Indian J. Fish., 63(1) : 152-155, 2016
DOI:10.21077/ijf.2016.63.1.49671-24

Note

Distribution of pathotypes of *Escherichia coli* in seafood from retail markets of Kerala, India

V. MURUGADAS, TOMS C. JOSEPH, AND K. V. LALITHA
ICAR-Central Institute of Fisheries Technology, Matsyapuri post, Willingdon Island, Kochi - 682 029
e-mail: murugadascift81@gmail.com

ABSTRACT

Escherichia coli is an indicator species of bacteria to determine faecal/sewage contamination in food and water. Some strains of *E. coli* have the potential to be pathogenic to humans. The food and its environment should regularly be monitored for the prevalence of pathogenic *E. coli*. A study was conducted to determine the prevalence of pathogenic *E. coli* from different fish markets and landing centres in three districts of Kerala, India. Isolated *E. coli* were pathotyped as enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC) and Shiga toxin producing *E. coli* (STEC) by PCR, targeting the genes *viz*., *bfpA, eaeA, stx1 stx2, Lt1, Lt2, ST, phoA* and *rfbeO157*. Pathogenic *E. coli* was found in 23.4% of the 123 seafood samples screened and ETEC, EPEC, and STEC were prevalent in 18.6, 4.0 and 0.8% of the samples respectively.

Keywords: *Escherichia coli*, Multiplex PCR, Pathotypes, Prevalence, Seafood

The coliform bacteria, *Escherichia coli* is used as the best faecal indicator (USEPA, 2011) of sewage contamination and have potential to cause a wide variety of diseases in human beings including infants. *E. coli* colonises and resides in the intestinal tract of almost all warm blooded animals including human beings (Katouli, 2010) with the predominant groups being harmless in healthy human intestinal tract. Transmission of *E. coli* occurs mostly by feco-oral route through ingestion of contaminated water or food. There are nearly about thousand serotypes prevailing in the environment which are categorised into different serogroups (Cui *et al*., 2013). *E. coli* causing disease in human beings especially diarrhoea are commonly called as pathogenic *E. coli* or diarrheagenic *E. coli.* Pathogenic *E. coli* are categorised in to six pathotypes *viz.,* enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAggEC), Shiga toxin producing *E. coli* (STEC) and diffusively aggregated *E. coli* (DAEC), based on presence of virulence determinants or markers (Feng *et al*., 2011). ETEC produces secretory toxins (enterotoxins); EPEC adhere intimately to epithelial cells and induce host cell transmembrane signaling; EIEC invade eukaryotic cells; and STEC produce Shiga toxins (Nataro and Kaper, 1988). Some strains of STEC attach to the intestinal wall of the host because of the protein intimin coded by *eae* gene. *E.coli* carrying both *stx* and *eae* genes are known as enterohemorrhagic *E. coli* (EHEC) and is responsible for uremic and haemolytic syndrome (UHS). Among the pathogenic *E. coli*, only ETEC, EPEC, EIEC and STEC including EHEC have been implicated in major food or water borne outbreaks (Feng *et al*., 2011).

Ever increasing human population in the country has resulted in increased release of sewage from different sources into the water bodies. Use of sewage contaminated water in the food production chain can lead to emergence of water borne diseases particularly by pathogens of public health importance (Jung *et al*., 2014).

Detection of pathogenic *E. coli* by conventional methods is often expensive; require special expertise and various detection systems. Application of such assays for enteric microbiological diagnosis is cumbersome (Lopez-Saucedo *et al*., 2003). Polymerase chain reaction (PCR) has increasingly been used to improve the rapidity in detection. Since there are different pathotypes of *E. coli,* multiplex PCR targeting more number of genes in a single PCR reaction is being used to categorise them in single attempt. The present study focused on screening of seafood samples and associated environments for presence of pathogenic *E. coli* employing PCR method targeting virulence associated genes.

One hundred twenty three seafood samples comprising of fish and shellfish were collected from various fish markets and landing centres in three districts of coastal Kerala *viz*., Ernakulam, Kottayam and Alappuzha. The samples were brought to the laboratory in chilled condition and processed within 4 h of collection. Isolation and identification of pathogenic *E. coli* was performed as per BAM (2011) employing two different protocols *viz*., protocol 1 for pathogenic *E. coli* except EHEC of

serotype O157 also called as O157 STEC and protocol 2 for EHEC of serotype O157 *i.e.,* O157 STEC serotype. In protocol 1, seafood samples were enriched in Brain Heart Infusion broth and subsequently in *E. coli* broth, serially diluted and 100 µl each was plated onto Levine-Eosin Methylene Blue agar and MacConkey agar. Typical lactose fermenting pink colonies on MacConkey agar and dark centered purple colonies with or without green metallic sheen on Levine-Eosin Methylene Blue agar were taken for further confirmation using biochemical tests such as triple sugar iron agar (TSI); urease; indole - methyl red - Voges - prouskauer - citrate (IMViC) tests. In protocol 2, homogenised samples were enriched in EHEC enrichment broth (EEB) and 100 µl was selectively plated onto CT-Sorbitol MacConkey agar. Non-sorbitol fermenting yellow colonies on CT-SMAC agar were picked and checked in EMB agar for dark purple centered colonies with or without green metallic sheen.

Two hundred and twenty five strains of *E. coli* obtained in this study were confirmed as *E. coli* by PCR and then characterised into different pathotypes by single and multiplex PCR using nine pairs of primers targeting virulence and housekeeping genes. Primers, cycling conditions and product size are presented in Table 1. Crude DNA extract was prepared by boiling the culture in 1X TE buffer (pH.8.0) and stored at -20^{O}C until analysis. Pathogenicity of *E. coli* strains isolated in this study

Table 1. Details of the PCR conditions used in the present study

Genes targeted in PCR/ Multiplex PCR	Primer sequence	Product length	Cycling condition	References
bfpA	bfpAF- AATGGTGCTTGCGCTTGCTGC bfpAR- GCCGCTTTATCCAACCTGGTA	326 bp	50˚C/2 m Initial denaturation 94˚C/5 m followed by 40 cycles of Denaturation, Annealing and Extension 94˚C/45 sec 52˚C/45 sec 68˚C/45 sec Final extension 68˚C/ 74˚C/α	Gunzburg *et al*. (1995)
rfbeO157	rfbeO157F-CGAGTA CAT TGG CAT CGT rfbeO157R-ATT GCG CTG AAG CCT TTG	500 bp	Initial denaturation 93˚C/3 m followed by 35 cycles of Denaturation, Annealing and Extension 93˚C/15 sec 60˚C/15 sec 72˚C/30sec Final extension 72˚C/5 4˚C/α	Abdulmawjood *et al*. (2003)
phoA	Pho-F GTGACAAAAGCCCGGACACCATAAATGCCT Pho-R TACACTGTCATTACGTTGCGGATTTGGCGT	903bp	Initial denaturation 94˚C/2 m	Kong *et.al.* (1999)
St1	St1-F CTTTCCCCTCTTTTAGTCAG St1-R TAACATGGAGCACAGGCAGG	175 bp	Followed by 40 cycles of Denaturation, Annealing and Extension	
Lt1	Lt1-F TTACGGCGTTACTATCCTCTCTA Lt1-R GGTCTCGGTCAGATATGTGATTC	275 bp	94˚C/1 m 55˚C/1 m	
Lt2	Lt2-F ATATCATTTTCTGTTTCAGCAAA Lt2-R CAATAAAATCATCTTCGCTCATG	720 bp	72˚C/1 m Final extension 72˚C/10 4˚C/α	
eaeA	eaeAF- AGGCTTCGTCACAGTTG eaeAR- CCATCGTCACCAGAGGA	570bp	Initial denaturation 94˚C/5 m	China *et al*. (1996)
stx1	stx1F- AGAGCGATGTTACGGTTTG stx1R- TTGCCCCCAGAGTGGATG	388bp	followed by 30 cycles of Denaturation, Annealing and Extension	
stx2	stx2F- TGGGTTTTTCTTCGGTATC stx2R - GACATTCTGGACTCTCTT	807 bp	94˚C/30 sec 50˚C/30 sec 72˚C/30 sec Final extension 72˚C/5 4˚C/α	

were confirmed by amplification of *bfpA* gene specific for EPEC. Two multiplex PCR (one targeting *phoA, Lt1, Lt2, st1* genes specific for ETEC and another targeting *stx1, stx2, eaeA* genes specific for STEC including EHEC and *rfbeO157* gene specific for *E. coli* O157 serotype of STEC/ EHEC.

Fifty five percent of the seafood samples analysed were found to harbour *E. coli* out of which 23.4% was found to carry pathogenic *E. coli*. PCR analyses of the pathogenic *E. coli* revealed that 18.6, 4.0 and 0.8% of the pathogenic *E. coli* were ETEC, EPEC and STEC respectively (Table 1; Fig.1, 2 and 3). ETEC was present in seafood from all the 3 districts while EPEC was detected in Alappuzha and Ernakulam and STEC in Alappuzha only. Forty strains (from 18 samples) were found to possess genes specific for ETEC (*St1* genes), 6 strains (5 samples) were found to possess gene specific for EPEC (*bfpA* genes) and one strain (1 sample) was found to harbour gene *stx1* specific for STEC and the rest of the strains were not harbouring any of the virulence genes (non-pathogenic *E. coli*). Even though STEC was present, none of the isolates were found to harbour genes specific for EHEC of O157 serotype *i.e.,* O157 STEC.

Although Surendraraj *et al*. (2010) reported the prevalence of EHEC strain from Cochin market in an earlier study, EHEC especially *E. coli* O157 was not detected from the samples screened in this study. Higher incidence of STEC has been reported in seafood as compared to ETEC (Singh and Kulshrestha, 1994; Theophilo *et al*., 2002). However, in our study ETEC incidence was highest, followed by EPEC and STEC. This study is in agreement with the findings of Kumar

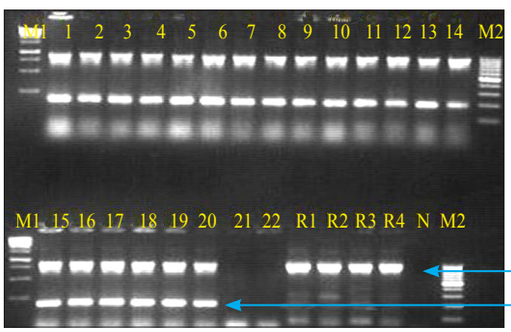

Fig. 1. Multiplex PCR for detection of enterotoxigenic *E.coli* (ETEC)
Lane1 to 20: Positive ETEC strains with both *phoA* gene and *slt gene*
Lane M1: 1kb plus marker (Fermentas)
Lane M2: 100 bp marker (Fermentas)
R1: *E. coli* NCTC 1113, R2: *E. coli* 12568, R3: VT3 positive lab strain, R4: O42 lab strain
N: Negative control

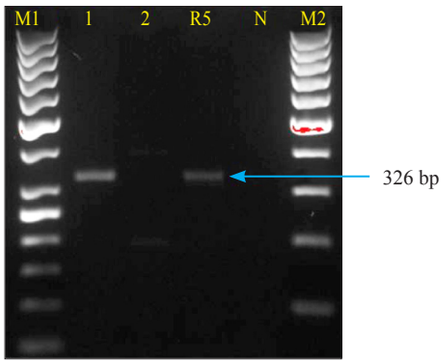

Fig. 2. PCR for detection of *bfpA* gene specific for EPEC
Lane M1 and M2: 50 bp molecular weight marker
Lane 1 and 2: Test culture
R5: *bfp*A positive lab strain; N: Negative control

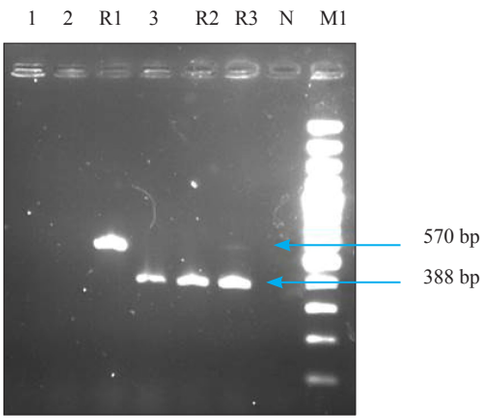

Fig. 3. Multiplex PCR for detection of *stx1, stx2* and *eaeA* genes
Lane M1: 100 bp Marker
Lanes 1, 2 and 3: Test *E. coli* isolates
N: Negative control
R1: *E. coli* NCTC 1113, R2: *E. coli* 12568,
R3:VT3 positive lab strain

et al. (2001) who reported only prevalence of STEC and not EHEC in fish and fishery products. The presence of STEC in food samples were insignificant in comparison to that of EHEC strains (Paton and Paton, 1998). Although prevalence of STEC was very low in this study, modifying the method of isolation by including immuno magnetic separation for concentrating the organism may improve the chances of detection of this pathotypes in seafood.

The study revealed prevalence of ETEC and EPEC pathotypes of *E. coli* in seafood from three districts of Kerala which clearly revealed the entry of this sewage indicator bacteria at any point starting from harvest onboard fishing vessel or aquaculture farms to the point of sale in retail markets for human consumption. Presence of pathogenic *E. coli* is usually linked with the use of

contaminated water in food production chain. Hence, there is a need for source tracking of these pathogens to identify the source of contamination and to implement control measures to reduce its incidence in seafood.

Acknowledgements

The authors are immensely thankful to the Director, ICAR-CIFT, Kochi for the facilities provided, Indian Council of Agricultural Research, New Delhi for funding and to the staff of ICAR-CIFT for kind support.

References

Abdulmawjood. A., Bulte, M., Cook, N., Roth, S., Schonenbrucher, H. and Hoorfar, J. 2003. Toward an international standard for PCR-based detection of *Escherichia coli* O157 Part 1. Assay development and multi-center validation. *J. Microbiol. Methods*, 55: 775-786.

BAM 2011. *Bacteriological analytical manual.* U. S. Food and Drug Administration, USA.

China, B., Pirson, V. and Mainil, J. 1996. Typing of boving attaching and effacing *Escherichia coli* by multiplex *in vitro* amplification of virulence associated genes. *Appl. Environ. Microbiol.,* 62(9): 3462-3465.

Cui, Y. J., Li, D. F. and,Yang, R. F. 2013. Shiga toxin-producing *Escherichia coli* O104: H4: An emerging important pathogen in food safety. *Chin. Sci. Bull.,* 58: 1625-1631.

Feng, P., Stephen D. Weagant and Karen Jinneman 2011. Diarrheagenic *Escherichia coli. Bacteriological analytical manual* Chapter 4A, U.S. Food and Drug Administration, USA.

Gunzburg, S. T., Tornieporth, N. G. and Riley, L. W. 1995. Identification of enteropathogenic *Escherichia coli* by PCR based detection of the bundle forming pilus gene. *J. Clin. Microbiol*., 33(5): 1375-1377.

Jung, Y., Jang, H. Matthews, K. R. 2014. Effect of the food production chain from farm practices to vegetable processing on outbreak incidence. *Microbial Biotechnol.,* 7: 517-527.

Katouli, 2010. Population structure of gut *Escherichia coli* and its role in development of extra-intestinal infections. *Iran. J. Microbiol*., 2(2): 59-72.

Kong, R. Y. C., So, C. L., Law, W. F. and WuA, R. S. S. 1999. Sensitive and versatile multiplex PCR system for the rapid detection of Enterotoxigenic (ETEC), Enterohaemorrhagic (EHEC) and Enteropathogenic (EPEC) strains of *Escherichia coli*. *Mar. Pollut. Bull.,* 38(12): 1207-1215.

Kumar, H. S., Otta, S. K., Karunasagar, I. and Karunasagar, I. 2001. Detection of shiga toxigenic *E.coli* (STEC) in fresh seafood and meat marketed in Mangalore, India by PCR. *Lett. Appl. Microbiol.,* 33: 631-635.

Lopez-Saucedo, C., Cerna, J. F, Villegas-Sepulveda, N., Thompson, R., Velazquez, F. R., Torres, J., Tarr P. I. and Estrada-Garcia, T. 2003. Single multiplex polymerase chain reaction to detect diverse loci associated with diarrheagenic *Escherichia coli*. *Emerg. Infect*. *Dis*., 9: 127-131.

Nataro, J. P. and Kaper, J. B. 1988. Diarrheagenic *Escherichia coli*. *Clin Microbiol. Rev.,* 11(1): 142-201.

Paton, A. W. and Paton, J. C. 1998. Detection and characterisation of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic *E. coli* hlyA, rfbO111, and rfbO157. *J. Clin. Microbiol*., 36(2): 598-602.

Singh, B. R. and Kulshreshtha, S. B. 1994. Incidence of *Escherichia coli* in fishes and seafood; isolation, serotyping, biotyping and enterotoxigenicity evaluation. *J. Food. Sci. Technol*., 31: 324-326.

Surendraraj, A., Thampuran, N. and Joseph, T. C. 2010. Molecular screening, isolation, and characterisation of enterohemorrhagic *Escherichia coli* O157: H7 from retail shrimp. *J. Food Protect*., 73: 97-103.

Theophilo, G. N. D., Fernandes-vierra, R. H. S., Prazeres-Rodriques, D. and Menezes, F. G. R. 2002. *Escherichia coli* isolated from seafood; toxicity, plasmid profiles. *Int. Microbiol.,* 5: 11-14.

USEPA 2011. *Using microbial source tracking to support TMDL development and implementation.* Prepared for U. S. Environmental Protection Agency, Region 10, Watersheds Unit, Tetra Tech, Inc. and Herrera Environmental Consultants, Seattle, WA.

Date of Receipt : 25.06.2015
Date of Acceptance : 21.12.2015