The Detection Method of *Escherichia coli* in Water Resources: A Review

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Abstract. This article reviews several approaches for *Escherichia coli* (*E. coli*) bacteria detection from conventional methods, emerging method and goes to biosensor-based techniques. Detection and enumeration of *E. coli* bacteria usually required long duration of time in obtaining the result since laboratory-based approach is normally used in its assessment. It requires 24 hours to 72 hours after sampling to process the culturing samples before results are available. Although faster technique for detecting *E. coli* in water such as Polymerase Chain Reaction (PCR) and Enzyme-Linked Immunosorbent Assay (ELISA) have been developed, it still required transporting the samples from water resources to the laboratory, high-cost, complicated equipment usage, complex procedures, as well as the requirement of skilled specialist to cope with the complexity which limit their wide spread practice in water quality detection. Recently, development of biosensor device that is easy to perform, portable, highly sensitive and selective becomes indispensable in detecting extremely lower consolidation of pathogenic *E. coli* bacteria in water samples.

1. Introduction

Some of the major health risks are caused by microorganisms such as bacteria or pathogen because it’s may survive, reproduce and disperse in water systems [1]. Approximately, 1.7 billion children in developing countries under age five died due to diarrhea, mainly by drinking contaminated water that has been reported by World Health Organization (WHO). Besides, 525,000 children deaths a year in world-wide because of the poor water quality, sanitation and hygiene mainly through infectious diarrhea [2, 3]. Human and animal waste in water resources can cause water to contaminate [4]. There is no method has been reported used to numerate or isolate all of the particular noteworthy pathotypes because there have so much element in water other than dangerous bacteria and directly testing a large variety of pathogens will be difficult, pricey, and time consuming [3]. Therefore, several approached are developed to pointedly numerate or isolate convinced pathotypes. *E. coli* as shown in Figure 1 is the best-known coliform to indicate the fecal contamination because it can be found almost exclusively in human and animal feces [2, 5].

Therefore, measuring *E. coli* bacteria in water resources become a critical issue to indicate fecal contaminant from human and animal. The monitoring device to detect the amount of *E. coli* bacteria is crucial to maintain the quality of water and protect public health. It remains a significant risk to people.
for minimizing the spreading of infectious disease from E. coli because it can lead to some diseases such as bloody diarrhea, kidney failure and can cause death if not treated immediately. E. coli is in a group of Enterobacteriaceae which is in Gram-negative bacteria family and known as most infecting organisms in that group. This bacteria are non-spore forming and rod shaped with approximately 0.5 µm in diameter and between 1.0 to 3.0 µm in length [7].

Figure 1. Image of an E. coli cell [6].

2. Conventional Method
There are conventional microbiological tests that have ability to numerate the bacteria levels in water or other substances such as Multiple Tube Fermentation (MTF) [8], Plate Count Enumeration Method and Membrane Filter (MF) technique [9]. These methods are labelled as conventional microbiological method introduced in 19 centuries that's already been approved by U.S. Environmental Protection Agency (EPA) because of high accuracy [10]. Nowadays, although the methods are still being used for routine detection of E. coli in drinking water, it became not practical since the result can only be confirmed in several days and required expert in the biological area to perform the cultivation step.

Multiple-tube fermentation Technique (MTF) is based on the principle of dilution by using Most Probable Number (MPN) to enumerate total bacteria form [11]. The MTF technique is made of a series of dilutions generally one millilitre are shifted into several fermentation tubes [12]. The total specific bacteria accommodate with liquid medium are very suitable for growing in the fermentation tube as shown in Figure 2. The inverted gas collected in the fermentation tube shown positive result. Furthermore, this method became particularly useful for Plate Count Enumeration Methods in Figure 3 that is more often and convenient to use especially for an approximation of the E. coli bacteria count since too much tube needed in MTF method. MF is another traditional technique that can be used for E. coli detection by delivering water sample over small pore grid paper filter to trap the bacteria and selected agar are added into plate for growing the E. coli [13]. After incubation process, the dot that represent colonies are counted by using Plate Count Enumeration Method [9].

![Figure 2](image2.png)

(a) (b)

Sample are too numerous to count
Bacteria count

5 x 10^5
5 x 10^6
Figure 2. Interpretation methods to determine the concentration of viable E. coli. (a) MTF method; (b) Plate Count Enumeration Method [14].

3. Emerging Methods

Although standard methods can provide reliable results in the presence or absence along with the concentration of the E. coli bacteria, they take at least two days for the tests, which limits their use in the prevention of waterborne disease. Therefore, several methods have emerged to realize the rapid detection and enumeration of E. coli bacteria in drinking water such as Immunological [15-18] and Polymerase Chain Reaction [19-21].

Immunological methods perform more rapid method compare to conventional MTF, Plate Count Enumeration Method and MF. The test measured amount of E. coli bacteria within a 24-hour period so it performs better than traditional methods that required at least two days to get the result. In immunological, the antigen-antibody reaction are performed and can be anticipated by several approaches, such as Enzyme-Linked Immunosorbent Assay (ELISA). ELISA took about 24 hours because a pre-enrichment culture was still needed, make it not applicable for on-field applications [22].

Nucleic acid-based techniques by using Polymerase Chain Reaction (PCR) became promising due to high sensitivity and rapidity. The conventional PCR seems to have several limitations because it detects both viable and dead cells. Besides, it is very complicated method where there need many tube variable to perform the process and still timely [23]. An advanced method has been developed to overcome the problem, namely Reverse Transcriptase (RT-PCR) [24]. The technology already solved the problem and became more distinct to differentiate between viable and dead cells besides performing rapidly process. The research on PCR are performed to produce less complicated and simple made by Soto-Munoz L. et al. called Real-Time PCR [25]. However, it contains an extremely toxic compound (e.g. Propidium monoxide) make it become not appropriate to use [26].

Although many advanced methods are used to detect E. coli bacteria in water, only PCR and ELISA approaches are declared as established methods [27]. Both methods are the most common tools used studied by Lusaka et al. [28]. A researcher found that both methods can be merged together to produce more robust results [23]. PCR is an excellent method of E. coli detection compared to the standard culture and ELISA method since it is very sensitive, accurate and a promise to real-time quantitative. Despite its highly sensitive, PCR is difficult to operate because the complex standardized protocols were needed during testing process [29]. Therefore, a skilled worker is required while running the machine to cope with its complexity.

4. Biosensors

Biosensors are small devices that employ biological/biochemical reactions for detecting target analytes. Basically, the device consists of a biocatalyst and a transducer. Biocatalyst may be a cell, tissue, enzyme or even an oligonucleotide [30]. There are many transducers which can be used to detect a range of chemical, optical, and biological signals generated by the targeted pathogen. Most detection technologies are revolving around the measurement of optical, electrochemical and piezoelectric [31]. Application and technologies in biosensors for E. coli detection water environmental analysis had reviewed in many articles [30-35]. The sensitivity of most biosensor seems has significantly increased in recent years, but it still has some shortcoming and needs more improvement to increase the sensitivity. In E. coli bacteria detection, selectivity also the main parameter to take account. To make the sensor became high selectivity, the molecular and bio-molecular recognition is employed on the receptive layer such as antigen-antibody binding (i.e. any chemicals, bacteria, viruses, or pollen binding to a specific protein) [32].
4.1. Optical biosensor
Optical detection method is the most widely operated in pathogen monitoring system due to highly sensitive. Such diverse methods include detection based on absorption, fluorescence, refraction, reflection and chemiluminescence. Among of all diversity, fluorescence and absorption techniques are the simplest technique to use for monitoring and enumerate E. coli. B. Heery et al. had produced an immunoassay fluorescence device using specific enzyme assays of E. coli that was capable to deliver results on-site as low as 250 CFU/100 mL within 75 min [36]. Although labelling fluorescence detection method has high selectivity for detecting E. coli, majority of these techniques involve sample filtration, lysing, incubation, and detection steps which is critical when substrate quantity is limited. Furthermore, the process of labelling of fluorescence signal suffer from a relatively bulky external equipment and longer time of analysis. Efforts to miniaturize this platform are currently underway. A simple method using absorption techniques is analysed by N. M. Salih et al. that detect E. coli bacteria suspension in Polydimethylsiloxane (PDMS) Glass [37]. The microfluidic based that are represented by the system are noble for miniaturizing the analytical instrumentation. However, the method suffers from lack of selectivity since it has possibility to detect other materials.

The demand for high throughput label-free multiplexed biosensors for biological sensing has increased in the last decades. Therefore, the circumstances have led to the development of fiber optics [38-41], planar waveguides [42-44] and surface plasmon resonance [45-50] in the area of optical biosensor. These techniques are modified by the interaction of bio-recognition element, such as enzymes, antibodies, antigens, or receptors of E. coli used on it to make it highly selective. The concept of fiber optics is that the transmission of light energy is passing through transparent fiber as shown in Figure 3. Fiber optic known as an advanced technology and perform good performance, high bandwidth, low loss and relatively cheap that make it suitable mechanism for biosensor. One of the limitation of employing optical fiber for bacteria identification is the change of temperature that can vary the material optical properties. Therefore, if the sample gets much colder or hotter it gives inaccurate readings so it only design for specific temperature [46]. In planar optical waveguides, thin optically transparent film with that has high refractive more than superstrate mediums and adjacent substrate is usually being used. Under this condition, light is combined into the thin waveguide film are constrained to the layer and generate to meaningful distances as shown in Figure 4 [47]. SPR is known as label-free biosensor that produce real-time result based on changes the refractive index on the surface of sensor part which is proportional to the biomolecule concentration. The operating regulation of a typical SPR instrument is presented in Figure 5 [48].

![Figure 3. Schematic illustration using fiber optics in E. coli detection [35].](image-url)
4.2 Electrochemical biosensors

The electrochemical based approach is another possible technique for identification and quantification of E. coli. The basic of electrochemical dealt with interconversion between chemical and electrical energy. This detection method was based on amperometric, impedimetric, potentiometric and conductometric with its parameters such as current, impedance, potential, and conductance respectively [52]. Amperometric is known as the most typical electrochemical detection method used for pathogen detection and it has superior sensitivity compare to other electrochemical method. Rochelet et al. measure β-D glucuronidase (GLUase) activity with disposable carbon sensors [53]. J.-j. Gau et al. has evolved a system of amperometric identification and enumeration of E. coli uses the consolidation of Self-Assembled Monolayers (SAM), microelectromechnical systems (MEMS), enzyme amplification and DNA hybridization. By employing ssDNA-rRNA hybridization and amplification of enzymatic, high specificity for E. coli was achieved [54]. In potentiometric detection-based, the conversion occurs from the bio-recognition into a potential signal. Generally, to measure the electrical potential difference or electromotive force between two electrodes at near zero current a high impedance voltmeter is used. The feasibility of detecting E. coli in water with simple flow injection are analyse using Chronopotentiometric Aptsensing by J. H. Lei et al. Aptamer is used as a bioreceptor and the polion-sensitive membrane electrode as a signal transducer [55]. Recently, Shaibani et al. developed a rapid, simple and low cost E. coli detection with the pH sensitive hydrogel nanofiber-light addressable potentiometric sensor (NF-LAPS) as shown in Figure 6 [56]. The system of NF-LAPS provides a theoretical limit of detection (LOD) of 20 CFU/ml show it very high sensitivity.

Then, the assimilation of impedance approach to recognition biological technology has driven by the impedance biosensors development that are boundless in the recent years. Colquhoun et al. has utilized a medium of trimethylamine N-oxide (TMAO) and glucuronic acid that are very specific for E. coli in imperometric based sensor to detect E. coli in water samples [47]. Promising result had shown in the research which indicates the change of impedance during the growing of E. coli bacteria. Compact E. coli biosensor Electrochemical Impedance Immunosensor Based on SAM been reported by Z. Li et al. with a detection limit of 10² CFU/mL as shown in Figure 7 [57]. The miniature of electrode to micro size in this report represent the usage of electrode nowadays that minimizing the volume of testing sample. The smaller size can make the sensor prototype become compact. Conductometric detection method is based on the connection between conductance parameter and bio-recognition applying method. Z. Muhammad-Tahir and E. C. Alocilja had evolved a conductometric biosensor that successfully detecting bacteria such as E. coli and Salmonella [58]. Research work shows a specific, high limit of detection, low sample volume used and near real time assay mechanism.
Figure 6. Nanofiber-light addressable potentiometric sensor (NF-LAPS) mechanism [56].

Figure 7. Microelectrode using Impedance-Based immunosensor [57].

4.3 Piezoelectric biosensors

Piezoelectric based detection methods using quartz crystal are mostly utilized that can oscillates at a defined frequency when apply an oscillating voltage. The rapid resulting assay performed in ten minutes are developed by Pohanka et al. [59]. The piezoelectric crystal surface are linked with a polyclonal antibody using glutaraldehyde to detect E. coli that had a limit of detection of $1 \times 10^6$ CFU/mL. In the research by X. L. Su et al., piezoelectric methods have been applied to enumerate E. coli immobilized on a SAM [60]. This finding showed potential to be combined 16-mercaptopropanoic acid (MHDA) with SAM for better sensitivity compare by using protein A in the Piezoelectric Immuno-sensor with limit of detection of $10^3$ CFU/ml within 30 to 50 min. Based on the finding, V.K.T Ngo et al. has developed Quartz Crystal Microbalance (QCM) biosensor by combined 16-mercaptopropanoic acid (MHDA) with SAM [61]. The result shown better sensitivity with limid of detection of $10^2$ CFU/ml E. coli O157:H7 within 50 min. Recently, F. Tong et al. developed the piezoelectric biosensor with an annular microelectrode as shown in Figure 8. By using micro-scale structure of microelectrode, the optimization in term of current density, rapid response time and mass transfer between the electrodes [62]. The benefit of using piezoresistivity method is that the detection mechanism can be integrated easily into lab-on-a-chip type devices. Moreover, it is more cooperative with small amount of sample.
5. Conclusion

One of the major problems in developing a rapid test for *E. coli* is the bulky and complicated machine used, including lengthy enrichment procedures. Therefore, biosensors became an attractive proposition for diagnostic and bacteria detection in the research area. It continuously interesting because of newly recognized, newly appreciated, and evolving agents. Developing a method to detect *E. coli* is not only benefits the consumer by ensuring the safety of the water supply, but also benefits the industry by preventing costly recalls. The requirement to increase the sensitivity is needed to identify and consider microbial pathogens for possible regulation in water supply since *E. coli* present at very low magnification in water resources. The research area by using the interaction of bio-recognition element in label-free biosensor became a trend nowadays. The main advantages offered by biosensors are the possibility of portability, of miniaturization and working on-site, and the ability to measure pollutants in complex matrices with minimal sample preparation. Although many of the systems developed cannot compete with conventional analytical methods in terms of accuracy they can be used by regulatory authorities and by industry to provide enough information for routine testing and screening of samples.

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