the need for the study i.e. to investigate the presence of any human pathogenic bacteria from live freshwater fishes that are popularly consumed in Kathmandu valley.

Materials and methods

Collection of fish sample

Simple random sampling method was used for selecting samples. The freshly slaughtered 9 fish samples were collected on different day from different retail markets of Kathmandu valley (Sundhara, Baneshwor, Thapagaun, Gairigaun, Ghatthaghar) and brought to the laboratory in an icebox. Fishes were killed by the retailers without causing any physical injury just before the sample collection. The three species of fish, *L. rohita* (rohu), *C. carpio* (common carp) and *C. batrachus* (mungri) were chosen based on popularity among the consumers and commercial availability.

Sample preparation and processing

Sample preparation was done according to Sichewo et al. (2014) [7]. Fish was cut ventrally to collect intestine and gills using sterile surgical blades and forceps in aseptic condition. One g of intestine and gills were taken and crushed into fine solution by adding 10ml of normal saline in sterile mortar, from where one ml of aliquot volume was taken and serially diluted up to 10^{-5}. Skin samples were taken by rolling sterile cotton swabs all over the skin surfaces of all 9 fish and then inoculated into 10ml of normal saline. It was then serially diluted up to a dilution of 10^{-5}.

Bacteriological analysis of fish samples

For total plate count (TPC), 0.1ml sample from 10^{-3} and 10^{-5} dilutions were taken and spread plating was done on PCA agar. The plates were incubated for 24 hours at 37°C. For total coliform count (TCC) and total fecal coliform count (TFCC), spread-plating was done from 0.1 ml of every sample of 10^{-3} dilutions in VRBA agar plates and incubated at 37°C and 44.5°C, respectively for 24 hours. For isolation of *Salmonella* spp. one ml of sample was inoculated in 9 ml of enrichment media, Selenite F Broth, from where a loopful of sample was taken and cultured on SS Agar. For *Vibrio cholerae*, one ml of sample was inoculated in 9ml of enrichment media, alkaline peptone water, incubated at 37°C for 24 hours. A loopful of sample was taken from enrichment broth and cultured on TCBS Agar. A loopful of original sample was streaked on MacConkey Agar (for *E. coli*) and on Mannitol Salt Agar plate (for *Staphylococcus aureus*) and the plates were incubated at 37°C for 24 hours. The colonies obtained from TCBS, SS, MA and MSA were further sub-cultured on NA [10] and identified by gram staining and biochemical tests (IMViC, TSIA, Urease, Catalase, Oxidase, Oxidative/fermentative test). For *Vibrio cholerae* string test was also performed while *Staphylococcus aureus* was confirmed by coagulase test.

Results

Table 1. Average TPC of fish samples

Sample	Organs (cfu/gm in average)			
	Skin	Gills	Intestine	Average
LR	6.5×10^6	8.2×10^7	8.05×10^7	5.6×10^7
CC	1.23×10^7	2.96×10^7	8.03×10^7	4.1×10^7
CB	1.2×10^7	6.6×10^7	2.3×10^8	1.02×10^8
Average	1.02×10^7	5.9×10^7	1.3×10^8	

Note: LR = Labeo rohita, CC = Cyprinus carpio and CB = Clarias batrachus

In this study, 9 fish sample of 3 different varieties, *L. rohita* (LR), *C. carpio* (CC) and *C. batrachus* (CB) were analyzed for its microbial quality and antimicrobial susceptibility testing was done for the isolated strain. The average TPC of LR, CC and CB ranged from 4.1×10^7 to 1.02×10^8 cfu/gm (**Table 1**). The highest bacterial load was found in *C. batrachus* whereas the lowest in *C. carpio*. Among the

Table 2. Average TCC and TFCC of fish samples

Sample	Total Coliform Count (cfu/gm)				Total Fecal Coliform Count (cfu/gm)			
	Skin	Gills	Intestine	Average	Skin	Gills	Intestine	Average
LR	1.5×10^5	3.5×10^5	2.4×10^5	2.47×10^5	No growth	9.4×10^6	2.6×10^5	1.77×10^5
CC	1.2×10^6	2.1×10^5	1.2×10^6	8.7×10^5	No growth	No growth	1.6×10^5	1.6×10^5
CB	7.6×10^5	2×10^5	TMTC	4.8×10^5	1.7×10^5	2.9×10^5	4.6×10^5	3.07×10^5
Average	7.03×10^5	2.53×10^5	7.2×10^5		1.7×10^5	1.92×10^5	2.93×10^5	

Note: LR = Labeo rohita, CC = Cyprinus carpio, CB = Clarias batrachus, TMTC = Too many to count

body parts, intestine was found to be highly loaded with the bacteria in all the fishes.

Total coliforms were present in all parts of the three species of fishes. TCC ranged from 2.47×10^5 to 8.7×10^5 cfu/gm while TFCC was found to be 1.6×10^5 to 3.07×10^5 cfu/gm (**Table 2**). Fecal coliforms were present in all parts of all the analyzed fishes except for skin of *L. rohita*, and skin and gills of *C. carpio*. All parts of *C. batrachus* showed presence of fecal coliforms.

Six different organisms were isolated from gills, skin and intestine of the three fish samples (**Table 3)**. In case of *L. rohita*, gills were found to harbor the diverse species of bacterial pathogens compared to skin and intestines. Gills were found to harbor *S. aureus* (66.67%), *V. cholerae* (33.33%), *E. coli* (100%) and CoNS (33.33%). *E. coli*, *S. aureus* and CoNS each were present in 66.67% of skin sample. *E. coli* was found in 100% and *S.* Paratyphi in 33.33% of intestine. *S.* Typhi was not isolated from any of the three body parts. As for *C. carpio*, *S. aureus* was present in 100% of gills, 66.67% of skin. *V. cholerae* was found in 66.67% of gills and 33.33% of intestine. *E. coli* was found in 66.67% of gills and 33.33% of both skin and intestine. CoNS were present in 33.33% of both skin and gills. *S.* Typhi was present in only 33.33% of intestine whereas *S.* Paratyphi was not isolated from any of the three body parts. In case of *C. batrachus*, gills showed the prevalence of most of the type of bacteria. *E. coli* was present in 100% of gills, 66.67% of intestines and 33.33% of skin. *S. aurues* was in 100% of skin and 33.33% of gills. CoNS was present in 66.67% of skin and 33.33% of gills. *S.* Typhi was present in 33.33% of gills and intestines each.

Discussions

This study was carried out in the quest to determine the microbial quality of raw and freshly killed freshwater fishes available in retail markets of Kathmandu valley. The skin, gills, and intestine of the samples were analyzed for TPC, TCC and TFCC.

The average total plate count of *L. rohita*, *C. carpio* and *C. batrachus* was found to be 5.6×10^6, 4.1×10^7, 1.02×10^8, respectively indicating that latter had higher microbial load than the other two (**Table 1**). As for organ-wise distribution, skin of *L. rohita* contained least microbial load compared to gills and intestine where as in *C. batrachus* intestine had the highest microbial load. However, in *C. carpio*, all three organs had similar

Table 3. Distribution of bacteria in skin, gills and intestine of fish samples.

Sample	Organ (N=3)	Isolates					
		S. aureus	S. Paratyphi	S. Typhi	V. cholerae	E. coli	CoNS
LR	Skin	2(66.67%)	ND	ND	ND	ND	2 (66.67%)
	Gills	2 (66.67%)	ND	ND	1 (33.33%)	3 (100%)	1 (33.33%)
	Intestine	ND	1 (33.33%)	ND	ND	3 (100%)	ND
CC	Skin	2(66.67%)	ND	ND	ND	1 (33.33%)	1 (33.33%)
	Gills	3 (100%)	ND	ND	2 (66.67%)	2 (66.67%)	1 (33.33%)
	Intestine	ND	1 (33.33%)	ND	1 (33.33%)	1 (33.33%)	ND
CB	Skin	3 (100%)	ND	ND	ND	1 (33.33%)	2 (66.67%)
	Gills	1 (33.33%)	1 (33.33%)	ND	2 (66.67%)	3 (100%)	1 (33.33%)
	Intestine	ND	1 (33.33%)	1 (33.33%)	ND	2 (66.67%)	ND

% = calculated according to total number of fish samples (3 each)
ND= Not detected
Note: LR = Labeo rohita, CC = Cyprinus carpio, CB = Clarias batrachus, TMTC = Too many to count

microbial load. High load of bacteria possibly resulted from keeping fishes in wells with contaminated water. Total bacterial count of more than 10^5 cfu per gram elevates the concern of hygiene. According to ICMSF (2011), for a newly caught fish, aerobic count may range from 10^4 to 10^7 cfu per cm^2. Much lower count is associated with properly skinned ones [11]. Detectable spoilage is usually associated with spoilage bacteria exceeding 10^7 cfu per gram. Goja (2013) did similar study in three freshwater fish and found that viable bacterial counts in intestine and skin ranged from 1.5 x 10^3 to 8.4 x 10^4 cfu per gram and 2.8 x 10^3 to 9.8 x 10^3 cfu per gram respectively which is much lower than in the three fish samples in this study[12]. Comparable research conducted on 150 fish samples from Nile bream by Sichewo et al. (2014) revealed the TPC of intestine, skin, gills ranging from 4.6 x 10^3 to 8.03 x 10^3 cfu per gram [7].

Every organ of the fish samples was found to contain total coliform (**Table 2**). Similarly, presence of fecal coliforms was also true for all samples analyzed except skin of *L. rohita* and skin and gills of *C. carpio*. All samples contained fecal coliform in at least one of the body part whereas the coliform count was the highest in the intestines. According to Liu et al. (2016), gut microbiota and their diversity is influenced by many independent factors as different niches have variation in diet availability [13]. In addition, gut microbiota is also influenced by metabolic capacity and gut content enzyme activity. Coliform or fecal coliforms are not considered to be normal flora of fish which reflects contamination of fish during transportation, handling or during rearing in water contaminated with human or animal waste [3]. Four of the fish samples had a very high count of coliform in their intestines (TMTC). The feed and trophic level (carnivores, omnivores and herbivores) might have some association with the intestinal microbiota. The salinities of water in fish habitat also have some influence on the microbiota of fish intestine [14].

Prevalence of microorganisms varied among the three fish samples analyzed with *E. coli* being most common isolate followed by *S. aureus* and CoNS (**Figure 1**). Prevalence of *V. cholerae* was higher among *C. carpio*, while *S.* Typhi is absent in *L. rohita* and *S.* Paratyphi was absent in *C. carpio*. *E. coli* was present in the gills of *L. rohita* (100%), *C. carpio* (66.67%) and *C. batrachus* (100%) respectively (**Table 3**). Similar study conducted by Yogoub (2009) revealed that *E. coli* was the most dominant isolate from fishes [2]. Enterobacteriaceae genera were isolated from gills, skin, intestine and muscles of 83 out of 150 randomly collected fishes which also included pathogenic *Salmonella* and *Shigella* spp. During rainy season due to rain surface runoff of organic matters into water bodies are increased which favors multiplication of bacteria. Previous study performed by Shabeeb et al. (2016) revealed that most isolates were Gram negative rods [15].

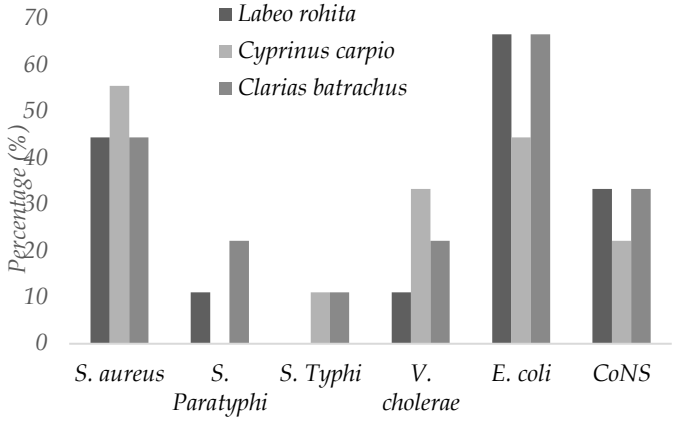

Figure 1: Distribution of pathogenic bacteria in *L. rohita*, *C. carpio* and *C. batrachus*
%= Calculated according to the total number of samples (9 each)

Salmonella being enteric bacteria, their presence in freshwater fish undoubtedly attributes to fecal contamination of such source from where it is harvested. Ponds with higher temperature or from hot regions harbors more *Salmonella* with high prevalence rates. According to Bibi et al. (2015) there has been occurrence of *Salmonella* Typhi in freshwater fish like *Labeo rohita* (skin, gills, intestine) and *Cyprinus carpio* (intestine)[16]. In our study, *S.* Typhi was isolated from one sample each of gills and intestine of *C. batrachus* and a sample of intestine of *C. carpio* while none of the sample from *L. rohita* showed the presence of the bacterium (**Table 3**). As for *S.* Paratyphi, it was not isolated from any of the tested samples of *C. carpio* but isolated from one sample of intestine of both *L. rohita* and *C. batrachus*. It was noted that none of the skin samples of all the three fish tested showed the presence of *Salmonella* spp. However, these isolates were not confirmed through serological tests.

All 3 gills samples of the *C. carpio* contained *Staphylococcus aureus* (**Table 3**). Intestines of *L. rohita* and *C. batrachus* were not found to contain the pathogen. *Staphylococcus* is related with unhygienic handling as these are inhabitant of human skin [17]. Presence of such opportunistic human pathogens advocates the possibility of cross contamination between handlers and fish. A study conducted in Andhra Pradesh, India by Bujjamma and Padmavathi (2015) in 192 fish samples disclosed that over 24.47% of fish were contaminated with *S. aureus* which also included similar samples [18]. Similarly, sampled skins were mostly found to harbor these

pathogens. However, all intestine samples were negative for CoNS. Skin was observed as the most contaminated area with CoNS and *Staphylococcus aureus*. Composition and count of microflora in fish is a function of water quality, age, type of species and fish [19] along with rearing density and diet impact [20]. In relation to this, hazard escalates when livestock manure is fed to fish which is also the case in Nepal. As an established fact, chicken has been remarkably associated with a high load of *Salmonella*.

In this study, *V. cholerae* was isolated from gills of all three types of fish and from intestines of *C. carpio* (**Table 3**). Samples were expected to yield positive results for *V. cholerae* as this bacterium is the indigenous member of fish associated aquatic habitats. However, their distribution was outnumbered by that of *E coli* and *S. aureus*. Gills bear a high load of bacteria as a consequence of water and organic substance precipitation[19, 21]. A study conducted in Bangladesh on Hilsha, a freshwater fish by Hossain et al. (2018) revealed that out of 48 fishes, 39 fishes tested positive for *V. cholerae* in specific OmpW gene assay [22]. Gills tested positive in highest number (79%) on market fish whereas the same happened for scale swab in case of fresh fishes which is similar to our findings as most gills tested positive for the pathogen. Furthermore, PCR results in this study, revealed (66.7%) higher prevalence of *Vibrio cholerae* in fish purchased from local markets than those collected from river banks. *E. coli* being highly prevalent in all intestinal samples, lower yield of *V. cholerae* can probably be attributed to glucose metabolism of *E. coli* yielding acid products which can potentially reduce survival chances of *V. cholerae* as evidenced by in-vitro studies [23]. Halpern and Izhaki (2017) emphasized the mutualistic relation between *V. cholerae* and fish with the evidence of the former helping fish to properly digest chitinous prey like zooplanktons [21]. However, fish serve as an important vehicle for *V. cholerae* and hence is the key to the dissemination process for epidemics. *Vibrio parahemolyticus* has been sporadically associated with freshwater fish and is seasonal. None of the freshwater fish contained *V. parahemolyticus* [22].

These findings and observations seem alarming and demand assessing of the freshwater fish and other aquatic products that are kept for sale in the markets. High load of bacteria in the samples including the presence of total coliforms, fecal coliforms and pathogenic bacteria can be attributed to the type of water used in the shops for keeping the fishes. The bacterial load in the fish can probably be the function of frequency of change of water. Besides, indigenous bacteria on surface as well as normal flora of other sites, rapidly multiply and invade the previously sterile part after death of fish and hence, spoilage is a consequence if proper keeping conditions are not maintained after harvesting or killing (after being bought). In addition to that, fish handlers at different stages of the supply chain are at greater risk apart from consumers eating the fish. Bacteria can pave a way into wounds, cracks or lacerated regions of skin of fish handlers and might potentially cause various infections. Moreover, the bad hygiene practice of the handler can potentially cause fish borne infection. Conventional cooking systems might reduce the bacterial load to acceptable or possibly kill pathogens. However, the risks remain high when consumers prefer it raw or smoked. In addition, undercooking of such contaminated fish can pose dangers to public health.

Conclusion

Nine (3 *L. rohita*, 3 *C. batrachus*, 3 *C. carpio)* fish samples were taken from retailers of Kathmandu which were processed for assessing their microbial quality and were assayed for presence of potential pathogenic bacteria. The samples were found to contain a high load of bacteria along with coliforms. The samples were also found to contain potential pathogens like *S. aureus*, *S.* Typhi, *S.* Paratyphi, *V. cholerae*, *E. coli* and CoNS with the most prevalent being *E. coli*. These findings suggested the poor microbial quality of freshwater raw fishes that are being sold in the market. *C. batrachus* was found to be the most contaminated fish. The research indicated that there is an immediate need for quality assessment of fish and such aquatic products on a large scale. To conclude, the fish whether alive, dead, dried or frozen should be consistently monitored for bacterial load and presence of pathogenic forms.

Author's Contribution

Project coordinator: KP
Conceptualization: SP, PS, SP, IA, SG and KP
Writing – Original Draft Preparation: SP, PS
Writing – Review & Editing: KP
All authors read and approved the final manuscript: Yes

Competing Interests

No competing interests were disclosed

Funding

No grants were involved in supporting this work

Acknowledgements

We would like to express our deepest gratitude to the Department of Microbiology, St Xavier's College,

Maitighar. We are also thankful to staff members for their help, support and their kind cooperation during the research period.

Ethical Approval and Consent

Not Applicable

References

1. Eizenberga I, Terentjeva M, Valciņa O, et al. Microbiological quality of raw fish at retail market in Latvia. In: *Food Quality and Safety*. NJF Latvia, 2015, pp. 16–18.
2. Yagoub SO. Isolation of Enterobacteriaceae and *Pseudomonas* spp. from raw fish sold in fish market in Khartoum state. *J Bacteriol Res* 2009; 1: 85–88.
3. Atwa EI. Bacteriological Study of Fish Samples Collected from Different Markets in Some Egyptian Governorates and Antimicrobial Sensitivity of Isolates. *Int J Curr Microbiol Appl Sci* 2017; 6: 2765–2776.
4. Megha P U and Harikumar P S. Isolation and Identification of Pathogenic Bacteria in Edible Fish: A Case Study of Mogral River, Kasargod, Kerala, India. *Bio Sci* 2016; 100: 43672–43677.
5. Jalal, K.C.A., Akbar John, B., Nurul Lyana, M.S., Faizul, H.N., Noor Isma, Yanti, M., Irwandi, J. and Mahbuba Bulbul. Comparative study on spoilage and pathogenic bacteria in selected commercial marine and freshwater fishes. *Int Food Res J* 2017; 24: S298–S304.
6. Tilahun A, Engdawork A. Isolation, Identification and Antimicrobial Susceptibility Profile of *E. coli* (O157:H7) From Fish in Lake Hawassa, Southern Ethiopia. 2019; 3: 14–19.
7. Sichewo PR, Gono RK, Muzondiwa J, et al. Isolation and identification of pathogenic bacteria in edible fish: A case study of rural aquaculture projects feeding livestock manure to fish in Zimbabwe. *IntJCurrMicrobiolAppSci* 2014; 3: 8897–8904.
8. Kumari SP, Prasad BN, Kumari G, et al. Microbiological quality of fish, rohu marketed in Patna and its public health significance. *J Food Sci Technol -Mysore-* 2001; 38: 607–608.
9. Xu M, Wu J, Chen L. Virulence, antimicrobial and heavy metal tolerance, and genetic diversity of *Vibrio cholerae* recovered from commonly consumed freshwater fish. *Environ Sci Pollut Res* 2019; 26: 27338–27352.
10. Cheesebrough M. *District Laboratory Practice in Tropical Countries Part 2*. Cambridge University Press, 2006.
11. International Comission on Microbiological Specifications for Food (ICMSF). *Microorganisms in Foods. 2. Sampling for Microbiological Analysis: Principles and Specific Applications.* 2nd ed. Toronto, Canada: University of Toronto Press.
12. Goja AM. Microbiological assessment of three types of fresh fish (*Tilapia niloticus*, *Labeo niloticus* and *Hydrocynus* spp.) sold in Ed Dueim, Sudan. *N Y Sci J* 2013; 6: 49–54.
13. Liu H, Guo X, Gooneratne R, et al. The gut microbiome and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels. *Sci Rep* 2016; 6: 0–12.
14. Wong S, Rawls JF. Intestinal microbiota composition in fishes is influenced by host ecology and environment: News and Views: Perspective. *Mol Ecol* 2012; 21: 3100–3102.
15. Al Shabeeb SS, Ibrahim MAM, Ramadhan GHA. A Comparative Microbial Quality Assessment among Fishes, Prawns and Cuttlefishes collected from Dammam Fish Market. *Int J Curr Microbiol Appl Sci* 2016; 5: 405–418.
16. Bibi F, Qaisrani SN, Ahmad AN, et al. Occurence of *Salmonella* in freshwater fishes: A review. *J Anim Plant Sci* 2015; 25: 330–310.
17. Mhango M, Mpuchane SF, Mpuchane BA. Incidence of indicator organisms, opportunistic and pathogenic bacteria in fish. *Afr J Food Agric Nutr Dev* 2010; 10: 4203–4218.
18. Bujjamma P, Padmavathi P. Prevalence of *Staphylococcus aureus* in Fish Samples of Local Domestic Fish Market. 2015; 4: 427–433.
19. Kluga A, Kacaniova M, Kántor A, et al. Identification of microflora of freshwater fish caught in the Driksna river and pond in Latvia. Foodbalt, 2017, pp. 164–168.
20. Wong S, Waldrop T, Summerfelt S, et al. Aquacultured Rainbow Trout (*Oncorhynchus mykiss*) Possess a Large Core Intestinal Microbiota That Is Resistant to Variation in Diet and Rearing Density. *Appl Environ Microbiol* 2013; 79: 4974–4984.
21. Halpern M, Izhaki I. Fish as Hosts of *Vibrio cholerae*. *Front Microbiol* 2017; 8: 282.
22. Hossain ZZ, Farhana I, Tulsiani SM, et al. Transmission and Toxigenic Potential of *Vibrio cholerae* in Hilsha Fish (*Tenualosa ilisha*) for Human Consumption in Bangladesh. *Front Microbiol* 2018; 9: 222.
23. Nag D, Breen P, Raychaudhuri S, et al. Glucose Metabolism by *Escherichia coli* Inhibits *Vibrio cholerae* Intestinal Colonization of Zebrafish. *Infect Immun* 2018; 86: e00486-18.