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SHORT COMMUNICATION

Evaluation of loop-mediated isothermal amplification assay along with conventional and real-time PCR assay for sensitive detection of pathogenic *Vibrio parahaemolyticus* from seafood sample without enrichment

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Abstract

The primary reason for foodborne illness is improper seafood safety testing, and hence, an appropriate tool for testing is the key to control the outbreaks. The current study aimed to develop a loop-mediated isothermal amplification (LAMP) assay to detect pathogenic *Vibrio parahaemolyticus*, important foodborne pathogen, targeting *tdh*, and *trh* genes. The specificity of the LAMP assay was good without any false-positive and false-negative results. The assay was highly sensitive and could detect the pathogenic *V. parahaemolyticus* as low as 1 CFU/reaction in spiked seafood samples and 1 pg of extracted DNA. Out of 62 seafood samples from India's southwest coastal region tested with LAMP assay, eight (12.9%) were positive for *trh*, and seven (11.29%) samples were positive *tdh* gene. LAMP-based on *tdh* and *trh* was found to be significantly more sensitive ($p < 0.05$) than conventional PCR and nearly equal sensitive as real-time PCR (RT-PCR) for the detection of pathogenic *V. parahaemolyticus*. Our study shows that LAMP assay can be a better approach as a point-of-care (POC) diagnostic tool and could detect pathogenic *V. parahaemolyticus* on seafood samples directly without enrichment and isolation. The high sensitivity and simplicity make LAMP assay a better alternative method than the conventional method and RT-PCR for the detection of pathogens. LAMP assay can be considered as a good alternative to PCR for the routine detection of pathogenic *V. parahaemolyticus* in seafood.

Keywords LAMP assay · Sensitive detection · *V. parahaemolyticus* · Without enrichment · Seafood

Introduction

Vibrio parahaemolyticus is a Gram-negative, halophilic bacterium, universally found in the coastal and estuarine marine waters. It is an opportunistic foodborne pathogen, often sheltered on marine reservoirs, predominantly in shellfish, serving as a vehicle for transmission of infection to humans, causing gastroenteritis. *V. parahaemolyticus* possesses different virulence properties, the major ones are thermostable direct hemolysin (TDH), and TDH-related hemolysins (TRH) encoded by *tdh* and *trh* genes, respectively [1]. These factors cause hemolysis and cytotoxicity to the host cells resulting in diarrhea. The virulence genes, *tdh*, and *trh* are considered to be a marker for detecting pathogenic *V. parahaemolyticus* [2]. Along with these virulence genes, secretion systems also play a major role in pathogenicity (T3SS2, T3SS6) [3–5]. Reports show the involvement of adhesion factors such as *VpadF* as a virulence marker with the potential to be used as vaccine candidates [6]. Seafood associated disease outbreaks are one of the major threats to public health. Worldwide gastroenteritis caused by *V. parahaemolyticus* is usually due to the consumption of contaminated shellfishes and other raw or undercooked seafood.

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According to the epidemiological data, most outbreaks occur after consuming oysters and clams [7]. *V. parahaemolyticus* is considered to be one of the major foodborne pathogens by the Centers for Disease Control and Prevention (CDC) [8]. *V. parahaemolyticus* infection has been increased worldwide in the last two decades, including outbreaks caused by pandemic strains [9].

For the detection of *V. parahaemolyticus*, several methods are available from conventional to the molecular level. In general, conventional culture-based techniques are time-consuming and often require multiple days to achieve the results. Moreover, these assays show less sensitivity when target bacteria are present in lower numbers [10]. The molecular methods include polymerase chain reaction (PCR), real-time (RT)-PCR (RT-PCR), and more advanced droplet digital PCR. However, these sophisticated techniques require expensive equipment, trained personnel, and expensive reagents [11–14]. Hence developing simple diagnostic tools without compromising simplicity and sensitivity would be very important. The loop-mediated isothermal amplification (LAMP) assay is one such method that has the potential to be a point-of-care (POC) detection tool. Recently LAMP assay has been customized and used worldwide to detect tuberculosis [15]. LAMP is a DNA based amplification method that amplifies the nucleic acid under isothermal conditions. As compared to existing molecular methods like PCR and RT-PCR, LAMP assay can be performed in a simple dry or water bath [16]. LAMP is known for the specificity and simplicity; hence it would be an excellent tool for detecting an important sea-foodborne pathogen viz *V. parahaemolyticus*. In this study, we have optimized LAMP assay for the sensitive detection of pathogenic strains of *V. parahaemolyticus* in seafood without enrichment. The assay was validated by detecting the organism from a direct seafood sample and comparing it with existing molecular methods.

Methods

Bacterial strains, culture condition, and genomic DNA

Details of the bacterial strains used in this study are listed in Table ST1, which includes *V. parahaemolyticus* strains, other *Vibrio*, and non-*Vibrio* strains. All these bacterial strains were tested to determine the specificity of the LAMP assay. The strains of *V. parahaemolyticus* and other bacterial strains were revived from the institutional deep freezer (−80 °C) (Thermo Scientific, USA) into 5 ml of tryptic soy broth (TSB) (HiMedia, Mumbai, India) and incubated at 35 ± 2 °C for 24 h. The overnight grown *V. parahaemolyticus* culture was streaked onto a selective medium, thiosulfate–citrate–bile salts–sucrose (TCBS) agar (HiMedia, India) obtain a single colony. Other bacterial strains were directly sub-cultured into 5 ml Luria Bertani (LB) broth. Genomic DNA was extracted from all the cultures used in this study as per the protocol described by Ausubel et al. [17] with minor modifications. DNA concentration and purity were checked using a spectrophotometer (BioSpectrometer® basic, Eppendorf, USA).

LAMP primers and assay optimization

The *tdh* and *trh* genes of *V. parahaemolyticus* were retrieved from NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) database. The sequences were further subjected to multiple sequence alignment tools, multi-align (http://multalin.toulouse.inra.fr/multalin/) [18] to find the consensus sequences. The best-matched sequence was chosen for primer designing. LAMP primer designing was done using PrimerExplorer software—V5 (Eiken Chemical Co. Ltd., Japan; http://primerexplorer.jp/e/).

The LAMP assay was optimized for the detection of *V. parahaemolyticus* targeting the *trh* and *tdh* gene. LAMP assay was performed in a 25 µl reaction mixture, containing 1× reaction mixtures (Eiken Chemical, Japan), template DNA (100 ng/µl), 1 µl of *Bst* DNA polymerase (Eiken Chemical, Japan), and 40 pmol of FIP and BIP, 20 pmol of F3 and B3, for the amplification of *tdh* gene 5 pmol of LF primer was used. Results were analyzed using LoopAmp real-time turbidimeter (LA-500, Eiken Japan) and 2% agarose gel, stained with ethidium bromide (0.5 µg/ml) and analyzed using a gel documentation system (Bio-Rad, CA, USA). The reaction was carried out using a simple dry bath (Genei, India) and real-time turbidimeter at different temperatures (60, 63, and 65 °C) for 15, 30, and 60 min, followed by inactivation at 80 °C for 5 min. Details of primers used in this study are listed in Table ST2.

Specificity test of *trh* and *tdh* LAMP primers

Various bacterial strains were used to test the specificity of LAMP assay. DNA extracted from the bacterial strains were subjected to both LAMP and conventional PCR amplification. The LAMP assay's specificity was determined by identifying the amplification of *trh* and *tdh* genes in all the bacterial strains (Table ST1).

Sensitivity of the LAMP assay

Determination of sensitivity using purified genomic DNA

The sensitivity of LAMP assay was carried out by preparing a ten-fold serial dilution of DNA extracted from *V. parahaemolyticus* standard cultures (AQ4037 and 81TDH2 for *trh*$^+$ and *tdh*$^+$ respectively) concentration ranging from 100 ng to 1 fg.

The LAMP outer primers, F3, and B3 were used for PCR amplifications to check the sensitivity.

Determination of sensitivity in spiked clam meat

The sensitivity of LAMP assay was also determined using clam meat spiked with *tdh*$^+$ and *trh*$^+$ *V. parahaemolyticus*. The clam sample was purchased from a local market, Mangalore, India. Initially, the collected clam was pooled and homogenized in a sterile container. One gram of sample was taken and inoculated into 9 ml of alkaline peptone water (APW). After 18 h of incubation, 1 ml of the enriched sample was then taken in the micro-centrifuge tube and centrifuged at $900 \times g$ for 1 min to separate the larger meat particles. The supernatant was transferred in a fresh tube and centrifuged at $10{,}000 \times g$ for 10 min. The pellet was gently mixed with 100 µl of molecular grade water. The mixture was then heated at 95 °C for 5 min and immediately transferred into ice for 5 min. Finally, centrifugation was performed at $2800 \times g$ for 5 min, and crude cell lysate (CCL) was collected as supernatant in a fresh tube and preserved at −20 °C for future use. The CCL was used as DNA template and tested for the presence of *tdh* and *trh* gene using PCR [19], and LAMP assay (protocol described before). Clam sample negative for conventional PCR & LAMP assay was further used for the sensitivity study. The *tdh*$^+$ and *trh*$^+$ *V. parahaemolyticus* cultures (2.88×10^8 and 2.35×10^8 CFU/g respectively) were serially diluted (ten-fold serial dilution). The 25 g of clam meat in 225 ml of APW was homogenized separately using a high-speed blender. Then, 1 ml from each different *V. parahaemolyticus* dilutions was inoculated to 9 ml of homogenate, mixed, and immediately subjected for CCL preparation (protocol described before). One microliter of CCL was further used for conventional PCR, RT-PCR, and LAMP assay.

LAMP assay to detect pathogenic *V. parahaemolyticus* in naturally contaminated samples

Clam ($n = 31$) and shrimp ($n = 31$) were collected from the local market in March 2018 to February 2019 (Table 1). The samples were brought to the laboratory, cleaned, and homogenized aseptically in the laboratory. The CCL was prepared from the samples and subjected to LAMP assay targeting *trh* and *tdh* genes. Similarly, the same CCL was also subjected to conventional PCR and RT-PCR assay.

Comparison of LAMP assay with conventional PCR and RT-PCR for the detection of pathogenic *V. parahaemolyticus*

Conventional PCR assay

Conventional PCR was carried out for all the seafood samples targeting *trh*, and *tdh* genes. The assay was performed in 30 µl volumes comprising LAMP F3 and B3 primers and Tada et al. [19] primers using CCL. Amplification was done using a T100™ thermal cycler (Bio-Rad, CA, USA) and visualized on 2% agarose gel and documented (described before).

RT-PCR

The RT-PCR assay was performed in a CFX96 real-time system (Bio-Rad, USA) for all the samples collected in the study. Reaction volume (25 µl) (in triplicate) consists of the supermix (Bio-Rad, USA), 2.5 pmol of each forward and reverse primer of *trh* and *tdh* genes (F3 and B3 of LAMP primers, Table ST2), and 2 µl of crude DNA template. Amplification was performed with an initial denaturation at 95 °C for 4 min, followed by amplification for 40 cycles at 95 °C for 20 s and 55 °C for 30 s. Melt curve analysis to check the random amplification of untargeted regions from 65 to 95 °C for 5 s with an increase of 0.5 °C in a stepwise manner as compared to a positive control (±0.5). A cycle threshold (Ct) value of less than 35 was considered to be positive. A no-template control and positive control were included in every reaction. The amplification for various samples (Ct values of 24–35) was validated by considering the Ct value of positive control. Representative amplicons were also confirmed by being visualized on 2% agarose gel and documented (described before).

Statistical analysis

The significant difference between two detection methods, i.e., LAMP and conventional PCR and LAMP and RT-PCR for each gene were calculated using two-sample proportion tests with the significance of $p < 0.05$. Two sample proportion tests are performed using an online software Mathcracker. Graphs were generated using Prism version 5.0 software (Graph Pad, Inc., La Jolla, USA). The percentage of agreement between three diagnostic assays was calculated using Cohen's Kappa coefficient in SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). The kappa coefficients' values were interpreted according to the criteria of Viera and Garrett [20].

Table 1 Details of the samples and results of the LAMP, real-time PCR, and conventional PCR assay

Sl. no.	Sample type	Place	Culture ID	PCR[a]		RT-PCR[a]		LAMP assay	
				trh	*tdh*	*trh*	*tdh*	*trh*	*tdh*
1	Clam	Kundhapur	SBCL0118/1	–	–	–	–	–	–
2	Clam	Kallapu	KCL0218/3	–	–	+	–	–	–
3	Clam	Thokkottu	THCL0218/4	–	–	+	–	–	–
4	Clam	Kuloor	KUCL0218/5	–	–	–	–	–	–
5	Clam	Tannirbhavi	TNCL0218/8	–	–	–	–	–	–
6	Clam	Ullal	UBCL0218/9	–	–	–	–	–	–
7	Clam	Kasargod	KSCL0318/10	–	–	–	+	–	+
8	Clam	Beeri Dakke	BDCL0318/11	–	–	+	+	+	+
9	Clam	Malpe	MKCL0318/12	–	–	–	–	–	–
10	Clam	Mulki	MMCL0318/13	–	–	–	–	–	–
11	Clam	Kannur	TKCL0318/14	–	–	–	+	–	–
12	Clam	Dakke, Mangaluru	CDCL0318/15	–	–	–	+	+	+
13	Clam	Kasaragod	KRCL0318/16	–	–	–	–	–	–
14	Clam	Kumbala	KMCL0318/17	–	–	–	–	–	–
15	Clam	Kannur	SWCL0318/18	–	–	+	+	–	–
16	Clam	Nethravathi	NBCL0318/19	–	–	+	+	+	–
17	Clam	Dakke, Mangaluru	ITCL0318/20	–	–	+	+	–	–
18	Clam	Kanhangad	SKCL0318/21	–	–	–	–	–	–
19	Clam	Dakke, Mangaluru	AMCL0318/22	–	–	+	–	–	–
20	Clam	Dakke, Mangaluru	EKCL0318/23	–	–	+	–	–	–
21	Clam	Kasaragod	UKCL0418/25	–	–	+	–	–	–
22	Clam	Mulki	CECL0418/26	–	–	+	–	–	–
23	Clam	Kanhangad	NICL0418/27	–	–	–	–	–	–
24	Clam	Kannur	TRCL0418/28	–	–	–	–	–	–
25	Clam	Kuppepadav	JSCL0418/29	–	–	–	–	+	–
26	Clam	Malpe	ZYCL0418/30	–	–	–	–	–	–
27	Clam	Mulki	MUCL0118/2	–	–	+	–	–	–
28	Clam	Kannur	KACL0218/6	–	–	–	–	–	–
29	Clam	Dakke	DHCL0218/7	–	–	–	–	–	–
30	Clam	Surathkal	PRCL0418/24	–	–	+	–	–	–
31	Clam	Thumbe	BLCL0418/34	–	–	–	–	+	–
32	Shrimp	Thokottu	SHT121118	–	–	+	+	+	+
33	Shrimp	Statebank, Mangaluru	SHSB131118	–	+	–	–	–	–
34	Shrimp	Kasaragod	SHKG131118	–	–	–	–	–	–
35	Shrimp	Beeri	SHBM131118	–	–	–	+	–	–
36	Shrimp	Deralakatte	SHD151118	–	–	–	–	–	–
37	Shrimp	Beeri	SHBI151118	–	–	–	–	–	–
38	Shrimp	Kumbala	SHKU151118	–	–	–	–	+	+
39	Shrimp	Mulki	SHMU211118	–	–	–	–	–	+
40	Shrimp	Deralakatte	SHDR211118	–	–	+	–	+	+
41	Shrimp	Kuttar	SHKU211118	–	–	–	–	–	–
42	Shrimp	Thokottu	SHTH211118	–	–	–	–	–	–
43	Shrimp	Ullala	SHUL231118	–	–	–	–	–	–
44	Shrimp	Deralakatte	SHDR231118	–	–	–	–	–	–
45	Shrimp	Kuttar	SHKR231118	–	–	–	–	–	–
46	Shrimp	Thokottu	TOSH051218	–	–	–	–	–	–
47	Shrimp	Deralakatte	DLSH051218	–	–	–	+	–	–
48	Shrimp	Deralakatte	SHDT171218A	–	–	–	–	–	–
49	Shrimp	Deralakatte	SHDT171218B	–	–	–	–	–	–

Table 1 (continued)

Sl. no.	Sample type	Place	Culture ID	PCR[a]		RT-PCR[a]		LAMP assay	
				trh	*tdh*	*trh*	*tdh*	*trh*	*tdh*
50	Shrimp	Thokottu	SHTT171218	–	–	–	–	–	–
51	Shrimp	Thokottu	SHTK191218	–	–	–	–	–	–
52	Shrimp	Ullala	SHYK191218	–	–	–	+	–	–
53	Shrimp	Manjeshwar	SHMA191218	–	–	–	–	–	–
54	Shrimp	Kasaragod	SHKS191218	–	–	–	–	–	–
55	Shrimp	Manjeshwar	MJSH060219	–	–	–	–	–	–
56	Shrimp	Dakke, Mangaluru	DKSH060219	–	–	–	+	–	–
57	Shrimp	Ullala	UASH080219	–	–	–	–	–	–
58	Shrimp	Thokottu	TUSH080219	–	–	–	+	–	–
59	Shrimp	Statebank, Mangaluru	SBSH110219	–	–	+	–	–	–
60	Shrimp	Dakke, Mangaluru	DESH110219	–	–	–	–	–	–
61	Shrimp	Statebank, Mangalore	SBSH150219	–	–	–	–	–	–
62	Shrimp	Deralakatte	DLSH150219	–	–	–	+	–	–

(+): Positive, (−): Negative

[a]PCR and RT-PCR were performed using F3 and B3 primers

Result and discussion

Specificity and sensitivity of LAMP assay

In LAMP assay, 63 °C temperatures for 60 min of amplification was found to be optimum for both *tdh* and *trh* genes. An increase in the turbidity due to LAMP reaction was measured using LoopAmp real-time turbidimeter as compared to negative controls. The reaction was considered positive when turbidity reached 0.1 within 60 min (Fig. SF1). Specificity assay of LAMP assay was tested using various bacterial strains, including both *V. parahaemolyticus* and other bacterial strains. Both the designed primers of *trh* and *tdh* genes showed good specificity with no false positive and negative results for the tested bacterial isolates (Fig. SF2). In general, the presence of *tdh* and/or *trh* is primarily associated with the pathogenic strains of *V. parahaemolyticus* and is considered a virulence marker [1, 2, 21]. It is also true that the presence of *tdh* or *trh* genes have been reported in other non-*V. parahaemolyticus* vibrionaceae species, including *V. mimicus*, *V. cholerae*, *V. hollisae*, *V. diaboilcus*, and *V. alginolyticus* [1]. Hence, further inclusion of these strains harboring *tdh* or *trh* can confirm the assay's high specificity. However, due to these strains' unavailability, we could not include them during our specificity study.

The sensitivity of LAMP assay was found to be high. In the DNA dilution method, LAMP assay was able to detect 1 pg of DNA (*trh* and *tdh*), whereas conventional PCR was able to detect 100 pg of DNA (Fig. SF3). Similarly, when the sensitivity of the assay was tested with spiked clam meat, it was able to detect 10^2 CFU/g (1 CFU per reaction) and 10^4 CFU/g (100 CFU per reaction) by LAMP and conventional PCR assay, respectively. The RT-PCR assay showed a similar degree of sensitivity of LAMP assay with a detection limit of 10^2 CFU/g (Fig. 1). Di et al. [22] developed LAMP assay targeting *tlh* and *tdh*, which could able to detect as low as 2 CFU/g of *V. parahaemolyticus* in seafood. Prompamorn et al. [23] reported the detection limit of 10^3 CFU/g for *V. parahaemolyticus* in spiked samples. LAMP assays with both loop primers can detect the target cells at concentration of 10 CFU per reaction [24]. Previously, LAMP assay was developed for *tdh* and *trh* to detect pathogenic strains of *V. parahaemolyticus* however, not been tested using seafood samples [2, 25, 26]. To date, all the LAMP-based assays developed for the detection of pathogenic *V. parahaemolyticus* targeting *trh* and *tdh* was not validated by direct seafood samples, and the studies involved either enrichment or use of pure cultures. The other available isothermal amplification methods for the detection of *V. parahaemolyticus* require enrichment for detecting 2 CFU/g of cells in the seafood sample [27]. The validation of an assay is important to confirm the sensitivity, specificity, and accuracy of the method. Therefore, in this study, we have tested the optimized LAMP assay using clam and shrimp samples collected from the local market for 1 year.

Detection of *V. parahaemolyticus* in naturally contaminated seafood sample

Reports on the presence of *trh* and *tdh* gene in environmental samples are always checked using conventional culture-based methods or standard conventional PCR based assays. The detection limit using these available methods is affected by low amount of template DNA, inhibitory agents present

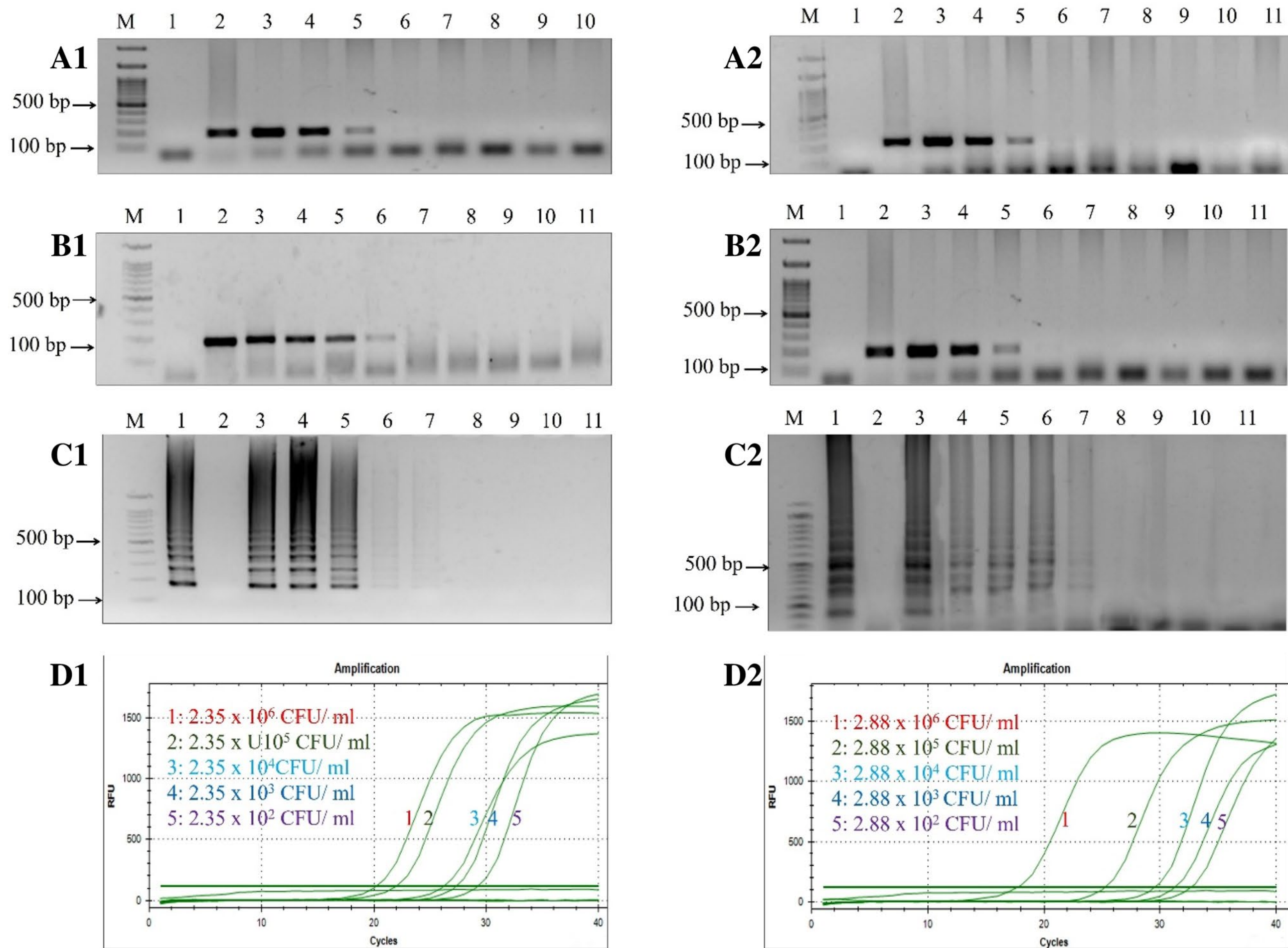

Fig. 1 Determination of sensitivity test by artificial contamination of clam targeting trh^{+} and tdh^{+} *V. parahaemolyticus* culture. (**A1**, **B1**) Conventional PCR amplification targeting *trh* using Tada et al. [19] and F3–B3. Lane M: 100 bp marker, Lane 1: Negative control, Lane 2: Positive control: 2.35×10^{7} CFU/ml. Lanes 3–9: Reaction carried out with spiked suspensions containing 10^{6}, to 0 CFU/ml cells. (**A2**, **B2**) Conventional PCR amplification targeting *tdh* using Tada et al. [19] and F3–B3. Lane M: 100 bp marker, Lane 1: Negative control, Lane 2: Positive control: 2.88×10^{7} CFU/ml. Lanes 3–9: Reaction carried out with spiked suspensions containing 10^{6}, to 0 CFU/ml cells. (**C1**, **C2**) LAMP amplification targeting *trh* and *tdh*. Lane M: 100 bp marker, Lane 1: Positive control (*trh*: 2.35×10^{7} CFU/ml and *tdh*: 2.88×10^{7} CFU/ml), Lane 2: Negative control, Lanes 3–9: Reaction carried out with spiked suspensions containing 10^{6}, to 0 CFU/ml cells. (**D1**, **D2**) Results of RT-PCR showing sensitivity for *trh* and *tdh* (F3 and B3) in the spiked sample

in the sample to perform conventional PCR [28]. Hence, the report of low prevalence may be due to the false-negative results by the tool used to detect the pathogenic strains of *V. parahaemolyticus*. Malcolm et al. [21] reported better LAMP assay sensitivity for the detection of *V. parahaemolyticus* over multiplex PCR, even when the targeted contaminant density was low in shellfish.

In this study, out of 62 samples screened (clam and shrimp), eight (12.9%) were positive for *trh*, and seven (11.29%) samples for *tdh* gene by LAMP assay (Fig. SF4, Table 1). Five (8.02%) samples were positive for both *trh* and *tdh*. However, three (4.83%) samples were positive for *trh*, and two (3.22%) samples were positive for *tdh* gene. The increase in the pathogenic strains of *V. parahaemolyticus* in the food chain is possibly due to cross-contamination in the seafood harvesting, processing and distributing centers, and changing cooking habits. Hence, these issues need to be addressed, and simple detection methods required to be implemented. A similar result was also observed by Raghunath et al. [29] where the number of trh^{+} *V. parahaemolyticus* was significantly higher in seafood samples as compared to tdh^{+} *V. parahaemolyticus*.

In different types of seafood samples, six (19.35%) clam, and four shrimp (12.9%) samples were carrying pathogenicity associated genes (*trh/tdh*). Further, LAMP assay detected the presence of *trh* gene in five (16.12%) clam and three (9.6%) shrimp samples. *tdh* gene was detected in three (9.6%) clam samples and four (12.9%) shrimp samples. Odeyemi [7] reported a meta-analysis of the incidence of *V. parahaemolyticus* from 2003 to 2015, which showed that

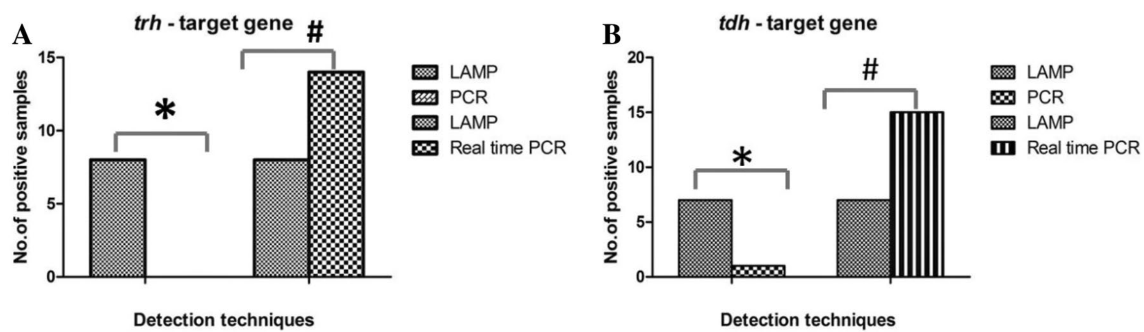

Fig. 2 Comparison between LAMP, conventional PCR assay, and RT-PCR of *trh* gene (**a**) and *tdh* gene (**b**). *Significant difference between LAMP and conventional PCR assay ($p < 0.05$). #No significant difference between LAMP and real-time PCR assay

the prevalence of *V. parahaemolyticus* was 52.9% for clam and 48.3% for shrimp. Besides, other studies also prove the fact that *V. parahaemolyticus* prevalence is relatively more in shellfish [30, 31].

Comparison of LAMP assay with conventional PCR and RT-PCR

None of the seafood samples were positive for *trh* gene in conventional PCR assay, and only one sample (1.6%) was positive for *tdh* gene. This is possibly due to CCL as a template for conventional PCR. Reports suggest that PCR amplification can be affected due to the presence of inhibitors in the reaction mix. However, this is not a major issue for LAMP assay since it is based on *bst* polymerase enzyme [32, 33]. In the RT-PCR assay, 15 (24.19%) samples were positive for *trh*, and 14 (22.58%) samples were positive for *tdh* genes (Fig. 2, Table 1). Results were analyzed using a two-sample proportion test to check the significant difference between the techniques compared in the study. A significant difference ($p < 0.05$) in the results was observed when samples were subjected to LAMP and conventional PCR for the detection of two different genes (*trh* and *tdh*). However, no significant difference was observed when LAMP and RT-PCR were used to detect *V. parahaemolyticus* using *trh* and *tdh* genes. Cao et al. [34] explained the equal sensitivity of LAMP and RT-PCR for the detection of viable but non-culturable (VBNC) *V. parahaemolyticus*. The agreement between LAMP and RT-PCR analysis was found to be fair with kappa value $k \geq 0.2$ for both the genes. There was no agreement between LAMP and PCR with kappa value $k \leq 0$. Samples showing positive amplification in LAMP, RT-PCR, and PCR methods are represented in the Venn diagram (Fig. SF5). Even though the RT-PCR (cutoff Ct value ≤35) was able to amplify *tdh* and *trh* genes in slightly more samples than LAMP assay, LAMP assay can be considered to be a better practical method to detect pathogenic strains of *V. parahaemolyticus* due to its simplicity and cost-effectiveness.

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Author contributions KPA designed the study, collected samples, performed the experiments, analyzed result data, and prepared the initial draft of the manuscript. AN carried out laboratory experiments and reviewed the manuscript. IK and IK supervised the study and revised the manuscript. BM conceptualized and designed the study, analyzed result data, supervised the study, mobilized the resource, and finalized the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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