Chapter

Molecular Diagnostic Platforms for Specific Detection of *Escherichia coli*

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Abstract

Developing countries due to socio-economic conditions are more prone to frequent pathogenic outbreaks; inadequate sanitation and water quality monitoring are also responsible for such conditions. Therefore, it is of paramount importance to provide microbiologically safe food/water in order to protect public health. Several flaws in traditional culturing methods have sparked a surge in interest in molecular techniques as a means of improving the efficiency and sensitivity of microbiological food/water quality monitoring. Molecular identification of water contaminants, mainly *Escherichia coli*, has been extensively used. Several of the molecular-based techniques are based on amplification and detection of nucleic acids. The advantages offered by these PCR-based methods over culture-based techniques are a higher level of specificity, sensitivity, and rapidity. Of late, the development of a biosensor device that is easy to perform, highly sensitive, and selective has the potential to become indispensable in detecting low CFU of pathogenic *E. coli* in environmental samples. This review seeks to provide a vista of the progress made in the detection of *E. coli* using nucleic acid-based approaches as part of the microbiological food/water quality monitoring.

Keywords: molecular diagnostics, *E. coli*, PCR, LAMP, CRISPR

1. Introduction

Public health protection is of paramount importance that demands the rapid and accurate detection and quantitation of microorganisms in potable water and in various raw and processed foods to prevent undesirable outbreaks of microbial contamination. Water quality has been assessed for potable and recreational activities using culture-dependent quantification and sensing of fecal indicator bacteria (FIB), such as total coliforms, *Escherichia coli*, or Enterococci, an approach that is used as a reference standard in the evaluation of microbial safety of water [1]. The presence of FIBs in large numbers in freshwater, particularly *E. coli* and *Enterococcus*, has been associated with the emergence of waterborne illnesses [2, 3]. Children as young as five years are particularly susceptible to diarrheal infections, with over 800 children dying every day [4, 5]. Amongst coliform bacteria, *E. coli* is commonly regarded as an indicator of fecal pollution of water supplies [6, 7].

Waterborne diseases have been one of the major causes due to the consumption of contaminated water affecting seriously the public health of a humongous number of people in quick succession. In the 2014–2016 survey, the detection

rate of pathogenic bacteria was 79.3%, followed by pathogenic *E. coli* (5009 cases, 90%), *Vibrio* spp. (264 cases, 5%), *Shigella* spp. (67 cases, 1%), and *Salmonella* spp. (48 cases, 1%) [8]. The distribution of *E. coli* amongst Korean children suffering from diarrheagenic *E. coli* showed that enteropathogenic *E. coli* (EPEC) was the most common, followed by ETEC and enterohemorrhagic *E. coli* (EHEC) [8]. Of the pathogenic *E. coli*, enteropathogenic *E. coli* (EPEC) was the most common (39%), followed by enterotoxigenic *E. coli* (ETEC) (36%). In a separate study, children suffering from diarrhea were reported [9] in Utah, USA wherein the most commonly detected pathogens included toxigenic *Clostridium difficile* (16%) and diarrheagenic *E. coli* (15%) whereas Shiga toxin-producing *E. coli* were detected in 4% samples [9].

Between 2013 and 2016, a monocentric hospital-based investigation showed that *E. coli* was responsible for about 15% of child infection cases of severe enteritis and EPEC (54%) was the most dominant *E. coli* pathotype, followed by other pathogenic *E. coli* including Shiga toxin-producing *E. coli* [10]. And on the heels of that, in another waterborne outbreak of *E. coli* infection associated with the drinking of contaminated potable water at three different school premises in Korea was reported [11]. As a result of this outbreak, a total of 188 patients with severe gastrointestinal symptoms were reported. The EHEC and EPEC strains isolated from clinical fecal specimens and water samples from water purifiers and water basins respectively were confirmed by the pulsed field gel electrophoresis method [11]. It is warranted therefore to develop rapid and sensitive methods for the detection and quantitation of waterborne bacteria.

Coliforms, particularly *E. coli* is regarded as a primary fecal indicator [12] and indicate the contaminating presence of enteropathogenic bacteria in water and foods supplies [13]. Though these enteric bacteria are abundant in human and warm-blooded animal feces, an umpteen number of the *E. coli* strains have been reported as pathogens [14]. Despite the fact that the wild type of *E. coli* strain is not pathogenic, it could emerge as an infectious agent in immunologically vulnerable people. Furthermore, several *E. coli* O157:H7 outbreaks have been documented in both industrialized and developing economies, resulting in human mortality, notably amongst children under the age of five [15]. *E. coli* serogroup O157:H7 is the most common cause of hemorrhagic colitis in foodborne illness. *E. coli* serogroup O104:H4 was first discovered as an emerging strain in the 2011 German pandemic and was designated a microorganism of serious concern [16]. Perna et al. [17] reported that *E. coli* O157:H7 caused 75,000 cases of foodborne infections per year, of which 85 percent incidences were related to *E. coli* O157 infections [18, 19] with contaminated fruits, vegetables, and water is the principal sources of *E. coli* O157:H7 outbreaks [19].

Traditional microbiological detection techniques consume time as *E. coli* cells require to be isolated, cultivated, and identified using a sequence of biochemical tests [20]. For example, for identification and quantification of *E. coli* in water, the water samples are filtered using the membrane filtration method, followed by the counting of *E. coli* colonies using the plate count method [21]. Furthermore, such processes necessitate 24 to 48 hours to generate observable results and frequently require water samples to be transported to a central laboratory and trained employees to conduct the testing [22].

It is necessary to develop new approaches for detecting *E. coli* in contaminating food and water samples. Optical or impedimetric biosensor systems have evolved as an alternative to the traditional tools for *E. coli* detection, enabling selective, specific, and cost-effective solutions. DNA-based sensing approaches have played an essential role in the development of sensing for the detection of *E. coli*. Due to their rapidity and accuracy, sensing technologies such as the polymerase chain reaction

(PCR), loop associated isothermal method of amplification (LAMP), DNA-based biosensors, and CRISPR/Cas platforms have evolved over time for *E. coli* detection and have been applied in numerous applications in various industries, agriculture, and health care sectors.

2. Polymerase chain reaction (PCR) method

PCR being a mighty and handy tool with molecular biologists showed enormous potential in various forms including multiplex PCR and quantitative real-time PCR. The advantage of PCR is that despite its inability to distinguish between live and dead cells, nonculturable cells may be detected rapidly. In the recent two decades, various PCR-based strategies have been introduced to improve the detection of indicator organisms [23, 24]. Genetic markers such as 23S rRNA and lacZ are often used to establish PCR tests for detecting *E. coli* in environmental samples [25, 26]. The uidA and tuf genes have been identified as potential targets for *E. coli*/Shigella detection using PCR [27, 28]. Most of the PCR assays were reported to amplify the virulence genes, such as *eaeA*, and *stx1, stx2* [29–33] or phenotypic genes, such as *rfbE* (O antigen), and *fliC* (H antigen), *uidA* and *lacZ* which are commonly shared [26, 28, 32]. The ability to generate these lesions is restricted to 43-kb loci of the *E. coli* O157:H7 chromosome [17]. Intimin encoded by eae locus is necessary for early bacterial cell attachment to host cells and the creation of A/E lesions [34, 35]. In a couple of studies, virulence genes like *stx1* and *stx2* were unable to accurately identify a species, owing to the fact that they are widely shared by different species or strains [33]. *Shigella dysenteriae* and *Aeromonas* spp. have been described as the two outliers as non-*E. coli* bacteria bearing Shiga toxin genes [36, 37]. Real-time PCR techniques targeting *Shigella* spp. in food or water utilizing *ipaH* as a target have also been developed to detect enteroinvasive *E. coli* (EIEC) that carries ipaH [36]. Therefore, phenotypic genes such as *rfbE* and *fliC* have been utilized as targets for confirmed identification of *E. coli* in PCR [30].

The *E. coli* genes such as uidA and tuf were used for the detection of *E. coli* and Shigella strains [27, 38, 39]. However, the uidA gene used as a marker was not reported in 3.4% of 116 *E. coli* strains [37]. In another work, Maheux et al. [27] detected Escherichia fergusonii in a PCR targeting the tuf gene. Albeit, it has been extensively reported, neither β-D-glucuronidase activity nor *uidA* gene amplification is the full proof for the accurate molecular detection *E. coli* in the presence of this enzyme or gene has been reported in Flavobacteria and to a great extent in *Shigella, Salmonella* and *Yersinia* [38, 40, 41]. Contrarily, Fricker & Fricker [42] using uidA primer pair detected five non-*E. coli* coliforms in water samples. Recently, Molina et al. [40] designed a set of primers targeting the *E. coli* orphan gene *yaiO* that encodes an outer membrane protein and succeeded in obtaining the *yaiO* amplicon of 115 bp size from unfermented and fermented dairy samples. These workers in terms of specificity claimed superiority of *yaiO* gene-based primers to uidA primers though the study was limited by small sample size. In another recent study, the xanQ-PCR using novel primer set for amplification of *xanQ* gene was demonstrated for specific detection of a large number of *E. coli* strains [41].

Li et al. [43] established a multiplex real-time PCR test that targets the *z3276* and Shiga toxin genes to specifically detect *E. coli* O157:H7 and screen for non-O157 STEC (*stx1* and *stx2*). The reaction mixture contained a primer set; four probes (*z3276, stx1, stx2,* and IAC), and the template DNA of appropriate concentrations. The optimized multiplex assay achieved the limit of detection (LOD) as low as 200 femto grams of bacterial DNA from beef and fresh spinach samples (40 CFU/reaction). In a separate study, a multiplex fluorogenic PCR assay was developed to

quantify *E. coli* O157:H7 in manure, soil, dairy wastewater, and cow and calf feces in an artificial wetland. Oligonucleotides were designed to amplify the *stx1* and *stx2* and the *eae* genes of *E. coli* O157:H7 in a simplex reaction [44].

Being a rapid, sensitive, and specific method enabling the detection of multiple pathogens simultaneously this method finds applications in different types of foods and poultry industries. Nguyen et al. [45] developed a multiplex PCR for the rapid and simultaneous detection of three epidemic food-borne pathogens: *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* in food samples.

In developing countries, the identification of enteric pathogens in food and other edible items are time-consuming process and often results in wrong and delayed diagnosis. Enteropathogenic *E. coli* (EPEC) has been reported to be frequently associated with outbreaks of infantile diarrhea and recognized as a causative agent for diarrheagenic ailments [46]. In order to detect and identify the Shiga toxin producing *E. coli*, enterohemorrhagic *E. coli* (EHEC), and EPEC primers were designed to amplify *eae* gene and long polar fimbriae (*lpfA*) variants, the bundle-forming pilus gene *bfpA*, and the Shiga toxin-encoding genes *stx1* and *stx2* [47]. This group demonstrated consistent amplification of genes specific to the prototype EHEC O157:H7 EDL933 (*lpfA1–3, lpfA2–2, stx1, stx2*, and *eae-γ*) and EPEC O127:H6 E2348/69 (*eae-α, lpfA1–1*, and *bfpA*) strains using the optimized mPCR protocol with purified genomic DNA (gDNA). A screen of gDNA from isolates in a diarrheagenic *E. coli* collection revealed that the mPCR assay was successful in predicting the correct pathotype of EPEC and EHEC clones grouped in the distinctive phylogenetic disease clusters EPEC1 and EHEC1, and was able to differentiate EHEC1 from EHEC2 clusters. The mPCR assay detection threshold was 2×10^4 CFU per PCR reaction for EHEC and EPEC. Thus, mPCR methodology permitted differentiation of EPEC, STEC, and EHEC strains from other pathogenic *E. coli* and the developed assay has the potential tool for rapid diagnosis of these pathogens. Wang et al. [48] demonstrated the ability of the mPCR assay to detect six bacterial pathogens viz., *E. coli*, *Pasteurella multocida, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella* spp. and *Staphylococcus aureus* in liver, spleen, and blood samples from experimentally infected chicks without cross-amplification with viruses or parasites. In the mPCR assay, gene targets were *phoA, KMT1, ureR, toxA, invA*, and *nuc* of these six pathogens, and six sets of specific primers were designed.

Toma et al. [49] used a single-tube mPCR for the identification of enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), and Shiga toxin-producing *E. coli* (STEC). In total six targets were chosen for (*eae*) enteropathogenic *E. coli*, (*stx*) Shiga toxin-producing *E. coli* enterotoxigenic *E. coli*, elt, and est. for enterotoxigenic *E. coli*, (*ipaH*) for enteroinvasive *E. coli* for, and *aggR* for enteroaggregative *E. coli*.

Chen et al. [50] developed a multiplex rtPCR assay for the identification of diarrheagenic *E. coli* (DEC) and claimed it to be a highly sensitive and specific and suggested the rapid identification of DEC in clinical and public health laboratories. Specific virulence genes were selected to identify specific pathogens: *ipaH* for EIEC, *stp/sth/lt* for ETEC, *eaeA/escV* for EPEC, *stx1/stx2* for EHEC, *aggR* for EAEC. The 5′ end of primers were added with a homo tail sequence to reduce the primer dimer formation and the addition of homo tail to 5′ end of primer sequences allowed proper annealing temperature that would fall into broad range in each individual PCR reaction. Molecular beacons were modified and designed using DNA folding form website (http://mfold.rit.albany.edu/?q=mfold/DNA-Folding-Form) [50]. Five categories of DEC were split into two tubes. For tube number one, *stp/sth/lt* for ETEC, *aggR* for EAEC and IAC were included, while *ipaH* for EIEC, *eaeA/escV* for EPEC, *stx1/stx2* for EHEC and IAC were included in tube number two. Carboxy fluorescein (FAM), Hexachloro fluorescein (HEX), Carboxy-X-rhodamine (ROX),

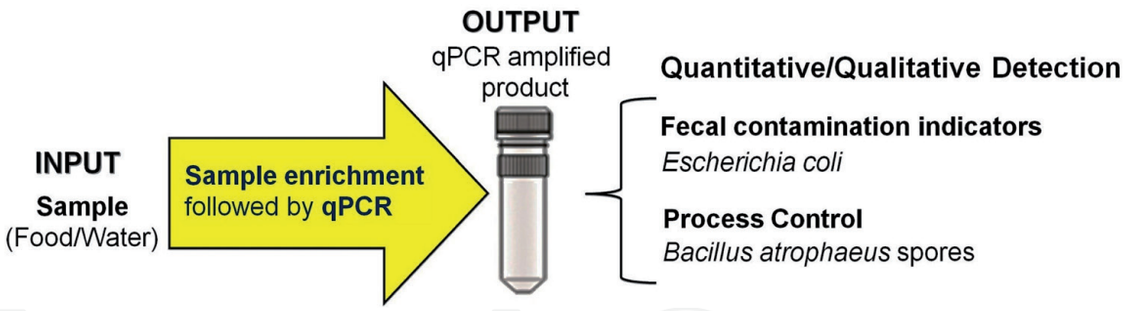

Figure 1.
Schematic depicting the steps in culture-independent detection of E. coli *in a sample using qPCR method. Bacillus atrophaeus Spores are used as an internal control for monitoring of possible PCR inhibition [52].*

Quasar 705, and indodicarbocyanine5 (Cy5) fluorescence were collected and recorded at the end of the annealing step during the third stage.

Detection of harmful bacteria with higher specificity, sensitivity, and reliability is the focus of nucleic acid-based approaches. The desired nucleic acid sequence is hybridized to a synthetic oligonucleotide for specific detection of the pathogen [51]. Nucleic acid-based approaches are routinely used to detect bacterial infections and their toxin-producing genes [51]. Nucleic acid-based methods are rapid and easy to use, and they do not require the pathogens to be cultured (**Figure 1**).

Even a decade ago, the identification and measurement of specific target genes with absolute accuracy and as little as a few copies in a matter of hours was a dream. In the area of water quality assessment, however, qPCR technology has proven to be a powerful technique [53]. Unlike the classical PCR, which needs agarose-gel electrophoresis to identify the end-point PCR products, the qPCR enables assessing PCR product amplification by measuring fluorescence signals released by specialized dual-labeled probes or the intercalating dyes. The fluorescence intensity generated during the qPCR is directly related to the quantity of PCR products produced [12, 54, 55]. The most often used fluorescent systems for qPCR include SYBR green, TaqMan probes, and molecular beacons [56]. The qPCR techniques, which have higher specificity, sensitivity, and reliability than classic culture methods and mPCR [57], allow for the time-efficient detection of harmful bacteria with higher specificity, sensitivity, and reliability [12, 56, 58]. Although the qPCR has been used to detect and quantify *E. coli* O157:H7 in food and clinical samples, it has not been thoroughly evaluated with environmental samples [57, 59, 60].

Utilizing TaqMan probes labeled with different fluorophores, microfluidic qPCR was shown to identify pathogens such as *Listeria monocytogenes, Vibrio cholerae, Vibrio parahaemolyticus, Pseudogulbenkiana* spp., *Salmonella typhimurium, Shigella flexneri, Clostridium perfringens*, and *E. coli* at a limit of detection of 100 CFU/L [56, 61]. Despite its high sensitivity, qPCR has significant drawbacks, such as the inability to provide information on the physiological status of target cells in environmental samples. Humic substances found in environmental samples such as water hinder DNA polymerase activity, and colloidal debris has been reported to have a DNA affinity [62, 63]. There is no universal answer to avert such problems. As a result, the existence of these compounds in environmental samples has the potential to adversely affect the amplification effectiveness of qPCR, which is used to detect small quantities of bacteria [60]. To overcome these issues in qPCR, several compounds such as bovine serum albumin, methoxsalen, dimethyl sulfoxide, and internal amplification controls have been proposed. However, these approaches may have certain drawbacks as well as benefits [64, 65]. Walker et al. [63] established a new qPCR technique for detecting and quantifying *E. coli* that targeted a segment of the ybbW gene, which encodes a potential Allantoin transporter. The *ybbW* gene

is part of the *E. coli* "core genome," which means that each gene is found in >95 percent of all sequenced strains. For this work, water samples were taken at monthly intervals from different locations in the southwest of England. The *ybbW*-qPCR was found to be 100% specific towards 87 *E. coli* strains tested. This work also reported that despite the theoretically low detection levels achievable by qPCR, the quantity of *E. coli* DNA has been the key issue in limiting the detection in real samples. This could be addressed in part by filtering greater quantities of water samples, but this is likely to be unfeasible for regular sample analysis and could result in the accumulation of higher inhibitory substance quantities.

In another study, Liu et al. [66] reported designing of the novel oligonucleotide primer set and TaqMan probes targeting the specific virulence genes of twelve common food pathogens such as *E. coli* O157:H7, *Salmonella enterica, L. monocytogenes/ivanovii*, β-*Streptococcus hemolyticus, Enterococcus faecalis, Yersinia enterocolitica, Shigella sp., P. mirabilis, V. fluvialis, V. parahaemolyticus, S. aureus* and *Campylobacter jejuni*. Liu et al. [66] reported the use of TaqMan in artificially spiked dilution series of each pathogen into meat to detect 12 strains. The TaqMan assays demonstrated expected amplification with no amplification inhibition. In spiked food samples, *V. parahaemolyticus* was found in concentrations ranging from 10^3 to 10^7 CFU/g, while the remaining 11 strains were from 10^4 to 10^7 CFU/g. The qPCR has been touted as a specific and sensitive method with high throughput sample analysis. Smati et al. [66] reported a rapid, sensitive, and reliable qPCR method to quantify *E. coli* phylogroup from 100 healthy human stool specimens and demonstrated the existence of subdominant clones. The new 16S-rRNA-qPCR assay was highly repeatable, with a detection limit of 10^5 CFU/g of feces.

3. Loop mediated isothermal amplification (LAMP) assay

In order to circumvent the use of thermocyclers that entail the time-consuming thermal cycling, an innovative method such as isothermal DNA amplification has been introduced which finds its application in the advanced Research & Development (R & D) unit of the food industry. The LAMP reaction that involves isothermal amplification chemistry has a good range of possible applications, including point-of-care testing with the potential of getting developed into portable diagnostic systems, and quick testing of food products, clinical and environmental samples.

The isothermal characteristics of LAMP enable the simplification of the detection process without involving any costly and complex instrumentation wherein a simple heating block or a precise digital water bath would work. Though conventional PCR and LAMP techniques were reported to be vulnerable to several inhibitors while testing various biological (for example urinary and plant materials) matrices [64], yet LAMP is much less sensitive to amplification inhibitors [64], potentially permitting its application bypassing the general requirement for cultural enrichment or DNA purification.

Despite some disadvantages like its qualitative nature of detection, the LAMP offers several advantages over PCR. LAMP assay emphasizes the requirement of a heating block and obviates the need for a thermal cycler. Unlike PCR that requires DNA extraction from samples for amplification, LAMP assay does not require DNA extraction step. The difficulties in amplifying DNA in PCR from unprocessed urinary samples in the presence of a high concentration of urea were reported by Khan et al. [65]. Therefore the LAMP assay, by rendering the DNA extraction step redundant, has made the process more rapid and facile [67]. The implementation of LAMP does not require any denatured template as due to the

use of Bst DNA polymerase from *Geobacillus stearothermophilus* with auto-cycling strand-displacement activity denatured template use has been eliminated. In the LAMP reaction, the nucleic acid amplification takes place at a fixed temperature (isothermal) through repetition of two types of elongation reactions occurring at the loop regions: self-elongation of templates from the stem-loop structure formed at the 3′-terminal and the binding and elongation of new primers to the loop region (**Figure 2**) [68]. LAMP reaction time is merely 60–65 min at 60–65°C involving four

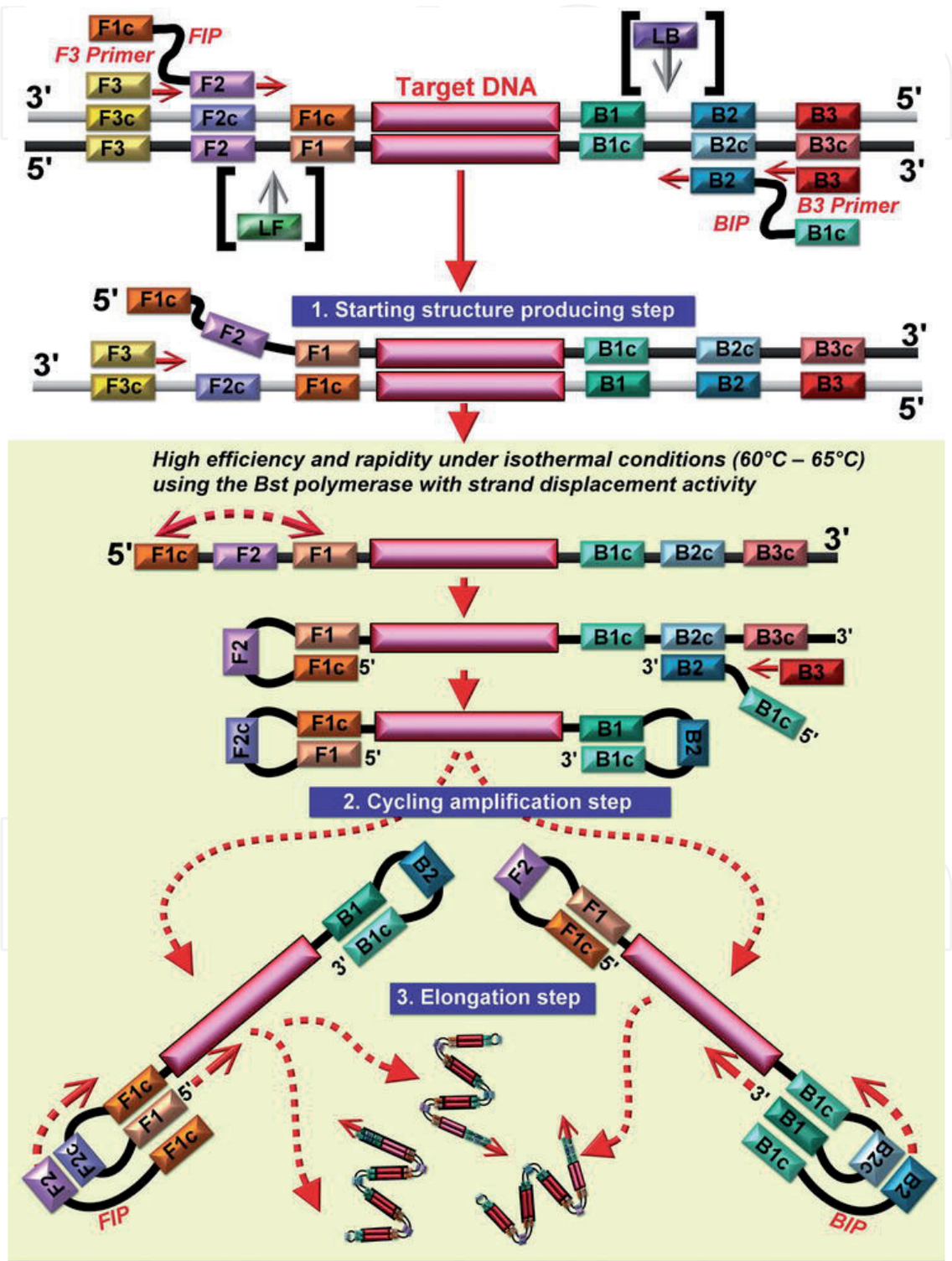

Figure 2.
Schematic representation of the principle steps in a LAMP assay and localization of the eight LAMP primers for specific amplification of target DNA. Adapted from Gallas-Lindemann et al. [68]. Copyright (2017). IntechOpen. Inner primer: FIP (consisting of F1c and F2), BIP (consisting of B1c and B2), typical length ~ 40 bp; outer primers: F3, B3 typical length ~ 20 bp; loop primers: LF and LB, typical length ~ 20 bp.

to six precisely designed primers to amplify DNA targets at the specific amplification temperature [68].

The very purpose of inner primers that consisted of two different sequences was to recognize a sense and antisense sequences of the target viral DNA, and the outer primers were designed to recognize an external sequence of the target viral DNA [69]. Additionally, in the LAMP assay, as an advantage, the identification of a positive reaction does not involve any special processing or electrophoresis. Only the visual observation of color change of the reaction mix in normal light is enabled when the appropriate DNA-binding dye is used. Thus, LAMP positive results could be better detected through visual observation of turbidity changes [70]. This visualization process can be improved by a UV transilluminator. Hill et al. [67] had demonstrated the use of propidium iodide for detecting the LAMP products.

In order to detect generic *E. coli*, *E. coli* O157, or different VTEC virulence genes a number of LAMP assays were developed and discussed in several previous studies [71–74]. In order to develop the LAMP assays for the simultaneous detection of an *E. coli*-specific gene and verotoxin-elaborating genes, and capable of distinguishing between generic *E. coli* and VTEC that would serve the purpose of simultaneous detection both *E. coli* and VTEC simultaneously in beef would allow the simultaneous monitoring of hygienic status/quality of beef. Therefore, the development of multiplex LAMP assay was of paramount importance. In the study, the LAMP assay was designed to detect nonpathogenic *E. coli* targeting the phoA and VTEC targeting the *stx1* and *stx2* without the need for a cumbersome culture enrichment process. The specificity of the *phoA* LAMP-based detection assay for *E. coli* showed 100% specificity (when a total of 58 bacterial strains were used for detection purposes) to determine with no false-positive or false-negative results with strains of any of the other bacterial genera tested. Interestingly only phoA gene-positive *E. coli* strains showed detectable amplification and non-*E. coli* showed no amplification. LAMP-false negative tests were reported by Stratakos et al. [75] while determining non-pathogenic *E. coli* and verocytotoxigenic *E. coli* (VTEC) in beef and bovine feces. Of note for the improvement of LAMP detection sensitivity, an enrichment step (which would not allow the post-enrichment quantification of *E. coli* or VTEC) prior to LAMP was suggested following the demonstration in previous studies by Wang et al. [76], and also a touchdown LAMP approach was suggested by Wang et al. [73].

It is to be noted that the LAMP assay reported by Hill et al. [67] was able to detect a large number of strains with very high sensitivity. Since biological samples such as cerebrospinal fluid and blood require very high sensitivity as compared to urine samples LAMP can be suitably modified for its clinical uses. LAMP has also been proposed to detect a lower copy number in partially treated infections (post-empirical antibiotic doses) [67].

4. DNA-based biosensors

A biosensor typically consists of a bioreceptor element with a transducer. The bioreceptor, interacts specifically with the analyte, whereas the transducer converts the biomolecular interaction into an electronic signal. Three basic parts of a biosensor are recognition material, transducer or detector system, and signal processor [74]. Monitoring the molecular interaction between the DNA-based bioreceptor and the analyte is an essential element of various DNA-based sensing strategies. The measurement methods of DNA–DNA interactions that take place on the various sensor surfaces are gaining much interest to improve sensor performance. The assays are applicable to the determination of low numbers of *E. coli* cells in various

matrices. In addition, the molecular detection of *E. coli* using single-stranded nucleic acids or aptamers coupled with the electrochemical impedance spectroscopy for sensing of DNA is a growing field of research and proving to be an alternative method of detection to traditional techniques [74].

Arora et al. [77] reported an electrochemical DNA biosensor for the detection of *E. coli*. In this study, avidin was modified with –COOH and then attached to the polyaniline (PANI)-modified platinum disk by the covalent binding between –COOH and –NH/NH_2 of PANI. Subsequently, the biotin-labeled DNA probe was functionalized on the electrode surface to achieve a LOD of 0.01 ng/uL for *E. coli* genomic DNA. Few studies reported the use of nanomaterials with graphene oxide (GO) to enhance the sensitivity of the DNA biosensor for *E. coli* detection. For example, a DNA biosensor for the detection of *E. coli* O157:H7 eaeA gene based on a novel sensing tag of GOx-Thi-Au@SiO2 nanocomposites is reported [78]. The combined use of GO and Au@SiO2 creates an environment for maintaining the appropriate conformation of DNA. These biosensor modalities led to wide linear response for *E. coli* O157:H7 eaeA gene in the range of 0.02 to 50.0 nM with LoD of 0.01 nM. In addition, Tiwari et al. [79] reported a DNA biosensor for *E. coli* O157:H7 using a DNA probe sequence. The DNA probe was immobilized onto GO modified iron oxide-chitosan hybrid nanocomposite (GIOCh) film. The DNA biosensor resulted in linear response to *E. coli* DNA in the range of 10^{-6} to 10^{-14} M with a LoD of 10^{-14} M.

Since its discovery in the 1980s, the system has demonstrated widespread applications in basic biotechnology research and disease treatment [80, 81]. A pressing need of the hour is the availability of a cost-efficient, rapid and selective molecular diagnostic platform to detect different pathogens and lethal diseases in the early stage of the infection. Quantitative PCR and metagenomic next-generation sequencing (mNGS) are the most commonly explored molecular platforms for the same; however, these methods have their disadvantages and limitations. Clustered Regularly Interspaced Short Palindromic Repeat/associated protein (CRISPR/Cas)-based diagnostic platform for the detection of nucleic acids has progressively demonstrated its potential as an ideal diagnostic approach for pathogens, cancer biomarker, and single-nucleotide polymorphisms (SNPs) detection. CRISPR systems have evolved in prokaryotes as a defensive mechanism against foreign viruses by cleaving their nucleic acids [82–84].

Additionally, the unique cleavage activity of Cas9 is often utilized for the development of ultra-low abundance DNA biosensors. A highly innovative and sensitive CRISPR/Cas9 system was developed by Huang et al. [84] that triggered isothermal exponential amplification reaction (CAS-EXPAR) strategy to detect DNA targets with attomolar (aM) sensitivity and single-base specificity [84]. CAS-EXPAR was primed by the target DNA fragment produced by cleavage of CRISPR/Cas9, and associated with the cyclical amplification reaction to produce numerous DNA replicates capable of getting detected by a real-time SYBR Green fluorescence signal [83].

Recently, Sun et al. [84] reported the detection of *E. coli* O157:H7 based on the CRISPR/Cas9 coupled with metal–organic framework platform (MoF) (**Figure 3**). In this approach, the virulence gene sequences of *E. coli* O157:H7 were identified and spliced by the CRISPR/Cas9 system leading to strand displacement and rolling circle amplification. Subsequently, amplified products were hybridized with the target-specific probes. The virulence genes were detected by the fluorescence quenching caused due to MoF platform. The method showed high sensitivity with LoD of 4.0×10^1 CFU mL^{-1} [84]. Although there is only one reported work available for CRISPR/Cas-based detection of *E. coli*, however, the CRISPR/Cas system can be exploited further for the detection of *E. coli* and other waterborne pathogens using novel strategies.

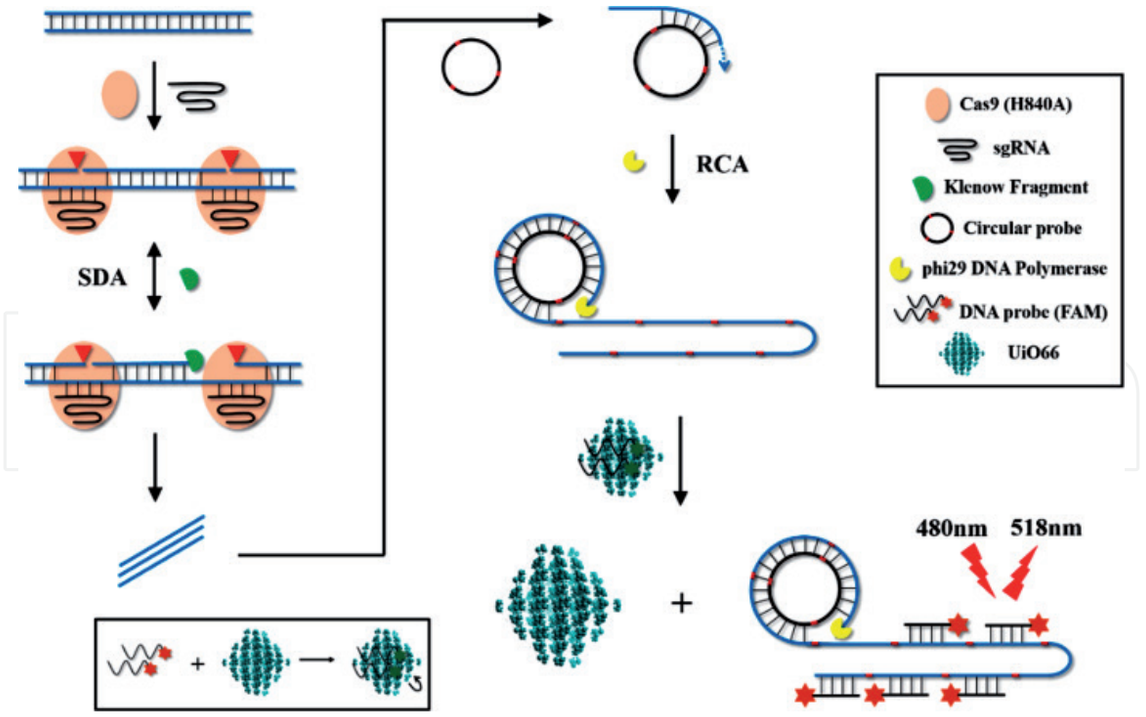

Figure 3.
CRISPR/Cas9 platform coupled with two-step isothermal amplification for detection of E. coli *O157:H7. Reprinted with permission from Sun et al. [84]. Copyright (2020) American Chemical Society.*

5. Conclusion

Molecular diagnostic platforms have become promising alternatives to traditional methods for *E. coli* detection. In particular, LAMP assay and DNA biosensors because of their advantages of lower detection limits, and high reproducibility are preferred for pathogen detection. In this review, we have discussed the recent advances in the development of PCR, LAMP assay, and DNA biosensors platforms applied to *E. coli* detection. In the case of CRISPR/Cas platforms, the major challenge associated with the CRISPR/Cas sensing platform is the time taken to produce the results. Therefore, the future perspective would be to reduce the assay turnaround time for CRISPR/Cas sensing. Nonetheless, CRISPR/Cas sensing platforms possess the potential to overcome the use of conventional molecular diagnostic platforms and become a promising tool for next-generation diagnostic platforms for sensitive and selective detection of DNA in clinical, food, and environmental samples. In the future, more, specific, sensitive, cost-sensitive, and portable biosensors will be required to detect *E. coli*, hence, further leading to controlling and monitoring the waterborne epidemics.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] José Figueras M, Borrego JJ. New perspectives in monitoring drinking water microbial quality. International Journal of Environmental Research and Public Health. 2010;**7**(12):4179-4202

[2] J. M. Colford et al., "Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination.," Epidemiology, vol. 18, no. 1, pp. 27-35, Jan. 2007.

[3] V. J. Harwood, C. Staley, B. D. Badgley, K. Borges, and A. Korajkic, "Microbial source tracking markers for detection of fecal contamination in environmental waters: Relationships between pathogens and human health outcomes," FEMS Microbiology Reviews 38, 1, pp. 1-40, Jan. 2014.

[4] Gorchev HG, Ozolins G. WHO Guidelines for Drinking-Water Quality. Vol. 38, no. 3. WHO Press; 2011

[5] UNICEF. Somalia - Water, Sanitation and Hygiene - Priority issues. Unicef; 2019. Available from: https://reliefweb.int/report/somalia/somalia-water-sanitation-and-hygiene-assessment-report-december-2019

[6] Graham N. Guidelines for Drinking-Water Quality, 2nd edition, Addendum to Volume 1 – Recommendations, World Health Organisation, Geneva, 1998, 36 pages. Urban Water. 1999;**1**(2):183

[7] Deshmukh RA, Joshi K, Bhand S, Roy U. Recent developments in detection and enumeration of waterborne bacteria: a retrospective minireview. Microbiology. 2016;**5**(6):901-922

[8] Ryoo E. Causes of acute gastroenteritis in Korean children between 2004 and 2019. Clinical and Experimental Pediatrics. 2021;**64**(6):260-268

[9] Stockmann C et al. Detection of 23 gastrointestinal pathogens among children who present with Diarrhea. Journal of the Pediatric Infectious Diseases Society. 2017;**6**(3): 231-238

[10] Y. Kim et al., "Community-acquired Escherichia coli enteritis in Korean children: The clinical application of a stool polymerase chain reaction assay," Infection & Chemotherapy, vol. 49, no. 4, pp. 275-281, Dec. 2017

[11] J. Park et al., "A waterborne outbreak of multiple diarrhoeagenic Escherichia coli infections associated with drinking water at a school camp," International Journal of Infectious Diseases, vol. 66, pp. 45-50, Jan. 2018

[12] A. Rompré, P. Servais, J. Baudart, M. R. De-Roubin, and P. Laurent, "Detection and enumeration of coliforms in drinking water: Current methods and emerging approaches," Journal of Microbiological Methods, vol. 49, no. 1, pp. 31-54, Mar. 2002

[13] Maheux AF et al. Molecular method for detection of total coliforms in drinking water samples. Applied and Environmental Microbiology. 2014;**80**(14):4074-4084

[14] Kaper JB, Nataro JP, Mobley HLT. Pathogenic *Escherichia coli*. Nature Reviews. Microbiology. 2004;**2**(2): 123-140

[15] Lupindu AM. Epidemiology of Shiga toxin-producing Escherichiaï¿¿coli O157:H7 in Africa in review. Southern African Journal of Infectious Diseases. 2018;**33**(1):24-30

[16] I. Son, R. Binet, A. Maounounen-Laasri, A. Lin, T. S. Hammack, and J. A. Kase, "Detection of five Shiga toxin-producing *Escherichia coli* genes with multiplex PCR," Food Microbiology, vol. 40, pp. 31-40, Jun. 2014.

[17] Perna NT et al. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. Nature. 2001;**409**(6819): 529-533

[18] Mead PS et al. Food-related illness and death in the United States. Emerging Infectious Diseases. 1999; **5**(5):607-625

[19] Ferens WA, Hovde CJ. *Escherichia coli* O157:H7: Animal reservoir and sources of human infection. Foodborne Pathogens and Disease. 2011;**8**(4):465-487

[20] Tok S, De Haan K, Tseng D, Usanmaz CF, Ceylan Koydemir H, Ozcan A. Early detection of: *E. coli* and total coliform using an automated, colorimetric and fluorometric fiber optics-based device. Lab on a Chip. 2019;**19**(17):2925-2935

[21] Stender H, Broomer AJ, Oliveira K, Hyldig-nielsen JJ, Sage A, Coull J. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Applied and Environmental Microbiology. 2001;**67**(1):142-147

[22] Rompre A, Servais P, Baudart J, De-Roubin M-R, Laurent PP. Detection and enumeration of coliforms in drinking water: current methods and emergin approaches. Journal of Microbiological Methods. 2002;**49**(2002):31-54

[23] Khan IUH et al. Development of a rapid quantitative PCR assay for direct detection and quantification of culturable and non-culturable *Escherichia coli* from agriculture watersheds. Journal of Microbiological Methods. 2007;**69**(3):480-488

[24] Luna GM, Dell 'anno A, Pietrangeli B, Danovaro R. A new molecular approach based on qPCR for the quantification of fecal bacteria in contaminated marine sediments. Journal of Biotechnology. 2011;**157**:446-453

[25] Ludwig W, Schleifer K-H. How quantitative is quantitative PCR with respect to cell counts? Systematic and Applied Microbiology. 2000;**23**(4): 556-562

[26] Foulds IV, Granacki A, Xiao C, Krull UJ, Castle A, Horgen PA. Quantification of microcystin-producing cyanobacteria and *E. coli* in water by 5′-nuclease PCR. Journal of Applied Microbiology. 2002;**93**(5): 825-834

[27] Maheux AF et al. Rapid concentration and molecular enrichment approach for sensitive detection of *Escherichia coli* and Shigella species in potable water samples. Applied and Environmental Microbiology. 2011;**77**(17):6199-6207

[28] Bej AK, DiCesare JL, Haff L, Atlas RM. Detection of *Escherichia coli* and Shigella spp. in water by using the polymerase chain reaction and gene probes for uid. Applied and Environmental Microbiology. 1991; **57**(4):1013-1017

[29] Bae S, Wuertz S. Discrimination of viable and dead fecal Bacteroidales bacteria by quantitative PCR with Propidium monoazide. Applied and Environmental Microbiology. 2009;**75**(9):2940-2944

[30] Y. Liu, A. Gilchrist, J. Zhang, and X.-F. Li, "Detection of viable but nonculturable *Escherichia coli* O157:H7 bacteria in drinking water and river water.," Applied and Environmental Microbiology, vol. 74, no. 5, pp. 1502-1507, Mar. 2008.

[31] Ercoli L et al. Prevalence and characteristics of verotoxigenic *Escherichia coli* strains isolated from pigs and pork products in Umbria and Marche regions of Italy. International Journal of Food Microbiology. 2016;**232**:7-14

[32] Hamner S et al. Isolation of potentially pathogenic *Escherichia coli*

O157:H7 from the Ganges River. Applied and Environmental Microbiology. 2007;**73**(7):2369-2372

[33] Chassagne L, Pradel N, Robin F, Livrelli V, Bonnet R, Delmas J. Detection of stx1, stx2, and eae genes of enterohemorrhagic *Escherichia coli* using SYBR Green in a real-time polymerase chain reaction. Diagnostic Microbiology and Infectious Disease. 2009;**64**(1): 98-101

[34] Alperi A, Figueras MJ. Human isolates of Aeromonas possess Shiga toxin genes (stx1 and stx2) highly similar to the most virulent gene variants of *Escherichia coli*. Clinical Microbiology and Infection. 2010;**16**(10):1563-1567

[35] K. E. Hyma et al., "Evolutionary genetics of a new pathogenic Escherichia species: Escherichia albertii and related Shigella boydii strains," Journal of Bacteriology, vol. 187, no. 2, pp. 619-628, Jan. 2005.

[36] Deer DM, Lampel KA. Development of a multiplex real-time PCR assay with internal amplification control for the detection of Shigella species and enteroinvasive *Escherichia coli*. Journal of Food Protection. 2010;**73**(9):1618-1625

[37] Feng P, Lum R, Chang GW. Identification of uidA gene sequences in beta-D-glucuronidase-negative *Escherichia coli*. Applied and Environmental Microbiology. 1991;**57**(1):320-323

[38] Frampton EW, Restaino L. Methods for *Escherichia coli* identification in food, water and clinical samples based on beta-glucuronidase detection. The Journal of Applied Bacteriology. 1993;**74**(3):223-233

[39] Feng P, Lum R, Chang GW. Identification of uidA gene sequences in beta-D-glucuronidase-negative *Escherichia coli*. Applied and Environmental Microbiology. 1991;**57**(1):320-323

[40] Molina F, López-Acedo E, Tabla R, Roa I, Gómez A, Rebollo JE. Improved detection of *Escherichia coli* and coliform bacteria by multiplex PCR. BMC Biotechnology. 2015;**15**:48

[41] Deshmukh RA, Bhand S, Roy U. A novel molecular quantitative method for rapid and sensitive detection of *Escherichia coli* from roof-harvested rainwater. Analytical Methods. 2019;**11**(25):3155-3167

[42] Fricker EJ, Fricker CR. Application of the polymerase chain reaction to the identification of *Escherichia coli* and coliforms in water. Letters in Applied Microbiology. 1994;**19**(1):44-46

[43] Li B, Liu H, Wang W. Multiplex real-time PCR assay for detection of *Escherichia coli* O157:H7 and screening for non-O157 Shiga toxin-producing *E. coli*. BMC Microbiology. 2017;**17**(1):215

[44] Ibekwe AM, Leddy M, Murinda SE. Potential human pathogenic bacteria in a mixed urban watershed as revealed by pyrosequencing. PLoS One. 2013;**8**(11): e79490

[45] Nguyen TT, Van Giau V, Vo TK. Multiplex PCR for simultaneous identification of *E. coli* O157:H7, Salmonella spp. and L. monocytogenes in food. 3 Biotech. 2016;**6**(2):205

[46] Ochoa TJ, Contreras CA. Enteropathogenic *Escherichia coli* infection in children. Current Opinion in Infectious Diseases. 2011;**24**(5): 478-483

[47] Botkin DJ, Galli L, Sankarapani V, Soler M, Rivas M, Torres AG. Development of a multiplex PCR assay for detection of Shiga toxin-producing *Escherichia coli*, enterohemorrhagic *E. coli*, and enteropathogenic *E. coli* strains.

Frontiers in Cellular and Infection Microbiology. 2012;**2**:8

[48] Wang Z et al. Development of a multiplex PCR assay for the simultaneous and rapid detection of six pathogenic bacteria in poultry. AMB Express. 2019;**9**(1):1-11

[49] Toma C et al. Multiplex PCR assay for identification of human diarrheagenic *Escherichia coli*. Journal of Clinical Microbiology. 2003;**41**(6): 2669-2671

[50] Chen Q et al. Rapid genetic typing of diarrheagenic *Escherichia coli* using a two-tube modified molecular beacon based multiplex real-time PCR assay and its clinical application. Annals of Clinical Microbiology and Antimicrobials. 2014;**13**(1):30

[51] Ramírez-Castillo FY et al. Waterborne pathogens: Detection methods and challenges. Pathogens. 2015;**4**(2):307-334

[52] Picard FJ et al. Internal control for nucleic acid testing based on the use of purified bacillus atrophaeus subsp. globigii Spores. Journal of Clinical Microbiology. 2009;**47**(3):751-757

[53] Girones R et al. Molecular detection of pathogens in water - The pros and cons of molecular techniques. Water Research. 2010;**44**(15):4325-4339

[54] Law JW-F, Ab Mutalib N-S, Chan K-G, Lee L-H. Rapid methods for the detection of foodborne bacterial pathogens: Principles, applications, advantages and limitations. Frontiers in Microbiology. 2014;**5**(January):770

[55] Bellin T, Pulz M, Matussek A, Hempen HG, Gunzer F. Rapid detection of enterohemorrhagic *Escherichia coli* by real-time PCR with fluorescent hybridization probes. Journal of Clinical Microbiology. 2001;**39**(1):370-374

[56] Ishii S, Nakamura T, Ozawa S, Kobayashi A, Sano D, Okabe S. Water quality monitoring and risk assessment by simultaneous multipathogen quantification. Environmental Science & Technology. 2014;**48**(9):4744-4749

[57] J. S. Way, K. L. Josephson, S. D. Pillai, M. Abbaszadegan, C. P. Gerba, and I. L. Pepper, "Specific detection of Salmonella spp. by multiplex polymerase chain reaction," Applied and Environmental Microbiology, vol. 59, no. 5, pp. 1473-1479, May 1993.

[58] Sharma VK, Carlson SA. Simultaneous detection of Salmonella strains and *Escherichia coli* O157:H7 with fluorogenic PCR and single-enrichment-broth culture. Applied and Environmental Microbiology. 2000;**66**(12):5472-5476

[59] Bustin SA et al. The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clinical Chemistry. 2009;**55**(4):611-622

[60] Straub TM, Pepper IL, Gerba CP. Removal of PCR inhibiting substances in sewage sludge amended soil. Water Science and Technology. 1995;**31**(5-6): 311-315

[61] Klerks MM, Zijlstra C, Van Bruggen AHC. Comparison of real-time PCR methods for detection of Salmonella enterica and *Escherichia coli* O157:H7, and introduction of a general internal amplification control. Journal of Microbiological Methods. 2004;**59**(3):337-349

[62] Shanks OC et al. Quantitative PCR for detection and enumeration of genetic markers of bovine fecal pollution. Applied and Environmental Microbiology. 2008;**74**(3):745-752

[63] Walker DI et al. A highly specific *Escherichia coli* qPCR and its comparison with existing methods for

environmental waters. Water Research. 2017;**126**:101-110

[64] Schrader C, Schielke A, Ellerbroek L, Johne R. PCR inhibitors - occurrence, properties and removal. Journal of Applied Microbiology. 2012;**113**(5):1014-1026

[65] Khan G, Kangro HO, Coates PJ, Heath RB. Inhibitory effects of urine on the polymerase chain reaction for cytomegalovirus DNA. Journal of Clinical Pathology. 1991;**44**(5):360-365

[66] Liu Y, Cao Y, Wang T, Dong Q, Li J, Niu C. Detection of 12 common food-borne bacterial pathogens by taq man real-time PCR using a single set of reaction conditions. Frontiers in Microbiology. 2019;**10**(FEB):222

[67] Hill J et al. Loop-mediated isothermal amplification assay for rapid detection of common strains of *Escherichia coli*. Journal of Clinical Microbiology. 2008;**46**(8):2800-2804

[68] Gallas-Lindemann C, Sureshkumar P, Noack MJ, Sotiriadou I. Loop-mediated isothermal amplification: An advanced method for the detection of giardia. Current Topics Giardiasis. 2017

[69] Notomi T, Mori Y, Tomita N, Kanda H. Loop-mediated isothermal amplification (LAMP): Principle, features, and future prospects. Journal of Microbiology. 2015;**53**(1):Jan

[70] Mori Y, Nagamine K, Tomita N, Notomi T. Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. Biochemical and Biophysical Research Communications. 2001;**289**(1):150-154

[71] Kouguchi Y, Fujiwara T, Teramoto M, Kuramoto M. Homogenous, real-time duplex loop-mediated isothermal amplification using a single fluorophore-labeled primer and an intercalator dye: Its application to the simultaneous detection of Shiga toxin genes 1 and 2 in Shiga toxigenic *Escherichia coli* isolates. Molecular and Cellular Probes. 2010;**24**(4):190-195

[72] Ravan H, Amandadi M, Sanadgol N. A highly specific and sensitive loop-mediated isothermal amplification method for the detection of *Escherichia coli* O157: H7. Microbial Pathogenesis. 2016;**91**:161-165

[73] Wang Y et al. Rapid and sensitive detection of Shigella spp. and Salmonella spp. by multiple endonuclease restriction real-time loop-mediated isothermal amplification technique. Frontiers in Microbiology. 2015;**6**:1400

[74] Wu Q, Zhang Y, Yang Q, Yuan N, Zhang W. Review of electrochemical DNA biosensors for detecting food borne pathogens. Sensors (Switzerland). 2019;**19**(22):4916

[75] Stratakos AC, Linton M, Millington S, Grant IR. A loop-mediated isothermal amplification method for rapid direct detection and differentiation of nonpathogenic and verocytotoxigenic *Escherichia coli* in beef and bovine faeces. Journal of Applied Microbiology. 2017;**122**(3):817-828

[76] F. Wang, L. Jiang, and B. Ge, "Loop-mediated isothermal amplification assays for detecting Shiga toxin-producing *Escherichia coli* in ground beef and human stools," Journal of Clinical Microbiology, vol. 50, no. 1, pp. 91-97, Jan. 2012.

[77] Arora K, Prabhakar N, Chand S, Malhotra BD. *Escherichia coli* genosensor based on polyaniline. Analytical Chemistry. 2007;**79**(16):6152-6158

[78] Li Y et al. A novel electrochemical DNA biosensor based on HRP-mimicking hemin/G-quadruplex

wrapped GOx nanocomposites as tag for detection of *Escherichia coli* O157: H7. Biosensors & Bioelectronics. 2015;**63**:1-6

[79] Tiwari I, Singh M, Pandey CM, Sumana G. Electrochemical genosensor based on graphene oxide modified iron oxide-chitosan hybrid nanocomposite for pathogen detection. Sensors and Actuators B: Chemical. 2015;**206**:276-283

[80] Makarova KS et al. Evolutionary classification of CRISPR–Cas systems: A burst of class 2 and derived variants. Nature Reviews Microbiology. 2020;**18**(2). Nature Research:67-83

[81] Li SY et al. CRISPR-Cas12a-assisted nucleic acid detection. Cell Discovery. 2018;**4**(1):20

[82] Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014;**157**(6):1262-1278

[83] Huang M, Zhou X, Wang H, Xing D. Clustered regularly interspaced short palindromic repeats/Cas9 triggered isothermal amplification for site-specific nucleic acid detection. Analytical Chemistry. 2018;**90**(3):2193-2200

[84] Sun X et al. CRISPR-Cas9 triggered two-step isothermal amplification method for *E. coli* O157:H7 detection based on a metal-organic framework platform. Analytical Chemistry. 2020;**92**(4):3032-3041