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# Occurrence and distribution of virulent strains of *Vibrio parahaemolyticus* in seafoods marketed from Cochin (India)

**Rekha D. Chakraborty · P. K. Surendran**

**Abstract** This study was aimed for the detection of *Vibrio parahaemolyticus* by biochemical and molecular methods in seafood samples collected from the markets of Cochin located at the southwest coast of India. A total of seventy-two *V. parahaemolyticus* cultures were isolated by selecting sucrose and cellobiose non-fermenting colonies. All the biochemically confirmed strains were found to have 368-bp *toxR* gene fragment, while an additional 24% of the samples were confirmed as *V. parahaemolyticus* by *toxR* based polymerase chain reaction (PCR) from enrichment broths. PCR based methods are used to detect *tdh*, *trh*, and *orf*8 genes for the identification of pathogenic and pandemic *V. parahaemolyticus*. Only one out of two urease positive isolates amplified the *trh* (500bp) gene. About 10% of the isolates showed weak haemolysis and none were found to amplify *tdh* (269 bp) and *orf*8 (746 bp) genes, thus indicating the meager incidence of pandemic strains from this area. The incidence of *trh* positive isolates from market samples signals towards the adoption of stringent seafood safety measures for the products meant for human consumption.

**Keywords** *Vibrio parahaemolyticus* · Seafood · Polymerase chain reaction · *toxR* · *trh*

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## Introduction

*Vibrio parahaemolyticus* is a prevalent seafood-borne pathogen in many Asian countries where marine foods are frequently consumed (Joseph et al. 1983). The organism has been reported as the causative agent of gastroenteritis (Fujino et al. 1953), wound infections, and septicemia because of the consumption of contaminated seafoods. Recently with the emergence of pandemic O3:K6 strain of *V. parahaemolyticus* that was reported to cause acute gastroenteritis, this pathogen has acquired greater significance (Matsumoto et al. 2000). Epidemiological studies revealed an association between the Kanagawa positive phenomenon (KP+) and gastroenteritis (Okuda et al. 1997a). In the 1980s few gastroenteritis cases were reported from the KP negative *V. parahaemolyticus* isolates, which led to the discovery of the TDH-related haemolysin (TRH) (Hervio-Heath et al. 2002). Studies revealed the thermostable direct haemolysin (TDH) and the TDH-related haemolysin (TRH), encoded by the *tdh* and *trh* genes, respectively, as the major virulence factors of this organism (Shirai et al. 1990). The test adopted for Kanagawa phenomenon using Wagatsuma agar consumes time and labor. Moreover, the test may not be determinative as many environmental strains are Kanagawa negative and do not produce TDH (Tada et al. 1992). Presently there is no in-vitro test for the detection of TRH in *V. parahaemolyticus*. Sensitive and rapid molecular method such as PCR has been applied to identify the presence of *tdh* and *trh* genes from *V. parahaemolyticus* (Wong et al. 1999; Kim et al. 1999).

A unique clone of *V. parahaemolyticus* O3:K6 was responsible for many of the recent *V. parahaemolyticus* outbreaks, reported in India, Russia, Southeast Asia, Japan, and North America (Matsumoto et al. 2000). During 1996

the strains of the O3:K6 serovar was reported to emerge in the Eastern province of India accounting for about 50–80% of *V. parahaemolyticus* infections annually. It was determined that a filamentous phage, f237 is specifically and exclusively associated with the O3:K6 serovar (Nasu et al. 2000), which was found to have 10 open reading frames (ORFs), including a unique open reading frame, ORF8. *Vibrio cholerae* has a similar filamentous phage CTX, which carries the cholera enterotoxin genes *ctxA* and *ctxB* (Iida et al. 2001). In *V. parahaemolyticus* O3:K6 serotype, the f237 phage possesses *orf*8 in place of *ctxAB* (Waldor and Mekalanos 1996). It was therefore suggested that *orf*8 gene might be associated with the virulence characteristics of the strains (Okuda et al. 1997b). Nasu et al. (2000) isolated a filamentous phage possessing *orf*8 in another recently emerging serovar (O4:K68), and reported the *orf*8 gene fragment as a useful genetic marker for the pandemic group. A toxin regulatory gene (*toxR*) sequence specific to *V. parahaemolyticus*, which is present in all the strains irrespective of their ability to produce TDH or TRH, has been applied for definitive identification of the bacterial isolates by several workers (Jacksic et al. 2002; Tada et al. 1992; Kim et al. 1999).

Cochin is a major fish-landing centre in south west coast of India, and accounts for over 90% of statewide exports. Earlier studies carried out at Cochin to identify virulent strains of *V. parahaemolyticus*, were based on the traditional methods using phenotypic assays and biochemical tests, which require several days for confirmation (Sanjeev and Stephen 1992). In this study an attempt has been made to determine the occurrence of *V. parahaemolyticus* in seafoods marketed in this area, and to detect the presence of virulent isolates carrying *tdh, trh*, and *orf*8 genes from sample isolates and their enrichment broths by biochemical and PCR methods.

## Materials and methods

### Sample collection and processing

A total of 12 finfish, 25 shellfish, and 5 cephalopod samples were collected over a period of one year from various retail fish markets in and around Cochin for microbiological examination. Finfish samples included *Oreochromis mossambicus*, *Mugil cephalus*, *Sardinella longipes*, *Etroplus suratensis*, *Pampus argentius*, *Megalaspis cordyla*, and *Labeo rohita*. Shellfishes consisted of *Penaeus monodon*, *Fenneropenaeus indicus*, *Parapenaeopsis stylifera*, *Panulirus homarus*, *Metapenaeus dobsoni*, *Metapenaeus affinis*, *Macrobrachium rosenbergii*, and *Scylla serrata*. Cephalopod samples included *Sepia pharonis* and *Loligo duvacelli*. All the samples were collected in sterile containers or

sterile polythene bags and processed within 2 h of their collection. In brief, heads and tails of the fish were cut off, and the guts were removed. In the case of shrimp, whole mass was taken and cut into small pieces with sterile scissors, while in case of cephalopods; the meat was picked from different parts.

### Enrichment, isolation, and identification of *V. parahaemolyticus*

*V. parahaemolyticus* was isolated and identified as described in the Bacteriological Analytical Manual of the Food and Drug Administration (Elliot et al. 1995) with modifications. Briefly, the process of identification was initiated with the selection of sucrose followed by cellobiose non-fermenting colonies, and further proceeded with other biochemical tests unlike the reported method (Elliot et al. 1995).

The samples (25 g) were homogenized with alkaline peptone water (APW) broth (225 ml) in a sterile polythene stomacher bag (Stomacher 400 Seaward medicals, UK) at 230 rpm for 1 min, and enriched in APW broth for 18–24 h. Following 8 h of incubation, samples from enrichment broth (1 ml) was drawn aseptically into a microfuge tube for PCR experiment. After 18–24 h of incubation the broth (0.5 ml) was aseptically pipetted into thiosulphate citrate bilesalt sucrose agar (TCBS) plates previously pre-set and dried (56°C, 45 min). The plates were incubated at 37°C for 24–30 h. About 3–4 typical colonies having green or bluish green color with dark blue or green centre measuring about 3–5 mm were picked from TCBS plate, and each one was inoculated into sterile sucrose broth followed by cellobiose medium supplemented with NaCl (3% w/v). Sucrose and cellobiose non-fermenting colonies were streaked onto sterile tryptone soy agar slants (TSA) supplemented with NaCl (3% w/v) and maintained at room temperature for further identification. Halophilism tests were performed using tryptone broth with different concentrations of NaCl (0, 3, 6, 8, and 10% w/v). Additional characterization tests for the identification of *V. parahaemolyticus* namely, Gram staining, catalase, cytochrome oxidase tests, triple sugar iron tests, lysine iron agar tests, arginine dehydrolase tests, lysine and ornithine decarboxylase test, and O/129 susceptibility tests were performed (FDA 1992). Tests for glucose oxidation-fermentation were carried out using Hugh-Leifson broth, and arabinose, lactose, mannitol, mannose, salicin and inositol fermentation tests were also performed. All the media were supplemented with NaCl (3% w/v) unless otherwise specified. Colonies that gave typical colony characteristics were picked up and confirmed by Kanagawa (Wagatsuma 1968) and urease tests (Andrews and Hammack 2001).

The isolates exhibiting atypical reaction for any one or two of the sugars or amino acids were also maintained for

further analyses. The presumed positive cultures were confirmed using RAPID Hi-Vibrio™ identification kit (KB007, Himedia, India) containing twelve different dehydrated medias (Voges Proskauer, Arginine, ONGP, citrate, ornithine, mannitol, arabinose, sucrose, glucose, salicin, cellobiose and salt concentration) in a single strip. In brief, individual colonies of *V. parahaemolyticus* grown on nutrient agar (3% w/v, NaCl) were isolated and inoculated in alkaline peptone water (APW, 5 ml) supplemented with NaCl (3% w/v). The broth was incubated at 37°C for 18–24 h until the turbidity was found to be greater than or equal to 0.5 OD at 620 nm. Each well, of the strip containing dehydrated media was aseptically inoculated with 50 µl of the inoculum by surface inoculation method, and the strip was incubated at 35–37°C for 18–24 h. Results were interpreted following standards given in the identification index supplied with the kit.

Haemolytic activity

The Kanagawa phenomenon (KP) or TDH phenotype detection was carried out on Wagatsuma agar (Wagatsuma 1968) as described by Elliot et al. (1992). Haemolytic activity was determined following the procedures of Kishishita et al. (1992). In brief *V. parahaemolyticus* strains were seeded in Wagatsuma agar by stab and surface inoculation methods, incubated at 37°C for 20–24 h. Isolates producing a clear zone (β-haemolysis) around the colonies were identified as Kanagawa positives while those with weak haemolysis (α–haemolysis) and no hemolytic zone (γ–haemolysis) were termed as Kanagawa negative strains.

Determination of urease activity

Urease activity was determined as described by Andrews and Hammack (2001) with modifications. Briefly, urea agar slants were prepared with an additional incorporation of NaCl (1% w/v) to the Christensen's urea agar (1 g of peptone, 20 g of NaCl, 2 g of $KH_2PO_4$, 1 g of dextrose, 15 g of agar, and phenol red 0.012 g in 1 l of distilled water; pH 6.8). Urea solution (0.5 ml of 40% w/v) was added to the sterile agar medium under aseptic conditions and allowed to cool to form slants. In order to characterize the TRH phenotype, bacterial cultures were streak inoculated and kept for incubation at $36 \pm 1$°C for 24–48 h. Development of pink color in the urea agar slant was indicated as positive reaction, while the negative cultures retained the media color (yellow).

Preparation of bacterial lysate and PCR assay

Bacterial lysate was prepared following established procedure (Ausubel et al. 1987) with modifications. The biochemically identified isolate was streaked on TSA plates and the colonies were inoculated in the Luria Bertane (LB) broth supplemented with NaCl (2% w/v), and incubated at 37°C under shaking (120 rpm) for 16–18 h. The broth cultures were centrifuged (10,000 rpm, 4°C, 1 min; 5804 R, Eppendorf, Germany) to obtain the pellet, which was washed with normal saline (0.8% NaCl w/v) and re-suspended with DNA-free sterile distilled water (200 µl). The resulting suspension was heated at $98 \pm 2$°C for 15–20 min in a water bath to lyse the cells, and release the DNA. The lysate was clarified by centrifugation (10,000 rpm, 4°C, 5 min), and the supernatant was stored (−20°C) until further use.

The PCR targeting *toxR*, *tdh*, *trh*, and *orf*8 genes of *V. parahaemolyticus* were performed separately both from the characterized isolates and the sample enrichment broths as described previously (Bej et al. 1999; Kim et al. 1999, Nasu et al. 2000) using a thermocycler (Eppendorf Mastercycler, Germany).

PCR amplification was optimized in a total reaction volume of 25 µl consisting of sterile Milli Q water (13.5 µl), 10× PCR buffer (2.5 µl), primer (1.5 µl), dNTP mix (0.5 µl, 200 mM), template (5 µl), and *Taq* DNA polymerase (0.5 µl). The components were mixed well and the PCR amplification of the target sequence was arranged in a thermocycler (Eppendorf Mastercycler, Germany), and programmed for 30 cycles of amplification. Each cycle consisted of three step reactions i.e., initial denaturation (94°C, 3 min) followed by 30 cycles of denaturation (94°C, 1 min), and extension (72°C, 1 min) followed by final extension (72°C, 5 min). Variation was found in the annealing temperatures for each gene as 60°C for *toxR*, 58°C for *trh*, 56°C for *tdh*, and 57°C for *orf*8 for 1 min.

The PCR products were resolved on agarose (1.5% w/v) gel electrophoresis. The gel was stained with ethidium bromide (0.5 mg/ml) and visualized under a UV transilluminator (Alpha Imager, Innotech Corporation, USA). GeneRuler™ 100 bp DNA Ladder (MBI Fermentas, USA) was used as a molecular size marker.

Primers

The primers used in this study to detect the targeted genes namely, *toxR* (toxin regulatory gene), *tdh* (thermostable direct hemolysin gene), *trh* (*tdh*-related hemolysin gene), and *orf*8 (open reading frame gene) are referred in Table 1.

DNA sequencing

The primers based on the *toxR* and *trh* gene fragments were used to amplify and sequence DNA of these amplicons using Beckman coulter CEQ 8000 genetic analysis system (Bioserve Biotechnologies, India). *V. parahaemolyticus*

**Table 1** PCR nucleotide sequences and size (in base pairs, bp) of the primers used in the study (references included)

| S. No. | Primers | PCR nucleotide sequences | Size (bp) | Reference |
|---|---|---|---|---|
| 1 | *toxR* | 5′ GTC TTC TGA CGC AAT CGT TG 3′ | 368 | Kim et al. 1999 |
| | | 5′ ATA CGA GTG GTT GCT GTC ATG 3′ | | |
| 2 | *trh* | 5′ TTG GCT TCG ATA TTT TCA GTA TCT 3′ | 500 | Bej et al. 1999 |
| | | 5′ CAT AAC AAA CAT ATG CCC ATT TCC G 3′ | | |
| 3 | *tdh* | 5′GTA AAG GTC TCT GAC TTT TGG AC 3′ | 269 | Bej et al. 1999 |
| | | 5′ TGG AAT AGA ACC TTC ATC TTC ACC 3′ | | |
| 4 | *orf*8 | 5′GTT CGC ATA CAG TTG AGG 3′ | 746 | Nasu et al. 2000 |
| | | 5′ AAG TAC AGC AGG AGT GAG 3′ | | |

isolates, which generated amplicons of the expected size i.e., 500 bp for *trh* and 368 bp for *toxR* were used for sequencing. Sequence assembly, analysis, and alignments were performed with DNA using Chromas LITE software (Version 2.01, Technelysium Pvt. Ltd., Australia). The nucleotide sequence data was analyzed for homology with the published sequence in the GenBank database using the Basic Local Alignment Search Tool (BLAST), and percent homology was noted. The nucleotide sequences were submitted to the GenBank to obtain the accession numbers. The accession numbers obtained from the GenBank for the selected isolates were bankit914935 EF640377 and bankit843662 DQ997814 for *toxR* and *trh* gene sequences, respectively.

## Results

The incidences of *V. parahaemolyticus* were found to be 41.6, 64, and 40% by conventional method in finfish, shellfish, and cephalopods, respectively. The isolates which were initially identified, as *V. parahaemolyticus* by

conventional biochemical tests were further confirmed by PCR targeting species specific *toxR* (368 bp) gene. An additional 25%, 20%, and 40% were noticed in the sample enrichment broths by PCR targeting *toxR* gene in finfish, shellfish, and cephalopods, respectively. Seventy-two of the isolates, which were confirmed to be *V. parahaemolyticus* by RAPID Hi-Vibrio™ identification kit were further confirmed by PCR targeting species specific *toxR* (368 bp) gene. All the isolates were found to amplify *toxR* gene by PCR analysis thus confirming the identity of the bacteria (Fig. 1). By conventional method twenty-three out of 42 samples (54.8%) were found to be positive for *V. parahaemolyticus*, while an additional 24% of the samples were confirmed for the same with *toxR* based PCR method using enrichment broths (Table 2).

The suspected *V. parahaemolyticus* isolates were identified by a battery of biochemical tests following the method listed in Bacteriological Analytical Manual (FDA 1992). However, certain isolates were reported to exhibit variation in one or two biochemical reactions (Dileep et al. 2003). Such atypical reactions may pose difficulties in identification of the organism (Karunasagar et al. 1997).



**Fig. 1** Agarose gel electrophoresis of PCR products amplified for *toxR* (368 bp) gene. Lane M: DNA ladder (100 bp); Lane 1: MTCC 451 (type strain); Lanes 2–7: biochemically characterized *V. parahaemolyticus* isolates; Lanes 8–13: enrichment broths from the samples showing atypical *V. parahaemolyticus* isolates, Lane 14: PCR negative control
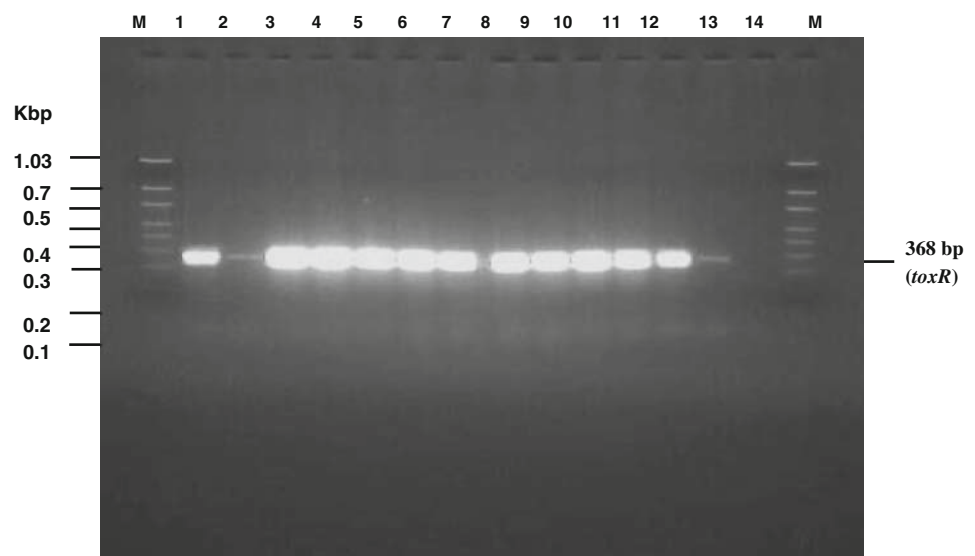
**Table 2** Incidence of *V. parahaemolyticus* from the samples collected from markets of Cochin by conventional and *toxR* based detection from enrichment broths

| Samples | Number of samples | Number of samples positive[a] | No. of additional samples positive[b] | Total incidence (%)[c] |
|---|---|---|---|---|
| Shellfish | 25 | 16 (64.0)[d] | 5 (20.0)[d] | 84.0 |
| Finfish | 12 | 5 (41.6) | 3 (25.0) | 66.6 |
| Cephalopods | 5 | 2 (40.0) | 2 (40.0) | 80.0 |
| Total | 42 | 23 (54.8) | 10 (23.8) | 78.6 |

[a] Number of samples positive for *V. parahaemolyticus* by conventional method

[b] Number of samples positive for *V. parahaemolyticus* by the *toxR* based PCR from the sample enrichment broth

[c] Total incidence (%) by both conventional and PCR methods

[d] Figures in parentheses indicate the percentage incidence by conventional method

The identification method based on the *toxR*-targeted PCR was developed to solve this problem (Kim et al. 1999). In this study, the isolates were tested for the fermentation of six different sugars and three amino acids. The isolates that exhibited variations in the utilization of one or two sugars or amino acids were considered as atypical isolates. A total of nine atypical isolates were recovered of which five were confirmed to be *V. parahaemolyticus* by the PCR targeting *toxR* gene.

In this study, 2 out of 72 (2.8%) isolates showed urease activity of which only one (1.4%) isolate, obtained from *Metapenaeus dobsoni* sample exhibited amplification for *trh* gene (Fig. 2). The other urease positive strain, which did not amplify *trh* gene, was isolated from another inshore shrimp, *M. affinis*. The *trh* gene was not amplified from the urease positive isolate of *M. affinis* was evident from the PCR of the biochemically confirmed strains and the sample enrichment



**Fig. 2** Agarose gel electrophoresis of PCR products amplified for *trh* (500 bp) gene. Lane M: DNA ladder (100 bp); Lane 1–3: isolates negative for the gene; Lane 4: type strain (NCMB 1902) positive for *trh* gene; Lane 5: isolate from *M. dobsoni* sample containing *trh* gene; Lane 6: isolate from *M. affinis* sample without *trh* gene, showing urease activity; Lane 7: PCR negative control

broths. Although 10% of the 72 *V. parahaemolyticus* isolates showed weak haemolysis, none of them were found to exhibit positive amplification for the *tdh* (269 bp) gene or *β*-haemolysis on Wagatsuma agar. Though *toxR* gene was amplified from all the isolates, none of these strains exhibited positive amplification for *orf*8 gene.
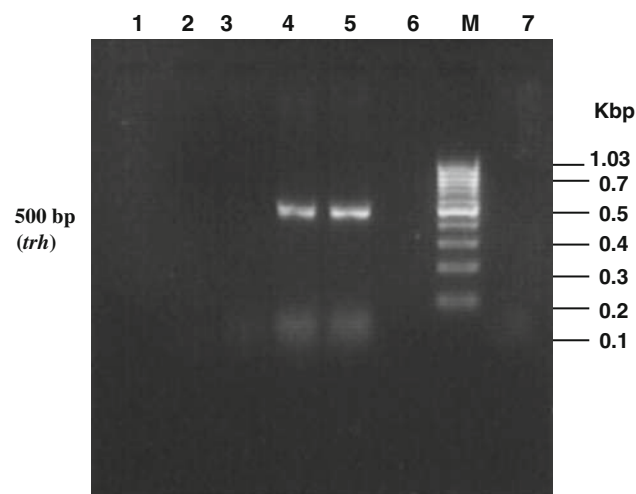
The nucleotide sequences of the amplified products were analyzed for homology with the published sequence in the GenBank database using the Basic Local Alignment Search Tool (BLAST) and percent homology was noted. The *toxR* and *trh* gene fragments exhibited 95–97% and 93–97% similarity, respectively with the published sequences.

## Discussion

The members of the genus *Vibrio* have been frequently defined as opportunistic and potential pathogenic bacteria of the tropical waters (Huss 1997). In India the incidence of *V. parahaemolyticus* causing food poisoning due to the consumption of contaminated seafoods was reported to be doubled in the last 5 years (Choudhury et al. 2000). Therefore detection of this organism in seafoods is very important.

cIn this study the detection of *V. parahaemolyticus* was carried out by conventional biochemical and modern PCR methods. By biochemical methods *V. parahaemolyticus* was detected in 55% of the samples, the highest being found in shellfish. The higher incidence of *V. parahaemolyticus* in shrimps can be correlated with the ability of this bacterium to utilize chitin, which is found abundant in shellfish.

Identification by biochemical method was initiated with the selection of sucrose followed by cellobiose non-fermenting colonies, and progressed with the other biochemical and microbiological tests as listed in the standard methods (FDA 1992). Earlier studies suggested that majority of the sucrose and cellobiose non-fermenting colonies could be *V. vulnificus* and *V. campbelli* other than *V. parahaemolyticus* (Elliot et al. 1995). The early

elimination of vibrios other than these three vibrios by selecting sucrose/cellobiose non-fermenting colonies considerably reduced the time and labor involved in screening of *V. parahaemolyticus* isolates. It was reported that cellobiose fermentation can distinguish *V. parahaemolyticus* from other vibrios (Thampuran and Surendran 1998). In the present study all the isolates were subjected to cellobiose fermentation test, which could further narrow down the process of identification of presumable *V. parahaemolyticus* colonies. The sucrose and cellobiose non-fermenting colonies were selected and subjected to a battery of biochemical tests. Some colonies exhibited deviation from the standard methodology (Dileep et al. 2003) in giving few biochemical reactions. Those isolates which deviated in giving the reactions from the usual procedure are termed as atypical isolates, and identification of such isolates pose a major challenge for the microbiologists (Bharathi et al. 1987; Karunasagar et al. 1997). In the present study nine atypical isolates have been found, which deviated in the utilization of few sugars (arabinose, salicin, lactose, and inositol) and amino acids (lysine, arginine, and ornithine). Five of these atypical isolates were confirmed to be *V. parahaemolyticus* by targeting species-specific *toxR* (368 bp) gene.

All the biochemically confirmed isolates (55% of total isolates) were found to possess *toxR* (368 bp) gene. Moreover an additional 24% of the samples enriched in APW broth were confirmed to be positive for the presence of *V. parahaemolyticus* by this species-specific *toxR* gene. Thus the *toxR* targeted PCR method was found to be sensitive and rapid in the detection of *V. parahaemolyticus* from environmental samples as well as atypical colonies. The superiority of the *toxR* targeted PCR over the biochemical method was supported by earlier studies (Dileep et al. 2003).

In general, the phenotypic character such as urease activity corresponds to the presence of *trh*, a virulent gene as reported earlier (Shirai et al. 1990). In the present study two of the isolates were found to exhibit urease activity, and 50% of these urease positive isolates amplified *trh* (500 bp) gene. However, the remaining urease positive isolates did not amplify the *trh* gene. These results are in contrast with the studies of Okuda et al. (1997a, b) who reported the presence of *trh* gene in urease negative isolates. This observation led us to conclude that urease test alone may not be suitable for correlating the presence of *trh* gene in *V. parahaemolyticus*, and the results must be validated by PCR targeting *trh* gene.

The pathogenic *V. parahaemolyticus* isolates were found to exhibit β-haemolysis on blood agar, termed as Kanagawa positives (KP+), and such isolates were found to amplify the virulent gene *tdh* (269 bp). In the present study, 10% of the isolates were found to exhibit weak haemolysis (Kanagawa negative) and none of them were found to exhibit positive amplification for the *tdh* gene. The direct correlation between the presence of *tdh* gene and Kanagawa reaction led us to conclude that both tests are mandatory for the confirmation of pathogenicity of *V. parahaemolyticus*. The weak haemolysis points towards the presence of other virulence factors other than *tdh* in *V. parahaemolyticus*. The capability of the strains to produce few extra cellular enzymes may also lead to the weak haemolysis (Lee et al. 2002). There are reports of Kanagawa phenomenon being correlated with the positive detection of urease by urea hydrolysis (Kaysner et al. 1994; Iida et al. 1998). However, in the present study none of the weak haemolytic strains were found to exhibit urease activity.

Another gene *orf*8 was found to be associated with the virulence, was reported to be an useful genetic marker for the detection of pandemic *V. parahaemolyticus* strains. A unique serovar of *V. parahaemolyticus* O3:K6 possessing a unique open reading frame, *orf*8 was reported to be responsible for several outbreaks (Matsumoto et al. 2000). Although in the present study all the isolates were found to possess *toxR* gene, none of them were found to amplify the *orf*8 gene thus indicating the meager occurrence of pandemic strains in seafoods marketed from this area.

In conclusion, the present study emphasizes the importance of molecular method like PCR using enrichment broth lysates for identifying both pathogenic and non-pathogenic isolates exhibiting atypical biochemical reactions. This study provides the first direct evidence for the presence of pathogenic *trh* positive *V. parahaemolyticus* strains in the marketed seafood samples from the areas in and around Cochin. However, the lower incidence of *trh* positive pathogenic isolates and the complete absence of *tdh* and *orf*8 strains indicate that the seafoods marketed from Cochin are safe for consumption. In summary, this investigation thrusts upon the need for enhancing seafood-borne disease control strategies, focusing the attention on pathogenic *V. parahaemolyticus* strains, which are often linked with acute infections, associated with seafood ingestion.

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