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Research Note

Occurrence of Faecal Indicators in Freshwater Fishes of Navi Mumbai in Retail Outlets

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Microbiological diversity of freshwater fish muscle depends on its habitat and other environmental factors (Cahill et al, 1990; Claucas & Ward, 1996). Reservoirs/ponds are sometimes contaminated by faecal material through surface runoff water. Faecal materials are prime sources of many enteric pathogens responsible for food and water borne infections. It is necessary to screen all food borne pathogens in fresh water fishes for food safety. But, screening of all pathogens in fish will be a time consuming and expensive procedure. Hence, assessment of faecal indicators will be a suitable alternative to identify the level of faecal contamination in fishes (Berg, 1978).

Faecal indicator bacteria (FIB) are commensal bacteria, ubiquitously present in gastrointestinal tract of human and warm blooded animals but not native flora of fish's intestinal tract. These are considered as index bacteria to indicate the presence of pathogenic bacteria or viruses (Tyagi et al., 2006). Hence, presence of these bacteria in fishes needs to be considered seriously to overcome the pathogenic contamination in fish (Ashbolt et al., 2001).

Escherichia coli is a primary indicator for faecal contamination and ubiquitously present in warm blooded animals forming 90% of coliforms (Hurst et al., 2002). Faecal streptococci (FS) are group of bacteria used to assess the quality of drinking water resources (EC, 1998). It is an additional indicator for faecal pollution (WHO, 1996). Majority of FS isolated from polluted water are of true faecal origin (Pinto et al., 1999). The predominant intestinal Enterococci are *E. faecalis*, *E. faecium*, *E. durans* and *E. hirae* (Ashbolt et al., 2001). Among the Enterococci *E. faecalis* and *E. faecium* are responsible for endocarditis, intra-abdominal infection, surgical wound infection and urinary tract infections in humans (Ross, 1998; Barrell et al., 2000). Sulphite reducing clostridia (SRC) are another group of bacteria monitored regularly in aquatic environment to find remote faecal contamination. Hence, these are recommended to be tested along with *E. coli* (Tyagi et al., 2006). *Clostridium perfringens* is key species in SRC, and its presence in water indicates the presence of Cryptosporium and Giardia cysts (Berg, 1978).

Staphylococcus aureus in the main habitant of upper respiratory tract of humans and animals. Healthy individual carrier rate may be upto 60% of with 25–30% of population being positive for enterotoxin-producing strains which is one of the major food poisoning bacteria, usually occurring in fish during handling and processing (Huss, 1994; Sindhu & Surendran, 2006).

Most of the studies carried out on faecal indicator bacteria (FIB) are related to monitoring of water samples (Kistemann et al., 2002; Desmarais et al., 2002; Rao & Surendran, 2000). Recent report on the

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presence of pathogenic FS in food samples gives a warning signal to initiate monitoring the level of FS and other FIB in fishes (Hinguita et al., 2014). Due to rainfall and surface runoff, most of the freshwater fishes are exposed to faecal contamination. Hence, the present study was conducted to assess the level of faecal contamination using faecal indicator bacteria in freshwater fishes in retail fishery outlet of Navi Mumbai, Maharashtra (India).

Fresh (unfrozen) fish samples of Catla (*Catla catla) (n=15),* Rohu (*Labeo rohita)* (n=14), Mrigal (*Cirrhinus mirgala*) (n=4), Pabda (*Ompok pabda)* (n=7), Tilapia (*Oreochromis mossambicus*) (n=7) and Murrel (*Channa striata*) (n= 3) were collected from Navi Mumbai retail market and brought to laboratory for micro-biological analysis. All fishes were analysed for aerobic plate count (APC), *S. aureus* and faecal indicator bacteria such as *E. coli,* faecal streptococci (FS) and sulphite reducing clostridia (SRC).

Twenty five grams of fish sample was aseptically weighed and blended with 225 ml of 0.1% peptone saline in sterile bags using stomacher (Seaward, UK). The blended homogenate was serially diluted and spread onto preset Tryptone Glucose Beef Extract (TGBE) agar plates (Hi Media, #M791). Then the plates were incubated at 37ºC for 2 days (Downes and Ito, 2001) for enumeration.

For rapid enumeration of *E. coli* from fish samples, ISO, 9308-1 protocol was followed with slight modification (ISO, 1990). Enumeration of *S. aureus* was carried out as per FDA (1995) using Baired Parker (BP) agar. The faecal streptococci were enumerated as per Downes and Ito (2001) using Kenner faecal (KF) streptococcal agar base. SRC numbers were enumerated by three tube most probable number (MPN) technique with Differential Reinforced Clostridial Broth (Collee et al. 1996).

In the present study, the aerobic plate count (APC) of all fish samples were within the limit i.e. <5,00,000 cfu g^{-1} (FSSAI, 2012; IS, 1978; ICMSF, 1986); but *E. coli* count was very high i.e., out of 50 samples, 21 samples showed higher levels of *E. coli* than recommended limit. Food safety and standardization authority of India (FSSAI) permits only 20 cfu g^{-1} of *E. coli* in fresh fish (FSSAI, 2012). So as per the recommendation, 42% of samples in retail market harboured higher level of *E. coli* (Table 1). Higher level of *E. coli* was encountered in Catla i.e., 12 out of 15 catla samples (80%) harboured higher level of *E. coli* than the limit.

Since Catla fish alone harboured higher levels of *E. coli,* further studies are necessary to accurately understand the relationship between *E. coli* and surface feeders. It was also observed that, similar to

Table 1. Incidence of faecal indicator bacteria and *S. aureus* in freshwater fishes in retail fish markets of Navi Mumbai region:

		APC		*E. coli*			*S. aureus*			FS		SRC		FI
Name	No.	Mean ± SD	Range	Mean ± SD	Range	No. and % of sample >20 cfu/g	Mean ± SD	Range	No. of samples >100 cfu/g	Mean ± SD	Range	Mean ± SD	Range	Avg.
Catla catla	15	85,133 ±38,915	$2.9X10^4$ – $1.64X10^5$	40.00 ±22.36	0 - 80	12 (80%)	54.00 ±25.85	20 - 80	0	28.67 ±20.3	0 - 80	11.47 ±11.11	0.9 - 45	80.14
Labeo rohita	14	67,214 ±19,965	$3.7X10^4$ – $9.3X10^4$	20.71 ±12.06	0 - 40	3 (21%)	31.43 ±14.06	10 to 60	0	18.57 ±5.34	10 - 30	8.62 ±10.07	0.6 to 25	47.9
Ompok pabda	7	79,214 ±13,849	$6X10^4$ – $9.4X10^4$	25.71 ±19.02	0 - 60	2 (28.5%)	31.43 ±10.69	20 - 40	0	18.57 ±12.14	0 - 40	21.07 ±40.16	0.9 - 110	65.35
Oreochromis mossambicus	7	63,942 ±26,701	$1.16X10^4$ – $8.7X10^4$	21.43 ±10.69	10 - 30	2 (28.5%)	40 ±11.54	30 - 60	0	44.28 ±25.07	0 - 70	16.21 ±9.7	4.5 - 25	81.92
Cirrhinus mirgala	4	63,500 ±34,229	$1.8X10^4$ – $1X10^5$	15.00 ±19.14	0 - 40	1 (25%)	22 ±9.57	10 - 30	0	17.50 ±17.07	0 - 40	7.83 ±11.58	0.4 to 25	40.33
Channa striata	3	1,28,333 ±1,07,742	$4.5X10^4$ – $2.5X10^5$	23.33 ±32.14	0 - 60	1 (33%)	40 ±20	20 - 40	0	56.66 ±49.32	0 - 80	51.66 ±55.3	0 - 110	131.65

higher *E. coli* count in the surface feeder; total faecal Indicator Bacteria count was also higher in Catla (80.14 cfu g^{-1}); lesser in Rohu (47.9 cfu g^{-1}) and least in Mirgal (40.9 cfu g^{-1}). Ironically, higher incidence of FIB was also observed in *Channa striata* i.e., 131.65 cfu g^{-1} which may be due its habitation. As these fishes prefer muddy water; during dry season they stays in the bottom of the lake by burrowing mud (FAO, 2016). Environmental protection agency (EPA) also reported that sand, sediment, and soil can serve as reservoirs of faecal indicator bacteria in many tropical, subtropical, and temperate pond and recreational waters (EPA, 2010).

In the present study, variation in *E. coli*/Faecal indicator count was observed in freshwater fish which may be due to the environmental factors such as pH, salinity, light exposure and temperature. In addition some other factors such as UV light (duration and intensity), rainfall, runoff, dispersal, suspended solids, turbidity, nutrients, organic content, organic foams, water quality, biological community in water column, water depth, stratification, mixing, presence of aquatic plants, bio-films, water temperature and inputs of soil particles during heavy rains and predation also play a significant role (EPA, 2010; Isobe et al, 2004; Vincy et al., 2015). Previous reports mentioned about the variations in *E. coli* and other bacterial count could be attributed to fish species, environment, method of catching and extent of handling while catching (Wang et al. 1994).

Estimation of more number of FIB would give accurate result than a single FIB testing (Tyagi et al., 2006). Hence in the present study *E. coli*, FS and SRC level were analysed in the freshwater fishes. In all samples, FS counts were within the limit. Still, the mean value (30.71cfu g^{1}) showed that presence of FS in fish, which need to be considered; because, these FS are heat resistant and withstand pasteurization temperature; moreover, these are not affected by the ingestion (Sorensen et al., 2001). Some of the Enterococcus are responsible of urinary tract infection in human especially lower urinary tract i.e., cystitis, prostatitis and epididymitis. Hence if the fishes are contaminated with these bacteria, it may spread to handlers and consumers (Higuita et al., 2014). As per European council recommendation, the level of *Enterococcus* sp. (sub set of FS) in potable water is 0/ 250 ml (EC, 1998). But, there is no international standard for the level of FS/Enterococcus in fish flesh. But, in India, Bureau of Indian Standard (BIS) – 1978 recommended level of FS as 100 cfu g^{-1} (IS, 1978). But, implementing and monitoring authority *viz.,* Food safety standards authority of India has not included the FS in their guidelines. So, maximum recommended level of FS has to be finalised and implemented at national/international level to control the FS in fish retail markets.

In the present study, SRC were observed in fish with an average value of 19.48 cfu g^{-1}. But no limit has been legalized for SRC in fish. Previously for potable water, the EC directive recommended level of SRC was 'absent in 20 ml'; but, recently 98/83/ EC Directives amended as the absence of *C. perfringens* in 100 ml of water (Barrell et al., 2000; EC, 1998). FSSAI (2012) has cited the absence of *C. perfringens* in 25 g fish. Earlier reports on SRC in inland and marine fishes in India (Visnuvinayagam et al., 2015a) suggested that the fishes are possible source of SRC contamination.

In the present study, *S. aureus* was analysed to identify the handling contamination. Since, *S. aureus* are inhabitant of upper respiratory tract of human and animal, presence of it in fish indicates personnel unhygienic handling. FSSAI (2012) has permitted 100 cfu g^{-1} of sample. In the present study, all samples contained *S. aureus*; but well within the limit. The average count of *S. aureus* (54 cfu g^{-1}) in catla fish indicates considerable number is present in the fish. Presence of highly hazardous methicillin resistant *S. aureus* (MRSA) and multi drug resistant (MDR) in retail fish markets are also reported in India (Visnuvinayagam et al., 2015b).

Based on the above discussions, it has been concluded that the variation in FIB count is due to various environmental and handling processes. Since, Catla fish alone had higher level of *E. coli,* further studies are necessary to explore the relationship between *E. coli* and surface feeders. In fresh water fishes, occurrence of noteworthy number of FS and SRC indicates the possible health hazard prevailing in fishes, which may get transferred to handlers and consumers. Hence, stringent hygienic practices have to be followed by fish handlers both at farm and retail markets. Regular monitoring of faecal indictors with strict implementation of GMP is very essential.

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