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Review

Diagnostic techniques for rapid detection of *Vibrio* species

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ABSTRACT

Background: *Vibrio* sp. has caused several foodborne outbreaks of gastroenteritis around the world. Given the mounting evidence that *Vibrio* sp. poses a significant public health threat, and that initiating effective treatment for *Vibrio* infection requires rapid identification of the causative organism, there is a need for reliable, cost-effective point of care diagnostic testing. Conventional detection techniques that are the current gold standard in detecting *Vibrio* sp., are typically resource-intensive, laborious, and time-consuming. These limitations greatly hinder their efficiency in under-developed countries, which are usually the regions most affected by *Vibrio* infections. Thus, the development of rapid, reliable point of care detection methods is crucial to overcome the limitations of conventional methods.

Aim of the review: This review aims to summarize and give insights into the array of diagnostic techniques for rapid detection of *Vibrio* sp.

Key scientific concepts of review: This review focuses on different rapid methods to detect *Vibrio* sp., including polymerase chain reactions, loop-mediated isothermal amplification, enzyme-linked immunosorbent assays, microarrays, lateral flow immunoassays, aptasensors, spot dye-based sensors and CRISPR/Cas-PCR systems. The rapid detection methods were found to have high specificity and sensitivity in detecting various species within the genus *Vibrio*. However, each detection method has its own advantages and limitations, thus continuous effort is needed to improve on what is currently available and develop techniques with high sensitivity, specificity, affordability with minimal effort to obtain consistent and accurate diagnoses.

1. Introduction

Vibrio species are Gram-negative, halophilic, facultative, γ-Proteobacteria that are autochthonous to estuarine and marine waters (Baker-Austin et al., 2018; Lee and Raghunath, 2018). Multiple species such as *Vibrio alginolyticus, Vibrio harveyi, Vibrio anguillarum, Vibrio cholerae, Vibrio parahaemolyticus,* and *Vibrio vulnificus* often interact with aquatic life within their shared environment. This results in *Vibrio* infections with high mortality rates in these marine animals both in the wild and in aquaculture systems (Baker-Austin et al., 2018; Dong et al., 2017; Frans et al., 2011; Ina-Salwany et al., 2019; Krupesha Sharma et al., 2012; Zhang et al., 2014; Zhang et al., 2015a). *V. alginolyticus, V. harveyi,* and *V. anguillarum* are important pathogenic *Vibrio* species that are responsible for disease outbreaks in aquaculture systems. The most commonly reported symptoms of vibriosis in marine life are enlarged internal organs, exophthalmos, hemorrhages on the body surface, and skin ulcerations (Abdullah et al., 2017; Dong et al., 2017; Ina-Salwany et al., 2019; Marudhupandi et al., 2017). The spread of vibriosis ultimately leads to major losses in the seafood industry and shortages of seafood supply in our food chain (de Souza Valente and Wan, 2021; Novriadi, 2016). Moreover, these pathogens are commonly transmitted to humans via the consumption of contaminated water or inadequately cooked aquatic food products (Emam et al., 2019; Letchumanan et al., 2019a). Infections can also occur if open skin wounds are exposed to water or aquatic animals contaminated by vibrios (Brehm et al., 2020; Osunla and Okoh, 2017; Sinatra and Colby, 2018). Most notably, *V. cholerae, V. parahaemolyticus*, and *V. vulnificus* possess the most medical significance from a perspective of food safety and public health (Letchumanan et al., 2019b; Loo et al., 2020). Therefore, they have a higher epidemic potential than the other members in the genus *Vibrio*. In addition, global

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warming and climate changes resulting in increased water temperatures, turbidity, salinity, and floods may further enable these pathogens to propagate and become more prevalent in the environment (Baker-Austin et al., 2017; Lara et al., 2009; Vezzulli et al., 2015). *V. cholerae* is a non-invasive organism in which they release enterotoxins in the small intestine, leading to the development of cholera. The serogroups O1 and O139 are the causative agents of epidemic cholera, while other non-O1 and non-O139 *V. cholerae* strains can occasionally cause diarrhea in humans. Meanwhile, *V. parahaemolyticus* and *V. vulnificus* are categorized as intrusive organisms which affect the colon. *V. parahaemolyticus* is the leading cause of gastroenteritis because of consumption of uncooked seafood while *V. vulnificus* can cause gastroenteritis, septicemia, and wound infections. *V. fluvialis* and *V. vulnificus* have also been linked to outbreaks, and sporadic cases of severe diarrhea (Bhattacharjee et al., 2010; Centers for Disease Control and Prevention, 2020; Liang et al., 2013). Exposure to these emerging foodborne pathogens can be very detrimental to our aquaculture systems and public health, therefore constant surveillance and monitoring are essential to prevent outbreaks of infections.

As *Vibrio* species are naturally diverse within the natural environment in which they reside, they can exchange genetic elements. For example, horizontal transfer of toxigenic genes can occur, resulting in non-toxigenic strains becoming equipped with epidemic potential (Azarian et al., 2016; Letchumanan et al., 2016). Thus, continuous surveillance of these *Vibrio* species' toxigenic and non-toxigenic strains is essential to prevent outbreaks. The lack of common characteristics between the vibrios due to intra-species variation of biochemical characteristics makes phenotypic identification challenging and time-consuming. During outbreaks or in low resource point of care (POC) settings, it is important to have easily deployable detection methods that offer real-time and simultaneous detection of multiple disease markers. Rapid, precise, and sensitive pathogen identification technologies can facilitate early prevention of *Vibrio* infections and provide timely treatment in healthcare facilities. In addition, easily accessible detection methods can be very useful in areas that lack access to laboratories where culture methods to detect these bacteria are done. The rapid diagnostic methods are also valuable for surveillance and confirmation of disease to ensure timely implementation of protective and preventive measures to protect the public from an outbreak.

Nevertheless, conventional methods have always been the gold standard used to identify causative pathogens. The US Food & Drug Administration's Bacteriological Analytical Manual (FDA-BAM) (FDA-BAM, 2004), ISO/TS 21872-1:2007 (International Organization for Standardization, 2007b) and ISO/TS 21872-2:2007 (International Organization for Standardization, 2007a) have been the recommended standardized microbiological guidelines to detect the presence of *Vibrio* sp. in the environment or seafood. To identify *Vibrio* sp., the ISO and FDA-BAM methods are used to detect the pathogens, but only the FDA-BAM methods include steps for both enumeration and detection of the bacteria. In brief, the FDA-method details the steps to identify *Vibrio* sp. from samples, which includes enrichment process in alkaline peptone water, enumeration by Most Probable Number (MPN) technique, isolation on selective mediums, and confirmation of the *Vibrio* sp. identity via a series of biochemical tests (FDA-BAM, 2004). Similarly, the ISO methods are also based on the three main steps, enumeration, isolation, and confirmation. The aforementioned ISO/TS 21872-1:2007 (International Organization for Standardization, 2007b) was developed to detect only *Vibrio cholerae* and *Vibrio parahaemolyticus* while the detection of other *Vibrio* sp. was done using the methods described in ISO/TS 21872-2:2007 (International Organization for Standardization, 2007a). Both of these ISO methods are horizontal methods mainly used to detect pathogenic *Vibrio* species in food. They both start off with enrichment in alkaline peptone water, followed by isolation using selective mediums. Lastly, confirmation is done using biochemical tests and the ISO method recommends that the species of the isolated vibrios should be further confirmed in a reference laboratory. The biggest discerning factor between ISO/TS 21872-1:2007 and ISO/TS 21872-2:2007 is the incubation temperature during enrichment which is dependent on the target *Vibrio* sp. and whether the food samples are fresh or frozen. However, the conventional methods such as plating methods, immunochemical or molecular-based methods could take hours to days to achieve conclusive results (Fig. 1) (Law et al., 2015). They also often require more effort and labour in processing the specimens and isolates to determine the causative organism of the infections accurately. Moreover, high-standard laboratory facilities and the complexities of the techniques required by the laboratory staff to carry out these conventional methods may affect the accuracy of the results.

Rapid detection methods have been very useful during outbreaks of infectious diseases caused by *Vibrio* sp. Considering the epidemiological significance of *Vibrio* sp. and their ability to cause outbreaks, it is crucial to detect and identify the causative agents of an outbreak in order to initiate an effective and efficient response to treat the disease. Rapid detection methods include polymerase chain reactions (PCR), loop-mediated isothermal amplification (LAMP), immunoassays, etc. In comparison to conventional methods, rapid methods are more specific, sensitive, reliable, time-efficient, and less laborious (Law et al., 2016). The ability to detect these foodborne pathogens more rapidly can potentially reduce the rate of spreading of the infection. Furthermore, timely administration of appropriate treatment can be facilitated to reduce the mortality rates among the population in the affected areas. This aims to review and compare the array of rapid detection methods for *Vibrio* sp. as well as providing an overview on the emerging rapid detection methods (Fig. 1).

2. Methodology

This narrative review searched for rapid detection methods of *Vibrio* sp. using the "preferred reporting items for systematic review and meta-analysis" (PRISMA) statement (Fig. 2). The literature search was performed across three databases, PubMed, Ovid Medline, and Scopus, using the keywords: "vibrio AND "rapid AND detection" OR isolation OR identification OR presence OR methods". Additional literature that was not captured in the aforementioned databases was uncovered using Google Scholar. Original research articles from year 2010-2022 that were written in English and demonstrated rapid detection methods of *Vibrio* sp. were included in this review. The titles and abstracts yielded by the search were independently screened by two authors to ensure the reliability of this narrative review. The full-text content of included articles were then screened by authors to ensure relevance and eligibility for inclusion, and any disagreements were resolved through discussions among the authors. As a result of the reviews, 201 studies that met the inclusion criteria were included in this narrative review.

3. Rapid detection methods

Rapid detection methods are developed based on the various genes and identification markers present in the *Vibrio* species. For instance, a gene sequence specific to *Vibrio* sp. can be chosen as the target gene in a polymerase chain reaction to identify the species level of the bacteria accurately. In addition, identification markers in these foodborne pathogens such as lipopolysaccharide of *V. cholerae* and secretory proteins of *V. parahaemolyticus* have been used in immunological-based rapid methods for species confirmation. The various gene sequences and identification markers used in the rapid detection methods are listed in Table 1.

Due to the potential of *Vibrio* sp. to cause epidemics and severe illnesses, there is a need for rapid detection and identification methods to ensure an effective response can be initiated early on during an infectious disease outbreak. Although conventional methods like phenotypic fingerprinting of *Vibrio* sp. are the gold standard for bacteria identification, these methods are more labor-intensive, time-consuming, and require experienced personnel. Remote areas around the world which

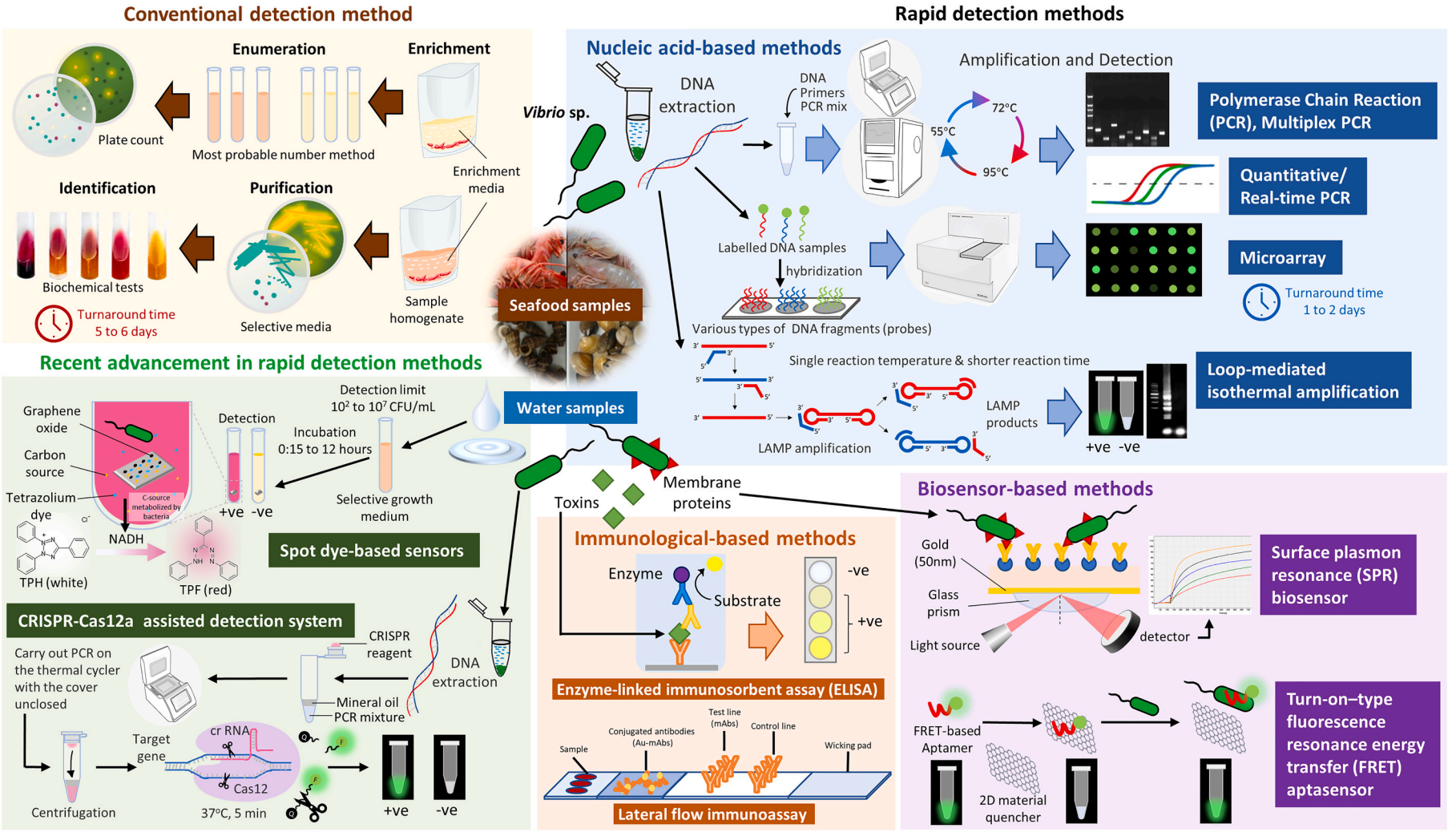

Fig. 1. Illustration of conventional and recent rapid detection methods for *Vibrio* sp. The conventional detection method is the gold standard to detect *Vibrio* sp. Due to the laborious and time-consuming conventional detection method, numerous rapid detection methods have been developed for *Vibrio* sp. detection in food and water samples, including nucleic acid-based, immunological-based and biosensor-based methods. Moreover, CRISPR-Cas12a system is one of the most recent nucleic-acid based rapid detection methods that can successfully detect *Vibrio* sp. in food samples.

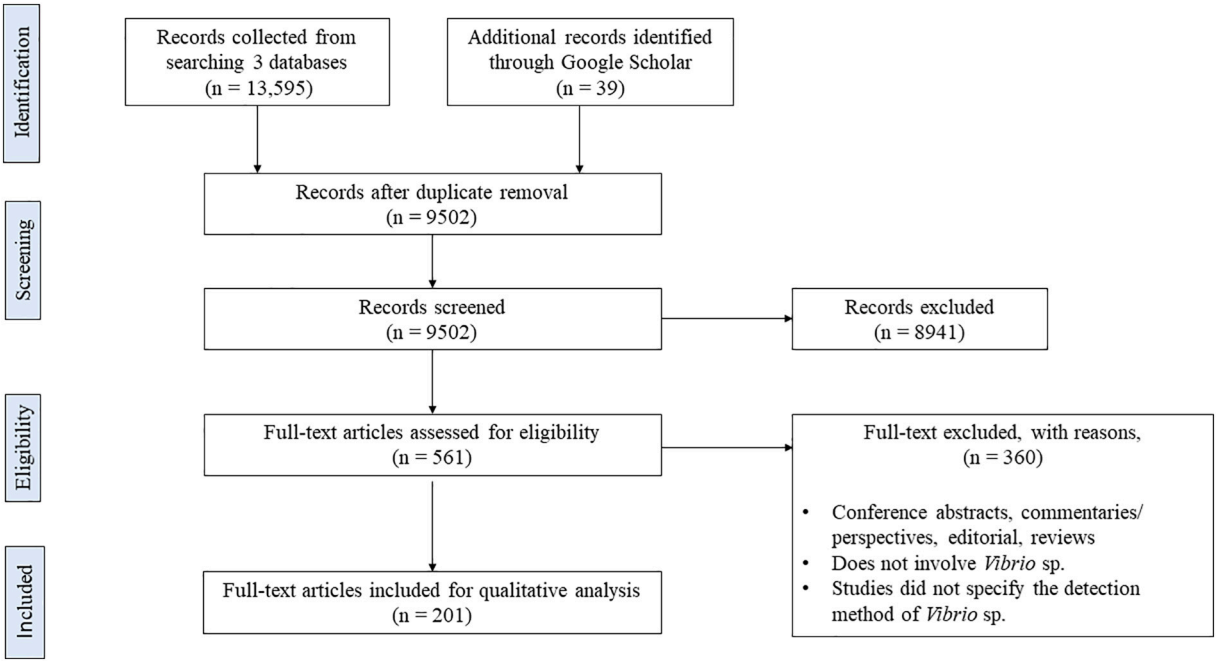

Fig. 2. PRISMA flow diagram of the study search and selection process.

are usually most affected by outbreaks caused by *Vibrio* sp. often lack the diagnostic facilities required to conduct tests based on conventional methods. As a result, the detection of this pathogen becomes a major roadblock that leads to delayed treatment and causes detrimental effects in patients with severe cases of infection. The introduction and utilization of rapid detection methods can overcome these setbacks by allowing timely identification of the *Vibrio* sp. Various detection methods have been effective in identifying *Vibrio* sp. from various sources that come with their own advantages and limitations. These techniques include nucleic acid-based methods such as PCR, loop-mediated isothermal amplifications, microarrays, biosensor-based methods, and immunological-based methods like enzyme-linked immune-sorbent assay (ELISA) and lateral flow immunoassays. Despite the abundance of well-established detection methods available for use, researchers are constantly developing novel detection techniques with improvements in terms of rapidity, specificity, and sensitivity to detect these foodborne pathogens. These methods can then be utilized by epidemiologists and health care authorities in the surveillance and monitoring of *Vibrio* sp. outbreaks. Upon the detection of a potential disease outbreak(s), appropriate preventative and early control measures can be deployed immediately.

3.1. Nucleic acid-based methods

Nucleic-acid-based molecular methods provide good resolution in differentiating *Vibrio* species, even in low-level infections. In general, these methods involve a hybridization process between the RNA or DNA sequence of the target *Vibrio* sp. to a synthetic, complementary oligonucleotide sequence and can be monitored via the detection of fluorescent signals in certain quantitative methods (Zhao et al., 2014). The specific primers used, which correspond to the various genes in the *Vibrio* sp. is shown in Table 2. Examples of nucleic acid-based methods include singleplex PCR, multiplex polymerase chain reaction (mPCR), real-time or quantitative polymerase chain reaction (qPCR), loop-mediated isothermal amplification (LAMP), and microarray technology.

3.1.1. Polymerase Chain Reactions (PCR)

PCR presents as a good alternative to conventional methods in pathogen detection as minimal biochemical tests are required to obtain a manageable number of strains for screening. The process is rapid, cost-effective, and can be completed within several hours. First invented in the 1980s, PCR assays primarily operate on the recognition of species-specific gene sequences followed by the amplification of these nucleic acids using specifically-designed primers. In PCR, double-stranded DNA is first denatured into single-stranded DNA at high temperatures. Next, the annealing of the specific forward and reverse primers to the targeted gene sequences on the DNA strands take place. Lastly, polymerization takes place in which the primers complementary to the DNA strands are elongated. This process requires thermostable DNA polymerase and deoxyribonucleotides. Once amplification is complete, the PCR products can be visualized on electrophoresis gel as bands using nucleic acid stains (Kadri, 2019; Lorenz, 2012). PCR has been widely applied to confirm the identities of foodborne pathogens and to screen for specific genes associated with pathogenicity by targeting specific genes of various *Vibrio* species (Table 1). Examples of *Vibrio* species detected through PCR assays include *V. cholerae, V. parahaemolyticus*, *V. harveyi*, *V. alginolyticus*, and *V. anguillarum* (Huq et al., 2012; Kim et al., 2010; Letchumanan et al., 2014; Paydar et al., 2013; Santhyia et al., 2015; Xie et al., 2020; Xu et al., 2017a).

Although PCR assays have been more successful in identifying total and/or pathogenic strains of *Vibrio* sp. than conventional biochemical tests, the need for electrophoresis in an agarose gel to visualize and analyze the PCR product can make the process tedious and time-consuming (Huq et al., 2012; Kadri, 2019). Besides, equipment failure, DNA polymerase inactivation and PCR inhibitors present in the samples could affect the efficiency of identifying *Vibrio* sp. This can be overcome by using an internal amplification control (IAC) such as 16s rRNA to prevent false-negative results and ensure PCR integrity (Wei et al., 2014). For PCR assays, the limiting factors are generally sampled purity and volume size. Aside from that, there is a possibility that PCR assays may not be an appropriate choice of detection methods if the number of pathogenic strains present in the samples falls below the detection limit. A pre-enrichment step in alkaline peptone water can be done to ensure a sufficient sample is present to facilitate the detection of *Vibrio* sp. using PCR assays (Huq et al., 2012). In short, PCR offers much higher sensitivity and detection power, this technique is still not the preferred tool in diagnostics settings as it requires operating space to fit advanced instrumentations that incur high maintenance and personnel costs.

Table 1
Examples of targeted genes and identification markers to detect *Vibrio* sp.

Species	Genes/ markers	Function	References
V. cholerae	*ctxA*	Cholera toxin subunit A	(Chua et al., 2011; Mehrabadi et al., 2012; Shirai et al., 1991)
	ace	Increases permeability of intestinal cell monolayers	(Fields et al., 1992; Koch et al., 1993; Rivera et al., 1995; Singh et al., 2001; Trucksis et al., 1993)
	lolB	Encodes outer-membrane lipoprotein	(Chua et al., 2011; Lalitha et al., 2008)
	ompW	Outer membrane protein W	(Kaper et al., 1995; Nandi et al., 2000)
	rtxA	Repeat in toxin subunit A	(Fields et al., 1992; Koch et al., 1993; Rivera et al., 1995; Singh et al., 2001)
	sto	Heat stable enterotoxin	(Fields et al., 1992; Koch et al., 1993; Rivera et al., 1995; Singh et al., 2001)
	tcpA	Encodes toxin-coregulated pilli	(Iredell and Manning, 1994; Mehrabadi et al., 2012)
	zot	Increases permeability of epithelial barrier	(Fields et al., 1992; Koch et al., 1993; Rivera et al., 1995; Singh et al., 2001)
	VCC	Cytolysin	(Honda and Finkelstein, 1979; Zhou et al., 2016)
V. parahaemolyticus	*tdh*	Thermostable direct hemolysin	(Nishibuchi et al., 1986; Nordstrom et al., 2007)
	tlh	Thermolabile hemolysin	(Haldar et al., 2010; Taniguchi et al., 1986)
	trh	TDH-related hemolysin	(Lee et al., 1992; Nordstrom et al., 2007)
	hut	Haem-utilizing gene	(Vinothkumar et al., 2013)
	ORF8	Genetic marker for O3:K6 strains	(Nasu et al., 2000; Ward and Bej, 2006)
V. vulnificus	*vvhA*	Cytolysin	(Neogi et al., 2010; Wright et al., 1985; Xu et al., 2017b)
	acfA	Encodes for accessory colonization factors	(Li et al., 2019; Miller and Mekalanos, 1988)
	flaA	Formation of filaments of bacterial flagella	(Li et al., 2019)
	fur	Encodes for ferric uptake regulation protein	(Li et al., 2019)
	rpoS	Elastase regulation	(Kim et al., 2008; Wang et al., 2016a)
V. anguillarum	*groEL*	Heat shock protein (phylogenic marker)	(Kim et al., 2010; Siddique et al., 2019)
V. harveyi	*chiA*	Encodes for chitinase A	(Ramaiah et al., 2000; Svitil et al., 1997; Xu et al., 2017a)
	luxR	Quorum-sensing master regulator	(Showalter et al., 1990; Xu et al., 2017a)
	mreB	Encodes for cytoskeletal protein	(Cano-Gomez et al., 2015)
	topA	Encodes for Topoisomerase I	(Cano-Gomez et al., 2015)
	vhh	*Vibrio harveyi* hemolysin	(Xu et al., 2017a; Zhang et al., 2001)
Vibrio sp.	*atpA*	Encodes ATP synthase subunit A (housekeeping gene)	(Izumiya et al., 2011; Thompson et al., 2007)
	dnaJ	Heat shock protein	(Nhung et al., 2007; Tall et al., 2012)
	ftsZ	Encodes for cell division protein	(Cano-Gomez et al., 2015; Mougin et al., 2020)
	gyrB	Encodes DNA gyrase subunit B	(Fu et al., 2016; Venkateswaran et al., 1998)
	hly	Hemolysin production	(Haldar et al., 2010)
	mdh	Encodes malate dehydrogenase	(Fischetti et al., 1999; Xu et al., 2017b)
	recA	Repair and maintenance of DNA	(Mougin et al., 2020; Tolmasky et al., 1992)
	toxR	Positive transcriptional regulator in virulence gene expression	(DiRita, 1992; Neogi et al., 2010; Xu et al., 2017a)

3.1.2. Multiplex Polymerase Chain Reactions (mPCR)

As opposed to singleplex PCR which detects only one gene at a time, mPCR detects several genes or species simultaneously, which offers great benefits in terms of analysis time during a large-scale screening. The core principle of mPCR is based on conventional PCR, but the main difference lies in the number of primers used, whereby multiple sets of specific primers are utilized to detect several genes at once. Thus, to produce a successful mPCR assay, it is crucial to design the primers with similar annealing temperatures. In addition, the interaction between multiple primer sets in mPCR may occur and result in primer dimers, leading to unreliable PCR products. Thus, the concentrations of the primer sets need to be adjusted to ensure the mPCR produces accurate and reliable results (Zhao et al., 2014). Researchers have reported using mPCR assay to detect pathogenic *Vibrio* sp., identifying their specific species or serotypes, and detecting virulence genes (Awasthi et al., 2019; Devadas et al., 2019; Gandhi et al., 2020; Guan et al., 2021; Li et al., 2021; Tao et al., 2020; Triwibowo et al., 2020; Tsai et al., 2019; Yáñez et al., 2015). In one study, a mPCR assay was designed to detect the *gyrB* gene of *V. alginolyticus,* collagenase of *V. parahaemolyticus, vvhA* gene of *V. vulnificus,* and outer membrane protein W (*ompW)* gene of *V. cholerae*, making it the first mPCR to combine five primer pairs for the simultaneous detection of four different pathogens. The detection limit for this mPCR was found to be 10^5 CFU/mL for *V. cholerae*, while the limit for the other *Vibrio* species in the study was 10 CFU/mL. The difference in detection limits were expected to be due to different amplification efficiency during reaction: a preferential amplification of smaller fragments in mPCR assay was observed. The *ompW* gene (427 bp) was described as more difficult to amplify owing to its longer nucleotide sequence and gene copy numbers. Nevertheless, this mPCR assay was still able to detect these foodborne pathogens with high specificity even at low CFU/mL (Wei et al., 2014).

Another study reported the development of an mPCR assay that was able to simultaneously detect *V. parahaemolyticus*, *Listeria monocytogenes*, and *Salmonella* spp. Primers targeting *tlh, hlyA* and *orgC* genes were used in the assay. The specificity of the mPCR was determined to be 100% as all 13 target strains were identified, and no positive signals were detected from the 15 non-target strains (Zhang et al., 2015b). A comparison between mPCR and standard culture methods also reflected that mPCR produced comparable results, with high specificity and sensitivity in detecting the presence of foodborne pathogens in contaminated samples. Moreover, excluding the time for DNA extraction, the results from the mPCR could be obtained within approximately 50 minutes, thus saving a considerably significant amount of time compared to cultural methods (Zhang et al., 2015b). In the same study, the limit of detection (LOD) of the mPCR for foodborne pathogens in artificially contaminated shrimp samples was 10^2 CFU/g without enrichment of the samples. This further emphasizes that mPCR assays

Table 2
Examples of various rapid detection method and the specificity, sensitivity, and detection limit.

Assay	Species	Sample source	Targets/requirements	Sensitivity (CFU/reaction) [%]	Specificity (%)	LOD (CFU/ml)	References
PCR	VH	Seafood	*luxR*, *toxRvh*, *chiA*, *vhh*, serine protease	-	100	$2x10^4$-10^6	(Xu et al., 2017a)
mPCR	VC, VP, VV	Clinical, environmental	*toxR* gene, *vvhA* gene	100	100	10	(Neogi et al., 2010)
mPCR	V	Clinical, environmental	*atpA* gene	100	100	-	(Izumiya et al., 2011)
mPCR	VA, VH, VP	Seafood	Collagenase, *toxR*	-	-	-	(Liu et al., 2016)
mPCR	VC	Clinical	*ctxA*, *lolB* genes	100	100	$2x10^4$	(Chua et al., 2011)
mPCR	VC	Reference strains	*ctxA*, *tcpA* and *ompW*	100	100	-	(Mehrabadi et al., 2012)
mPCR	VA, VP, VV, VC	Environmental, clinical, reference strains	*ompW*, *gyrB*, *collagenase*, *vvhA*, 16S rRNA	100	100	10 CFU 10^5	(Wei et al., 2014)
mPCR-CE-LIF	VC	Marine products	*VCC*, O1-serogroup specific O1 gene, O139-specific O139 gene, *ctxA*	-	100	5-20	(Zhou et al., 2016)
DPO-based mPCR	VC, VA, VP, VV	Clinical, seafood	*mdh*, *vvhA*, *colH*, *toxR*	-	100	1.2-$1.5x10^2$	(Xu et al., 2017b)
mPCR	VB, VH, VP	Reference strains	*hly*, *tlh*	100	100	1-10	(Haldar et al., 2010)
mPCR	VP	Seafood	*tlh*, *hlyA*, *orgC*	-	100	10^2	(Zhang et al., 2015b)
mPCR	VC, VP, VF	Clinical, environmental	*toxR*, *glyA*, *hut*	100	100	-	(Vinothkumar et al., 2013)
mPCR	VH	Environmental, diseased aquatic animals	*topA*, *ftsZ*, *mreB*	-	100	0.3	(Cano-Gomez et al., 2015)
mPCR	VV	Diseased aquatic animals	*16S rRNA*, *fur*, *ompU*, *acfA*, *flaA*, *vvhA*, *vvp*, *tcp*	-	100	$2.4x10^3$	(Li et al., 2019)
Triplex reverse transcription-PCR	VC	Enriched environmental Non-enriched	*ompW*, *ctxA*, *tcpA*	-	100	6.7 $3.2x10^5$	(Kanoktippornchai et al., 2014)
qPCR	VV	Seafood	*gyrB*, *vvhA*	-	100	10	(D'Souza et al., 2019)
qPCR	VH	Environmental	*mreB*, *topA*, *toxR*, *ftsZ*, *recA*, *gyrB*, *16S rRNA*	-	90.6-100	-	(Mougin et al., 2020)
qPCR	VV, VC, VA	Environmental, seafood	*16S rDNA*, *dnaJ*	98.4	100	-	(Tall et al., 2012)
qPCR	VA	Seafood	*groEl*	-	-	-	(Ahmed et al., 2016)
qPCR	VC	Clinical	*tcpA*	100	100	-	(Grembi et al., 2020)
qPCR	VC	Reference strains, environmental	*viuB*, *rfbO1*	100	-	3	(Nasreen et al., 2022)
qPCR	VP	Seafood	*toxR*	-	100	$5x10^1$	(Ling et al., 2020)
LAMP	VC	Clinical	*ctxA* gene	100	100	0.54	(Okada et al., 2010)
LAMP	VP, VV	Seafood	*toxR*, *rpoS*	-	100	-	(Wang et al., 2016a)
LAMP	VV	Reference strains, clinical, environmental	*vvhA*	100	100	4	(Zhang et al., 2018)
LAMP	VA, VN	Reference strains	*groEL*, *fklB*	-	100	-	(Siddique et al., 2019)
LAMP	VA	Environmental	*toxR*	-	100	10^2	(Fu et al., 2016)
LAMP	VA	Diseased aquatic animals	*gyrB*	-	100	$3.7x10^2$	(Cai et al., 2010)
LAMP	VP	Reference strains, seafood	*toxR*	-	-	$4.5x10^0$	(Huanteng et al., 2019)
LAMP	VC	Viable but non-culturable strains	*ctxA*, *ompW*, *rfbN*	-	100	-	(Chamanrokh et al., 2022)
LAMP	VC	Reference strains	*ace*, *zot*	-	-	-	(Chen et al., 2021a)
LAMP	VP	Seafood	*toxR*, *tlh*	-	-	-	(Mendoza-Avilés et al., 2021)
RPA	VP	Seafood	*toxR*	-	-	$1.29x10^1$	(Ma et al., 2020)
RPA	VP	Reference strains	*toxR*	100	100	$7x10^3$	(Feng et al., 2022)
RCA	VP	Seafood	*toxR*	100	95.88	$2.3x10^0$	(Yuan et al., 2022)
ELISA	VC	Clinical	IgY antibodies against α-*ompW* and α-*ctxB*	-	100	$9.33x10^2$	(Bayat et al., 2018)
ELISA	VP	Reference	Anti-*trh* antibodies	100	100	10^3	(Kumar et al., 2011)
ELISA	VP	Reference strains	Anti-*omp* antibodies		100	($6.2x10^5$ cells/ml)	(Seo et al., 2010)
Immunoassay	VP	Reference strains	Conjugated monoclonal antibodies	-	100	10	(Liu et al., 2017)
Dot blotting Immunoassay	VH	Seafood	Monoclonal antibodies	-	100	10^7-10^8	(Thongkao, 2015)
Nucleic acid lateral flow biosensor	VC	Clinical	DNA probes	100	100	10	(Ang et al., 2012)
Immunochromatographic (dipstick)	VC			-	100%	10^6	

(*continued on next page*)

Table 2 (*continued*)

Assay	Species	Sample source	Targets/requirements	Sensitivity (CFU/reaction) [%]	Specificity (%)	LOD (CFU/ml)	References
		Clinical, reference strains	Anti O139 monoclonal antibodies				(Thattiyaphong et al., 2013)
Crystal Vc dipstick	VC	Environmental	Anti-LPS monoclonal antibodies	89	100	10^7	(Chakraborty et al., 2013)
Lateral flow biosensor	VC	O139	Anti-O1 LPS, anti-O139 LPS monoclonal antibodies	100	98-100	10^7	(Yu et al., 2011)
Luminescent hybridoma-based biosensor	VC	Clinical, environmental	IgM antibody against LPS of *V. cholerae* O1	-	100	50	(Zamani et al., 2016)
Graphene Oxide-DNAzyme-based biosensor	VA	Reference strains	VA-specific DNAzymes	-	-	$2.1x10^3$	(Tian et al., 2022)
MSN/NA-doped nanoflower on-site single cross-priming fluorescnence (SCPF) sensor	VP	Reference strains	*tlh*	-	100	$2.4x10^1$	(Su et al., 2022)
Dual-mode aptasensor with PBA-Fc@Pt@MXenes nanoprobe	VP	Seafood	VP	-	-	5	(Wang et al., 2022)
Crystal VC	VC	Clinical, environmental	Antibodies against O1 *V. cholerae*	89.3	98.9	-	(Debes et al., 2016)
Crystal VC	VC	Clinical	Antibodies against O1 *V. cholerae*	86.1	100	-	(Ontweka et al., 2016)
Crystal VC	VC	Clinical, environmental	Monoclonal antibodies for O1/O139 LPS	65.6	99.6	-	(Rashid et al., 2017)
Lateral flow dipstick	VA	Clinical, environmental, seafood	*rpoX* gene hybridized with a fluorescein isothiocyanate-labeled probe	-	100	$1.8x10^2$	(Plaon et al., 2015)
Lateral flow dipstick	VV	Reference strains	Anti *V. vulnificus*-H monoclonal antibodies	-	100	10^4	(Jadeja et al., 2015)
Lateral flow dipstick	VP	Reference strains	*toxR* gene hybridized with biotin and fluorescein isothiocyanate	-	100	$4.2x10^2$	(Wang et al., 2016b)
CARID	VC	Clinical, environmental	*ctxA*	-	100	-	(Lu et al., 2022)

†VC: *Vibrio cholerae*; VP: *Vibrio parahaemolyticus*; VV: *Vibrio vulnificus*; V: *Vibrio sp.*; VA; *Vibrio alginolyticus*; VH: *Vibrio harveyi;* VB: *Vibrio campbellii;* VF: *Vibrio fluvialis;* VN: *Vibrio anguillarum*. ** LOD: Limit of detection; E: Enrichment

can detect *Vibrio* sp. at high specificity and sensitivity within a shorter timeframe as compared to conventional culture methods. However, mPCRs require post-amplification processing of the amplicons via gel electrophoresis to obtain conclusive results and the method is not suitable for samples containing high levels of PCR inhibitors.

3.1.3. Quantitative/real-time Polymerase Chain Reactions (qPCR)

Quantitative real-time polymerase chain reactions (qPCR) can provide a quicker and more sensitive detection of a diverse range of microorganisms. The quantitative power of the assay relies on the detection of fluorescent signals resulting from the amplification of specific genetic sequences. The intensity of the fluorescence emitted in the assay is directly proportional to the number of genes present/expressed (Zhao et al., 2014). As reflected in the name of this technique (i.e., real-time), the process of amplification and visualization of the newly formed DNA amplicons occurs simultaneously in a qPCR, thus shortening the acquisition time for results. Fluorescent dyes such as SYBR Green I and fluorogenic DNA stains have been used in qPCR to rapidly and effectively detect *Vibrio* species (D'Souza et al., 2019; Jing-jing et al., 2011). SYBR green emits fluorescence when it binds to the minor groove of a double stranded DNA (Tajadini et al., 2014). SYBR green-based qPCR assay has also been developed to target *gyrB* gene to detect *V. vulnificus* in seafood. The qPCR was able to specifically amplify *V. vulnificus* isolates efficiently; other vibrios and non-vibrios were not amplified for the *gyrB* gene. This detection method is highly sensitive as it can discern between *Vibrio* species and only identify *V. vulnificus* from the pure culture samples. When spiked clam meat homogenates were examined, the assay was able to identify *V. vulnificus* isolates with LOD of 10^2 CFU/g without any enrichment. This can be useful in prescreening seafood for *V. vulnificus* isolates even in small amounts to prevent exposure and spread of *V. vulnificus* infections (D'Souza et al., 2019).

Other fluorescent systems include Taqman probes which are commonly used as they have greater specificity than conventional SYBR green assays. In a Taqman system, PCR products formed are detected by fluorogenic probes via exonuclease activity of the Taq polymerase. Taqman probes are oligonucleotides with a reporter dye on the 5' end and a quenching dye on the 3' end. The quenching dye prevents any fluorescence from being emitted before the hybridization and cleavage processes. During the PCR cycle, the Taqman probes will specifically bind to its complementary nucleic acid sequence and consequently the Taq DNA polymerase will cleave the probe and separate the fluorescent dye and the quenching dye. A fluorophore signal is then generated and detected by the fluorescence detector. An increase and accumulation of the PCR products will lead to a directly proportional increase in the fluorescence emitted (Botes et al., 2012). Many studies have also been successful in utilizing Taqman probes in qPCR assays to detect *Vibrio* species such as *V. parahaemolyticus*, *Vibrio splendidus*, *Vibrio fluvialis* (Han et al., 2015; Liu et al., 2019; Saulnier et al., 2017; Schikorski et al., 2013).

Unlike conventional PCR and mPCR, gel electrophoresis is not required in qPCR post-amplification, thus reducing the time required for analysis. Furthermore, qPCR can be automated, allowing fast and high throughput runs (Kralik and Ricchi, 2017). However, qPCR requires a more costly and advanced thermal cycler with a fluorescence detector, hence they may not be suitable for settings with limited resources. In order to mitigate this disadvantage, Grembi et al. has developed a high throughput low cost nanoliter-qPCR (nl-qPCR) for enteropathogens including *V. cholerae* that deploys nl-qPCR on a 5184-well chip (SmartChip™ Real-Time PCR, TakaraBio Inc.) (Grembi et al., 2020). In comparison to the more commonly used Taqman Array Cards (TAC) in

qPCR, nl-qPCR developed by Grembi et al. aims to reduce the cost by 6-fold while maintaining high quality diagnostic capabilities of the qPCR (Grembi et al., 2020). This nl-qPCR was able to detect *V. cholerae* at 100% sensitivity and 100% specificity, but it was less sensitive than qPCR using TAC when detecting other enteropathogens. The limit of detection is also comparatively greater due to its use of nanofluidic volume. Nevertheless, nl-qPCR is still an attractive, new method in detecting *Vibrio* sp. under conditions with limited resources as it can produce high quality results at a significantly lower cost at an estimated cost of 8-12 USD per sample compared to TAC-qPCR which cost around 60 USD per sample (Flaherty et al., 2021).

Multiplexing of amplification of several target genes in a single reaction is also a viable option when utilizing qPCR. Garrido-Maestu et al. reported a novel multiplex qPCR to simultaneously detect the *ompW* gene of *Vibrio cholerae, tlh* gene of *V. parahaemolyticus,* and *vvhA* gene of *V. vulnificus*. The study found that the multiplex qPCR developed was able to correctly identify the *Vibrio* species with a low LOD (Garrido-Maestu et al., 2014). This assay can potentially be applied to efforts of monitoring and surveillance of the presence of pathogenic *Vibrio* in seafood. The advantages of qPCR set off the development of qPCR test kits, which simplifies the process to detect *Vibrio* sp. while producing accurate results. Some examples of commercially available test kits include BAX® System Real-Time PCR Assays for *Vibrio* (Hygiena), *Vibrio* sp. Real-Time PCR Detection Kit (BioPremier), iQ-Check *Vibrio* PCR Detection Kit (Bio-Rad), foodproof® Vibrio Detection Lyokit (BIOTECON), *Vibrio cholerae* Pathogenicity Microbial qPCR DNA Multi-Assay Kit (Qiagen), and *Vibrio cholerae* Detection Kits (Norgen Biotek Corp).

When comparing qPCR and conventional PCR assay specifically designed to detect *V. vulnificus*, qPCR showed higher sensitivity compared to conventional PCR. The qPCR was able to identify *Vibrio vulnificus* in naturally incurred seafood samples, producing results of 40.67% or 20 out of 59 seafood samples that tested positive for the microorganism six to eight hours post enrichment (D'Souza et al., 2019). However, with the conventional PCR, only 11 out of 59 (18.64%) of the samples were positive. In addition, the qPCR assay developed in this study was completed within 9-12 hours, including enrichment of seafood samples rendering it a rapid and sensitive assay to be used to detect and screen for *Vibrio vulnificus* isolates in seafood (D'Souza et al., 2019). It is recommended to be used as a routine test in the seafood and aquaculture industry to screen for *V. vulnificus* as a measure to ensure product safety.

3.1.4. Loop-mediated isothermal amplification

Loop-mediated isothermal amplification (LAMP) is a molecular detection technique developed by Notomi et al. (Notomi et al., 2000) that relies on nucleic acid amplification. LAMP assays operate based on the principle of auto-cycling strand displacement DNA synthesis, which uses *Bst* DNA polymerase and a set of four specially designed primers. The four primers consist of two inner primers and two outer primers that targets six independent sequences on the target DNA (Tian et al., 2011; Zhao et al., 2014). LAMP occurs continuously under isothermal conditions at approximately 60°C to 65°C, and this isothermal reaction enables the assay to be highly efficient in amplifying the gene sequences as there is no thermal change (Siddique et al., 2019; Tian et al., 2011). Following the amplification process, amplicons of many different sizes of stem loop DNAs are generated with cauliflower-like structures with multiple loops. LAMP assays synthesize a large amount of DNA and it is correlated to the formation of a white precipitate made up of magnesium pyrophosphate, which will appear when a positive sample is identified (Wang et al., 2011). The turbidity allows the results to be easily assessed by the operator as positive and negative results are determined by the presence or absence of the white precipitate, respectively. The LAMP amplicons can also be visualized using agarose gel electrophoresis and SYBR Green dye (Zhao et al., 2014).

LAMP assays have been able to successfully detect *Vibrio* species such as *V. cholerae, V. alginolyticus, V. parahaemolyticus, V. vulnificus,* and *V. splendidus* from various seafood, clinical, and environmental samples (Anupama et al., 2020; Chamanrokh et al., 2022; Fu et al., 2016; Izumiya et al., 2019; Lee et al., 2020; Yi et al., 2014; Zhong et al., 2016; Zhong et al., 2020). LAMP assays function at high specificity, sensitivity, and efficacy can be utilized for screening for specific *Vibrio* species in various settings. A LAMP assay designed by Zhang et. al. targeting the *vvhA* gene was able to identify *V. vulnificus* from clinical and environmental samples at 100% specificity. The assay produced positive results only for *V. vulnificus* isolates while other isolates from the *Vibrio* genus produced negative results. Other species of bacteria were also used to determine the specificity of the assay and negative results were produced indicating the designed LAMP assay is highly specific toward the detection of *V. vulnificus* (Zhang et al., 2018). However, the setbacks of LAMP are that primer designs can be complicated as multiple primers are needed, and LAMPs are unsuitable to detect unknown or *de novo* targets (Zhao et al., 2014).

An alternative to *in situ*-nucleic acid amplification technique that relies on LAMP is *in situ*-LAMP which has improved specificity and sensitivity. In comparison with traditional culture methods and *in situ*-PCR, *in situ*-LAMP has several advantages over its predecessors. *In situ*-LAMP only requires mild permeabilization conditions and low isothermal reaction temperature rapidity with shorter experiment cycles. To ensure all reagents can enter the cell without diffusing the amplified products outside, it is crucial that the permeabilization conditions for *in situ* gene amplification are optimized (Ye et al., 2011). It also causes less damage to the samples than PCR and is unaffected by various inhibitory components. Nucleic acid extraction and cell destruction are also not required for *in situ*-LAMP (Kaneko et al., 2007; Maruyama et al., 2003). In both LAMP and *in situ*-LAMP, the *Bst* polymerase has offered both detection methods robustness, especially in the presence of inhibitory substances (Li et al., 2009; Wang et al., 2008). In a regular LAMP assay, nucleic acid extraction and cell destruction is required, thus complicating the detection and pre-processing steps (Okada et al., 2010). On the other hand, *in situ*-LAMP allows the amplification of target sequences to be done within intact cells. It is a combination of high sensitivity and high specificity in localizing specific DNA in the cells to be amplified. This could be very useful for the detection of bacteria (including *V. parahaemolyticus*) that are difficult to culture, enrich or extract DNA from (Cremonesi et al., 2006).

The favourable properties of LAMP makes it a good candidate it to be combined with other advancements in technology to better detect the target organisms (Jin et al., 2021; Kampeera et al., 2019; Varona et al., 2021; Zhou et al., 2021). Recently, a paper embedded microchip based on LAMP was developed to detect multiple foodborne pathogens simultaneously. The reaction chambers on the microchip were embedded with paper which contained LAMP reagents and primers specific to the pathogens (*Escherichia coli, Salmonella sp, Staphylococcus aureus,* and *V. parahaemolyticus*). The sample containing the target pathogens was injected into the center chamber of the microchip and distributed into the respective reactive chambers. (Zhang et al., 2021) After undergoing the LAMP process for approximately 30 minutes, the products will be placed under UV fluorescence to detect green fluorescence which indicates positive results. This assay is relatively inexpensive, user- friendly, and can produce results with high specificity.

LAMP assays are very useful in detecting *Vibrio* at high specificity as four primers are utilized to target the desired sequence specifically and large amounts of DNA can be synthesized more rapidly. However, LAMP assays are generally limited by the requirement of elaborate reagents for the DNA amplification process and expensive equipment. These factors could potentially inhibit its potential to be applied in a broader spectrum of use in detecting microorganisms in the environment. Other types of LAMP assays, such as real-time LAMP and multiplex LAMP have also been developed to detect *Vibrio* sp (Siddique et al., 2019; Yi et al., 2014). By applying real-time monitoring of LAMP amplification products, the need for gel electrophoresis is eliminated, thus allowing for high-throughput analysis while retaining the high specificity and sensitivity

of the assay.

When comparing LAMP and conventional PCR assays, LAMP assays have an advantage as they are not hindered by inhibitors such as stool, urine, or blood that may have been present in the clinical specimens unlike PCR assays (Schrader et al., 2012). This was shown in a study to identify *V. cholerae* from rectal swabs of cholera patients and healthy volunteers, the LAMP method detected *V. cholerae* at detection limits of 5 fg of purified genomic DNA/reaction and 0.54 CFU/reaction. It was shown to be 10- to 100- fold more sensitive than the PCR method used on the same specimens. In the same study, rectal swabs of 21 household contacts of cholera patients were taken and examined with the same LAMP method without enrichment of the samples. The assay was able to identify five asymptomatic carriers, meaning the method is sensitive in detecting the presence of *V. cholerae* even when in low cell counts and before significant propagation of the bacteria has occurred in the host to cause disease. The LAMP method used produced the same results as the culture methods, whereby it detected all 19 *V. cholerae* isolates. However, when the PCR method was used, it did not detect all the positive samples (Okada et al., 2010). In addition, LAMP methods require fewer steps than conventional PCR and qPCRs to detect *Vibrio* sp. henceforth results can be obtained within a shorter timeframe (Yamazaki et al., 2010). LAMP methods can be run at constant temperature in a water bath, while PCRs or qPCRs need dedicated equipment to run cyclothermal amplification (Niessen et al., 2013).

3.1.5. Recombinase polymerase amplification

Recombinase polymerase amplification (RPA) is an isothermal DNA amplification method founded by Piepenburg et al. in 2006 (Piepenburg et al., 2006). RPA has been used in various studies to detect the presence of *Vibrio* sp. in samples from various sources (Feng et al., 2022; Pang et al., 2019; Wang et al., 2021a; Yang et al., 2020a; Zhu et al., 2021).The mechanism behind RPA couples isothermal recombinase-driven primer which targets the template material with strand-displacement DNA synthesis (Piepenburg et al., 2006). The process begins with the binding of recombinase proteins to primers to form a recombinase-primer complex. The recombinase protein-primer complex then scans the double-stranded DNA to promote strand exchange at the cognate sites. Once the primers are inserted, single- stranded binding proteins are present to stabilize the displaced DNA strand to prevent dissociation of the primers. The recombinase will then disassemble, exposing the 3' end of the DNA strand, allowing binding of a strand displacing DNA polymerase which then elongates the strand for amplification (Lobato and O'Sullivan, 2018; Piepenburg et al., 2006). RPA has been utilitized to detect *V. parahaemolyticus* by targeting the *toxR* gene. The isothermal amplification process was completed within 30 minutes in a single run, producing results with 100% specificity and sensitivity (Feng et al., 2022).

RPA can be widely exploited in kits and assays for the detection of microorganisms due to its simplicity, high sensitivity and selectivity, rapid amplification, and ability to be multiplexed. RPA can also be combined with other rapid detection methods to be deployed as an assay to detect pathogens (Chen et al., 2021b; Dong et al., 2020; Jiang et al., 2020; Ma et al., 2020; Wang et al., 2021b). As shown by Ma et al., the team developed a multiplex RPA coupled with colloidal gold labeled with anti-digoxin monoclonal antibody lateral flow dipsticks to simultaneously detect foodborne pathogens in seafood. The whole process from DNA amplification to the reading of positive results was completed within 15 minutes at 37°C, indicating the application of RPA yields rapid results. For *V. parahaemolyticus,* the detection limit was $1.29x10^1$ CFU/mL as the red test lines were not visible to the naked eye for bacterial concentrations below these levels (Ma et al., 2020). In addition, RPA can also be combined with lateral flow immunoassays to better visualize the positive test results for the presence of the target pathogen (Fang et al., 2021; Yang et al., 2020b).

3.1.6. Rolling circle amplification

DNA polymerase can synthesize long linear strands containing multiple copies of a given sequence by using circular templates which is a process known as rolling circle amplification (RCA)(Fire and Xu, 1995). The detection of nucleic acids can be done by combining RCA with a padlock probe. In short, the padlock probe contains both 3'- and 5'- end sequences that bind to target sequences to facilitate circularization of the padlock probe by ligase if the 5'- end is phosphorylated. The primer and DNA polymerase will then produce long, linear, repeating DNA sequences which can be detected by various methods once the padlock probe circularization is complete (Yan et al., 2014). Yuan et al. have developed a novel real-time fluorescence saltatory rolling circle amplification technique (RF-SRCA) with a portable scanner to detect *V. parahaemolyticus* in seafood. The RF-SRCA targets the *toxR* gene and effectively determined the presence of *V. parahaemolyticus* within 20-60 minutes via real-time fluorescence curve. When compared to SRCA by electrophoresis, RF-SRCA can detect the targets in a shorter time with greater accuracy with minimal cross-contamination (Yuan et al., 2022). One study found that RF-SRCA was 100-fold more sensitive than qPCR with a sensitivity of $3.2x10^0$ CFU/mL and a detection limit of $2.08x10^1$ CFU/g (Dong Jing et al., 2021). RCA can also be multiplexed to detect multiple target pathogens concurrently including *V. cholerae* by targeting the *vrrA* gene whilst maintaining high sensitivity and specificity (Yeap et al., 2021).

3.1.7. Microarray

Microarrays are used for genotyping thousands of different loci simultaneously at a time as it works by having many different DNA sequences deposited on a surface such as a glass slide. The glass slide acts as a "chip" and the identity of each DNA sequence on the chip is represented by its position on the array (Govindarajan et al., 2012). In order for microarrays to work, complementary binding of nucleic acid sequences will occur between the immobilized probes and the target DNA. Two main steps are involved in microarray analysis which are probe production and target (cDNA) production. First, the probes are deposited on the DNA chip. Secondly, restriction endonucleases are used to cut unknown DNA into fragments and these fragments are then labelled with fluorescent markers to produce cDNA. The fragmented DNA is then reacted with the immobilized probes and the remaining DNA fragments are washed away. A fluorescence emission will occur when a laser beam passes the target DNA. The fluorescence pattern is then recorded in a computer and the target DNA can be identified (Govindarajan et al., 2012). Feng et. al. was able to use microarrays to simultaneously detect 13 bacterial and 11 viral pathogens of aquatic animals. The array developed by the team consisted of 853 oligonucleotide probes which were complementary to various virus-specific sequences and for chromosomal loci of bacteria including 16S rRNA, *gyrB, dnaJ,* and *recA*. Their results show that in DNA and RNA extracted from diseased fish or shrimp, evidence of strong fluorescent signals for various *Vibrio* sp. were found. The identified *Vibrio* sp. are *V. parahaemolyticus, V. harveyi, V. vulnificus, V. fluvialis, V. alginolyticus,* and *V. cholerae* (Feng et al., 2020).

To detect *V. cholerae*, a hybrid double molecular marker (DBM) microarray containing a 16S rRNA-based DNA capture probe and GM1 pentasaccharide capture probe were used to detect *V. cholerae* and cholera toxin, respectively. The DBM microarray was also able to detect the *V. cholerae* isolates and cholera toxin produced without cross-reactivity to other species of bacteria or other toxins and proteins produced. However, the sensitivity of DBM is low thus pre-enrichment of the bacterial cells is necessary to obtain results. In the study, pre-enrichment for 90 minutes was the minimum time needed for results to be observed on the microarray. This enrichment step was required for *V. cholerae* and the cholera toxin to be detected simultaneously (Shin et al., 2016). With reference to the developed microarrays, newer probes can be developed to specifically detect other *Vibrio* sp. and their respective toxins. This method is a rapid, sensitive, and specific approach in identifying microorganisms as it can target many DNA

sequences at one time. Microarrays have an advantage over PCR as they are cheaper to operate, require shorter processing time while still having high sensitivity and specificity to detect microorganisms. However, probe signals need to be corrected to account for the background fluorescence of the array substrate. Transient hybridization with noncomplementary or partially matching DNA molecules causes additional noise contributions when reading the results (Kooperberg et al., 2002). Hybridization between the expected nucleic acid sequences can potentially be hindered if probes and target DNAs form hairpins or other secondary structures (Ratushna et al., 2005). If most or all of the oligonucleotides in a probe feature are bound by target DNAs, chemical saturation occurs which creates a nonlinear relationship between target concentration and probe intensity (Burden et al., 2004).

4. Immunological-based methods

Other than detecting gene sequences in the *Vibrio* species, antibodies against the antigens present on the *Vibrio* strains can also be incorporated into detection methods to successfully detect *Vibrio*. Immunological-based methods can be applied in identifying, quantifying, and visualization of cells *in situ* (Ferone et al., 2020; Law et al., 2015). These methods are rapid, simple and can provide high specificity and sensitivity when detecting microbes in comparison to conventional methods. Immunological-based methods include enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassays (dipsticks and strips).

4.1. Enzyme-linked immunosorbent assay (ELISA)

ELISA has been employed since the 1970s for the detection of microorganisms and is regarded as rapid and efficient in detection (Denmark and Chessum, 1978). The principle of ELISA is based on a specific antigen-antibody reaction, by utilizing the interaction between antibodies produced by white blood cells and pathogen antigens. This allows for highly specific and sensitive reactions to accurately identify the target pathogens. In ELISA, the antigen or the antibody is labeled using enzymes and immobilized onto a solid phase such as a microtiter plate. Then, the antigen is reacted with a specific antibody which will be detected by an enzyme-labeled secondary antibody. The presence of the antigen is determined by the development of colour in the solid phase which contains a chromogenic substrate (Sakamoto et al., 2018). In one study, avian antibodies (IgY) targeting the OmpW protein W and cytotoxin B (CtxB) antigens of *V. cholerae* were utilized to develop a sandwich ELISA. The specificity of this ELISA was determined by using bacterial reference strains which consisted of non-*Vibrio* species. Subsequently, both the α-OmpW and α-CtxB ELISA assays were used to detect the presence of *V. cholerae* and cholera toxin respectively. Both assays showed high specificity as only positive results were shown with *V. cholerae* and the cholera toxin, indicating no cross-reactivity occurred with the other tested bacteria (Bayat et al., 2018). Another monoclonal antibody-based ELISA was developed to detect pathogenic *V. parahaemolyticus,* from seafood. IgM antibodies that recognized the TRH protein of *V. parahaemolyticus* were used in the ELISA. The monoclonal antibodies were able to detect *tdh* and *trh* positive *V. parahaemolyticus* from seafood samples with a detection limit of 10^3 cells after 16 hours of enrichment. As the target for the antibodies is a secretory protein, only viable strains of *V. parahaemolyticus* were able to be detected by the assay (Kumar et al., 2011). Species-specific monoclonal antibodies for rapid detection of *V. vulnificus* have also been developed and based on Dot-enzyme linked immunosorbent assay, the limit of detection for these monoclonal antibodies were 10^5 to 10^7 CFU/mL. When compared to the detection limit of PCR (10^4 CFU/mL), the monoclonal antibodies were significantly more sensitive in successfully detecting *V. vulnificus* (Wangman et al., 2021). ELISA is a useful tool in identifying *Vibrio* species at high specificity and sensitivity, thus producing accurate and reliable results (Mai et al., 2020). The assay is also relatively easy to perform compared to conventional methods or PCRs, and a shorter time is required to obtain results.

ELISA can be incorporated with other detection techniques to detect *Vibrio* species. Seo et. al. developed an ELISA-on-a-Chip (EOC) biosensor system coupled with immunomagnetic separation in order to detect *V. parahaemolyticus* within a single working day. The ELISA uses IgG which targets the outer membrane proteins of *V. parahaemolyticus*. The assay was found to be highly specific as it only produced positive results for *V. parahaemolyticus* which indicates that it can discern *V. parahaemolyticus* from other bacterial species that were tested. This system was also able to minimize the risk of infection by the foodborne pathogen as well as shorten the time required to obtain positive results from the samples. The ELISA-on-a-Chip biosensor system could detect *V. parahaemolyticus* at a detection limit of 6.2×10^5 cell per ml, however this could only be achieved with at least one day of enrichment of the collected samples. Thus, they introduced an immunomagnetic separation technology (IMS) to shorten the time for enrichment and they were able to obtain results within six to nine hours depending on the inoculum size (Seo et al., 2010). This could potentially be very useful as the pre-enrichment step is commonly used to ensure enough bacterial cells are present to be detected. As pre-enrichment steps can be time-consuming, the introduction of IMS into ELISA can reduce a significant amount of time for pre-enrichment and results can be obtained more rapidly.

ELISA has high sensitivity and specificity as it utilizes the antigen-antibody reaction along with being highly efficient as simultaneous analyses can be done without complicated sample pre-treatment. It is also generally safe to use because large amounts of organic solvents are not needed. Though it can be labor-intensive and expensive as the preparation of antibodies is a complicated process that requires expensive culture cell media. False positives or negatives can also occur if the surface of microtiter plates immobilized with antigens are insufficiently blocked. As antibodies are proteins, they are vulnerable to degradation risk during transport and storage, thus antibody instability could be a contributing factor to inaccurate results of ELISA (Sakamoto et al., 2018).

During outbreaks or epidemic studies, Dot-ELISA is a good screening test to be used for rapid identification as it only requires a significantly shorter execution time compared to conventional ELISA, which requires 24 hours to execute. One study demonstrated that Dot-ELISA was able to detect all the *V. cholerae* toxin in supernatants of isolates from both human and environmental samples in a short amount of time. The Dot-ELISA developed is also 10 times cheaper than ELISA and the results can be interpreted by the naked eye, thus requiring less equipment than ELISA to analyze results. This Dot-ELISA had an analytical sensitivity of as low as 1 ng/mL of cholera toxin, thus making it very useful when detecting *V. cholerae* in epidemiological conditions in a rapid manner (Meza-Lucas et al., 2016). Nevertheless, when compared to PCR, immunoassays have been shown to have a lower sensitivity in *Vibrio* pathogen detection. For instance, to detect *V. parahaemolyticus* from seafood sources, a sandwich ELISA was developed with monoclonal antibodies, which target the TRH protein of *V. parahaemolyticus*. The ELISA was able to detect all the 30 pathogenic reference strains of *V. parahaemolyticus.* The sensitivity of this assay after 16 hours of enrichment was found to be 10^3 cells whereas the PCR method could detect 10^1 cells after the same amount of time for enrichment. This is because the sandwich ELISA could only detect viable *V. parahaemolyticus* as the secretory protein TRH is the target. The monoclonal antibody-based ELISA was then used to detect TRH and/or TDH in 34 naturally incurred seafood samples. A total of 22 (64.7%) samples were found positive for *V. parahaemolyticus*, six (17.65%) of the samples contained the *tdh* gene while *trh* gene was present in nine (26.47%) of the seafood samples. The results of these samples were examined with PCR, and the positive results (either tdh or trh) were the same as the ELISA except that the ELISA failed to detect one fish sample that was positive for *trh* gene. However, as the PCR assay was not able to differentiate between live and

dead cells, it can be suggested that both methods are used when screening for the presence of *Vibrio* species in seafood to accurately identify both living and dead bacterial cells even at a low cell number (Kumar et al., 2011).

4.2. Lateral flow immunoassay

Lateral flow immunoassays are able to identify pathogens (Pengsuk et al., 2021; Wangman et al., 2020) through the conjugation of antigens or proteins with monoclonal antibodies that are usually coupled to colloidal gold nanoparticles in the assay. This antigen-antibody immunoassay depends on the accumulation of colored reagents at the test and/or control regions to produce visible results. Lateral flow immunoassays include dipsticks and immunochromatographic strips. They are typically made up of four sections: a sample pad, conjugate pad, nitrocellulose membrane and an absorbent pad. The test sample fluid flows along the four sections via capillary action. The test sample will migrate from the sample pad towards the conjugate pad and encounter the conjugate such as antibodies or antigens labeled with a colored reagent. After the test sample mixes with the conjugate, it further travels along the nitrocellulose membrane where there are lines or sections with immobilized antibodies or antigens. Depending on the constituents of the sample fluid, the colored reagent will bind to the test zone. After two to ten minutes, the results can be visualized with the naked eye. Lateral flow immunoassays are very simple to use and do not require expensive equipment to obtain results in a short period of time. Thus, they can be very useful during outbreaks to quickly identify the causative *Vibrio* sp. or in POC settings where resources are scarce.

Lateral flow immunoassays can be combined with other detection techniques to detect *Vibrio* sp. To detect *V. alginolyticus*, a lateral flow dipstick (LFD) combined with LAMP to target the *rpoX* gene which is specific to the pathogen was deployed. Biotin-labeled LAMP reactions were performed using primers that targeted the *rpoX* gene. The LAMP reaction utilized DNA probes which were developed with fluorescein isothiocyanate (FITC). The lateral flow dipstick was able to detect biotinylated DNA products that had hybridized to the FITC-labeled DNA probe. The test results were presented as reddish-purple colour at the test line indicating a positive reaction. This LAMP-LFD method only obtained amplicons from *V. alginolyticus* during the LAMP reactions, thus indicating the primers used to target the *rpoX* gene of the foodborne pathogen was highly specific. PCR method targeting the *rpoX* gene produced the same results as the LAMP-LFD assay, further validating the specificity of the detection method developed in the study. The LAMP-LFD method was also more sensitive than that of the PCR method as it was able to detect *V. alginolyticus* in pure samples at $1.8x10^2$ CFU/mL compared to 1.8×10^3 CFU/mL in the PCR method. In the spiked whiteleg shrimp samples, the LAMP-LFD method was also more sensitive than the PCR method by one order (Plaon et al., 2015). Lateral flow immunoassays can also incorporate nucleic acids for the sequence-specific detection of single-stranded polymerase chain reaction (PCR) amplicons at the ambient temperature of 22-25°C. A study reported the detection of *V. cholerae* by using capture probes immobilized on the rapid nucleic acid lateral flow biosensor (NALFB) which can hybridize with the complementary fluorescein-labeled single-stranded amplicons. The positive results are in the form of visible red lines as conjugated gold nanoparticles bind to the fluorescein label of the captured amplicons. NALFB has been found to have a detection limit of 10 CFU/mL with 100% specificity and sensitivity for *V. cholerae* strains found in clinical samples. This serves as an easy-to-perform two-step-assay that is able to generate results in minutes (Ang et al., 2012).

In a separate study done by Wu et al., four nanomaterial: traditional colloidal gold (AuNP), new colloidal gold (N-AuNP), multibranched gold nanoflowers (AuNF) and luminol-reduced Au nanoparticles (L-AuNP) labeled with a monoclonal antibody against *V. parahaemolyticus* were used to fashion immunochromatographic strips to detect *V. parahaemolyticus* (Wu et al., 2021c). The study aimed to develop and compare the efficiency of each nanomaterial. The results of these strips were highly consistent with the PCR results in the actual samples in detecting *V. parahaemolyticus*. These immunochromatographic strips are highly stable during storage for up to 14 weeks in laboratory conditions. Although AuNP is commonly used in lateral flow immunoassays as it can be easily conjugated with antibodies, these traditional AuNPs can cause oily floating matter in the solution, indicating the structure is unstable and finally precipitating out after a period of time. Thus, a more suitable nanomaterial is needed to overcome these disadvantages. In their study, when comparing the sensitivity of the four nanomaterials, the N-AuNP-labelled strips had a higher consistency and sensitivity and better stability than the others. N-AuNP also required fewer antibodies and gold-labeled antibodies to work. The study concluded that N-AuNP is the best candidate for labels in lateral flow immunoassays because they are more effective, rapid, and affordable to be deployed on-site. A novel bacterial coloration immunofluorescence strip (BCIFS) for antibody detection was recently developed by Fang et al. The strip was designed to utilize inactivated colored bacteria as the tracer and goat anti-mouse IgG was used as the test line. Once BCIFS is inserted into the microwell, the monoclonal antibodies in the sample will first bind to bacteria-labeled FITC on the conjugate pad. The complex will then flow through the pad via capillary action and will be captured by goat anti-mouse IgG on the test line, resulting in the formation of a tetramer goat anti-mouse IgG which will show up as a green line on the test strip. This method was found to be highly specific and 4-fold more sensitive than indirect ELISA (Fang et al., 2022).

Disptick-based forms of immunoassays are simple, user-friendly, and cost-effective. It has been shown that these strips involve minimal labour and expenses. Material costs of each strip was reported to be less than THB 25 (USD0.80), compared to the culture method which costs THB 200-300 (USD 6.45-9.68) (Thattiyaphong et al., 2013). In another report, a dipstick test kit cost 19 USD and contains 10 test strips which make up to 1.90 USD per test (Rashid et al., 2017). In addition, as dry reagents are typically used in lateral flow immunoassays, a cold-chain is not required during transportation or storage which makes it useful during *Vibrio* outbreaks in poor resource settings. Thus, it is an appealing approach to be used in point-of-care settings and in places where laboratory resources are lacking.

5. Biosensor-based methods

Biosensors are useful analytical devices that are made up a bioreceptor and a transducer. Bioreceptors can be biological material (antibodies, enzymes, nucleic acids), biologically derived material (aptamers, recombinant antibodies), or a biomimetic (imprinted polymers, synthetic catalysts). Biosensors are further categorized into optical biosensors, electrochemical biosensors, and mass-based biosensors. They are highly sensitive, low-cost, user friendly, portable, and allow for multi-pathogen detection and real-time analysis of samples (Da-Silva et al., 2018; Law et al., 2015; Zhao et al., 2014). Therefore, biosensors can be deployed during *Vibrio* outbreaks to accurately identify the causative species.

Optical biosensors are compact analytical devices with high specificity and sensitivity in detecting foodborne pathogens. They are made up of a bioreceptor with an optical transducer system (Da-Silva et al., 2018; Zhao et al., 2014). *Vibrio* species such as *V. cholerae, V. alginolyticus,* and *V. parahaemolyticus* have been detected in various studies by utilizing optical biosensors (Liu et al., 2014; Park and Choi, 2017; Taheri et al., 2016). An example of an optical biosensor is a surface plasmon resonance (SPR) biosensor that has been used to detect *Vibrio* species (Ahn et al., 2018; Taheri et al., 2016). It is widely used to detect foodborne pathogens as it is a rapid, sensitive method which provides real-time analysis of samples (Taheri et al., 2016). In SPR, bioreceptors are immobilized on the surface of a thin metal film to detect the target pathogens. The electromagnetic radiation of a specific wavelength will interact with the electron cloud of the thin metal to produce a strong

resonance. As the target pathogen binds to the metal surface, there is a shift in the resonance whereby the shift will determine the concentration of bound pathogens (Zhao et al., 2014).

Electrochemical biosensors include amperometric, potentiometric, impedimetric, and conductimetric biosensors. They are dependent on the antigen-bioreceptor interactions, which affect the changes in current, voltage, impedance, and conductance respectively. They have also been used to detect various *Vibrio* species as they are rapid, easy to use, low cost and easily miniaturized (Laczka et al., 2014; Sha et al., 2016; Tam and Thang, 2016). However, low cell count of target pathogens and food matrices present in the sample may interfere with an accurate analysis. Sha et al. developed a one-step label-free electrochemiluminescence (ECL) immunosensor to detect *V. parahaemolyticus* in seawater and seafood. The ECL immunosensor was determined to be highly specific, stable and reproducible for the detection of *V. parahaemolyticus*. The detection limit of this biosensor for the pathogen was 5 CFU/mL for seawater and 5 CFU/g for seafood, further indicating that the ECL biosensor is highly sensitive in detecting *V. parahaemolyticus* even at very low cell counts (Sha et al., 2016).

Mass-based biosensors are able to detect target pathogens by using piezoelectric crystals to detect small changes in mass. When electric signals of specific frequency are induced, the crystal vibrates at a certain frequency. Bioreceptors are immobilized on the crystal and once there is a binding of target antigens to the bioreceptors, there is an increase in the crystal mass causing a measurable frequency shift. The results can then be interpreted to determine the identity of the bacterium in the test sample (Velusamy et al., 2010). A study done in Korea reported a piezoelectric immunosensor which was able to detect *V. vulnificus* within 5 minutes by monitoring frequency shifts in real time (Hong and Jeong, 2014).

Fluorescence resonance energy transfer (FRET) aptasensors are a type of biosensor that employs fluorescence substances as signal sources that exhibit higher signal intensity which easily label aptamers (Zhao et al., 2018). A FRET biosensor was developed for *V. parahaemolyticus* detection using hybrid chain reaction (HCR). This biosensor detected *V. parahaemolyticus* isolates at 10 CFU/mL in water samples within 90 minutes (Ren et al., 2019). Recently, Hong et al. developed a turn-on-type FRET aptasensor for *Vibrio* detection, which uses an aptamer-modified polyhedral oligomeric silsesquioxane-perovskite quantum dots (POSS-PQDs-Apt) as signal probes while titanium carbide (Ti3C2) MXenes as quenchers (Hong et al., 2021). This turn-on-type FRET aptasensor consists of an aptamer probe as a fluorescence source and another quench probe. The aptamer used in this detection method is a sequence of DNA/RNA fragments with a high affinity towards targets to make the ultrasensitive aptasensors by DNA amplification (Zhang et al., 2015c). When used to detect *Vibrio* species, the aptasensor developed by Hong et al. could detect *V. parahaemolyticus* at 10^3 CFU/mL as the fluorescence could almost all be recovered after incubation of the bacteria and the composite probes for 50 minutes (Hong et al., 2021). This sensor was also able to detect *V. parahaemolyticus* in spiked aquatic waters with good precision as the relative standard deviation (RSD) for inter and intra tests of 10^3 CFU/mL of *V. parahaemolyticus* were 2.7% and 6.7%. This aptasensor also had a high specificity towards *V. parahaemolyticus* as a strong fluorescent signal was present when the foodborne pathogen was present while other bacteria only triggered weak to no fluorescence signal. Furthermore, the aptasensor has high storage stability as it still maintained approximately 92% of its initial intensity after being stored at 4°C for a week. This aptasensor is not only highly specific and sensitive in detecting *V. parahaemolyticus*, but it also has good stability and anti-interference capacity in aquaculture environments.

6. Recent advancement in rapid detection methods

There is a need for new and emerging rapid detection methods that work based on different principles and mechanisms to be applied for use in various settings to detect *Vibrio* species. Better sensitivity, specificity, affordability, and simplicity of new detection techniques could simplify the species confirmation process allowing for more rapid diagnosis and treatment or control measures. A simpler rapid detection technique based on spot dye-based sensors was developed to detect *Vibrio* load in shrimp culture farms (Kumar et al., 2021). The test kit involves only two steps and is generally easy to operate without skilled personnel. First, a selective media is added into the water sample, which selectively promotes the growth of *Vibrio,* and the growth of other bacteria is inhibited. Next, the dye-based sensor is added into the sample to detect the active *Vibrio* present in the water sample, whereby a positive result is shown with the change in colour of the sample to red or pink. The dye previously developed by the research group comprises tetrazolium dye and a carbon source for the detection of live bacteria in different water sources (Kumar et al., 2018). The optimum incubation time was chosen to be at 15 minutes as too short of an incubation time would not inhibit the growth of non-targeted bacteria, while prolonged incubation might yield inconsistent results. This highly specific kit was able to successfully determine the concentration of *V. cholerae, V. parahaemolyticus, V. campbellii, V. harveyi,* and *V. proteolyticus* in the aquaculture farms. The kits can specifically detect *Vibrio* species as other bacteria such as *Escherichia coli* and *Bacillus subtilis* were not detected with the kit. The principle behind this spot-dye test kit is simple, efficient, affordable, and does not require any skilled personnel for the operation. It is also a sensitive detection method as it can detect low concentrations of *Vibrio* sp. at 10^2 CFU/mL within six to seven hours (Kumar et al., 2021).

In recent years, novel rapid detection techniques have been developed to target the clustered regularly interspaced short palindromic repeat (CRISPR) and CRISPR-associated (Cas) proteins immune defence system. It is a naturally-occurring defence mechanism in bacterial species to fight against foreign DNA such as phages and plasmids (McDonald et al., 2019). A CRISPR-Cas system contains three functional components, namely the *cas* genes, a leader sequence and a CRISPR array. CRISPR-Cas systems are categorized into two classes according to Cas protein content and arrangements in CRISPR-Cas loci. The types of CRISPR-Cas systems are further differentiated based on the type of CRISPR RNA (crRNA)-effector protein complexes that are utilised, and thus far six types have been identified (McDonald et al., 2019). Class I systems use a multi-subunit crRNA–effector complex in which a large protein complex is formed from the binding of Cas proteins to crRNA. This large subunit complex can bind to crRNA to target complementary foreign DNA and cleave the targeted sequences via its nuclease ability (Garneau et al., 2010; Wiedenheft et al., 2011). On the other hand, Class II systems have only one protein such as Cas9, Cas12, and Cas13 which function similarly as the multiprotein effector complex in Class I systems (McDonald et al., 2019). CRISPR-Cas systems can be combined with other amplification methods to produce rapid diagnostic capabilities of high sensitivity and specificity (Zhang et al., 2020).

Zhang et al. reported successful detection of *V. parahaemolyticus* with CRISPR-Cas12a assisted PCR for on-site application with the end products easily visualised by the naked eye (Fig. 1). The team utilised CRISPR-Cas12a system in the assay under the guidance of a specifically designed crRNA which targets the amplification sequence. In the CRISPR-Cas12a system, the Cas12a enzyme recognises a short T-rich protospacer-adjacent motif (PAM) which results in the crRNA maturation, forming an enzyme-RNA complex and ultimately causing a PAM-distal double stranded DNA breakage (Zetsche et al., 2015). Subsequently, once the crRNA binds to the target DNA, the single stranded DNA will be cleaved by the Cas12a enzyme, producing target amplicons (Singh et al., 2018). To better visualise the end products of the reaction, a fluorophore quencher (FQ)-labeled reporter was employed. As the crRNA binds to the target DNA, the FQ-labeled reporter would be cleaved by the Cas12a enzyme, generating obvious green fluorescence for target amplicons. Upon testing with spiked shrimp samples with gradient concentrations of *Vibrio parahaemolyticus*, this detection method was found to have a detection limit of 10^2 to 10^6 CFU/g. There

was little difference in observation of the fluorescence generated after 5 minutes of incubation. Moreover, after 40 minutes of incubation, there was similar intensity of fluorescence for all concentrations of spiked samples (Zhang et al., 2020). This shows that the method is sensitive in detecting *V. parahaemolyticus* even at low concentrations. Moreover, this method is advantageous as it uses a mini thermal cycler which reduces the need for bulky, expensive instruments. The compatibility and portability also make it a useful tool for on-site application. Besides, a Cas12a-assisted rapid isothermal detection (CARID) system was also developed for the detection of toxigenic *V. cholerae* serogroups O1 and O139 by combining CRISPR-Cas and recombinase-aided amplification. CARID was shown to be able to rapidly produce results within an hour and the results were easily visualized using fluorescent signals or lateral flow dipstick which simplifies the user's experience. CARID was able to detect the *ctxA* gene of *V. cholerae* and identified the isolates with a high specificity and sensitivity with a minimum detectable genomic concentration of 20 copies/reaction (Lu et al., 2022).

All the methods described were able to detect a variety of *Vibrio* sp. either by using primers that target a wide array of genes in the *Vibrio* species or using antibodies to target the pathogens or the protein they produce. These rapid detection methods can be combined to further improve diagnostic capability, sensitivity, specificity, ease of use, and efficacy (Chen et al., 2021c; Wu et al., 2021a; Wu et al., 2021b; Xiao et al., 2021; Xing et al., 2020; Yang et al., 2021a; Yang et al., 2021b; Ying et al., 2021). For instance, the combination of RPA and CRISPR-Cas12a was effective in detecting *pirA* and *pirB* produced by *V. parahaemolyticus*. In order to improve the usability and visualize the results from this assay, a lateral flow strip was added. The assay can be easily completed in two hours without cross-reactivity with other pathogens (Li et al., 2022). LAMP has also been combined with LFD to better visualize the results from the detectin of *V. parahaemolyticus* in aquatic foods (Hu et al., 2021). Table 3 illustrates examples of the various methods and their respective sensitivity, specificity, and detection limits when identifying *Vibrio* sp. Each detection method has its advantages and disadvantages that should be taken into consideration before being deployed for use in identifying these bacterial isolates. The sensitivity, specificity, efficiency, cost, and time required for the respective methods are important factors to be considered depending on the varying circumstances and the amount of samples needed to be assessed at one time. This is to ensure that the best method suited for the situation can be used to obtain results in an efficient and timely manner. The summary of the advantages and limitations of each detection method is illustrated in Table 3.

Table 3
Summary of the advantages and disadvantages of each detection method.

Detection method	Advantages	Disadvantage
Simple PCR	• High sensitivity and specificity • Reliable results • Automated	• Time-consuming due to gel electrophoresis • PCR inhibitors hinder detection • Requires skilled personnel
Multiplex PCR	• High sensitivity and specificity • Reliable results • Automated • Simultaneous detection of multiple *Vibrio* species	• Time-consuming due to gel electrophoresis • PCR inhibitors hinder detection • Requires skilled personnel • Potential formation of primer dimers causing unreliable results
Real-time/ Quantitative PCR	• High sensitivity and specificity • Reliable results • Automated • Can be multiplexed for simultaneous detection of multiple *Vibrio* species • Does not require gel electrophoresis • Real-time analyses of amplification products	• PCR inhibitors hinder detection • Requires skilled personnel • Expensive • Potential for cross contamination
LAMP	• High sensitivity and specificity • High throughput • Can be multiplexed for simultaneous detection of multiple *Vibrio* species • Does not require processing of post-amplification products • Results easily visualized • Does not require thermal cycler • Compatible with other detection methods to better detect *Vibrio*	• Complicated primer design • Unsuitable for detection of unknown or *de novo* targets
RPA	• Short amplification time • Able to be multi-plexed • Sensitive • Low incubation temperature required • Exponential amplification	• Background noise can affect results • Requires purification following amplification
RCA	• Short amplification time • Low incubation temperature required • Power saving	• Unable to be multiplexed • Linear amplification • Complex primer • Only works with circular nucleic acid template
Microarray	• High sensitivity and specificity • High throughput • Simultaneous detection of multiple *Vibrio* sp. • Allows detection of specific serotype • Less labour intensive	• Requires skilled personnel • Requires specifically designed probes and labelling of target genes • Presence of background fluorescence of array substrate • Potential hybridization of noncomplementary or partially matched DNA causes inaccurate results
ELISA	• High sensitivity and specificity • Can be automated • Time efficient • Direct detection of *Vibrio* toxins • Suitable for large sample sizes	• Require skilled personnel • Requires labelling of antibodies and antigens • False positive or false negative results • Antibody instability may cause unreliable results • Potential cross-reactivity with closely related antigens

(*continued on next page*)

7. Conclusion

The ground-breaking developments and advancements made in microbiology have successfully improved the detection of *Vibrio* sp. from various sources. The constant improvements in molecular detections have facilitated progressively higher throughput analysis with high accuracy and sensitivity, which in turn leads to reduction in cost-per-sample and allows higher number of samples to be processed for pathogen detection. These rapid detection methods come with the potential for automation and customization options, molecular techniques which dramatically reduce turnaround time for results. Although they may bring many benefits and advantages, these techniques do possess certain limitations, especially pathogen amount and nucleic acid integrity (e.g., degradation, presence of PCR inhibitors). For instance, samples with very low bacterial counts may need to be subjected to a pre-enrichment step prior to detection. This may expose samples to further contamination risk, but this can be prevented/eliminated with personnel training and an appropriate sample management system (Blanco-Abad et al., 2009). In summary, each rapid detection method has its advantages and disadvantages, which should be considered when choosing the most appropriate method for screening for *Vibrio* sp. isolates depending on the nature of the samples and circumstances. Combining conventional

Table 3 (*continued*)

Detection method	Advantages	Disadvantage
Lateral Flow Immunoassay	• High sensitivity and specificity • Time efficient • Direct detection of *Vibrio* toxins • User friendly • Low cost • Suitable for low resource areas	• Requires labelling of antibodies and antigens
Optical Biosensor	• High sensitivity and specificity • Real-time detection of *Vibrio* sp. • Automated • Label-free detection system	• Expensive
Electrochemical biosensors	• High sensitivity and specificity • Automated • Suitable for large sample sizes	• Unsuitable for samples with low concentration of *Vibrio* • Food matrices may hinder analyses • Many washing steps (Zhao et al., 2014)
Mass-based biosensor	• Real-time detection of *Vibrio* sp. • Time efficient • Ease of use • Label-free detection	• Low sensitivity and specificity • Many washing and drying steps • Regeneration of surface may be problematic (Zhao et al., 2014)
Spot-dye based sensors	• High sensitivity • Ease of use • Time efficient • Cost effective • Does not require skilled personnel • Suitable for low resource areas	• Low specificity
CRISPR/Cas-PCR systems	• High sensitivity and specificity • Time efficient • Cost efficient • Suitable for low resource areas	• Requires skilled personnel • Small sample sizes

detection methods and molecular detection methods can be beneficial in studying the ecology and epidemiology of the organisms that are causing diseases.

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Compliance with ethics requirements

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Ke-Yan Loo: Formal analysis, Resources, Writing – original draft, Writing – review & editing. **Jodi Woan-Fei Law:** Supervision, Writing - review & editing. **Loh Teng-Hern Tan:** Methodology, Visualization, Writing – review & editing. **Priyia Pusparajah:** Writing – review & editing. **Vengadesh Letchumanan:** Validation, Supervision, Writing - review & editing. **Learn-Han Lee:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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