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Assessment of Bacterial Contamination and Antibiotic Resistance Patterns among *Escherichia coli* in Finfishes of Kolkata, India

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Abstract

Bacterial contamination and safety of finfishes of commercial importance and the frequency of antibiotic resistance in *Escherichia coli* in fishes of Kolkata retail markets were evaluated. Finfishes available in the retail markets recorded high levels of total viable counts (4.30-7.93 log cfu g^{-1}), coliforms (1.60-4.38 log MPN g^{-1}), faecal coliforms (≤4.38 log MPN g^{-1}) and *E. coli* (≤4.04 log MPN g^{-1}). Majority of the *E. coli* was resistant to oxytetracycline (73%) and exhibited multiple antibiotic resistance (52%). Mutation frequencies to oxytetracycline were higher ($1.3x10^{-9}$-–$7.3x10^{-4}$) compared to chloramphenicol ($4.6x10^{-8}$-–$6.8x10^{-8}$). Seven serotypes were recorded, of which O-type 95 was the most common. Shiga-like toxin-producing *E. coli* serotypes such as O2, O22 and O114 were recorded. The results revealed that most of the commercially important fishes did not meet the national and international quality standards and were contaminated with antibiotic resistant and shiga-like toxin-producing *E. coli*, which represent a health risk to the consumers.

Keywords: Food safety, faecal contamination, bacterial quality, antibiotic resistance, shiga-like toxin producing *Escherichia coli*, mutation frequency

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Introduction

Fish being highly perishable, require proper handling and preservation to increase shelf life and to retain quality and nutritional attributes (Huss et al., 2003). Estimation of bacterial numbers in fish is frequently used to retrospectively assess microbiological quality or safety of the product. When total viable count reaches 10^6 g^{-1} (6.0 log cfu g^{-1}), the product is assumed to be at, or nearing, spoilage (ICMSF, 1986). Food products that show evidence of faecal contamination are generally regarded as a greater risk to human health, as they are more likely to contain human-specific enteric pathogens. Among the enteric bacteria, *E. coli* is always considered to be of faecal origin and exists only transiently in other environments. It is universally present in the faeces of warm-blooded animals at densities of 10^8-10^9 g^{-1} (8.0-9.0 log cfu g^{-1}) and comprises ca. 95% of the coliforms in faeces (APHA, 1992; Huss et al., 2003; Todar, 2007). *Escherichia coli* can cause diarrhea, dysentery, haemolytic uremic syndrome and bladder and kidney infections. Enteric *E. coli* are classified on the basis of serology and virulence properties (Todar, 2007). At least five biotypes, viz., enteropathogenic *E. coli* (EPEC), entero-invasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC) and enterohemorrhagic *E. coli* (EHEC) have been implicated in disease outbreaks, of which EHEC (*E. coli* O157:H7) is the most important in terms of food-borne disease (Temelli, 2002; Todar, 2007).

Antibiotic resistance has grown to become a global problem. The apparent increase of the occurrence of antibiotic resistance among bacteria from various areas of fish production during the past years and its possible implications for public health in many countries have led to an intensified surveillance of bacterial resistance. Antibiotic resistant bacteria of domestic sewage discharged into the water bodies or the use of antibiotics for preventing and controlling bacterial pathogens might transfer their antibiotic resistant determinants to indigenous

bacterial flora of fish, thus, provoking their spread and prevalence (Serrano, 2005; FAO/OIE/WHO, 2006). West Bengal is a leading freshwater fish producing state in India, which produced about 1.443 million t of inland fishes in 2011-12 (DAHDF, 2012). Demand for freshwater finfish is quite high, as majority of the people of West Bengal are fish eaters and show preference to freshwater fishes. Cultured freshwater finfishes in and around Kolkata are sold mostly in live condition or fresh, uniced. Besides, this state receives considerable quantities of iced freshwater fishes from neighbouring states to cater to the needs of the people. Since the quality of fish is variable (Manna et al., 2008), this investigation was aimed to evaluate the bacterial contamination in finfishes of commercial importance retailed in Kolkata market; to assess the sanitary conditions of fish markets and to determine the frequency of antibiotic resistance in *E. coli*.

Materials and Methods

A total of 50 finfish samples comprising five each from 10 commercially important species of freshwater origin such as *Catla catla* (Catla), *Labeo rohita* (Rohu), *Cirrhinus mrigala* (Mrigal), *Labeo bata* (Bata), *Clarias batrachus* (Magur), *Mystus tengara* (Tengara) and *Oreochromis mossambicus* (Tilapia), and of marine fish such as *Pampus argenteus* (Silver pompret), *Liza parsia* (Gold spot mullet) and *Lates calcarifer* (Asian sea bass) were collected from 12 retail fish markets in and around Kolkata (Lat. 22° 34' 10"N; Long. 88° 22' 10"E), West Bengal, India. Not more than five finfish samples, either live, fresh or improperly iced, were collected at a time. Fish samples were individually wrapped in UV sterile polythene bags, placed in insulated containers and brought to the laboratory within 2 h of collection. Water samples from all the retail fish markets were also collected in 300 ml capacity autoclavable plastic containers.

Finfish and water samples were analyzed for bacterial contamination within an hour of reaching laboratory. Media for the enumeration of bacteria from marine fishes were supplemented with 1% (w/v) sodium chloride. Briefly, aseptically cut fish meat (25 g) placed in UV sterile Stomacher Lab system Circulator Model 400 closure bags 6141/CLR was homogenized using Stomacher 400 Circulator (Seaward Limited, London) at 230 rpm for 3 min and ten fold serial dilution prepared. Aliquots (0.1 ml each) of appropriately diluted fish and water samples were spread on tryptic soy agar (TSA) plates in duplicates for the enumeration of total viable counts (TVC). The plates were incubated at 35ºC for 48 h and the colonies counted. The total coliforms (TC) from fish and water samples were enumerated by 3-tube MPN technique using single strength lauryl sulphate tryptose broth (LSTB). Tubes containing LSTB were inoculated With aliquots (1.0 ml each) of appropriately diluted samples. All the tubes were incubated at 35ºC for 48 h and tubes showing turbidity and gas in Durham tubes were recorded as positive for TC. Corresponding labeled tubes containing EC broth were then inoculated with loopful of broth from positive LSTB tubes. The EC broth tubes exhibiting turbidity and gas production following 24 h incubation at 44.5±0.5ºC in a water bath were considered positive for faecal coliforms (FC). Inocula from positive EC broth tubes were then streaked on eosine methylene blue (EMB) agar plates. After 24 h incubation at 35ºC, typical colonies with greenish metallic sheen on EMB agar were aseptically picked, purified on TSA and subjected to standard biochemical tests for the identification of *E. coli* (EC). McCardy table was referred for the MPN counts of TC, FC and EC (APHA, 1992).

A total of 44 fish-borne *E. coli* strains were tested for their sensitivity to chloramphenicol (30 μg), ciprofloxacin (5 μg), co-trimoxazole (25 μg), gentamycin (10 μg), nitrofurantoin (300 μg) and oxytetracycline (30 μg) by agar disc diffusion technique (Bauer et al., 1966) on antibiotic assay medium no. 37 with 1.5% agar (AAMA). The resistance profiles and pattern were determined from the antibiogram data. Oxytetracycline and chloramphenicol-resistant mutants were selected by plating $\geq 10^9$ susceptible *E. coli* cells on AAMA with or without respective antibiotic at 25 μg ml^{-1}. The seeded agar plates were incubated at 35ºC for 5 days. Growing colonies on the plates were counted and mutation frequency was calculated. Representative strains of fish-borne *E. coli* were serotyped at the National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, India by serum agglutination assay for the presence of O-antigens. Analysis of variance was employed, after log transformation of bacteriological data, to find out the significance of difference in bacterial counts between fish species, followed by Duncan's multiple range test. Students' 't' test was carried out to test the significance of difference in the bacterial counts of freshwater and saltwater fishes using MSTAT-C software.

Results and Discussion

TVC is indicative of general fish quality and to a lesser extent of handling and storage procedures. As seen in Table 1, the TVC of commercially important freshwater and saltwater finfishes of Kolkata retail markets ranged from 4.30 log cfu g^{-1} to 7.93 log cfu g^{-1}, with the mean of 6.23 log cfu g^{-1}. Significantly higher ($p<0.05$) levels of TVC were recorded in *C. catla* than that of *O. mossambicus, P. argenteus, L. bata* and other species possibly because of improper icing during transportation and retailing. The differences in bacterial counts among other species were insignificant ($p>0.05$). The results of TVC of carps are comparable to those of Singh et al. (2009), who recorded TVC ≥8.0 log cfu g^{-1} carp. On the contrary, earlier studies from India and Sri Lanka recorded lower viable bacterial counts (3.0–5.5 log cfu g^{-1}) in carps (Jeyasekaran & Ayyappan, 2003; Jayasinghe & Rajakaruna, 2005). Al-Harbi & Uddin (2005) reported TVC of tilapia in the range of 6.83-7.88 log cfu g^{-1} in Saudi Arabia, which is higher than the TVC recorded in *O. mossambicus* of the present study (5.39-6.41 log cfu g^{-1}). The quantitative bacteriology of saltwater fishes (Table 1) revealed the mean TVC in the following order: 6.49±0.80 log cfu g^{-1} in *L. parsia,* 6.01±0.68 log cfu g^{-1} in *L. calcarifer* and 5.91±0.83 log cfu g^{-1} in *P. argenteus.* Jeyasekaran et al. (2006) reported comparatively lower TVC in freshly caught *P. argenteus* ranging from 5.0 to <6.0 log cfu g^{-1} in Tuticorin, India.

Contamination of fish products is almost always due to poor personal hygiene, poor processing hygiene or poor water quality (Huss et al., 2003). The maximum recommended bacterial counts for marginally acceptable quality fish is 10^7 g^{-1}. Fish flesh containing 10^8 bacteria g^{-1} is considered as unsuitable for food (ICMSF, 1986). The results of the present study indicate that only 28% of the finfish samples had TVC within the ICMSF's maximum recommended limit (≤$5.0x10^5$ g^{-1}) for acceptable quality products, 70% of the samples had TVC within the marginally acceptable limit (≥$5.0x10^5$–$1.0x10^7$ g^{-1}) and 28% of the samples were unacceptable with counts >$1.0x10^7$ g^{-1}. The Export Inspection Council of India recommended a level of ≤$5.0x10^5$ g^{-1} for fresh fishery products (EIC, 2012), and only 28% of commercially important finfishes of Kolkata complied with this standard (Table 2). An increase in TVC to levels in excess of 6.0 log cfu g^{-1}, observed in this study, is usually indicative of long storage in ice or temperature abuse during retailing. Invariably in all retail markets, the fishes were stored openly, uniced or insufficiently iced, sprinkled with poor quality water to keep the fish moist and sold after descaling, degutting and chopping into pieces using the same knife. Fresh fish and fishery products from

Table 1. Range and mean counts of total viable bacteria (TVC), MPN total coliforms (TC), faecal coliforms (FC) and *Escherichia coli* (EC) in commercially important fishes retailed in Kolkata, India

Fish species	TVC log cfu g^{-1}		TC log MPN g^{-1}*		FC log MPN g^{-1}		EC log MPN g^{-1}	
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
Catla catla	6.23-7.65	7.12±0.56^{abc}	3.04-4.38	4.04±0.58^{ab}	2.59-4.38	3.45±0.86	0.00-2.81	1.37±1.35
Labeo rohita	5.97-7.32	6.61±0.49	3.04-4.38	3.97±0.61^{cd}	1.60-4.38	3.00±1.02	0.00-1.60	0.32±0.71^{a}
Cirrhinus mrigala	5.04-7.04	6.08±0.84	2.04-4.04	3.21±0.89	1.48-3.38	2.48±0.87	0.00-1.60	0.62±0.84
Labeo bata	4.30-7.32	5.71±1.14^{a}	1.60-4.38	2.64±1.15^{ac}	0.00-3.06	1.85±1.25	0.00-2.17	0.87±1.19
Clarias batrachus	5.39-6.87	6.22±0.54	1.60-4.04	3.01±1.00	0.00-3.38	2.31±1.35	0.00-2.36	1.22±1.15
Mystus tengara	5.15-7.93	6.22±1.15	1.95-4.38	3.31±0.88	0.00-3.66	2.09±1.37	0.00-1.60	0.32±0.72^{b}
Oreochromis mossambicus	5.39-6.41	5.90±0.36^{b}	2.63-3.66	3.20±0.40	0.00-3.38	2.11±1.32	0.00-1.48	0.30±0.66^{c}
Pampus argenteus	5.30-6.95	5.91±0.83^{c}	2.18-3.66	2.70±0.57^{bd}	0.00-2.63	1.96±1.11	0.00-1.60	0.32±0.72^{d}
Liza parsia	5.54-7.20	6.49±0.80	1.95-4.38	2.79±0.93	1.60-3.66	2.40±0.80	0.00-1.60	0.32±0.72^{e}
Lates calcarifer	5.20-6.96	6.01±0.68	1.60-4.38	3.39±1.05	1.60-4.38	3.28±1.02	0.00-4.04	1.81±1.45^{abcde}

MPN: Most Probable Number; a-e: Values within the column showing common superscripts differed significantly ($P<0.05$). cfu: Colony forming unit. Mean values are average of five sample observations for each species.
*: On the basis of 3-tube MPN technique using single strength lauryl sulphate tryptose broth.

Table 2. Compliance of counts of total viable bacteria (TVC) and *Escherichia coli* (EC) isolated from fish samples of Kolkata retail market, to national and international standards

Organization	ICMSF			EIC	
Bacterial group	Acceptable (%)	Marginally acceptable (%)	Unacceptable (%)	Within the limit (%)	Exceeding the limit (%)
Total viable counts	28	70	2	28	72
Escherichia coli	62	34	4	62	38

ICMSF: International Commission for the Microbiological Examination of Foods; Acceptable: $\leq 5.0 \times 10^5$ g^{-1}; Marginally acceptable: $\geq 5.0 \times 10^5 - 1.0 \times 10^7$ g^{-1}; Unacceptable: $\geq 1.0 \times 10^7$ g^{-1} for TVC and Acceptable: ≤ 11 g^{-1}; Marginally acceptable: 11-≤ 500 g^{-1}; Unacceptable: ≥ 500 g^{-1} for FC (ICMSF, 1986); EIC: Export Inspection Council of India. Limit for TVC and FC are $\leq 5.0 \times 10^5$ g^{-1} and ≤ 20 g^{-1}, respectively (EIC, 2012).

tropical regions often have TVC of 10^4-10^6 g^{-1}, although there are also examples of seafood and freshwater fish with TVC of 10^6-10^8 g^{-1} without objectionable quality changes (Huss et al., 2003; Singh et al., 2009). Improper icing, use of poor quality water and handling of fish with contaminated hands or casually rinsed hands could be the reason for the observed high bacterial contamination in Kolkata retail market finfishes. Likewise, Manna et al. (2008) reported that the microbiological quality of most of the fresh and ice-preserved retail fishes of Kolkata were within acceptable or marginally acceptable limits.

Coliform bacteria are the commonly used bacterial indicator of sanitary quality of foods and water. The results revealed high levels of coliforms (1.60-4.38 log MPN g^{-1}) in Kolkata retail market fish. The TC counts were observed to range from 1.60 log MPN g^{-1} in *L. bata, C. batrachus* and *L. calcarifer* to 4.38 log MPN g^{-1} in *C. catla, L. rohita, L. bata, M. tengara, L. parsia* and *L. calcarifer* (Table 1). Significant differences existed between the MPN TC counts of species *viz., C. catla* and *L. bata, C. catla* and *P. argenteus, L. rohita* and *L. bata,* and *L. rohita* and *P. argenteus* ($p<0.05$). Likewise, coliforms ranging from $<10^1$ to 10^5 g^{-1} were reported in retail market seafoods of USA (Abetya, 1983), freshwater fish of Sri Lanka (Jayasinghe & Rajakaruna, 2005), Vietnam (Lan et al., 2007) and Brazil (Da Silva et al., 2010). The presence of large number of coliforms in foods is highly undesirable, but it would be almost impossible to eliminate all forms. The highest FC counts (4.38 log MPN g^{-1}) were recorded in *C. catla, L. rohita* and *L. calcarifer*. Differences in the MPN counts of FC within species ($p>0.05$; $F=1.38$; $df=4$) and between species ($p>0.05$; $F=1.31$; $df=9$) were insignificant. The highest EC count (4.04 log MPN g^{-1}) was recorded in *L. calcarifer*. Significant differences existed between the MPN EC counts of species *viz., L. calcarifer* and *L. rohita, L. calcarifer* and *M. tengara, L. calcarifer* and *O. mossambicus, L. calcarifer* and *P. argenteus,* and *L. calcarifer* and *L. parsia* ($p<0.05$). Of the 50 finfish samples tested, 46 were positive for faecal coliforms (92%) at an elevated incubation temperature of 44.5°C. Only 19 finfish samples contained *E. coli* (38%). Observations on FC counts to the tune of 4.38 log MPN g^{-1} in *C. catla, L. rohita* and *L. calcarifer,* and *E. coli* counts of 4.04 log MPN g^{-1} in *L. calcarifer* suggested high levels of faecal contamination and poor sanitary quality of Kolkata retail market fishes. Differences in the counts of viable bacteria ($t=0.54$; $df=29$; $p>0.05$), TC ($t=1.41$; $df=27$; $p>0.05$), FC ($t= -0.22$; $df=29$; $p>0.05$) and EC ($t= -0.43$; $df=20$; $p>0.05$) between commercially important freshwater and saltwater fishes of Kolkata were insignificant. These results are expected as the water used by the fish mongers in the retail markets had high levels of viable bacteria, faecal coliforms and *E. coli* (Table 3) indicating the poor status of market sanitation.

Apart from the microbial load that fishes have at the time of capture, more are added via unhygienic practices and contaminated equipment such as storage facilities (Huss et al., 2003). In the present study, the FC counts recorded in fishes of different markets ranged from nil to 4.38 log MPN g^{-1} and that of *E. coli* ranged from nil to 4.04 log MPN g^{-1} (Table 1). In general, the counts of FC and *E. coli* recorded in commercially important finfishes of Kolkata were higher than those reported elsewhere (Jayasinghe & Rajakaruna, 2005; Sifuna et al., 2008), possibly due to faecal cross-contamination during handling and processing. Results showed that majority of the finfishes sold in Kolkata markets were unhygienically stored or processed and grossly contaminated with

Table 3. Range and mean counts of total viable bacteria, MPN total coliforms, faecal coliforms and *Escherichia coli* in water used in Kolkata retail fish markets (n=12)

Bacterial group	Log counts	
	Range	Mean±SD
Total viable counts ml^{-1}	4.08-5.19	4.49±0.43
MPN total coliforms 100 ml^{-1}*	1.60-3.08	2.34±0.49
MPN faecal coliforms 100 ml^{-1}	0.60-3.04	1.93±0.68
MPN *Escherichia coli* 100 ml^{-1}	0.60-2.48	1.21±0.61

* On the basis of 3-tube MPN technique using single strength lauryl sulphate tryptose broth

Table 4. Antibiotic resistance of *Escherichia coli* strains (n=44) isolated from fish samples of Kolkata retail markets

Antibiotics	Resistance (%)
Chloramphenicol, 30 μg	11.36
Ciprofloxacin, 5 μg	15.91
Co-trimoxazole, 25 μg	27.27
Gentamycin, 10 μg	11.36
Nitrofurantoin, 300 μg	36.36
Oxytetracycline, 30 μg	72.72

bacterial flora of animal and human faecal origin during handling or retailing. The results are in agreement with Manna et al. (2008).

Among the marine fishes, *P. argenteus* and *L. parsia* recorded low levels *E. coli* compared to *L. calcarifer*. Presence of detectable levels of *E. coli* indicated faecal contamination; while relatively large numbers suggested post-harvest contamination, temperature abuse and unhygienic practices in fish handling. The quality and/or storage condition of the ice used at the market may be an additional source of contamination. Likewise, the market seafoods in the USA were contaminated with *E. coli* (Samadpour et al., 1994). The recommended levels of *E. coli* for acceptable and marginally acceptable quality fresh fish are ≤11 and ≤500 g^{-1}, respectively (ICMSF, 1986). The results of the present study revealed that 38% of the finfish samples of Kolkata market had *E. coli* counts higher than the acceptable levels. The Export Inspection Council of India recommended a level of ≤20 *E. coli* g^{-1} for fresh fish (EIC, 2012) and only 62% of commercially important finfishes of Kolkata complied this standard (Table 2).

The results depicted in Table 4 revealed that the *E. coli* strains examined in this study exhibited varying degrees of resistance to antibiotics with the maximum towards oxytetracycline (72%). Higher proportion of *E. coli* strains was resistant to nitrofurantoin. Five *E. coli* strains exhibited resistance to five antibiotics. Eight *E. coli* strains were highly susceptible to all the tested antibiotics. Cross resistance was noticed among ciprofloxacin, chloramphenicol and

Table 5. Antibiotic resistance profile and multiple antibiotic resistance (MAR) of *Escherichia coli* (n=44) isolated from fish samples of Kolkata retail fish market

Resistance profile	Number of isolates	Resistance profile	Number of isolates
FTGNO	2	CG	2
FCTNO	3	CT	2
FCNO	1	FC	1
TGNO	1	T	2
FTO	1	N	1
CTO	1	O	10
TNO	1	None	8
NO	8	MAR	23 (52.27%)

C: Chloramphenicol, 30 μg; F: Ciprofloxacin, 5 μg; G: Gentamycin, 10 μg; N: Nitrofurantoin, 300 μg; O: Oxytetracycline, 30 μg; T: Co-trimoxazole, 25 μg; None: Sensitive to all the tested antibiotics; MAR: Resistant to at least two broad spectrum antibiotics

gentamycin resistant *E. coli* strains. These results corroborate the observation of earlier studies (Komp et al., 2003; Matyar et al., 2004).

A major concern of antibiotic usages is the acquisition of multiple antibiotic resistance (MAR), *viz.*, resistant to two or more antibiotics. In the present study, MAR was observed in *E. coli* strains to the tune of 52% (Table 5) indicating that majority of *E. coli* strains isolated from finfishes have already developed resistance to human medicines. The MAR was also reported in *E. coli* strains of retail fishes of Turkey (Matyar et al., 2004), Vietnam (Van et al., 2007) and Kenya (Sifuna et al., 2008). Food-borne infections due to antibiotic resistant enteric bacteria (Temelli, 2002) and resistance of *E. coli* to antibiotics with associated treatment failure (Blondeau, 2004) have been reported, which is a serious cause for concern.

Mutation frequencies of *E. coli* (Table 6) to oxytetracycline were higher ($1.3x10^{-9}$–$7.3x10^{-4}$) compared to chloramphenicol ($4.6x10^{-8}$–$6.8x10^{-8}$). Generally, in bacteria, spontaneous mutation occurs at a rate of 10^{-9}-10^{-10}. It has been reported that the rate of adaptative mutations in *E. coli* is on the order of 10^{-5} genome^{-1} generation^{-1}, a finding which may have significance for the study and management of

Table 6. Frequency of mutants among *Escherichia coli* strains resistant to oxytetracycline and chloramphenicol (25-μg ml^{-1}) isolated from fish samples of Kolkata retail fish market

Bacterial strain	Source	Mutation frequency	
		Chloramphenicol	Oxytetracycline
Escherichia coli-A_2	*Labeo bata*	6.80×10^{-8}	7.30×10^{-4}
Escherichia coli-B_2	*Clarias batrachus*	1.03×10^{-8}	1.29×10^{-9}
Escherichia coli-D_3	*Clarias batrachus*	4.61×10^{-8}	7.64×10^{-8}
Escherichia coli-C_4	*Lates calcarifer*	2.32×10^{-8}	3.09×10^{-8}
Escherichia coli-A_6	*Catla catla*	3.20×10^{-8}	3.00×10^{-8}

All *E. coli* strains were sensitive to chloramphenicol (30 μg), ciprofloxacin (5 μg), co-trimoxazole (25 μg), gentamycin (10 μg), nitrofurantoin (300 μg) and oxytetracycline (30 μg).

Table 7. Serotyping of fish-borne *Escherichia coli* strains isolated from fish samples of Kolkata retail market

E. coli Strain no	NSEC no	Source	Antigenic structure
B_2	E-1003/10	*Clarias batrachus*, Garia market	O95
C_3	E-1005/10	*Labeo bata*, Jadavpur market	O95
D_3	E-1006/10	*Clarias batrachus,* Jadavpur market	O95
A_4	E-1007/10	*Catla catla,* Salt Lake market	O71
B_4	E-1008/10	*Pampus argenteus*, Salt Lake market	O71
D_5	E-1011/10	*Lates calcarifer*, Gariahat market	O95
A_6	E-1013/10	*Catla catla,* Park Circus market	O114*
E_6	E-1014/10	*Clarias batrachus,* Park Circus market	O52
A_7	E-1015/10	*Labeo rohita*, Patuli market	O2*
A_{11}	E-1016/10	*Lates calcarifer*, Howrah market	O22*
B_{11}	E-1017/10	*Oreochromis mossambicus*, Howrah market	O95
A_{13}	E-1019/10	*Cirrhinus mrigala*, Ajoynagar market	O42

NSEC: National *Salmonella* and *Escherichia* Centre; *: Shiga-like toxin producing *Escherichia coli* serotypes

bacterial antibiotic resistance (Perfeito et al., 2007). Bacteria with increased mutation rates have been found among pathogenic isolates of several bacteria, which may be beneficial to the bacteria for adaptation to environmental changes but becomes deleterious in secondary environments (Komp et al., 2003; Perfeito et al., 2007). These resistant bacteria may function as a potential source in the transportation of antibiotic resistance to human pathogens (Serrano, 2005).

Results of serotyping of 12 strains of *E. coli* (Table 7) revealed the presence of seven serotypes namely O2, O22, O42, O52, O71, O95 and O114. The serotype O95 was the most common. The serotypes O42, O71 and O95 were of non-human origin. However, the detection of shiga-like toxin-producing *E. coli* serotypes such as O2 and O22 of EHEC group and O114 of EPEC group (Samadpour et al., 1994) suggested that finfishes of Kolkata are contaminated with pathogenic *E. coli* capable of causing human diseases. In a similar study by Manna et al. (2008) few fish samples of Kolkata market were reportedly contaminated with non-O157 serotypes of shiga toxin- and enterohaemolysin producing *E. coli*. The risk of transfer of antibiotic resistance to human bacterial flora is probably low in countries where the use of antimicrobials is limited (Serrano, 2005; FAO/OIE/WHO, 2006). However, in countries with less restrictive legislation, the risk of contaminating fish with resistant bacteria is greater as has been observed in this study. Surveillance of resistance patterns among indicator bacteria can, therefore, be of great value in detecting trends in resistance.

The results of the present study revealed that most of the commercial fishes sold in the retail markets of Kolkata did not meet the national and international bacterial and sanitary quality standards and were contaminated with antibiotic resistant shiga-like toxin-producing *E. coli*. More research work is, therefore, needed to determine the distribution of pathogenic *E. coli* in nature, their mode of entry into the food chain and potential pathogenicity.

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