and Goswami, 2013). The absence of pathogenic bacteria makes the shidal safe for human consumption. Further studies are needed to ascertain the protective and functional attributes of the shidal associated microorganisms.

Table 1. Microbial quality of shidal

| Microbial parameters | Count cfu/g |
|---|---|
| Total aerobic count | $6.98 \times 10^7$ |
| Total anaerobic count | $1.17 \times 10^8$ |
| Lactobacillus spp. | $1.4 \times 10^8$ |
| Bacillus spp. | $4.76 \times 10^4$ |
| Yeast & mould | $8.7 \times 10^4$ |

**Keywords:** Shidal, Fermented Fish, Microflora

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# Molecular characterization of diarrheagenic *E.coli* from seafood of Gujarat

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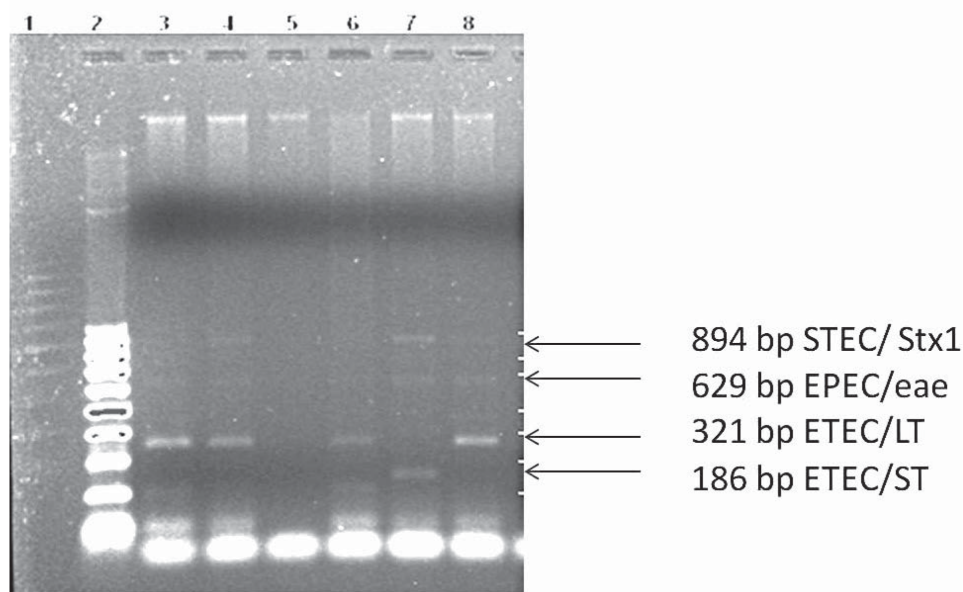
*Escherichia coli* are member of normal microbial flora of human gut. *E.coli* can be pathogenic and cause various infections (Nataro and Kaper 1998). Typically in developing countries *E.coli* is considered as the main etiologic agent of diarrhoea posing a major threat to public health (WHO, 2017). A group of Diarrheagenic *E coli* (DEC) plays an important role in causing enteric and diarrheal diseases. Based on distinct clinical and epidemiological features, associated with certain serotypes and virulence determinants, DEC can be further sub-categorized into six major pathotypes *viz.*, Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Shiga toxin producing *E. coli* (STEC), Enteroaggregative *E. coli* (EAggEC), Diffusively aggregated *E. coli* (DAEC) (Feng et al., 2011).

Due to increase in human population the amount of sewage released in water bodies have increased exponentially (Vermeulen *et al.*, 2015). The released sewage is mostly untreated along with mixture of various domestic as well as clinical waste lead to contamination of water bodies and coastal areas. This also contaminates the food production chain and may lead to disease outbreaks. Contamination of seafood with patho-

genic *E. coli* strains is mainly due to sewage contamination of water and also due to handling of seafood in processing plants by infected workers (Sivaraman et al., 2017). Detection of pathogenic *E. coli* by conventional methods is rudimentary, time consuming and often more expensive. Therefore molecular methods such as DNA hybridization and PCR have been developed and utilized in recent years for identification of pathogenic *E. coli* (Tobias and Vutukuru 2012). Multiplex Polymerase Chain Reaction (PCR) is a very efficient method that simultaneously detects several genes related to pathogenicity of DEC *E. coli.* The present study was aimed to monitor the incidence of diarrheagenic *E. coli* strains from seafood samples by multiplex PCR method (CDC, 2009).

A total of 120 fresh and processed fish samples were collected in and around Veraval region (Gujarat) and brought to laboratory immediately with suitable sterile polythene bags for enumeration of *E. coli* by ISO, 9308-1 method and 20E BioMerieux identification kit was used for further confirmation (ISO, 1990) as per the manufacturer directions. Deoxyribonucleic acid (DNA) was extracted from the confirmed *E.coli* isolates with GenElute™ Bacterial Genomic DNA Kit and a set of 8 PCR primer pairs were employed for the detection of virulence genes such as *eaeA* for EHEC and EPEC, *bfpA* for EPEC, vt1 and/or vt2 for shiga toxins 1 and 2 of EHEC, ST and/or LT enterotoxins for ETEC, ia for EIEC and pCVD432 of EAEC.

Out of 120 seafood samples screened 54 of isolates from 12 samples were confirmed as *E. coli* and these strains were subjected to pathotyping. A multiplex PCR was standardized for pathotyping of diarrhoeagenic *E. coli* isolates to screen for ETEC, EPEC, EHEC and EAEC. 14 *E. coli* strains (25.92%) were identified as Entero Toxigenic *E. coli* due to the presence of LT and ST genes with PCR band at 321 bp and 186 bp, respectively. 3 *E. coli* strains (5.54%) were confirmed to be Entero Pathogenic *E. coli* (EPEC) the presence *eae* gene (629 bp). Two isolates were positive for Shiga toxin producing *E. coli* (STEC). It was also noted that none of the *E. coli* strains showed presence of pCVD432 plasmid gene from seafood products. Hence no EAEC were present in seafood. The present comprehensive evaluation of pathogenic *E. coli* in seafood from Veraval region revealed that ETEC type was highly prevalent among seafood with highest percentage of 25.92%.




*Multiplex PCR for pathotyping of E.coli isolates from seafood*

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# Sequence variant *tdh* gene in environmental strains of *Vibrio parahaemolyticus*

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*V. parahemolyticus* is a natural inhabitant of coastal-marine environment. Food poisoning due to *V. parahaemolyticus* is associated with the consumption of raw or partially cooked seafood, especially the shellfish such as clams and oysters. Among the different virulence factors described in *V. parahaemolyticus*, chromosomal *tdh* and *trh* genes are well studied pathogenic determinants and their presence poses serious health risk to humans. The presence of these virulence factors has been found to correlate with the hemolytic toxins as evidenced in various mouse bioassays. Studies have shown that the detection rate of *trh* gene varies between 0% to 59.3% and *tdh* gene from 0% to 8.4% in different seafood and marine samples. Moreover, the detection rates of these genes were higher in clinical samples compared to the environmental and seafood samples. A Study was carried out to ascertain the pathogenic characteristics of *V. parahaemolyticus* from 140 samples comprising fish (40), shellfish (32), coastal sediment (23) and coastal water (45) from different fish markets and landing centers located in and around North Western Mumbai, Maharashtra, using *tdh* and *trh* targeted polymerase chain reactions (Honda et al., 1991; Okura et al., 2003). The *tdh*-specific PCR yielded non-specific amplifications visible as strong bands in the agarose gel and did not produce the expected amplicon size of 263 bp