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Molecular Detection of Marine-Borne Pathogen *Vibrio cholerae* in Traditional Fermented Shrimp Paste from East Lombok using Polymerase Chain Reaction

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ABSTRACT

Terasi is a traditional Indonesian fermented shrimp paste produced by local MSMEs, often under handmade conditions that raise concerns regarding the presence of marine-borne pathogens. This study aimed to perform molecular surveillance of *Vibrio cholerae* in matured solid block *terasi* from three prominent MSMEs in East Lombok using Polymerase Chain Reaction (PCR). Six samples from different production batches were evaluated. The methodology integrated physicochemical analysis (a_w and pH), and PCR detection targeting the *F0316_RS13020* gene. Results showed that all samples possessed a low a_w ranging from 0.63 to 0.66 and alkaline pH levels between 7.72 and 8.07. Molecular detection yielded negative results for *V. cholerae* across all samples, while the positive control was successfully amplified at the 637 bp target size. The absence of this pathogen is attributed to the synergistic hurdle technology of high salinity, low a_w, and high-temperature roasting (up to 260°C), which inhibits *Vibrio* survival. This study confirms that matured *terasi* from East Lombok meets the microbiological safety criteria. Future monitoring should focus on raw materials using viability-based PCR to further enhance regional food safety surveillance, especially from fish and fishery products.

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1. INTRODUCTION

Shrimp paste, known locally as *terasi*, is a crucial ethnic fermented condiment in Indonesia that serves as a primary flavor enhancer in various traditional cuisines. Shrimp paste is traditionally produced through a spontaneous fermentation process of planktonic shrimp, such as *Acetes japonicus*, or small fish mixed with salt and followed by a series of drying stages to develop its characteristic umami flavor and savory aroma [1], [2]. In the region of Lombok, particularly East Lombok, *terasi* holds a significant socio-economic role for Micro, Small, and Medium Enterprises (MSMEs), which produce diverse varieties ranging from pure shrimp to mixtures of various marine materials [3]. Despite its popularity, the traditional

production methods often rely on handmade practices with limited regulatory oversight, raising concerns regarding its microbiological quality. The traditional processing of *terasi*, which involves open-air sun drying and manual grinding, poses a risk of environmental contamination. Ensuring the microbiological safety of these products is crucial, as they are often consumed with minimal additional cooking. Several Indonesian Regulations strictly mandate that *terasi* products must be free from any pathogenic contamination, including *Vibrio cholerae* and other enteric pathogens [4], [5], [6].

Foodborne diseases caused by marine-borne pathogens represent a substantial global public health threat, especially in tropical maritime countries like Indonesia [7], [8]. Among these pathogens, *Vibrio cholerae* is a major concern as it is indigenous to estuarine marine environments where shrimp are harvested and can easily contaminate seafood products during handling or distribution [9]. Ingestion of contaminated seafood can lead to severe gastroenteritis and cholera outbreaks, which are characterized by rapid dehydration and can be fatal if not promptly treated [10]. The prevalence of *Vibrio* species in tropical shrimp culture and processed seafood highlights the necessity for rigorous monitoring programs to mitigate health risks [11], [12], [13]. The risk of *V. cholerae* contamination in shrimp paste is highly dependent on the initial microbial load of the raw materials and the hygiene practices during the fermentation and drying stages [5]. However, systematic surveillance of *V. cholerae* in traditional fermented products from specific MSMEs in East Lombok remains insufficiently documented in scientific literature.

The conventional detection of *V. cholerae* in food matrices typically relies on microbiological culture methods. Conventional identification generally involves enrichment in alkaline peptone water (APW), isolation on TCBS agar, followed by biochemical, serological, and pathogenicity confirmation tests. Although culture-based methods are considered the "gold standard", they often lack the sensitivity required to detect bacterial cells in a Viable But Non-Culturable (VBNC) state. These traditional approaches are also time-consuming, often requiring up to five days to yield results, and rely on well-trained laboratory personnel and adequate microbiological facilities [14], [15], [16], [17], [18]. This condition is particularly relevant for fermented products like *terasi*, where high salt concentrations and thermal processing (roasting) may induce physiological stress in bacteria, making them undetectable by conventional plating while remaining potentially pathogenic. Some *Vibrio* species exhibit halotolerant characteristics that can enter a VBNC state when exposed to environmental stress [19].

To overcome these limitations, molecular-based technologies, particularly Polymerase Chain Reaction (PCR), have emerged as superior alternatives for rapid, highly specific, and sensitive pathogen detection from food samples [15], [20], [21]. The application of molecular tools allows for more effective surveillance of marine pathogens, ensuring that traditional fermented products meet the microbiological safety criteria established by national authorities. PCR-based assays target unique genetic biomarkers, allowing for the direct identification of pathogens from complex food matrices with high sensitivity [22], [23], [24], [25]. Recent advancements have demonstrated the efficacy of PCR in monitoring the safety of various fermented seafood products across Southeast Asia [26], [27], [28], [29], [30]. By utilizing specific primers researchers can accurately verify the presence or absence of *V. cholerae* even at low contamination levels [31]. This technological approach is essential for enhancing the quality control systems of local MSMEs and supporting environmental health surveillance, especially in Lombok Island.

This study aims to conduct a molecular surveillance of *V. cholerae* in *terasi* products produced by three major MSMEs in East Lombok using the PCR method. By focusing on the qualitative detection of this marine-borne pathogen, the research

evaluates the safety of these traditional products against national and international food safety standards. The findings are expected to provide scientific evidence regarding the safety of Lombok's *terasi* and serve as a reference for the application of biotechnology-based monitoring in the traditional food industry.

2. LITERATURE REVIEW

2.1 Vibrio cholerae as a Marine-Borne Pathogen

V. cholerae is a Gram-negative, comma-shaped bacterium that naturally inhabits marine and estuarine ecosystems. As an indigenous member of the marine microbiota, it frequently colonizes the surfaces and digestive tracts of crustaceans, such as the rebon shrimp used in *terasi* production, through specific chitin-binding proteins [9]. While many environmental strains are non-toxigenic, the potential presence of virulent serogroups in raw seafood poses a significant risk of cholera outbreaks, characterized by severe watery diarrhea and rapid dehydration [10], [32]. The prevalence of this pathogen in tropical coastal waters makes its monitoring a top priority for food safety authorities in archipelago nations like Indonesia.

V. cholerae can persist in fermented seafood products despite the presence of preservation factors such as elevated salt concentrations and reduced water activity [9], [10]. The ability of this bacterium to survive under stressful conditions is associated with its adaptive responses, including osmotic stress tolerance, biofilm formation, and transition into a VBNC state. Environmental conditions generated during fermentation of seafood products and subsequent thermal processing may not completely eliminate *V. cholerae*, particularly when bacterial cells have entered a dormant physiological state. Cells in the VBNC state remain metabolically active and may retain virulence-associated genes, allowing them to resuscitate under favorable conditions and potentially pose a food safety risk [18], [19], [31]. Consequently, the persistence of *V. cholerae* in fermented seafood highlights the limitations of conventional culture-based detection methods and underscores the need for sensitive molecular approaches capable of detecting stressed or non-culturable bacterial cells.

2.2 Terasi Fermentation and Pathogenic Inhibition

The safety of *terasi* is largely attributed to its unique environmental barriers created during the fermentation and drying processes. During spontaneous fermentation, halophilic Lactic Acid Bacteria (LAB), such as *Lactobacillus plantarum*, often dominate the matrix, producing organic acids and other antimicrobial metabolites that lower the pH [2], [33], [34], [35]. Indonesian national standards require *terasi* to have a salt content between 12% and 20%, which generates high osmotic pressure that effectively inhibits the growth of non-halotoleran pathogens like *V. cholerae* [4]. This combination of chemical hurdles acts as a primary bio-preservative mechanism that characterizes traditional fermented seafood products.

Physical parameters, specifically water activity (a_w), serve as the final barrier against microbial proliferation in dried shrimp paste. *Vibrio* species generally require a minimum a_w of 0.90 to 0.95 to proliferate, whereas the intensive drying and oven-roasting processes practiced by MSMEs in East Lombok can reduce the a_w to levels significantly below this threshold [36]. Furthermore, many MSMEs apply high-temperature roasting as a Critical Control Point (CCP), often reaching temperatures between 150°C and 260°C, which is sufficient to eliminate most heat-sensitive enteric pathogens [3], [5], [37].

2.3 PCR Technology for Rapid Pathogen Detection

The application of Polymerase Chain Reaction (PCR) has revolutionized food surveillance by providing a rapid, sensitive, and highly specific alternative to traditional microbiological assays. PCR technology allows for the direct amplification of target DNA sequences, enabling the identification of pathogens within hours rather than days [20]. In complex food matrices like *terasi*, where high levels of salt and proteins can act as PCR inhibitors, the use of specialized DNA extraction kits and optimized enrichment stages is essential to ensure high-quality templates for detection [18]. This molecular approach is particularly valuable for verifying the absence of pathogens in accordance with international trade and safety regulations.

The specificity of PCR depends heavily on the design of primer sets that target unique conserved regions of the pathogen's genome. For *V. cholerae*, targeting specific chromosomal loci ensures that there is no cross-reactivity with other marine *Vibrio* species, such as *V. parahaemolyticus* or *V. vulnificus* [17], [38], [39]. To further enhance the reliability of the results, the integration of synthetic gene fragments as internal positive controls serves as a vital tool to validate the success of the reaction [25]. Thus, PCR is currently regarded as the best standard method for pathogen analysis due to its unmatched speed, specificity, and sensitivity. It detects active or non-viable pathogens in hours by exponentially amplifying targeted DNA or RNA sequences, allowing for early identification even in low-concentration samples [40], [41], [42], [43], [44], [45].

3. METHODS

3.1 Sample Collection and Preparation

The research employed a non-probability total sampling technique, focusing on finished shrimp paste products from three prominent obtained from three prominent shrimp paste-producing MSMEs in East Lombok, designated as MSME-A, MSME-B, and MSME-C. A total of six samples were collected, representing two different production batches for each MSME to account for process variability. The samples consisted of matured, oven-dried solid block *terasi* with a shelf life of 10 to 14 days at the time of collection. Each sample was aseptically handled and transported to the Laboratory of Food Microbiology, University of Mataram, for further analysis [46]. To provide a comprehensive environment profile, the water activity (a_w) and pH of each sample

were measured according to standard procedures [28], [47].

3.2 DNA Extraction

Genomic DNA was extracted using the Presto™ Food DNA Extraction Kit (Geneaid, Taiwan) according to the manufacturer's protocol. The concentration and purity of the extracted DNA were quantified using a NanoDrop 2000/2000c spectrophotometer (Thermo Scientific, USA) to ensure the templates met the minimum requirements for PCR amplification (ratio A_{260}/A_{280}) between 1.6 and 2.0 [28], [48], [49].

3.3 Primer Preparation

The PCR primers were specifically designed to target the *F0316_RS13020* gene of *V. cholerae* (Accession No: NZ_CP043554.1). The primer sequences used were based on validated in silico primer design and verification in the previous research, with a predicted amplicon size of 637 bp [50].

1. Forward 5′ TCGTGATGACAGCGAAAAAG-3′
2. Reverse 5′ TCTTGGAAAAATCGCAATCC-3′

3.4 PCR Conditions and Visualization

The PCR amplification was performed in a final volume of 20 µL, containing 2.0 µL of DNA template, 10.0 µL of 2x SensiFAST SYBR™ No-Rox Mix (Bioline Meridian Bioscience, US), 1.0 µL of each primer (20 µM), and 6.0 µL of sterile water. The reaction was conducted in a thermocycler (PCRmax ECO 48™, US) with the following optimized conditions: initial denaturation at 95°C for 2 minutes, followed by 40 cycles of denaturation at 95°C for 5 seconds, annealing at 60°C for 10 seconds, and extension at 72°C for 15 seconds. A final extension at 72°C for 5 minutes was performed to ensure complete DNA synthesis. The PCR products were visualized using 2% agarose gel electrophoresis at 90V for 35 minutes, stained with Ethidium Bromide, and observed under a UV transilluminator. A 5 µL aliquot of DNA GenLadder 100 bp + 1.5K (Genaxxon, Germany) was used to estimate the sizes of DNA fragments during agarose gel electrophoresis. The presence of a distinct fluorescent target band on the agarose gel under UV illumination was considered a positive PCR result, while the absence of the corresponding band indicated a negative result.

4. RESULTS AND DISCUSSION

4.1 Physicochemical Barriers and DNA Quality

The analysis of environmental factors within the *terasi* matrix revealed significant barriers to the survival of *Vibrio cholerae*. The water activity (a_w) of all samples ranged from 0.63 to 0.66, while the pH values were recorded between 7.72 and 8.07 (Table 1). These values indicate a highly unfavorable environment for *Vibrio* species, which typically require a minimum a_w of 0.90 to 0.95 for proliferation [10]. The low a_w in East Lombok's *terasi* is a direct result of the intensive sun-drying and high-temperature roasting processes (up to 260°C) practiced by local MSMEs [3], [36]. In addition, the slightly alkaline pH observed in all samples may further limit bacterial persistence by imposing physiological stress and reducing the active cell multiplication. The combined effects of low water activity and thermal processing create multiple hurdles that decrease the probability of survival of *V. cholerae*.

Furthermore, the extracted DNA showed sufficient concentration (4.2–33.5 ng/µL) and high purity (1.64–1.86), confirming that the templates were suitable for PCR amplification. High-quality DNA is a critical prerequisite in molecular surveillance to avoid false-negative results, especially in complex food matrices containing high salts and proteins that could potentially inhibit the *Taq* polymerase [21], [48]. The satisfactory DNA quality obtained in this study indicates that the extraction procedure effectively minimized inhibitors and yielded DNA suitable for reliable molecular detection.

Table 1. Physicochemical Properties and DNA Quality of *Terasi* Samples

MSME Code	Batch	Sample ID	a_w	pH	DNA Concentration (ng/µL)	Purity (A_{260}/A_{280})
MSME A	1	T_1U_1	0.66	7.77	33.5	1.83
MSME A	2	T_1U_2	0.66	7.86	5.3	1.81
MSME B	1	T_2U_1	0.66	8.07	4.2	1.79
MSME B	2	T_2U_2	0.66	8.05	30.7	1.86
MSME C	1	T_3U_1	0.64	7.73	12.5	1.68
MSME C	2	T_3U_2	0.63	7.72	12.4	1.64

Source: Processed Primary Data

4.2 Molecular Detection and Hurdle Effect

The PCR results for all six samples from three different MSMEs in East Lombok were negative for *V. cholerae*. There is no amplicon bands were observed at the target size of 637 bp for the samples, while the positive control of *V. cholerae* exhibited a sharp, distinct band at the expected position (Figure 1). This confirms that the PCR system, including the designed primers and optimized thermal conditions, was fully functional and specific [9]. The successful amplification of the positive control of *V. cholerae* at 637 bp validates the PCR sensitivity and confirms that the negative results in MSME samples are representative of the actual absence of *V. cholerae* DNA, thereby minimizing the likelihood of false-negative results caused by reaction or instrument failure.

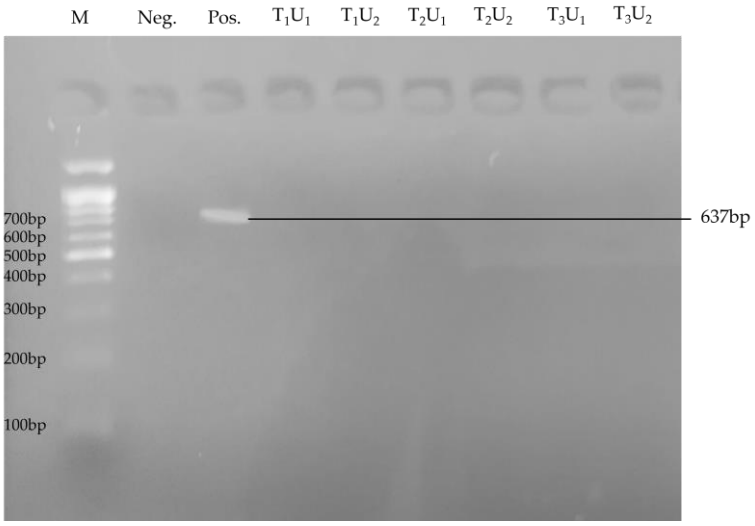

Figure 1. Detection results of *V. cholerae* in DNA extracted from *terasi* samples.
Lanes: M, DNA size marker; Neg., negative control (DNA-free sterile water); Pos., positive control of *V. cholerae*; T_1U_1 to T_3U_2, *terasi* samples

The absence of *V. cholerae* in Lombok's matured *terasi* can be explained by the "*hurdle technology*" effect. The combination of high salt content, low a_w (< 0.70), and final roasting acts as a synergistic elimination process [2]. Similar findings in Thailand and Vietnam reported that *Vibrio* species are rarely found in matured shrimp paste due to the osmotic stress and organic acids produced by halophilic Lactic Acid Bacteria (LAB) during the 30-day fermentation period [33]. This research reinforces the conclusion that the traditional processing methods used by three MSMEs in East Lombok are effective in maintaining the safety of the product from *V. cholerae*.

4.3 Challenges and Future Opportunities

Despite the negative results in this study, the potential for *Vibrio* to enter a VBNC state remains a challenge for food safety surveillance. In a VBNC state, pathogens may persist without being detectable by culture methods, although they can still be detected by PCR if the DNA remains intact. Future monitoring should consider the use of viability PCR (v-PCR) to distinguish between DNA from live and dead cells, providing a more accurate assessment of viable risks in raw or semi-processed seafood [19].

There is a significant opportunity to integrate PCR-based surveillance into the quality assurance protocols for Indonesian MSMEs. This technology would not only enhance local food safety but also increase the competitiveness of traditional products in the international market, which demands strict adherence to microbiological standards. Future research could focus on developing multiplex PCR assays that simultaneously detect *Vibrio* with other pathogens such as *Salmonella* and *Staphylococcus* within a single test. It is highly valued for food safety quality control due to its speed, sensitivity, and cost-effectiveness as screening tool for the industry [51]. It is also recommended to conduct similar surveillance on raw or semi-processed *terasi* across different traditional markets in Lombok to better understand the microbial dynamics before the final roasting stage. Furthermore, MSMEs are encouraged to consistently maintain their roasting protocols as a CCP to ensure that every production batch remains free from pathogenic threats throughout the supply chain.

5. CONCLUSION

The molecular surveillance conducted in this study confirms that *Vibrio cholerae* is not detected in the matured solid block *terasi* produced by MSMEs in East Lombok. The negative results obtained through the amplification of the *F0316_RS13020* gene at the 637 bp target size demonstrate that the finished products from all sampled batch from all MSMEs used in this research. The absence of this *V. cholerae* is effectively ensured by the synergistic *hurdle technology* inherent in the traditional processing methods of *terasi*, where low water activity (a_w 0.63–0.66), alkaline pH, and high-temperature roasting (150°C–260°C) create an environment that inhibits the survival of *V. cholerae*. Thus, these traditional fermented seafood products are considered safe for consumers regarding the risk of cholera contamination.

Future studies are recommended to the development of a multiplex PCR detection system is encouraged to simultaneously monitor multiple marine-borne pathogens, thereby enhancing the speed and cost-effectiveness of routine large-scale surveillance for the traditional fermented food industry. Furthermore, incorporate viability-based molecular assays, such as v-PCR, to further ensure the detection of live bacterial cells in raw seafood materials before processing. Additionally, it is suggested that local food safety authorities provide standardized technical training for MSMEs to maintain high-temperature roasting as a consistent CCP in their production chain.

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