Microbial Risk Assessment of Freshwater Fish for Internal Trade : A Preliminary Study

G. Padmaja, B. Madhusudana Rao and P.K. Surendran[1]

Research Centre of Central Institute of Fisheries Technology, Ocean View Layout, Pandurangapuram, Visakhapatnam-530 003 Andhra Pradesh, India

E-mail: cift.vsp@itpvis.ap.nic.in

Andhra Pradesh ranks second in inland fish production and almost the entire quantity is channeled to internal markets within the country. A preliminary study was carried out to assess the microbial risks associated with freshwater fish meant for domestic markets. *Catla catla* and *Labeo rohita* procured from aquaculture farms and domestic markets in Andhra Pradesh were analysed for bacteriological quality. Total Plate Count (TPC) in the fish samples ranged between $1x10^3$ and $2.3x10^7$ cfu.g^{-1} and about one-third of the fish had TPC values above the maximum acceptable limit of $5x10^5$ cfu.g^{-1}. Faecal *Streptococci* was the most frequently encountered faecal indicator bacteria in the fish samples with prevalence of ≥ 90% and counts ranging between $3x10^2$ and $5.2x10^4$ cfu.g^{-1}. The incidence of sulphite reducing *Clostridia* was 50% in *C. catla* and 57% in *L. rohita* with MPN counts ranging from 0.4 to 45 in *C. catla* and from 0.4 to 110 in *L. rohita*. *E. coli* was detected in 28% of *L. rohita* with counts ranging from 20 to 190 cfu.g^{-1} and 40% in *C. catla* with counts ranging from 20 to 1800 cfu.g^{-1}. One-third of *C. catla* and one-fifth of *L. rohita* samples had *E. coli* counts above the maximum permissible level of 20.g^{-1}. Fish samples were negative for the presence of *Salmonella* and *Vibrio parahaemolyticus*, but *Vibrio cholerae* was detected in one sample of *C. catla* and one sample of *L. rohita*. Antibiotic resistance pattern of *E. coli* isolates from *C. catla* (17 isolates) and *L. rohita* (16 isolates) showed no resistance to chloramphenicol, gentamicin and norfloxacin; showed intermediate resistance to amoxycillin (6%), ciprofloxacin (6%), co-trimoxazole (9%) and ampicillin (8%) but showed relatively higher resistance to nalidixic acid (24%), tetracycline (30%) and nitrofurantoin (34%). The *E. coli* isolates from fish samples showed Multiple Antibiotic Index of 0.13. The results of the preliminary study suggest that the microbial risk with respect to human pathogens in freshwater fish was minimum. However, the hygienic quality of freshwater fish needs improvement. The risk associated with the development of resistance in *E.coli* isolates from freshwater fish to medically important antibiotics was negligible.

Key words : Microbial risk assessment, antibiotic resistance, *Labeo rohita*, *Catla catla*

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[1]Central Institute of Fisheries Technology, P.O. Matsyapuri, Cochin – 682 029, Kerala, India

Fisheries is one of the fastest growing food sectors in India. The 10th Five Year Plan (2002-2007) envisages an 8% growth rate for inland fisheries (Ayyappan and Biradar, 2004). Of the 6.13 million t of fish production in India in 2001-2002, nearly 53.8% was contributed by inland fisheries. India is the second largest producer of freshwater fish in the world of which about 70% is contributed through aquaculture. Freshwater fish production has registered an average growth rate of over 5% in the last two decades (Tripathi, 2004). Andhra Pradesh ranks second in inland fish production and the state targets about 0.6 million t of inland fish production by the year 2010 (Murthy, 2002). Freshwater fish production in Andhra Pradesh is concentrated mostly on the culture of Indian major carps, *viz.*, rohu, catla, and mrigal and to a certain extent, the culture of exotic carps, *viz.*, common carp and grass carp. Almost, the entire quantity of freshwater fish produced in Andhra Pradesh is channeled through internal markets for consumption within the country. Major quantity is supplied to other states mainly West Bengal and North-eastern states while significant quantity is consumed within Andhra Pradesh. The current per capita consumption of fish in India is around 9 kg. The proportion of fish eating people in India, increased from 27.7% in 1987-88 to 39.7% in 1996-97 (Rao, 2004).

Rohu (*Labeo rohita*) and catla (*Catla catla*) are generally marketed either as fresh fish or iced fish. Fish and fishery products meant for export are subjected to stringent microbial quality specifications that are strictly enforced and monitored by government agencies. However, freshwater fish meant for internal trade are not regulated by microbial quality standards and the domestic consumer purchases the fish based on his judgement of fish quality. The quality of freshly caught fish is generally good but improper handling can adversely affect consumer acceptance. Unhygienic handling renders the fish unsafe while inadequate preservation causes spoilage of the fish. The contamination of water used for aquaculture with sewage, usage of animal manure as fertilizer in fish ponds, time-temperature abuse during preservation, transport and unhygienic handling in fish markets can result in transmission of pathogenic bacteria to fish, thereby posing risk to the consumer. Another area of concern is the use of antibiotics in fish and shellfish culture which can lead to the development of antibiotic resistance in aquatic microorganisms, thereby exposing the human population to the risk of transfer of antibiotic resistance from aquatic bacteria to pathogenic bacteria causing serious medical consequences. In the present study, an assessment of the microbial risks associated with two freshwater fishes, *viz.*, rohu and catla, produced in Andhra Pradesh, is given.

Materials and Methods

Twenty-four samples of freshwater fish comprising of ten samples of catla (*Catla catla*) and fourteen samples of rohu (*Labeo rohita*) were procured from aquaculture farms and domestic markets located in West Godavari, East Godavari, Visakhapatnam, Vizianagaram and Krishna districts in Andhra Pradesh. Fish samples were analyzed for Total Plate Count (TPC), faecal Streptococci (FS), sulphite reducing *Clostridia* (SRC), *E. coli* and pathogens (*Vibrio cholerae*, *Vibrio parahaemolyticus* and *Salmonella*) as per the methods described in AOAC (1995) and Surendran *et al.* (2003). Screening of *E. coli* isolated from catla (17 *E. coli* isolates) and rohu (16 *E. coli* isolates) for antibiotic resistance pattern was done as per Bauer *et al.* (1966) using ten antibiotics, *viz.*, amoxycillin (10 µg), ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), co-trimoxazole (25 µg), gentamicin (10 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), norfloxacin (10 µg) and tetracycline (30 µg). An inoculum of 100 µl of 16-18 h old *E. coli* culture grown in Nutrient broth was evenly spread on preset plates of Muller-Hinton agar and six antibiotic discs were placed on each plate. After incubation at 37°C for 24 h, the zones of clearance for each antibiotic was measured and the *E. coli* isolates were classified as resistant or sensitive to that particular antibiotic based on manufacturer's (Hi Media, Mumbai) interpretative chart.

Results and Discussion

Freshwater fish, *Labeo rohita* and *Catla catla* produced in Andhra Pradesh for internal trade were analyzed for microbiological quality. The microbiological quality criteria for freshwater fish sold in domestic markets are not defined and hence the results of the analysis of freshwater fish were compared with the specifications given for fresh fish meant for export (GOI, 1995).

TPC and faecal indicator bacteria in the freshwater fish samples are given in Table 1. TPC of freshwater fish ranged from $1x10^3$ to $2.3x10^7$ and there was no significant difference in the average TPC values of catla and rohu. In 28% rohu and 30% catla samples, TPC was found to be above the maximum acceptable limit of $5x10^5.g^{-1}$. Bandekar *et al.* (2004) reported that 33% of whole rohu obtained from Kakinada had TPC above $5x10^5.g^{-1}$. 72% of fresh fish and 77% of frozen marine fish available in retail markets of Cochin had TPC above $10x10^5.g^{-1}$. (Nambiar and Surendran, 2003). Faecal indicator bacteria serve as a measure of faecal contamination and indicate the possible presence of enteric pathogens. Faecal *Streptococci* was the most frequently encountered faecal indicator bacteria in freshwater fish samples, with prevalence of more than 90% followed by sulphite reducing *Clostridia* (53%) and *E. coli* (25%). The count of faecal *Streptococci*

ranged between $3x10^2$ to $5.2x10^4.g^{-1}$ in freshwater fish while the counts of SRC ranged between 0.4 to 110 $MPN.g^{-1}$. The levels of *E. coli* ranged between 20 to 190 in rohu and 20 to 1800 in catla. 21% of rohu and 30% of catla had *E. coli* counts above the maximum permissible level of $20.g^{-1}$. Initial time delay between harvesting, improper handling and inadequate icing during transport, use of poor quality water and uncleaned surfaces during retail trade contribute to higher microbial loads. Adoption of good manufacturing practices and hygienic handling, during culture, harvest, transport and retail marketing help in improving microbiological quality of freshwater fish. Varma *et al.* (2003) reported that only 13% of processed seafood exported from India during 2001-2002 had the values exceeding stipulated limits. Rohu processed in EU approved plants were found to be free from *E. coli*, *Vibrio cholerae* and *Salmonella* and TPC values were below $5x10^5.g^{-1}$ (Bandekar *et al*, 2004).

All the twenty-four samples of freshwater fish screened for the presence of pathogens were found negative for the presence of *Salmonella* and *Vibrio parahaemolyticus*. However, *Vibrio cholerae* was detected in one sample of catla and one sample of rohu. The high level of faecal indicators in freshwater fish suggests that faecal pollution might be responsible for the detection of *Vibrio cholerae* in freshwater fish. Nambiar and Surendran (2003) detected *Salmonella* in 17.3% of fresh fish and 6.3% of frozen fish from Cochin markets.

Table 1. Total bacteria and faecal indicator bacteria counts in freshwater fish samples

	Catla catla	*Labeo rohita*
Number of samples analyzed (n)	10	14
Total Plate Count,		
Range, $cfu.g^{-1}$	$1x10^3$ to $2.3x10^7$	$1x10^3$ to $1.6x10^7$
Mean, $cfu.g^{-1}$	$2.8x10^6$	$2.53x10^6$
Percentage of samples with counts above $5x10^5$ $cfu.g^{-1}$	30	28
Faecal *Streptococci*, $cfu.g^{-1}$		
Range, $cfu.g^{-1}$	$4x10^2$ to $5.2x10^4$	$3x10^2$ to $3.7x10^4$
Mean, $cfu.g^{-1}$	$1.08x10^4$	$1.0 x10^4$
Sulphite Reducing *Clostridia*		
Range, $MPN.g^{-1}$	0.4 - 45	0.4 to 110
Mean, $MPN.g^{-1}$	9.6	12.5
Escherichia coli		
Range, $cfu.g^{-1}$	20 to 1800	20 to 190
Mean, $cfu.g^{-1}$	56	30
Percentage of samples with counts above 20 $cfu.g^{-1}$	30	21

Table 2. Antibiotic resistance pattern of *E. coli* isolated from freshwater fish samples

Antibiotic	*Catla catla* (17 *E. coli* isolates)	*Labeo rohita* (16 *E. coli* isolates)	*Catla catla* and *Labeo rohita* (33 *E. coli* isolates)
	Percentage of *E. coli* strains resistant		
Ampicillin	11	6	8
Amoxycillin	6	6	6
Chloramphenicol	0	0	0
Ciprofloxacin	6	6	6
Co-trimoxazole	6	12	9
Gentamicin	0	0	0
Nalidixic Acid	30	18	24
Nitrofurantoin	12	56	34
Norfloxacin	0	0	0
Tetracycline	30	31	30

Table 3. Number of antibiotics to which *E. coli* isolates were resistant

Antibiotic	*Catla catla* (17 *E. coli* isolates)	*Labeo rohita* (16 *E. coli* isolates)	*Catla catla* and *Labeo rohita* (33 *E. coli* isolates)
	Percentage of *E. coli* strains resistant to antibiotic		
Resistant to 0 antibiotic	35	19	27
Resistant to 1 antibiotic	35	56	45
Resistant to 2 antibiotics	6	24	15
Resistant to 3 antibiotics	6	6	6
Resistant to 4 antibiotics	13	0	7
Resistant to > 4 antibiotics	0	0	0

Investigation on antibiotic resistance transmission between commensal and pathogenic representatives of *Enterobacteriaceae* showed that genetic material conferring antibiotic resistance is readily transmitted between the members of *Enterobacteriaceae* through multi-resistant transconjugants. R- plasmids were transferred at a slow rate from *Salmonella* to *E. coli*, whereas transfer occurs at a higher frequency from *E. coli* to *E. coli* O_{157}:H_7 (Mizan *et al.*, 2002). A total of 33 *E. coli* strains that included 17 *E. coli* isolates from *Catla catla* and 16 *E. coli* isolates from *Labeo rohita* were tested for antibiotic resistance pattern to ten antibiotics (Table 2). *E. coli* isolated from rohu and catla did not show development of antibiotic resistance to medically important antibiotics such as

chloramphenicol, norfloxacin and gentamicin. *E. coli* showed mild development of resistance towards ciprofloxacin (6%), amoxycillin (6%), ampicillin (8%) and co-trimoxazole (9%). *E. coli* isolates showed relatively higher development of resistance to nalidixic acid (24%), tetracycline (30%) and nitrofurantoin (34%). Twenty-seven per cent of *E. coli* isolates did not show development of resistance to all the ten antibiotics tested, while 45% of the *E. coli* isolates from freshwater fish samples showed development of resistance to one antibiotic (Table 3). The levels of resistance observed with the *E. coli* isolates from freshwater fish were less than the values of antibiotic resistance reported by Harish *et al.* (2003) for *E. coli* isolates from brackishwater shrimp farms. The MAR index of *E. coli* isolates from freshwater fish samples was only 0.13. MAR indices of *E. coli* isolated from brackishwater shrimp farms were reported to vary between 0.25 and 0.5 and all the *E. coli* isolates had MAR index value higher than 0.2 (Harish *et al.*, 2003).

Microbial risks with respect to pathogens in freshwater fish produced in Andhra Pradesh were minimal. However, the hygienic quality of freshwater fish needs to be improved. The risks associated with the development of antibiotic resistance in *E. coli* isolates from freshwater fish towards medically important antibiotics was negligible.

The authors thank the Director, Central Institute of Fisheries Technology, Cochin for giving permission to publish this paper. This work was carried out under the *Network Project on National Risk Assessment Programme for Fish and Fish Products for Domestic and International Markets.*

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