

Prevalence of *Escherichia coli* in Fish and Shrimps obtained from Retail Fish Markets in & around Kolkata, India

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Abstract: The work was aimed to study prevalence of *Escherichia coli* contamination in fish and shrimp sold in retail markets at Kolkata, India in different seasons. *E. coli* was identified by standard microbiological, biochemical tests, and further confirmed by 16S rRNA PCR. The faecal coliform loads in fish and shrimp samples were assessed. The bacterium was detected in 138(80.70%) samples out of 171 numbers of samples collected from different retail fish market in and around Kolkata. *E. coli* was detected in fish (65%) and shrimps (85%). *E. coli* had increased remarkably in summer months than winter.

Keywords: Prevalence, Faecal contamination, *Escherichia coli*, 16SrRNA PCR, Fish and Shrimp

1. Introduction

Fish and shrimps are generally considered as major vehicles for several bacterial disease transmissions [1]. *Escherichia coli* has been generally recognized as an indicator organism for faecal contamination of water and seafood [1]. The poor unhygienic conditions of the landing centres, storage and domestic retail markets exacerbate the problem of poor hygiene and consumer safety of fish & shrimps [2,7]. Most strains of *E. coli* or faecal coliform are harmless, but some may cause diarrhoea. Strains of bacteria those carry typically virulent properties have emerged as a serious health hazard in human, nevertheless consumption of even low numbers of these organisms bears the risk for life-threatening illness [3,4]. *E. coli* strains that cause diarrhea, acute gastroenteritis or colitis in humans are referred to as diarrhoeagenic or entero-virulent. These strains have been identified by the clinical symptoms of the diseases they trigger and the virulence traits they carry. At present there are several classes of entero-virulent *E. coli*, namely enterotoxigenic *E. coli* (ETEC), entero-pathogenic *E. coli* (EPEC), entero-haemorrhagic *E. coli* (EHEC), entero-

invasive *E. coli* (EIEC), entero-aggregative *E. coli* (EAggEC), diarrhoea-associated haemolytic *E. coli* and cytotoxic distending toxin (CLDT)-producing *E. coli* [6].

E. coli contamination of tropical seafood is quite common. In India, *E. coli* has been isolated from coastal beach and seawaters round the year [2]. Estuaries and coastal waters are the major sources of seafood in India and are contaminated with partially treated or untreated sewage water. Inadequately cleaned and disinfected boat decks and fish containers are also known to contaminate the catch with *E. coli* [9]. *E. coli* contamination can also occur from ice, unclean workers and handling after catching of fish and shellfishes. Kumar *et al.* (2008) reported that the salt-dried fishes sold in Tuticorin fish markets are contaminated with fungi and pathogenic bacteria like *E. coli* [10]. The objectives of our study were to assess the prevalence of *E. coli* in fish and shrimps sold at retail fish markets in and around Kolkata in different seasons and to enumerate the faecal coliform loads in fish and shrimps.

2. Material Methods

Fish species like Indian Major Carps (IMC) (*Labeo rohita*, *Catla catla*, *Cirrhinus mrigala*), minor carp (*Labeo bata*),

catfish(*M.tangra*, *C. batrachus*) and shellfish species like *Penaeus monodon* and *Penaeus indicus*, totalling 171 samples, were randomly collected from domestic retail fish markets in and around Kolkata, West Bengal India over a period of 6 months during November 2011 to April 2012. IMC contributes approximately 65% of the total inland fish production in West Bengal. The minor carps and catfishes are also economically important fresh water fishes in this State. The shrimps are locally cultured and /or caught and marketed fresh. The samples were collected individually in sterile polyethylene bags, brought to laboratory under ice-cover and processed within 2 hours.

All the samples were collected in sterile containers and processed within two hours of their collection. In case of shrimp sample, whole mass was taken and cut into small pieces with sterile scissors. Shrimp muscles including exoskeleton, finfish flesh were cut and homogenized with normal saline (0.85 NaCl) to 10% (w/v) suspension in a sterile blender inoculated into Lauryl Sulphate Tryptose Broth (LSTB) (DIFCO, USA) and incubated at 37°C for 24–48 h. The LSTB tubes indicating turbidity and gas production in Durham tubes were considered as positive for total coliform presence. Two loopfuls of broth culture from positive LSTB tubes were inoculated into corresponding labelled tubes with 5 ml of EC (*E. coli*) broth medium(DIFCO). EC medium tubes which developed turbidity and gas production following 24 h incubation at 44.5°C were considered positive for the presence of faecal coliforms. For isolation of *E. coli*, two loopfuls from positive EC broth tubes were streaked onto Eosin methylene blue (EMB) DIFCO agar plates. A minimum of five typical colonies were picked up, re-streaked on Tryptone soya agar (TSA) plates repeatedly to obtain pure cultures which were subjected to standard tests like gram staining, oxidation fermentation reaction, sugar (lactose, mannitol and cellobiose) utilization and IMViC reaction[12].

For quantitative assessment of faecal coliform contamination in fish and shrimp samples, the 10% w/v suspensions of samples were serially diluted in normal saline and 10^{-1} & 10^{-2} dilutions were membrane filtered and the membrane was put off mFC agar plate [membrane faecal coliform agar (mFC) agar(Difco laboratories, Detroit) without rosolic acid. The plates were inverted and incubated for 20 - 24 hours in bacteriological incubator (WTB BINDER) at $44.5 \pm 0.2^\circ\text{C}$. The blue colonies were counted in order to obtain faecal coliform counts [5].

2.1. Identification of Isolates by 16S rRNA Gene Sequence Analysis

The gene encoding 16S rRNA was amplified by Polymerase Chain Reaction (PCR) using universal bacterial primers 27f(5'-GAG TTT GAT CCT GGC TCA G-3') and 1492r (5'-TAC GGT TAC CTT GTT ACG AC -3'). The PCR reaction contained 25 µl of Sigma Red Taq ready mix (Jump start, REDTaq Ready Mix Kit, Catalog No.P0982), 22 µl water, 0.2 µM each of forward and reverse primers and template DNA. The template DNA was obtained by

extracting bacterial genomic DNA using the Gen Elute Bacterial Genomic DNA Kit (Sigma- Aldrich) from the bacterial colony. The following cycle was used for PCR reaction: initial denaturation at 95°C for 1 minute, followed by 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 2 minutes, final extension at 72°C for 5 minutes. Reaction was carried out in a thermal cycler (MJ Research PTC-200 Peltier thermo cycler, USA) All PCR amplified DNA were analysed on a 1% agarose gel with 0.5 X TBE as the running buffer. A 100 bp standard DNA ladder (Sigma) was included on each gel for base pair size comparison. The gel was then stained with 0.5 µg of ethidium bromide for 10 minutes and visualized on a UV transilluminator (BIO-RAD) and photographed with Image Analyzer. PCR products were sequenced following Sanger sequencing method. The sequence data were aligned with Codon Code Aligner (CodonCode Corporation, US) and compared with matching sequences in NCBI GenBank and Ribosomal Database Project (RDP), to identify our strains to the specific taxonomic groups.

2.2. Statistical Analysis

The statistical analysis of bacterial loads of all samples of finfish and shellfish was performed using SAS 9.2 software at $\alpha = 0.05$ significance. Figures and graphs were generated using JMP 8.0.2 software.

3. Results

The faecal coliform load in fish and shrimp samples collected from various retail markets of surroundings of Kolkata are depicted in Figure-1 which showed insignificant variations ($P > 0.05$) in bacterial load in fish from different retail markets, whereas significant variations ($P < 0.05$) between fish and shrimp samples (Figure. 2). The prevalence of *E.coli* in fish and shrimps obtained from different retail markets of Bengal is given in the Figure. 3. The bacterium was detected in 138(80.70%) samples collected from different retail fish market in and around Kolkata. *E.coli* was detected in fish (65%) and shrimps (85%). Faecal coliforms load in fishes from different markets were $0.29 \pm 0.7 \times 10^2$ CFU/g in Barrackpore, $0.19 \pm 0.2 \times 10^2$ CFU/g in Garia, $0.43 \pm 0.7 \times 10^2$ CFU/g in Howrah, $0.54 \pm 0.9 \times 10^2$ CFU/g in Kolkata, $0.15 \pm 0.4 \times 10^2$ CFU/g in Kalyani and $0.23 \pm 0.6 \times 10^2$ CFU/g in Ranaghat. Similarly, faecal coliforms load in shrimps from different markets were $4.9 \pm 1.1 \times 10^2$ CFU/g in Barrackpore, $5.6 \pm 0.9 \times 10^2$ CFU/g in Garia, $6.8 \pm 0.9 \times 10^2$ CFU/g in Howrah, $6.2 \pm 0.7 \times 10^2$ CFU/g in Kolkata, $3.4 \pm 0.7 \times 10^2$ CFU/g in Kalyani and $2.0 \pm 0.4 \times 10^2$ CFU/g in Ranaghat.

The seasonal fluctuation of prevalence of *E. coli* was given in Figure.4 and the occurrence of the bacterium was higher in fish and shrimp samples during warmer months (March to April) in comparison to winter (December to February).

The *E.coli* isolates were identified by biochemical test and confirmed by 16S rDNA sequencing. The 16S rDNA sequences have been submitted to NCBI GenBank vide Acc No. JQ266004.1 and JQ266006.1 (Table-1).

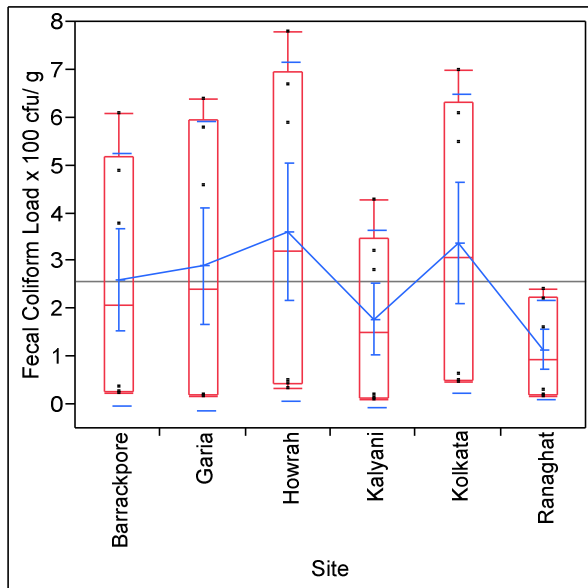


Figure 1. Total faecal coliform load in fish and shellfish samples collected from various retail markets of surroundings of Kolkata showed insignificant variation ($P > 0.05$) among retail market sites.

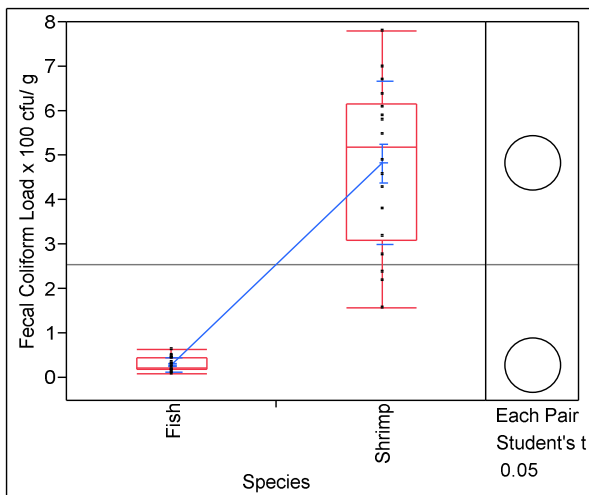


Figure 2. Total faecal coliform in fish and shellfish samples collected from various retail markets of surroundings of Kolkata showed significant variation ($P < 0.05$) among samples.

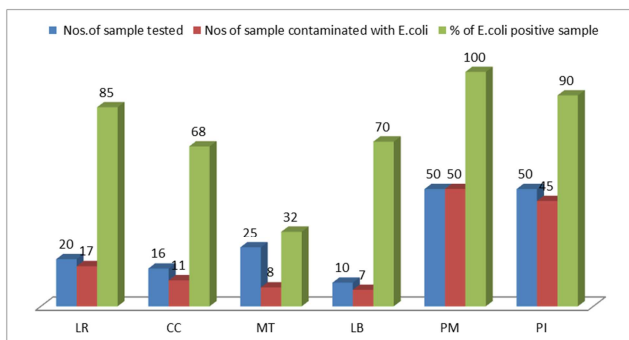


Figure 3. Percentages of samples contaminated with *Escherichia coli*.

LR- Labeorohita, CC – Catlacatla, MT – Mystustangra, LB- Labeobata, PM- Penaeusmonodon, PI – Penaeusindicus.

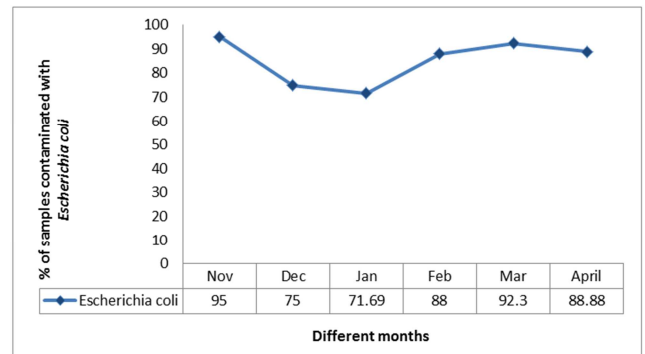


Figure 4. The occurrence of *Escherichia coli* in fish and shrimps in different months.

Table 1. Nucleotide sequences of bacteria obtained from fish and shrimp.

Identified bacteria	Necleotide Sequences	Gen Bank ACC No.
<i>E. coli</i>	AACAGGAAGAAGCTTGCTTCTTTGCTGACGAGTG GCGGACGGGTGAGTAATGTCTGGGAA ACTGCCTGATGGAGGGGGATAACTACTGGAAACG GTAGCTAATACCGCATAACGTCGCAA GACCAAAGAGGGGGACCTTCGGGCCTCTTGCCAT CGGATGTGCCAGATGGGATTAGCTA GTAGGTGGGGTAACGGCTCACCTAGGCGACGATC CCTAGCTGGTCTGAGAGGATGACCAG CCACACTGGAAGTGAACACCGTCCAGACTCCTA CGGGAGGCAGCAGTGGGGAATATTGC ACAATGGGCGCAAGCCTGATGCAGCCATGCCGCG TGTATGAAGAAGGCCCTTCGGGTTGTA AAGTACTTTTCAGCGGGGAGGAAGGGAGTAAAGTT AATACCTTTGCTCATTGACGTTACCC GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAG CCGCGGTAATACGGAGGGGTGCAAGCG TTAATCGGAATTACTGGGCGTAAAGCGCACGCAGG CGGTTTGTAAAGTCAGATGTGAAAT CCCCGGGCTCAACCTGGGAAGTGCATCTGATACTG GCAAGCTTGAGTCTCGTAGAGGGGG GTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAG ATCTGGAGGAATACCGGTGGCGAAG GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGC GAAAGCGTGGGGAGCAAACAGGATTA GATACCCTGGTAGTCCACGCCGT	JQ26606.1

Identified bacteria	Necleotide Sequences	Gen Bank ACC No.
<i>E. coli</i>	CGGTAACAGGAAGCAGCTTGCTGTTTTGCTGACG AGTGGCGGACGGGTGAGTAATGTCTGJQ26604.1 GGAAACTGCCTGATGGAGGGGGATAACTACTGGA AACGGTAGCTAATACCGCATAACGTC GCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTG CCATCGGATGTGCCAGATGGGATTA GCTTGTGGTGGGGTAACGGCTCACCAAGGCGAC GATCCCTAGCTGGTCTGAGAGGATGA	JQ26604.1

CCAGCCACACTGGAAGTGGAGACACGGTCCAGACT
 CCTACGGGAGGCAGCAGTGGGGAATA
 TTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC
 GCGTGTATGAAGAAGGCCTTCGGGT
 TGTAAGTACTTTACGCGGGGAGGAAGGGAGTAA
 AGTTAATACCTTTGCTCATTGACGTT
 ACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCA
 GCAGCCGCGTAATACGGAGGGTGCA
 AGCGTTAATCGGAATTACTGGGCGTAAAGCGCACG
 CAGGCGGTTTGTAAAGTCAGATGTG
 AAATCCCCGGGCTCAACCTGGGAAGTGCATCTGAT
 ACTGGCAAGCTTGAGTCTCGTAGAG
 GGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGT
 AGAGATCTGGAGGAATACCGGTGGC
 GAAGGCGGCCCCCTGGACGAAGACTGACGCTCAG
 TCGAAAGCGTGGGGAGCAAACAGGA
 TTAGATACCCTGGTAGTCCACGCCGTAAACGATGTC
 GACTTGGAGGTTGTGCCCTTGA

4. Discussion

The presence of faecal coliform bacteria, including *E. coli* in most of the samples examined indicates poor hygiene and sanitary condition. This finding substantiates with the study of Feng et al [21]. Quality of sea foods depends on the quality of waters from where the fishes are captured and the sanitary conditions of the landing centers. Proper Sanitation facilities at the retail markets play an important role in the overall quality of the fish. Even if the seafood samples collected from fish catch is landed in prime condition, contamination at poor landing sites and cross contamination may cause faecal contamination [15]. However, *E. coli* does not thrive in the marine environment for long period of time and so this organism cannot be expected to harbor in fish harvested from the sea. The detection of *E. coli* in some fishes might represent post harvest cross contamination [16].

Ideally *E. coli* should not be detected at a level of <3 CFUg⁻¹ which has been given as the satisfactory criteria for this organism. Levels exceeding 100CFU g⁻¹ are unacceptable and indicate level of contamination [18]. Antony et al. [19]. Jeyasekaran et al. [8] reported that *E. coli* in shrimp ranged from 0.6 to 240 CFU g⁻¹ in Indian fish market.

The average faecal coliform counts of fishes of different markets did not differ significantly ($P > 0.05$) but the loads in shrimp samples was significantly higher ($P < 0.05$) than the fish samples. This may be due to shrimp samples originated from water resource which is contaminated with sewage and faecal matter of the adjacent locality. The incidences of faecal coliform and *E. coli* in fish and shrimp samples were reported by Kumar et al. (2005) [2]. According to Thampuran et al. (2005) [13], *E. coli* in different fish samples collected from the retail market in Cochin (India) was recorded in high numbers. Moreover in the present study, among various markets surveyed, faecal coliform bacteria load was higher in fishes from Kolkata markets, in comparison to those from sub-urban areas (viz. Kalyani & Ranaghat); however the

difference was statistically insignificant. This indicate that the fish culture areas from the studied area were more or less equally contaminated with sewage matter or post harvest contamination was also uniform due to similar fish handling process practiced in this region. Faecal coliform loads were estimated in the range from 10 ± 1 (MPN/100ml) to 300 ± 80 (MPN/100 ml) during March, 2013 in lower stretches of Hooghly estuary by Bose et al [20]. A total number of 25 faecal coliform, 50 *E. coli* bacteria were identified out of 110 isolates from the Ganga river by Kaberi et al [24].

Occurrence of *Escherichia coli* in various species of fish and shrimps were examined and detected in 138 (80.70%) numbers out of 171 numbers of samples collected from different retail fish market in and around Kolkata. Similarly, the high prevalence of *E. coli* (69%) was also observed in fish samples collected from fish markets in Tuticorin by Jeyasanta et al [14]. According to Jeyasanta et al [14] the shrimp samples collected from fish market had 30% incidence of faecal coliform but the prevalence of *E. coli* in these samples was just 10%. Shrimp samples collected from landing center of Tuticorin showed 40% faecal coliforms and 20% *E. coli* contamination. Fresh shrimp such as *P. monodon* collected from landing center and *P. indicus* collected from fish market showed contamination by *E. coli* due to poor handling and sanitary condition. The occurrence of bacteria was higher in shellfish (*P. monodon*, 100%, *P. indicus* 90%) as compared to fish. This is to be expected since these shellfish are present in the estuarine environment where contamination with faecal matter is often recorded. Besides, our findings have corroborated well with earlier observation made by several authors [14, 15 & 16].

According to Karunasagar et al. (1992) faecal coliforms contents in shrimps and other food may vary depending on the sanitary and hygienic condition of the landing centers [22]. Besides Ekanem and Adegoke (1995) opined that the level of contamination in shellfish depends on the extent of pollution in the growing waters [23].

Another important finding of this study is that the quantity of *E. coli* had increased remarkably at the months of summer (November, March and April) than winter months (December to February). This is due to reduced water volume of culture areas in summer & the increased temperature effects on the population dynamics of *E. coli*. High temperature favours the bacterial growth and peak is observed in summer period [11]. Iyer et al. [17] also claimed that season plays an important role in controlling the bacterial quality in raw shrimps and he observed that the bacterial counts were higher in certain specific seasons.

5. Conclusion

This study revealed that raw fish and shrimp sold in fish market in Kolkata surroundings could be a source of food borne bacterial pathogens. Improvements in handling and processing and good sanitation and hygienic condition at landing centres and domestic retail markets are urgently needed to minimize seafood contamination.

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