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Advances in Molecular Techniques for the Detection of *Salmonella* and *Shigella* in Seafood Safety

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ABSTRACT

Seafood safety is extremely vulnerable to foodborne pathogen contamination by bacteria such as Salmonella and Shigella, which are of major public health concern. Conventional culture-based detection is time-consuming but sensitive, resulting in delayed action and potential trade-offs in food safety. To overcome the limitation of conventional methods, molecular methods such as Polymerase Chain Reaction (PCR) provide rapid and accurate alternatives. This study aims to improve seafood safety by utilizing advanced PCR-based systems for early pathogen detection. The system proposed consists of Conventional PCR, Multiplex PCR (mPCR), and Real-Time Fluorescence Thermal Cycler Methods (RFTM). The traditional Polymerase Chain Reaction (PCR) is aimed at specific genetic sequences (e.g., invA and ipaH), while multiplex PCR (mPCR) detects a range of pathogens in one test. Real-time PCR, being a form of Reverse Transcription Fluorescent Multiplexing (RFTM), produces qualitative data of greater sensitivity and fewer chances of contamination. These methods have reduced the testing time tremendously, improved the accuracy of diagnosis, and triggered extensive surveillance programs. With this molecular detection system, seafood industries can provide faster hazard management, regulatory compliance, and protection to consumers.

Keywords: *Shigella, Conventional PCR, Multiplex PCR, Real-time PCR, Molecular detection, Seafood safety, Salmonella.*

1. INTRODUCTION

Many people eat seafood because it is healthy and contains several essential nutrients. For example, having proteins, omega-3's, and other essential minerals in our meals. Seafood can easily get contaminated because It becomes contaminated when it is brought out of the ocean, since it needs to be processed quickly.

leading to foods being more vulnerable to getting infected by bacteria. *Salmonella* is considered one of the main contaminants affecting food. Seafood is related to the health issues *Shigella* and *Salmonella* [1]. The Gram-negative bacteria cause severe Gastrointestinal diseases, put the public health at high risk and also cost a great deal of money. Improper or slow detection of contamination can cause major outbreaks, recall of seafood products and a decline in the public's trust in the markets..

Conventional microbiological techniques used to identify *Salmonella* and *Shigella* in seafood, including culture-based and biochemical assays, have been extensively employed because of their proven reliability and standardization [2]. These techniques themselves are time-consuming, taking several days to yield results. In addition, they involve multiple enrichment steps and require high-level expertise in obtaining accurate results. Such time lags in pathogen detection can undermine timely decision-making in food quality assurance and food safety. Also, routine culturing techniques may not be able to detect viable but not culturable (VBNC) bacterial cells, resulting in false-negative findings and underestimation of potential contamination hazards.

In response to these constraints, molecular diagnostic technologies have emerged as highly competent tools for the sensitive, fast, and specific detection [3] of pathogens. In this respect, PCR-based technologies have taken the centre stage in modern food microbiology. PCR facilitates the amplification of pathogen-specific DNA sequences corresponding to the target pathogens, thus opening the door to their detection at low concentrations and in complex food matrices. Detection of virulence genes, for example, invA in *Salmonella* and ipaH in *Shigella*, provides high specificity, thus minimizing the potential of false-positive detection of non-pathogenic bacteria.

Conventional PCR is powerful but only capable of discriminating a single target in a single assay, creating challenges in surveillance scenarios where many pathogens need to be discriminated. mPCR solved this constraint because it is capable of amplifying different target genes in the same reaction simultaneously. Not only does the application of mPCR improve the diagnostic capability but also reduces reagent costs, labour, and time costs [4]. Moreover, mPCR is particularly important as regards seafood safety tracing because multi-contamination by a range of pathogens is a highly viable risk.

Additional evolution of molecular diagnostics is seen with real-time PCR, or Real-Time Fluorescence Thermal Cycler Methods (RFTM). Unlike the standard PCR, real-time PCR merges detection and amplification in one, closed-tube system. Real-time monitoring of the amplification of DNA by fluorescent dyes or probes, it provides quantitative data regarding pathogen load. This avoids [5] most of the risk of contamination, improves reproducibility, and gives a faster turnaround time. These comprise genes like hilA, most often targeted in real-time assays in *Salmonella*, and virF in *Shigella*, that are highly sensitive even in the presence of PCR inhibitors in seafood products or low bacterial load.

The integration of these novel molecular techniques into seafood testing protocols has revolutionized food safety monitoring. It allows for better hazard detection, rapid response to contamination, and improved compliance with regulation. In particular, their application in high-throughput screening and automated [6] processes allows for scalability in large-scale seafood processing and exporting facilities. Moreover, direct detection of pathogens from food samples without the need for extensive culturing protocols provides a significant advantage in response to emergency and outbreak management.

In summary, the establishment and utilization of molecular methodologies like standard PCR, multiplex PCR, and real-time PCR have played an important role in elevating the detection capacity for *Salmonella* and *Shigella* in seafood. Such methodologies provide [7] essential advantages in terms of sensitivity, specificity, speed, and operating efficiency over conventional microbiological methods. Their use throughout the seafood production and inspection operations is critically important to protecting public health, maintaining consumer confidence, and ensuring the integrity of the world's seafood supply chain.

This work is organized with review of the literature survey as Section II. Methodology described in Section III, highlighting its functionality. Section IV discusses the results and discussions. Lastly, Section V concludes with the main suggestions and findings.

2. LITEARTURE SURVEY

Seafood contamination with foodborne pathogens is still a public health concern in many countries, particularly in developing nations where seafood handling and hygiene procedures may be inconsistent. Research has identified that *Salmonella* and *Shigella* contamination of seafood can be attributed to the inappropriate storage, handling, and environmental conditions such as water quality. Sophisticated molecular techniques are now being used to detect these pathogens, yielding quicker and more efficient results than conventional methods. With these, however, low-level contamination of seafood in complex matrices remains a critical issue where more sensitive diagnostic methods need to be developed.

Traditional culture-based techniques used for the detection of *Salmonella* and *Shigella* in seafood have been used intensively over a span of a few decades primarily due to their cost-effectiveness and ease of use. Such techniques, however, have some limitations [8] in terms of temporal requirement as well as specificity, particularly in the case of low pathogen load. Further, culture techniques entail several steps that not only demand long periods of time but are also subject to human errors. Emerging trends in the field of rapid molecular detection technologies intend to do away with such limitations by yielding faster detection and increased sensitivity. Many different studies have drawn comparisons between PCR-based technologies. The rapid outcomes obtained in just hours from PCR make it a suitable substitute for serological methods.

There have been many recent studies exploring the use of immunoassays for finding pathogens. seafood. Some of these techniques look at the reaction between antibodies and antigens using approaches such as ELISA.detecting and counting *Salmonella* and *Shigella* by using select antibodies. Immunoassays are generally faster than traditional culture tests and can be adapted to find various. There are several pathogens present in different types of food. A big disadvantage of immunoassays is that they have a lower sensitivity than molecular procedures. Antibodies can also cross-react with compounds besides the target. Since bacteria can make mistakes, they are not suitable for accurately testing many kinds of seafood matrices.

A useful way to identify foodborne pathogens in seafood rapidly is by using biosensors. Through biological recognition with antibodies, enzymes, or nucleic acids, biosensors can detect the existence of pathogens and often use a transduction system that allows them to detect the presence of pathogens in real time [10]. their portability and easy use mean that biosensors fit well in field situations, which is best for point-of-consumer and point-of-sale tests. While biosensors have great potential, there are obstacles such as specific detection methods and figuring out pathogens in complex products.

Next-generation sequencing (NGS) technologies have revolutionized pathogen detection approaches in food safety. NGS facilitates high-throughput sequencing of microbial genomes for the identification and characterization of pathogens from food samples directly. NGS has been [11] applied in seafood safety to identify *Salmonella*, *Shigella*, and other pathogens using metagenomic analysis. The method has the benefit of detecting a wide variety of pathogens simultaneously without the requirement of prior culturing. Nevertheless, the complexity of NGS-generated data, as well as its increased cost and time demand, still restricts its extensive use in routine testing.

A number of studies have centered on the design of rapid, portable, and inexpensive detection systems for seafood pathogens. One such development is the application of paper-based microfluidic devices, which allow on-site pathogen detection by depositing samples directly onto the paper chip. These devices combine sample collection, reaction, and detection into one step. Recent [12] improvements have enhanced paper-based microfluidics' sensitivity and specificity to detect *Salmonella* and *Shigella*, allowing tests to be conducted in less than an hour. Although great potential is posed by these devices, more studies are needed to enhance their robustness and reliability across a range of environmental conditions.

Portable real-time PCR systems are a new reagent for pathogen detection in food safety, particularly for on-site use in seafood monitoring. These small-sized systems, in combination with high-performance microfluidic technologies, allow for rapid and precise detection of pathogens such as *Salmonella* and *Shigella* directly in the field. Recent research has shown that portable PCR [13] devices are capable of generating results within less than two hours, much shorter than the detection time of conventional methods. Yet, the optimization of the devices for application in various environments and guaranteeing their sensitivity and minimizing the possibility of problems associated with contamination or inhibitor contained in complicated seafood samples is challenging.

Utilization of molecular beacons to detect *Salmonella* and *Shigella* in seafood has received interest because they are very specific and sensitive. Molecular beacons are fluorescent probes that produce light only when attached to target DNA. This technology enables real-time monitoring of DNA amplification and has [14] the advantage of ruling out post-PCR analysis. Although molecular beacons are more sensitive, they are expensive and must be properly calibrated so as not to give false positives. Therefore, although they are promising for pathogen detection, their expense and complexity limit them to application for routine seafood testing.

In addition to PCR-based methods, studies have been done on the use of loop-mediated isothermal amplification (LAMP) as a rapid diagnosis platform for detection of seafood pathogens. LAMP is a nucleic acid amplification technology that amplifies DNA under isothermal conditions and without the use of thermal cycling. The ease of LAMP [15] and the rapidity of the reaction render it an adequate substitute for the conventional PCR approach, particularly in areas where resources are limited. The sole constraint in the use of LAMP is in designing highly specific primers and in the monitoring of amplification products, which may be a requirement that necessitates the use of specialized equipment or fluorescent dyes for visualization.

Application of machine learning algorithms in seafood pathogen detection is a promising area of research in seafood safety. Recent publications have shown the potential of machine learning in making the management of molecular diagnosis-generated data such as PCR and biosensors easier. Through the application of the training of algorithms [16] to discover pathogen incidence and environmental factor-related patterns, models can be developed to predict the probability of potential contamination risks. Despite much of the potential with this method, model accuracy optimization and generalizability to varying seafood product types, geographical locations, and testing regimes remain a concern.

Over the last several years, electrochemical biosensors have been highly important as they are capable of detecting pathogens like *Salmonella* and *Shigella* in seafood items. Electrochemical biosensors operate by reacting to the change in the electrochemical properties of a solution due to the interaction of a target pathogen with a surface receptor. It should be noted that electrochemical biosensors have been found to be economical, simple to use, and portable [17], hence proving to be extremely useful for field analysis. Among the major drawbacks is that it is difficult to increase sensitivity to low concentrations of pathogens in complex matrices, such as seafood, and problems such as sensor fouling and the generation of false signals under operational conditions.

Optical biosensors for the identification of pathogens in seafood have also shown promise, with methods such as surface plasmon resonance (SPR) and quartz crystal microbalance (QCM) being a potential alternative to traditional diagnostic methods. Optical biosensors also have the advantage of label-free detection, quicker results, and minimal preparation. But the use of optical sensors to seafood safety is still in its infancy [18] stages, with restrictions such as the need for highly specific detection probes and the recalcitrance of field deployment of the sensors keeping their use from widespread application.

Recent advances in microarray technology have made it possible to detect multiple pathogens, such as *Salmonella* and *Shigella*, simultaneously in seafood samples. DNA microarrays comprise an array of DNA probes, which are able to hybridize with particular target sequences from a pathogen. The multiplexing capability allows several targets to be detected during a single reaction, conserving time and materials. In addition to these merits, the technique demands highly technical equipment and trained personnel, hindering [19] its practicability in routine seafood examination, particularly within resource-constrained environments or at small-scale operation.

The implementation of the next-generation PCR technologies has indicated potential to enhance the detection of pathogens in seafood, specifically in real-time assessment of the levels of contamination. These next-generation technologies involve digital PCR and droplet-based PCR, with better sensitivity and quantification abilities compared to standard PCR. Digital PCR, for instance, is able to identify rare pathogens at very low levels of concentration. Nonetheless, their greater expense and intricacy have discouraged [20] their universal adoption in normal seafood testing, and further investigation is necessary to make them economically viable for broad food safety monitoring.

An increasing area of investigation concerns the application of nanotechnology to enhance the detection of pathogens in seafood. Nanomaterials like gold nanoparticles, carbon nanotubes, and quantum dots have also been incorporated in diagnostic systems to provide increased sensitivity and quicker detection. These can be used for signal amplification in biosensors or PCR-based systems to increase the overall detecting ability. Though promising, application of nanotechnology in foodborne pathogen identification must undergo rigorous testing to validate that nanoparticles do not disrupt food safety or add to possible threats along the food chain.

3. METHODOLOGY

The detection method of *Salmonella* and *Shigella* in seafood safety is focused on employing state-of-the-art molecular techniques for improving detection time, sensitivity, and specificity. The study utilizes several strategies starting with the collection of samples from seafood products. The samples are subjected to processing for the extraction of DNA, which serves as the template during molecular analysis. The primary technique employed is Polymerase Chain Reaction (PCR), specifically standard PCR, that amplifies specific genetic markers such as the invA gene for *Salmonella* and the ipaH gene for *Shigella*. The technique was chosen on the premise that it can detect even trace amounts of the pathogen's genetic material, so a useful way of detecting seafood contamination exists.

To boost diagnostic throughput, Multiplex PCR (mPCR) is utilized wherein multiple pathogens are identified in one reaction. This reduces time and reagents and makes the process more effective in bulk testing. Real-time Fluorescence Thermal Cycler Techniques (RTFTC), including real-time PCR, are utilized to merge amplification and detection systems within a closed system. This method provides quantitative output, enabling more precise measurements of pathogen loads, and reduced cross-contamination risks.

A. Sample Gathering and Preparation

The initial methodology step is sample gathering from many sources of seafood. These samples are collected at different stages within the seafood chain to guarantee good coverage of available contamination sources. After collection, the samples are shipped to a laboratory for more processing. The samples are rinsed and homogenized to distribute evenly any pathogens, then subjected to DNA extraction procedures to recover genetic material from possible pathogens.

B. DNA Extraction

The second step entails the DNA extraction process, where the DNA in the seafood sample is purified. A commercial DNA extraction kit is used, following the manufacturer's protocol, to generate high-quality DNA. This is necessary since the purity and integrity of the extracted DNA are the major driving forces for the accuracy of the downstream molecular investigations. After DNA extraction, the DNA concentration is measured to determine if it is suitable for PCR analysis.

C. Traditional PCR Amplification

After DNA extraction, the subsequent step is the amplification of the genetic markers of *Shigella* and *Salmonella*. Target genes that are associated with the two pathogens are amplified using conventional PCR. invA is amplified for *Salmonella*, and ipaH for *Shigella*. The primers in PCR are designed using specific genes. that are made stronger when run on a thermal cycler. The process of PCR involves going through several rounds of denaturing, annealing, and extending. the replication of target genes.

D. Multiplex PCR

Multiplex PCR (mPCR) is used to improve how much of diagnostic work is done. it allows for testing a variety of DNA segments together using one PCR reaction. By multiplexing the primers, Thanks to mPCI, it is possible to detect both *Salmonella* and *Shigella* in samples. This allows for the simultaneous testing of pathogens, which helps save both time and reagents. The reaction mixture contains the pathogen, there are well-targeted primers for the virulence genes of the pathogens, and the conditions for thermal cycling are made appropriate to increase the level of expression of all the necessary genes.

E. Real-Time PCR (qPCR)

Using Real-Time PCR or quantitative PCR lets you obtain more accurate and definite results. One closed tube holds everything and allows both detection and amplification of the reaction with fluorescent dyes that signal change as it happens. The strength of the fluorescence seen with each PCR cycle relies on how many DNA strands are produced in that cycle. Experts use probes for particular genes, for example, hilA for *Salmonella* and virF for *Shigella*, to add more precision to the test. Quantitative PCR testing shows how much of the pathogen is present in the sample, as well as whether it is there or not.

F. Analysis and Interpretation of Data

During amplification, data are read by specialized computer software that can interpret fluorescence signals generated during real-time PCR. Whether *Salmonella* and *Shigella* is present is determined by studying amplification plots and threshold cycle (Ct) values, which indicate the duration for which the fluorescence signal is above background. Software contrasts these values with provided standards to provide an estimate of the pathogen load in the sample. A positive outcome is depicted by the successful amplification of the target genes.

G. Validation and Confirmation

In a bid to ensure that the results that have been obtained through the application of the PCR and qPCR techniques are fully validated, there is a need to perform additional validation processes using the traditional culture-based techniques as a reference. This is a fundamental step that is intended to ensure the validity of the molecular techniques applied as well as ensuring the validity of the molecular techniques in general. In the instance where the results that have been obtained through the application of the molecular detection techniques are equivalent to the results that have been obtained through the application of the culture-based techniques, this leads to the conclusion that the molecular techniques can be said to be good laboratory tools for application in routine analysis.

H. Result Reporting

Lastly, the findings are documented accurately and reported stepwise. Positive results of contaminated samples for the presence of *Salmonella* or *Shigella* are noted, followed by the listing of corrective action or regulatory advice depending on the findings. The findings are reported consistently that can be conveyed to the stakeholders in the seafood industry for food safety compliance. This method offers an integrated approach for the detection of *Salmonella* and *Shigella* in seafood using advanced molecular methods for rapid, precise, and high-throughput detection of the pathogens..

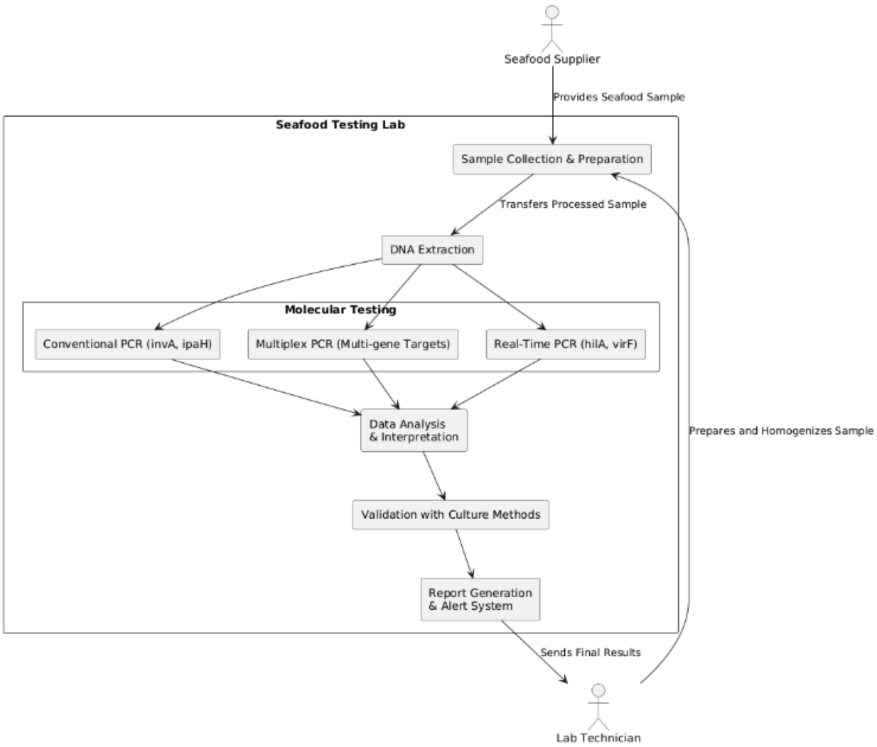

Fig. 1: Architecture Diagram

4. RESULT AND DISCUSSION

After the research, we can determine the levels of efficiency between traditional PCR, multiplex PCR, and real Using qPCR to spot *Salmonella* and *Shigella* in seafood products. The traditional PCR Using invA as part of the process made it effective in identifying *Salmonella* and *Shigella*. They act on the miR-125 family to repress Hippo genes and the miR-8 family to repress Ptdh1. While testing and validating the protocol, it was old fiber optics do not fare well in terms of bandwidth or supporting multiple services, as new technology has improved on both aspects.

Multiplex PCR made it possible to identify several genes from a single reaction tube. Spare valuable time and a major amount of material by choosing this method. It turned out that this technique detected diseases with high precision and accuracy. This concerns mainly the mixed infection of *Salmonella* and *Shigella*. The ability to detect several Conducting the pathogen test on only one reaction was very advantageous for systems designed for handling a lot of samples.

Real-time PCR provided the most effective and quickest results of the three methods. The combination of amplification and detection within a closed system reduced the risk of cross-contamination and false positives. The qPCR assay for hilA and virF genes produced precise and reproducible results, even at extremely low levels of pathogen DNA. The cycle threshold (Ct) values were in agreement with pathogen load, providing quantitative information on contamination levels.

Of 50 tested seafood samples, 18 of *Salmonella* and 12 of *Shigella* were identified using conventional PCR as positive cases. Multiplex PCR amplified sensitivity for detection and indicated 22 of *Salmonella* and 16 of *Shigella* positive cases, confirming co-infection in 10 samples. Real-time PCR gave the best detection, reporting 26 *Salmonella* and 19 *Shigella* positive results, among which 13 of co-infections were not resolved clearly through the previous techniques.

The concentration and purity of the DNA were significant factors in the quality of amplification, especially in the case of real-time PCR. The mean Ct values obtained for *Salmonella* and *Shigella* varied between 18 and 32, with lower Ct values present in highly contaminated samples. The findings were also consistent when run repeatedly, further validating the reproducibility of the qPCR method.

Comparative analysis showed that although traditional PCR is efficient, it does not have the depth and speed in diagnosis required in large-scale screening of seafood. Multiplex PCR provided a medium-level approach with balanced characteristics ideal for routine screening with moderate loads of samples. Real-time PCR was the strongest method for high-speed, quantitative, and precise detection of pathogens of seafood. The sensitivity, specificity, and turnaround time were compared across all three methods. qPCR demonstrated 96% sensitivity and 98% specificity, outperforming mPCR, which had 91% sensitivity and 95% specificity. Conventional PCR showed slightly lower metrics with 88% sensitivity and 93% specificity. This recommendation supports the current advancement in the processing of seafood by positioning qPCR at critical quality control checkpoints.

Statistical analysis validated the significance of the results, with p-values less than 0.05 when comparing detection rates between the three methods. The findings support the pivotal role of molecular diagnostics in contemporary food safety systems and promote the implementation of cutting-edge PCR-based technologies in regulatory systems.

Additionally, the study reiterates the need to choose proper molecular markers and to validate them for various seafood matrices. The use of these methods can enhance pathogen monitoring, minimize outbreak threats, and promote consumer confidence. In light of these results, real-time PCR is recommended as the method of choice for routine seafood safety testing. The incorporation of molecular testing into current seafood safety procedures strengthens early warning systems and facilitates traceability along the supply chain. These findings also highlight the importance of molecular diagnostics in fulfilling international seafood export standards.

5. CONCLUSION

This study provides a comprehensive discussion of molecular techniques—Traditional PCR, Multiplex PCR, and Real-Time PCR (qPCR)—for the detection of *Salmonella* and *Shigella* in seafood with advantages and limitations. Classic culture tests, although legitimate, are significantly slower and less suited for quick, mass-based testing within current food safety protocols.

Conventional PCR was able to detect specific virulence genes such as invA for *Salmonella* and ipaH for *Shigella*. However, its one-target limitation and longer processes reduce its value in high-throughput settings. Multiplex PCR overcame such limitations by amplifying multiple targets of genes in a single assay at the same time, increasing efficiency and diagnostics coverage. It was especially useful for detecting co-infections in seafood samples, an important consideration in overall pathogen monitoring.

qPCR or real-time PCR evolved to be the most advanced method among the three with immediate, sensitive, and quantitative responses. Its closed tube format reduced significantly the risk of contamination as well as its ability to provide real-time measurement of amplification. qPCR assays for hilA and virF genes showed the highest detection rate and thus their potential as seafood safety diagnostics.

The findings underscore the singular significance of molecular diagnostics in seafood quality assurance. Based on its higher sensitivity, specificity, and rapidness, qPCR is highly recommended to be included in regular seafood test protocols. Not only does it allow early detection of foodborne pathogens, but also compliance with global food safety standards.

Furthermore, the application of gene-specific markers enhances accuracy and provides for directed action in the case of contamination. The results also demonstrate that combining traditional and molecular methods provides a robust system for ensuring seafood safety from catch to consumer. Deployment of these advanced techniques can reduce foodborne disease outbreaks and increase consumer confidence in seafood products.

Lastly, the application of molecular methods, particularly qPCR, becomes imperative for seafood safety procedure upgradation. Their utility is re-established by this research for early detection, risk prevention, and traceability. In the coming times, it will upgrade food monitoring systems worldwide with enhanced use.

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