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Molecular Diagnostic Platforms for Specific Detection of *Escherichia coli*

Rehan Deshmukh and Utpal Roy

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, *stx1/stx2* for ETEC, *eaeA/escV* for EPEC, *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, *stx1/stx2* 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 per cent 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 Stratakis 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 $-\text{COOH}$ and then attached to the polyaniline (PANI)-modified platinum disk by the covalent binding between $-\text{COOH}$ and $-\text{NH}/\text{NH}_2$ 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 turn-around 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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Author details

Rehan Deshmukh¹ and Utpal Roy^{2*}

¹ Faculty of Science, School of Biology, MIT World Peace University, Pune, India

² Department of Biological Sciences, Birla Institute of Technology and Science, Pilani, Goa, India

*Address all correspondence to: utpalroy@gmail.com

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