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Research Note

Effect of Monsoon on Sulphite Reducing Clostridia Levels in Fish from Retail Outlets in Maharashtra

S. Visnuvinayagam[1]*, P. Viji[1], P. K. Binsi[2], R. Chakrabarti[1], Thriveni Adiga[1], C. G. Joshy[2] and K. V. Lalitha[2]

[1] *Mumbai Research Centre of Central Institute of Fisheries Technology, Vashi, Navi Mumbai - 400 703, India*

[2] *Central Institute of Fisheries Technology, P.O. Matsyapuri, Cochin - 682 029, India*

Pathogens present in aquatic systems are a threat to human health. Microbial indicators are commonly used for the assessment of public health risks associated with faecal contamination of aquatic ecosystems. Bacteria such as *Escherichia coli* and Enterococci are the most common indicators for faecal contamination in sewage polluted waters (Tyagi et al., 2006). Sulphite reducing clostridia (SRC) are a compilation of different clostridium species. These gram-positive, anaerobic spore-forming rods reduce sulphite to sulphide, which has been suggested as an alternative indicator of faecal contamination in aquatic environments. Owing to their wide distribution and their ability to form spores that withstand very harsh environmental conditions, they survive much longer time than vegetative cells. This group of Clostridia includes mainly *Clostridium perfringens, C. bifermentans, C. difficile, C. sporogenes, C. botulinum* and *C. septicum* (Kouassi et al., 2011). The genus Clostridium is considered as a potential indicator because its presence in sediment can be either natural or due to anthropogenic discharges. *C. perfringens* is a key species in SRC and is commonly found in human and animal faeces (though in much smaller numbers than *E. coli*). Presence of these bacteria in water and fish clearly indicates faecal contamination (John et al., 2004).

C. perfringens are most widely used as SFIB (Selective cultivation of standard faecal indicator bacteria) (Bisson & Cabelli, 1980; Leclerc et al., 2001).

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**E-mail:* visnuvinayagam@yahoo.co.in

Most of the studies carried out on SRC are related to monitoring of water samples (Robles et al., 2000; Kistemann et al., 2002; Desmarais et al., 2002; Rao & Surendran, 2000), while limited reports are available on SRC in the fish muscle. So, the present study was undertaken with the objectives of analyzing the occurrence of SRC in the muscle of marine and freshwater fish species and also to find out the variations in their count with respect to different seasons.

Fish samples including 140 marine and 42 freshwater fish (n=182) were procured from local fish markets of Vashi region (Table 1). The samples were collected in sterile polythene bags and were transported to the laboratory in an insulated ice-box for analysis within 2-4 h of collection. Samples were randomly collected during pre-monsoon, monsoon and post-monsoon seasons and 58, 36 and 40 marine fish samples were collected respectively during these seasons. Likewise 13, 14 and 15 fresh water fish samples were collected in pre-monsoon, monsoon and post-monsoon season respectively during 2008 to 2012.

Ten grams of fish meat was aseptically weighed in a sterile petri plate and transferred to sterile polyethylene bags and 90 ml of phosphate buffer saline (PBS) was added. The meat was properly blended in a stomacher (Seaward, UK) for 2 min. SRC numbers were determined by tube MPN procedure using Differential Reinforced Clostridial (M547, Himedia) broth (West, 1989) and confirmed by streaking on to Tryptose Sulfite Cycloserine (TSC) agar and characteristic colonies of SRC were confirmed by biochemical reactions as described by FDA (1995).

Table 1. Sulphite reducing clostridia level in different fishes

	Scientific Name	No. of Samples	MPN of SRC	
			Mean	Range
Marine fish	*Arius* sp.	9	10.50	1.5 - 140
	Chirocentrus dorab	14	17.97	0.3 - 110
	Johnius dussumieri	4	1.20	0.4 to 2
	Epinephelus sp.	2	0.75	0 – 1.5
	Congresox sp.	2	1.80	1.1 - 2.5
	Tylosurus crocodilus	4	14.32	0.3 - 25
	Parastromateus niger	7	19.60	3 - 110
	Rastrelliger kanagurta	6	18.44	0.9 - 45
	Mugil cephalus	18	16.55	0.4 - 45
	Sillago sihama	3	4.37	1.1 – 9.5
	Nemipterus japonicus	10	18.89	0.4 - 45
	Eleutheronema sp.	12	24.78	0.3 -110
	Dasyatis sp.	14	27.08	0.7 – 9.5
	Sardinella longiceps	7	21.80	0 - 110
	Scomberomorus sp.	6	28.57	0 - 140
	Pampus argenteus	4	14.32	0 - 110
	Cynoglossus sp.	12	10.08	1.5 - 45
	Harpadon nehereus	1	9.5	9.5
	Loligo duvauceli	5	14.37	0.7 - 45
Freshwater fish	*Catla catla*	14	8.85	0.3 - 110
	Labeo rohita	12	10.32	0.9 - 20
	Cirrhinus mrigala	4	15.83	0.4 - 25
	Ompok pabda	2	55.01	1.6 - 110
	Channa striatus	3	51.66	0.9 - 110
	Oreochromis mossambicus	7	16.21	4.5 - 25
		182		

Seasonal differences in SRC counts were analyzed using one way ANOVA for marine and freshwater fish samples. Student's t test analysis was used to evaluate the significance of differences between means of SRC level in marine and freshwater fish in each season; $p<0.05$ was considered as statistically significant.

Out of 182 samples, 35 showed SRC counts of <1 MPN g^{-1}, 128 samples showed SRC levels between 1.1 to 100 MPN g^{-1} and 14 samples had SRC count of >100 MPN g^{-1} while 5 samples showed the absence of SRC (Table 1). Among marine fish samples, the mean SRC level was less in Grouper species (0.75 MPN g^{-1}); whereas seer fish presented the highest mean value of 28.57 MPN g^{-1}. Among freshwater fish, catla showed the lowest mean count (8.85 MPN g^{-1}) and pabda fish showed the highest mean count (55.08 MPN g^{-1}). Wide variations were observed in the levels of SRC within collected species during the three different seasons.

Variations in SRC level of marine and freshwater fish collected in each season was compared using t-test (Table 2). It clearly shows that the counts were significantly lower in freshwater fish during monsoon season compared to that in marine fish. The results further indicate that in freshwater fish, SRC

counts were not affected by season. Marine fish samples showed significant difference ($p<0.05$) in SRC level between monsoon and pre-monsoon seasons. However, there was no significant difference in SRC levels between pre-monsoon and postmonsoon seasons (Table 2). This apparently indicates that, during monsoon season due to surface run off, there will be an increased contamination of SRC in the coastal waters and hence, the higher value during monsoon season. In the case of freshwater fish, no significant difference in SRC counts was found between the seasons.

Table 2. Comparison of SRC count between marine and freshwater fish in different seasons

Season	Marine Fish Samples	Freshwater Fish Samples
$SRC_{(Pre\text{-}monsoon)}$	$_a11.95^B$	$_a14.18^A$
$SRC_{(Monsoon)}$	$_a26.40^A$	$_b12.76^A$
$SRC_{(Post\text{-}monsoon)}$	$_a13.25^A$	$_a14.35^A$

Means with letters on the superscript compares the SRC count in different seasons and letters on the subscript compares SRC count in marine and fresh water in each season. Means with different letters differ significantly at 5% level of significance ($p<0.05$).

The results of this study are in agreement with that of Zhang et al. (2013) who reported a higher level of microbial and faecal indicator contamination in the bathing beaches of China in rainy season than in other seasons. Faecal contamination in coastal regions is a major concern especially in the tropical developing countries (Byamukama et al., 2005; Tyagi et al., 2006; Sabae, 2006; Rosenfeld et al., 2006). Studies carried out by Sudhanandh et al. (2012) showed that Cochin coastal waters are highly contaminated with fecal pathogenic bacteria than in other areas. It has been reported that *C. perfringens* is present in the faeces of humans, livestock and carnivores in high concentration *viz.,* 4.7–7.0 log cfu g-1 (Farnleitner et al., 2010). Increased human activities and urbanization in the coastal region lead to huge amount of sewage, thus potentially contaminating the coastal region. In Mumbai city, with high density of population and increased anthropogenic activities in the coastal area causes faecal contamination in coastal waters. Peter Cox et al. (2005) reported that domestic dogs, cats, poultry and pigs excrete *C. perfringens* in high concentration (4.6×10^3 to 3.3×10^5 cfu g^{-1}) than other animals including wild and feral animals. Use of the coastal water for washing of fish in landing centres is a great source of contamination of SRC. Though SRC can withstand cooking temperature (Bala Saraswathy et al., 2006), application of food processing techniques such as addition of salt and nitrates, additions of agents to lower water activity and reduced temperature can control the outgrowth of SRC (Houben et al., 2005). World Health Organization recommends *C. perfringens* as an useful indicator of faecal pollution in water quality surveys (WHO, 1978); this microorganism has been adopted in Europe exclusively as an additional source of water quality information (Cabelli, 1978; Olivieri, 1982). Previously EC directive recommended the level of SRC as absent in 20 ml, but 98/83/EC Directives recommend absence of *C. perfringens* in 100 ml of water (Barrell et al., 2000). However, no regulations have been established for SRC or *C. perfringens* levels in fish.

The study revealed wide variations in SRC levels in different fish species. SRC levels were significantly high in marine fishes during monsoon season. In order to protect the consumers from this potential health hazard, regulations have to be established for SRC levels in fish. Since, SRC spores can withstand cooking temperature; care must be taken to reduce/ eliminate contamination during processing of fish. Studies on the occurrence of SRC in fish samples from various ecosystems, water and ice should be undertaken to determine the contamination level and to develop control measures.

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