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Pathogens detection and identification in drinking water

Khadija Hanif[*1], Muhammad Riaz[2], Iqra Zahoor[1]*

[1]*Department of Chemistry, University of Agriculture, Faisalabad-38040-Pakistan.*

[2]*Department of Basic and Applied Chemistry, Faculty of Science and Technology University of Central Punjab, Lahore 54000, Pakistan.*

Abstract

The detection and identification of waterborne pathogens is crucial for ensuring public health globally. Drinking contaminated water with hazardous pathogens like bacteria, fungus, protozoa and viruses causes a significant effect to human health and leads to diseases like cholera, ulcers, Typhoid fever, hepatitis, nausea, Aspergillosis, and dermatitis. The World Health Organization (WHO) estimated that *Escherichia coli*, linked to water contamination, is associated with 485,000 deaths from diarrheal diseases annually. Therefore, continuous monitoring and effective detection methods are essential. Numerous techniques have been employed for pathogen identification, ranging from traditional to advanced detection. Traditional methods like culture-based methods, immunosorbent assays and nucleic acid base methods have played an important role in the identification of pathogens. However, these methods often suffer from limitations like sensitivity, specificity, and turnaround time. Advanced methods include polymerase chain reaction (PCR), DNA microarray, next-generation sequencing (NGS), and biosensors. These methods are sensitive, specific, time-effective, and capable of detecting emerging pathogens. However, emerging technologies are gaining attention for their potential in pathogen recognition. These technologies include nanosensors, microfluidics, wireless sensors, artificial intelligence, and CRISPR-Cas systems which have revolutionized the ability to detect and monitor waterborne pathogens.

Keywords: Waterborne pathogens, drinking water safety, advanced methods, biosensors, emerging technologies.

Full length review article **Corresponding Author*, e-mail: *khadijahanif284@gmail.com*

1. Introduction

Access to safe and drinking water is a fundamental human right. The presence of pathogens poses a significant challenge worldwide, including fungi, bacteria, protozoa, viruses, and prions [1]. Water is the most important resource, as it forms the basic medium for life. It is estimated that only about 2.5% of the Earth's water is fresh water. "Safe drinking water" as defined by the World Health Organization (WHO) that, water, when ingested over a period of time, offers no substantial risk to health. Rivers, lakes, ponds, groundwater, and streams are some of the freshwater sources [2]. Untreated, undertreated, or accidental release of sewage allows pathogens like *E. coli*, Salmonella, Giardia, Norovirus, Rotavirus, Cryptosporidium and Campylobacter to contaminate these water sources. It is estimated that children under the age of five are highly susceptible to diarrheal diseases, which account for over 90% of annual deaths, with about 5000 children dying per day. Waterborne disease outbreaks is challenging not only to developing nations but also to developed countries [3]. Figure 1 illustrates the rate of outbreaks due to pathogens varies across different countries and regions [4]. It is predicted that freshwater resources will decline, and drinking contaminated water raises the chance of dying from fatal diseases (e.g., fever, diarrhea, systemic disorders and gastrointestinal disorders) [5-6].

A WHO evaluation (Organization 2004) found that 80% of human infections in impoverished nations are caused by microbial pollution of water [7]. Hence, it is challenging to discover pathogens due to invisibility and unpredictable behavior. To identify bacterial infections, a variety of techniques are required that rely on growing the pathogens in various alternative or specific mediums [8]. The identification, isolation, and detection of pathogens might be a difficult, time-consuming, and very costly process if done on a regular basis. Traditional methods, such as culture-based methods, enzyme linked immunosorbent assays, lateral flow immunoassays (LFIA), polymerase chain reaction (PCR), and flow cytometry are effective to some extent. These methods have serious shortcomings [9]. These limitations are linked to the number of pathogens in a sample, the accuracy of the detection, the lack of sensitivity, specificity, and the

time required to isolate or identify the pathogen. Due to these challenges, a faster pathogen detection technique is needed to ensure human health [10].

In response to these needs, technological advancements were made and rapid and accurate identification of pathogens was developed. There are a number of reliable methods, like dPCR, multiplex PCR, DNA microarray, metagenomics, next-generation sequencing, and biosensors, that deliver high quality results, give quick analysis and efficient in the detection of emerging pathogens [3]. Among these cutting-edge methods, biosensors may be considered a dependable and accurate instrument for detecting bacterial contamination when compared to more traditional approaches. These biosensors have shown potential for detecting bacterial illnesses in contaminated drinking water [11]. Furthermore, with the appearance of emerging pathogens and the increasing complexity of water contamination, researchers are developing innovative technologies like microfluidics, nanosensors, wireless sensors, artificial intelligence-machine learning, and CRISPR-Cas system. These emerging technologies revolutionized the ability to detect and monitor waterborne pathogens [12].

2. Hazardous Pathogens in Drinking Water

2.1 Typical Pathogens in Water Sources

When it comes to the quality of drinking water, major concerns are the contamination of water sources with microbial pathogens. Water contains a variety of well-known contaminants, such as bacteria, protozoa, viruses, and fungi. Numerous microbes possess the ability to trigger epidemics and lead to illnesses transmitted via water. About 1400 distinct kinds of pollutants, including a few fungus or helminth species, as well as bacteria, viruses, and parasitic protozoa, have been related to several life-threatening disorders. Figure 2 illustrates a comprehensive overview of common waterborne pathogens and their associated diseases [13].

2.1.1 Bacteria

Bacteria are common pathogens found in water sources. Plasmids related to drinking water quality have probably been studied the most, with the exception of bacteria. They don't always do damage. However, a vast majority of bacterial species are dangerous and may cause damage to humans. *Escherichia coli,* Salmonella enterica, *Clostridium, Bacillus cereus, Vibrio species,* and *Staphylococcus aureus* produce toxins [14]. Foodborne diseases and waterborne illnesses may arise from consuming contaminated food or water, either by ingestion or consumption of their toxins. These might include a broad range of cuisines and various surroundings. *Escherichia coli* is the most common pathogen and may cause hemorrhagic colitis and hemolytic uremic syndrome in humans [15].

2.1.2 Viruses

Viruses are microscopic pathogens found in water and wastewater, with a diameter ranging from 18 to 1500nm. Pathogenic viruses include the polyomavirus, DNA viruses, Hepatitis A and E viruses, rotaviruses, noroviruses, enterovirus, and astroviruses. It is reported that the prevalence of rotavirus, norovirus and astroviruses varies. The hepatitis A virus is often found in urban sewage. Hepatitis E virus is more abundant in poor sanitation countries [16]. Viral infections are the most serious kind of disease that people may get from drinking water. Actually, drinking water contaminated with viruses may cause potentially dangerous diseases, such as diarrhea, encephalitis, hepatitis, and gastroenteritis. The purification stage recovers viruses in quantities ranging from less than 1 to over 1000 liters. The detection method must be technically feasible, highly recoverable, generate tiny quantities of concentrate, be economically priced, and be suitable for a broad spectrum of viruses. There are several strategies, like polymerase chain reaction, and enzyme-linked immunosorbent assays, that may be used to extract viruses from environmental specimens [17].

2.1.3 Protozoa

Protozoa constitute a varied collection of single-celled, eukaryotic organisms (flagellates, ciliates, amoebae, and sporozoa). Some watery protozoa are well known, such as *Giardia and Cryptosporidium* [18]. *Cryptosporidium* is considered a significant waterborne pollutant as it is found in numerous water sources. There are 20 valid *Cryptosporidium* species, and over 40 genotypes of this parasite cause human cryptosporidiosis. *Cyclospora cayetanensis, Balantidium coli, Blastocystis hominis, Toxoplasma gondii,* and *Isospora belli* are different types of protozoa present in contaminants. These parasites are the main causes of diarrheal illness in humans as well as animals worldwide, they may even reduce the lifespan of hosts with weakened immune systems. Waterborne protozoan parasite infections are a health risk to the public and the root cause of many worldwide epidemics in the whole world [16-19].

2.1.4 Fungi

Fungi are heterotrophic, ubiquitous organisms present in water sources. Unlike other pathogens such as viruses, bacteria, and protozoa, fungi have not traditionally been recognized as harmful organisms. Filamentous fungi, including *Aureobasidium pullulans, Cladophialophora spp., Exophiala dermatitidis, E. jeanselmei, E. mesophila, Rhinocladiella similis, and Graphium sp.,* are recognized[20]. Studying fungi in water is crucial because they influence the tastes and fragrances of drinking water and may lead to fatal diseases. The biosecurity of drinking water significantly affects human health. Health problems might arise from allergies, mycotoxins, and animal infections. Many fungal taxa cause infections under certain conditions [21]. The immune system can only partly stop the invasion of fungal infections, and this capacity fluctuates during life and is dependent on the environment. Fungi are omnipresent in the environment and may grow to huge sizes in water. Hence, fungi and their metabolites are generally recognized as the most harmful pollutants. Fungi may exist in different water sources, like tank water, groundwater, surface water, and waterworks [22-23].

2.2 Health Hazards Linked to Particular Pathogens

Drinking contaminated water from groundwater sources may be the result of several interconnected problems (for example, microbial biofilms, pollution in sewage, poor infrastructure of water pipelines, and inefficient water

treatment systems). Water-borne diseases in humans are mostly brought on by direct or indirect contact with water tainted with excrement-carrying pathogens, as is often the case in developing nations. In developed nations, waterborne pathogens are also linked to illness epidemics in particular rural areas [24]. Most outbreaks of waterborne illnesses are primarily caused by infections connected to microbial contamination. Water is home to a variety of pathogens, like bacteria, viruses, fungi, and parasites (including protozoa) [25]. Various effects result from different causative agents found in water sources such as rivers, lakes, surface-level settling water, coastal seawaters, marine waters, and urban water. Table 1 shows a comprehensive overview of the waterborne diseases posed by contaminants [16-26-28].

2.3 Emerging waterborne pathogens

Urban runoff, leaky sewage pipes, effluent discharges, and agricultural wastewater are some examples of water-borne pathogenic organisms. Enteric viruses are prevalent and deadly aquatic pathogens transmitted through the ingestion of contaminated food or water and may cause infections linked to outbreaks as well as solitary instances. The viruses investigated the most include noroviruses, astroviruses, enteroviruses, and the hepatitis A viruses [29]. In addition to enteric viruses, additional potentially emerging pathogens are found, including the origins of adenoviruses, Picobirnaviruses, parvoviruses, circoviruses, polyomaviruses, coronaviruses, mycobacterium, and microsporidia. Figure 3 shows a diverse range of emerging pathogens that contaminate water sources [30].

3. Traditional Methods for Pathogens Detection

Pathogens are a major hazard to public health worldwide, thus developing sensitive, precise, and focused detection methods is essential. Conventional methods include many culture and non-culture based methods. Non-culture methods are classified into nucleic acid base methods and immunology based methods. Figure 4 highlights the classification of traditional techniques employed in pathogen detection [31].

3.1 Culture based Methods

The phrase "culture methods" describes the in vitro development of pathogens on a specific medium that is high in nutrients. Microorganisms proliferate on these culture media, giving rise to colonies that exhibit variation in shape, size, and color. When discriminating between fungal and bacterial genera, this is considered the straightforward approach for visual identification [32]. Numerous culture-based methods, are used for cultivation, and identification of pathogens [33]. Despite the method's cheap cost, ease of use, and ability to quantify pathogens, its deficiency of sensitivity makes it unsuitable for application in specimens with very low concentrations of target. Moreover, a multitude of factors, including complex matrices containing inhibitors, might impact the pathogen's growth, perhaps leading to false-negative findings. To identify the pathogen with a turnaround time of two to three days, many labor- and time-intensive processes are required, including specific improved coating on selective medium, previous enrichment, and biochemical or serological confirmation tests. A speedy testing result is thus not achievable [34].

3.2 Non-Culture Methods

3.2.1 Immunology Based Methods

The immunological identification approach is based on the specific binding between antigens and antibodies [11]. Various antibodies are used in a variety of tests developed to identify various bacteria and microbial toxins. The degree of specificity shown by these antibodies largely determines their suitability. Numerous immunological methods, including lateral flow immunoassay (LFIA), enzyme-linked immunosorbent assay (ELISA), and flow cytometry, may be used to identify waterborne microorganisms [5].

3.2.1.1 Enzyme Linked Immunosorbent Assays (ELISA)

By using an enzyme-linked immunoglobulin (known antibody) that binds to the antigen, an unknown bacterial antigen may be identified by ELISA. A good binding will produce a visible change. One important aspect influencing ELISA is the specificity of the antibody employed. Low specificity and sensitivity may occur from the antibody's impact on the reaction process, which might cause false-positive findings. In order to circumvent this, ELISA is often used in combination with different detection techniques. Additionally, some researchers examined ELISA's non-specificity in an attempt to eliminate the false positive and also negative outcomes that are sometimes linked to the process [11].

3.2.1.2 Lateral Flow Immunoassays (LFIA)

Lateral Flow Immunoassay (LFIA), is a popular analytical technique. The main reasons why LFIA technology is so popular are its low cost, quick turnaround time, and simplicity of usage. LFIA devices are beneficial in underdeveloped countries where regular analytical gear is too expensive to conduct analyses. This is the reason for their great commercial success, as well as the fact that they may be used in non-laboratory settings by unskilled people. Strip tests are used to detect different analytes in a variety of applications. Moreover, it almost entirely eliminates the need for solvents and doesn't need sample transportation or a cold chain [35-36].

DNA, antibodies, enzymes, and synthetic chemicals are used to detect receptors. Tags such as enzymes, nanoparticles, redox molecules, and fluorophores are utilized to detect tiny, neutral substances. LFIA formation consists of a membrane with several pads linked and placed on adhesive and backing cards. LFIA uses the capillary force between an antigen and a tagged antibody to provide a visual result in predetermined regions known as test lines and control lines. Despite all of its wonderful characteristics, typical LFIAs only detect a single target analyte at a time. Forensic surveillance, clinical diagnosis, environmental control, food safety, and environmental control are just a few of the applications that sorely need multiplexing capacity. Moreover, multiplex LFIA makes testing more affordable without sacrificing its effectiveness [37].

3.2.1.3 Flow cytometry

Historically, flow cytometry has primarily been used for research and diagnostic purposes and relies on specialized antibodies labeled with organic fluorophores. However, recent works have shown the identification of pathogens like

bacteria and parasites by light scatter parameters. These parameters include forward scatter, sideward scatter, and various fluorescent wavelengths, depending on the complexity of the instrument. It consists of three main systems: fluidics, optics, and electronics. Results are typically displayed on a graph. Forward scatter and sideward give information about the size, shape, and complexity of the sample. Forward scatter measures narrow-angle light scatter influenced by the size of the sample and refractive index, while sideward scatter measures right-angle light scatter, internal complexity, and shape. During analysis, the instrument can be triggered by one of its parameters [38-39]. Flow cytometry is constrained by the necessity for cells under analysis. Furthermore, flow cytometry often generate massive amount of data, analysis complicated [40].

3.2.2 Nucleic acid-based Methods

3.2.2.1 Polymerase chain reaction (PCR)

PCR is a commonly used method to detect pathogens [41]. PCR functions by amplifying a target DNA sequence through a cyclic process comprising denaturation, annealing, and extension [42]. The first known use of PCR for diagnosis was the identification of Mycobacterium tuberculosis (M. tb) in 1991. Specific pathogenic viruses may be identified using PCR. The DNA polymerase uses the first polymerization cycle to copy the target sequence from pathogens. The created copy of the target sequence is then used as a template to make more copies of it. The real-time PCR method makes use of a fluorescent dye that becomes luminous as it binds to the amplified DNA sequence. Further fluorescence tuning may be carried out in order to quantify the pathogen present in the sample. In order to extract the DNA from the cells, the sample is set and managed using the amplification procedure. The extracted DNA pool is then heated via the processes of denaturation, annealing, and extension [43]. The PCR method has the advantage of quick analysis with high sensitivity and is capable of detecting trace amounts of target DNA with a small amount of starting material. However, PCR also presents limitations, including the inability to distinguish between viable and non-viable organisms and susceptibility to inhibit impurities [9].

3.2.2.3 Loop-mediated isothermal amplification (LAMP)

It is a DNA amplification technique that operates under isothermal conditions. It requires a set of four to six primers and a DNA polymerase. LAMP generates stem-loop DNA structures with multiple replications of the target sequence. This method efficiently synthesized number of DNA strands in a short time. Phosphate ions are produced that form a white precipitate of magnesium pyrophosphate. It allows easy determination of successful amplification. However, other methods like gel electrophoresis, real-time terbidimetry, and fluorescence probes are employed for LAMP detection. Despite its specificity and effectiveness, LAMP presents challenges, including the intricate design of multiple primers and potentially complicated matters. Moreover, the resulting product comprises a complex mixture of stem-loop DNA structures with different sizes [44].

3.2.3 Molecular Imprinting Method

A quick and sensitive method is "molecular imprinting." It combines molecularly imprinted polymers (MIPs) with mass-sensitive, optical, and electrochemical transducer platforms. With this method, microorganisms may be detected very quickly and sensitively [45]. MIPs are synthetic receptors. The target analyte acts as a template and may be removed to create holes in MIPs that maintain form, size, and functionality [46]. MIPs are more stable and reusable than other receptors. MIPs are used to detect a broad variety of hazards, such as larger analytes like pathogenic bacteria, smaller chemicals like mycotoxins, and macromolecules like allergenic proteins [47]. Due to their inherent characteristics, which include their enormous size, poor stability in chemical solvents, fluidity, and delicate structure, bacteria are more challenging to detect scientifically than other tiny substances. A number of novel imprinting methods, including stamp imprinting and cell membrane molecular imprinting, have been documented in an attempt to improve the rationale behind MIPs' molecular design for pathogen detection. Moreover, the idea that the pathogen and polymer interact non-covalently is the basis for the application of MIPs in pathogen-bacterial detection, a notion that needs further clarification [48].

4. Limitations of Traditional Methods

Detection and identification of pathogens by traditional methods are found to be time-consuming, labor-intensive, and less specific. Certain techniques depend on the capacity to cultivate pathogens, which might not be appropriate for all microbes [49]. PCR and LAPM techniques offer rapid and sensitive detection but limit their utility in accessing microbial viability and can be prone to false positive and negative results, as well as shown non-specific amplification [9]. ELISA may lack sensitivity for detecting low concentrations of analytes and can exhibit cross-reactivity with structurally similar molecules. Its labor-intensive nature can increase the risk of errors [11]. Molecular imprinting is cost-effective, but poor imprinting efficiency can result in decreased binding affinity and specificity of the imprinted polymers. Flow cytometry often gives complicated analysis due to extensive datasheets [40]. Table 3 summarizes the advantages and limitations of conventional methods. To overcome these challenges, faster and more sensitive techniques are required.

5. Advanced Pathogen Detection Methods

Traditional methods of detecting pathogens, such as culture-based methods, PCR, and ELISA, are labor-intensive and insensitive [49]. Modern biological technologies allow for speedier detection with simpler methods and improved analytical sensitivity. These technologies include molecular techniques like qualitative polymerase chain reactions, i.e., dPCR and multiplex PCR, next-generation sequencing, biosensors and molecular imprinting. Their range of use in the field where samples are obtained is constrained by their continued reliance on expensive supplies and laboratory plans. Commercial pathogen monitoring products, such as Colilert, are also available. Most still need laboratory setup, even if they do simplify processes and save time and effort. Figure 5 illustrates a comparison among traditional, advanced and emerging technologies [50].

5.1 Qualitative Polymerase Chain Reaction (qPCR)

5.1.1 Digital PCR

It takes a lot of time and effort to identify target infectious pathogens using traditional culture-dependent techniques. Distinct growth environments or culture media are needed to recognize and enhance certain pathogens. Aquatic environments are increasingly using culture-independent methods to monitor pathogen abundance because they are more sensitive and quicker than culture-dependent methods, such as PCR and quantitative polymerase chain reaction. Furthermore, dPCR, which has better sensitivity than conventional qPCR, has been utilized to count bacteria in aquatic settings. Digital PCR represents an advancement in PCR technology offering sensitive and specific detection and quantification of nucleic acids. This method enables absolute quantification with significantly enhanced precision compared to qPCR. Unlike qPCR, the signals of are measured after the complete amplification [17]. In dPCR, poison statistics are used to determine the number of DNA fragments with a certain sequence that are present in a sample. Underestimation may occur when a gene is redundantly encoded in the genome and many copies of the gene are discovered on a single DNA fragment. After extracting genomic DNA from the enterococcus genome using two commercial DNA extraction kits, it was confirmed that dPCR could detect unique copies of the redundant 23s rRNA gene [51-52].

The results of dPCR quantification showed good accuracy (p-value <0.0001) compared to the nominal concentration estimated from fluorimeter data. This means that dPCR data is closely related to the expected values; showing a slop of 0.98, an intercept of 0.03, and an R^2 value of 0.99 indicates that dPCR results explained most of the variability. Additionally, when cell counts obtained from dPCR were compared to those obtained from qPCR in 24 environmental samples, the dPCR quantification agreed with the qPCR. The slope of 1.08 and R^2 of 0.96 suggested that dPCR results closely matched those of qPCR. When comparing the average results from both methods, dPCR measurements were slightly lower (0.19 log units) than qPCR, with a 95% confidence interval indicating higher precision. Thus, dPCR quantification proved to be more accurate than qPCR [52].

5.1.2 Multiplex PCR

Given the importance of distinguishing between the many types of Otitis externa (OE), using genus- or species-specific primers, a PCR-based assay was developed. It is tested step-by-step on ear aspiration specimens from individuals who were clinically suspected. Following tissue digestion in a lysis buffer, 120 ear aspiration specimens suspected of having otomycosis underwent manual phenol-chloroform extraction. Initially, the multiplex PCR was conducted using hand-made primers for both bacteria and pan-fungi. Primers are used concurrently to identify the bacterial taxa. Every multiplex PCR result was analyzed using the amplicon size [53]. Stepwise multiplex PCR is proven to be less time-consuming, quicker, and more sensitive than culture in identifying and detecting bacterial and fungal OE [54].

5.2 DNA Microarray

The powerful genomic technique Oligonucleotide microarrays enable the characterization of bacteria in environmental samples and enzyme-specific mutation detection. Using nucleic acid hybridization, a single experiment may detect hundreds of genes at once. Microarrays are made up of short, chemically generated sequences that range in length from 25 to 80 bp and are coated on glass slides or chips. Many genes from many species may be quickly identified in a sample due to the high throughput, automation, and massive number of sequences that can be screened using microarray technology. For this reason, large-scale, data-intensive studies are conducted on microarrays. Using microarrays to characterize water pollution also has the advantage of identifying antimicrobial resistance to different medications. The probes may also be used to determine which host the pollutants are coming from. However, complex molecular technologies such as microarrays may be costly, suffer from non-specific hybridization, which would diminish specificity and sensitivity, and struggle to distinguish between non-viable and living cells [41].

5.3 Next Generation Sequencing

Next-generation sequencing is a flexible technique that has the potential to study viruses, bacteria, fungi, parasites, animal vectors, and human hosts. It is a useful method for detecting HAdV in wastewater samples [55]. It has been shown that research using outdated technologies might significantly overstate the quantity of certain types. Many of the "less prevalent" kinds could not be identified using standard Sanger sequencing. Using the chain-termination approach, Frederick Sanger created DNA sequencing technology in 1977. This technique is often known as Sanger sequencing. The smallest genomes, such as those of viruses and organelles, were the first to be sequenced using DNA. Sequencing a bacterium's whole genome was not feasible due to technical and budgetary limitations. Later, this method allowed for the completion of the sequencing of the whole genome of bacteria. The shotgun technique is considered the gold standard and has been used to sequence the whole genomes of many bacteria throughout the years [56].

5.5 Biosensors

Biosensors have become more and more popular in recent years as a way to detect pathogens due to their very sensitive, fast, low-cost, and specific analysis. In order to identify target pathogens, biosensors transform biological information into optical or electrical signals [6]. Utilization of low cost, quick turnaround, and the possibility of field testing is critical to long-term development, especially in developing countries. Biosensors may be used for food hygiene and water quality. The biosensor is one of the most important tools for very sensitive analytical detection [11]. Molecules such as aptamers, proteins, peptides, and antibodies may constitute the bio-receptor or recognition element. Aptamers seem to be a promising tool for pathogen identification due to their higher specificity, sensitivity, and stability compared to traditional detection methods. Aptamers are short, single-stranded DNA or RNA with the capacity to bind to a wide range of target molecules, including

ions, proteins, and tiny chemical compounds. In addition to being easier to synthesize, store, and label than antibodies, aptamers also have lower manufacturing costs [57]. Biosensors are classified in several ways based on the transduction principle to identify pathogens. Some of them discussed below are optical biosensors, surface-plasmon resonance biosensors, and electrochemical biosensors.

5.5.1 Optical biosensors

Individual cells inside complex matrices are examined using optical biosensors. Microbes floating in a liquid medium are illuminated by a laser beam. By gathering the scattered light using a set of lenses and photocells, one may examine the amount and type of the scattering to evaluate the size, shape, and quantity of the pathogens. The method is highly sensitive, as 102−103 bacterial cells, and 102 yeast cells per milliliter can be identified. Colorimetric bio-sensors are optical bio-sensor systems because they change color. Colorimetric biosensor systems come in two varieties: solution-based and flat substrate-based. Flat substrate-based sensors are popular because of their small sample and analysis volume, convenience of use, and common usage of paper and glass. These products take advantage of the sample's capillary movement across the membrane and the agglomeration of gold nanoparticles (Au NPs), which results in a change in color [58].

5.5.2 Surface plasmon resonance (SPR) biosensors

Surface plasmon resonance is a sub-type of surface Plasmon's (SPs) that possesses unique characteristics, including variable resonant frequencies with different refractive indices, high sensitivity, and real-time analysis. These characteristics have led to the practical applications of SPR in biosensors for identifying and characterizing bacteria, viruses, and other microorganisms in water samples [59]. SPR is based on the principle of detecting changes in the refractive index of a sensing layer as a result of molecular interaction on the sensor surface. Conventional SPR sensors activate SPs to absorb chemical and biological substances [60]. A prism's top is usually coated with a thin coating of metal. However, due to metal's poor absorbability, scientists are looking for alternatives to enhance the performance of biosensors. The ability of a SPR biosensor to detect resonance fluctuations depends on its sensitivity, and the low-sensitivity problem in SPR structures is yet unsolved [61].

5.5.3 Electrochemical microbial biosensors

A chemical sensor transforms chemical data into a signal for analytical purposes, including compositional analysis or the concentration of a specific sample component. The sample handling plan and sensor signal readout of the microbial biosensor set it apart from the electrochemical technique. In electrochemical biosensors, the recognition element may be any biomolecule, such as an enzyme, antibody, aptamers, DNA, or another, that has a specific binding affinity towards the target analyte. This interaction determines the biosensor's activity and specificity. After analyzing the analyte on the electrode surface, it may attach to the recognition element, allowing changes in electrical properties such as voltage or current to be monitored. The biosensor's ability to provide precise and sensitive detection is made possible by the special interaction between the biological molecule and the analyte during the electrochemical sensing process [1]. Electrochemical biosensors can be classified into two categories; affinity sensors and bio-catalytic devices, depending on how they identify biological signals. Bio-catalytic devices employ enzymes, tissues, or entire cells to detect specific analytes and generate signals. In contrast, affinity sensors function by establishing specific binding interactions between analytes and biomolecules, such as an antibody, receptor, or nucleic acid [52]. For electrochemical biosensors, enzymes are the main biocatalysts due to their high selectivity and bio-catalytic activity. These low-cost, portable, and easily manipulated enzyme-based bio-catalytic sensors have simple designs and don't need complex apparatus [62].

6. Emerging Technologies of Pathogen Detection

The complexity and heterogeneity of microbial communities in aquatic systems present challenges to traditional detection methods. Harmful bacteria cannot be identified by pure culturing-based detection when it comes to pollutants. For primer-based targeted sequencing approaches, the demands of microbial identification in low-concentration environmental specimens have proved to be intractable. The rapid development of high-throughput sequencing methods has made it possible to identify dangerous microorganisms in water systems with speed and accuracy [63].

6.1 Nanosensors

Many of the water quality monitoring systems in use today may be replaced with sensors enabled by nanotechnology. Some publications state that in order to monitor an analyte, the nanosensor may link to it quickly and reversibly. However, the Nano-probe may be selective in detecting certain chemicals or bacteria with greater sensitivity and does not need reversibility [64]. To be explicit, a "nanosensor" is any sensor platform that makes use of the special optical, electrical, or magnetic capabilities of nanomaterials (NM) to enhance analyte detection. More sensitive nanosensors that are capable of identifying illnesses, toxins, and water pH have been proposed on many occasions. A signal processing mechanism, a recognition element, and a suitable nanoscale are the three main parts of a nanosensor [65]. A detectable signal that is recorded is created when the target analytes and the recognition element comes into contact. The electrical, optical, or magnetic properties of the material and related signal processing methods may be employed to evaluate the sensitivity of the nanosensors when signals are detected during analyte contact [66].

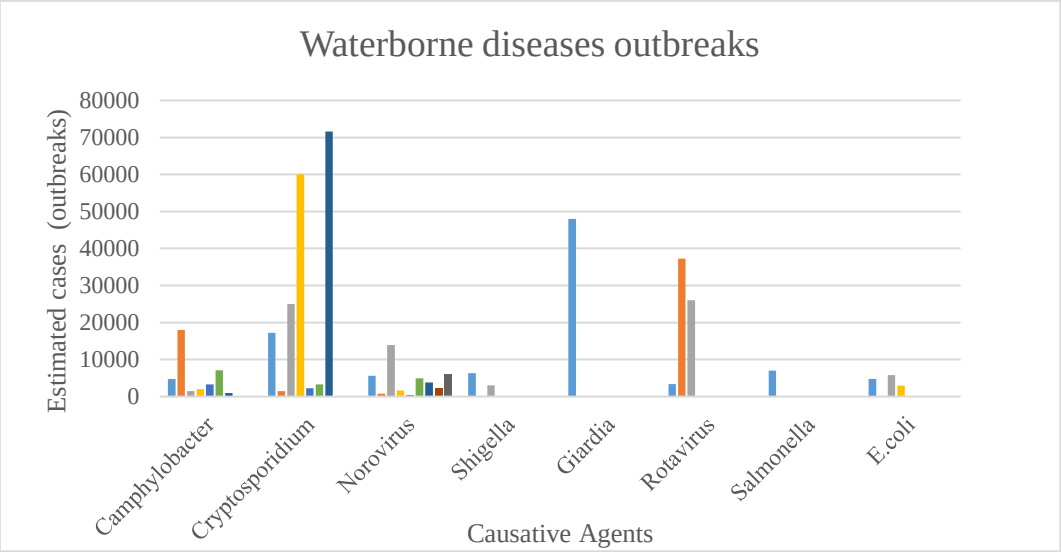

Figure 1: Waterborne diseases outbreaks variation due to different microbes.

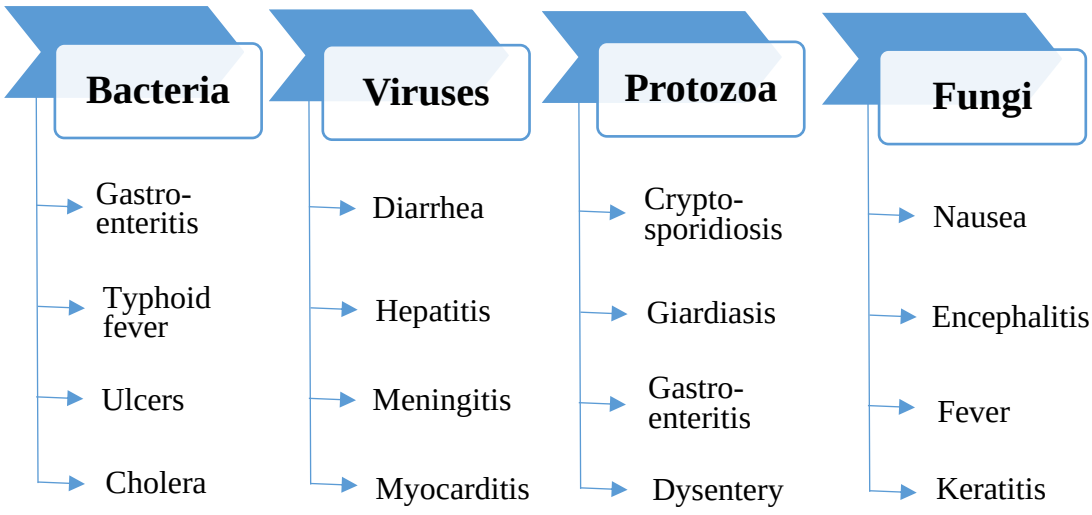

Figure 2: Common waterborne pathogens and their respective associated diseases.

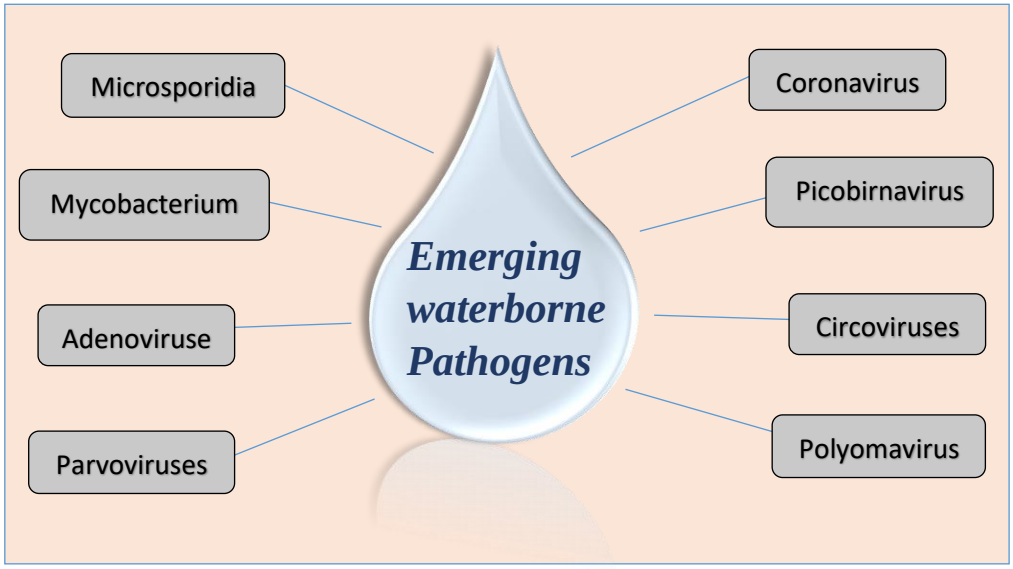

Figure 3: *Emerging waterborne pathogens: A Global Perspective.*

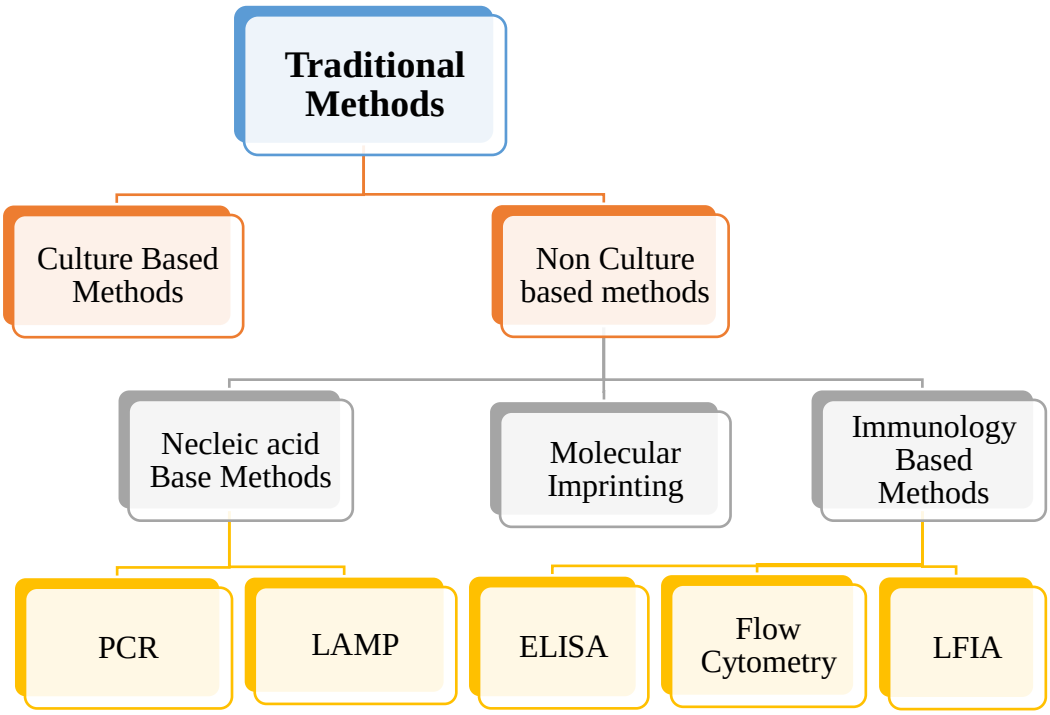

Figure 4: Schematic overview of traditional methods for pathogen identification.

Table 1: Types of microbial contaminants-associated diseases

Pathogens	Causative agents	Symptoms/Diseases
Bacteria	• *Escherichia coli* • Salmonella enterica • Clostridium • Bacillus cereus • *Staphylococcus aureus* • Vibrio species	Neonatal meningitis, hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), gastrointestinal infections, diarrhea, fever, botulism, tetanus, eye infections, pneumonia, bacteremia, toxic shock syndrome, cholera etc.
Viruses	• Hepatitis A and E viruses • Enteroviruses • Rotaviruses • Noroviruses • Sapovirus • Astroviruses	Fatigue, fever, jaundice, nausea, HIV/AIDS, skin cancer, acute flaccid paralysis, aseptic meningitis, myocarditis, encephalitis, poliomyelitis, rotavirus gastroenteritis etc.
Fungi	• *Exophilia dermatitidis* • Aureobasidium • *Exophilia jeanselmei* • *Cladophialophora spp.* • *Rhinocladiella similis*	Chromoblastomycosis, Aureobasidium pullulans, Phaeohyphomycosis, meningitis, Cladophialophora mycosis, Rhino cerebral mucormycosis etc.
Protozoa	• Amoeba • Giardia • Cryptosporidium • *Balantidium coli* • *Blastocystis hominis* • *Toxoplasma gondii* • *Isospora belli*	Neurological symptoms, Acanthamoeba keratitis, cryptosporidiosis, balantidiasis, diarrhea, toxoplasmosis, HIV/AIDS, Isosporiasis, fever, weight loss, abdominal pain etc.

Table 2: Emerging Contaminants, Associated Diseases and Sources of Transmission.

Contaminants	Pathogen type	Infections/Diseases	Size/Diameter	Sources
Microsporidia	Protozoa, fungi	AIDS	1-3µm	Ground water, drinking water
Mycobacterium	Rod shaped bacteria	Cough, fatigue, low-grade fever, tuberculosis	1-10µm	Natural and drinking water
Adenoviruses	Double-stranded DNA virus	Pneumonia, eye infection, gastroenteritis	70µm	Drinking water
Parvoviruses	Single-stranded pathogenic virus	Gastroenteritis	18-25µm	Feces, ground water
Coronavirus	Spherical pandemic virus	SARS-CoV-2	60-140µm	Drinking water
Polyomavirus	Non-enveloped double stranded virus	Kidney infections, cancer	38-43µm	Sewage water
Picobirnaviruses	Non-enveloped double stranded RNA virus	Gastroenteritis	30-40µm	Lakes water, ground water
Circoviruses	Circular single-stranded DNA virus	AIDS	30-32µm	Feces, Silva, sewage water

Table 3: Advantages and limitations of traditional methods.

Detection Methods	Advantages	Limitations	References
Culture based methods	• Quantification • Characterization • Versatility	• Labor intensive • Time-consuming • Limited scope • Requiring 24-48 h of culture	[34]
PCR	• Quick analysis • Minimal sample requirement • Ease of use	• unable to distinguish live and dead organisms • susceptibility to inhibit sample impurities	[9]
ELISA	• Low cost • Short training time • Less medical waste	• Detection of *E. coli* only • False negative outcomes	[11]
Flow cytometry	• High throughput • Single-cell analysis • Rapid results	• Extensive sample preparation • Limited detection sensitivity • Expensive	[38-40]
LFIA	• Simple and ease of use • Cost effective • No instrumentation required	• Limited multiplexing • Detection of single target at a time	[37]
LAMP	• Isothermal amplification • High specificity • Generate large amounts of DNA	• Complex primer design • Complex product mixture • Dependent on primer specificity	[44]
Molecular Imprinting	• Cost effective • Reusability • Selectivity	• Limited binding kinetics • Limited adaptability	[48]

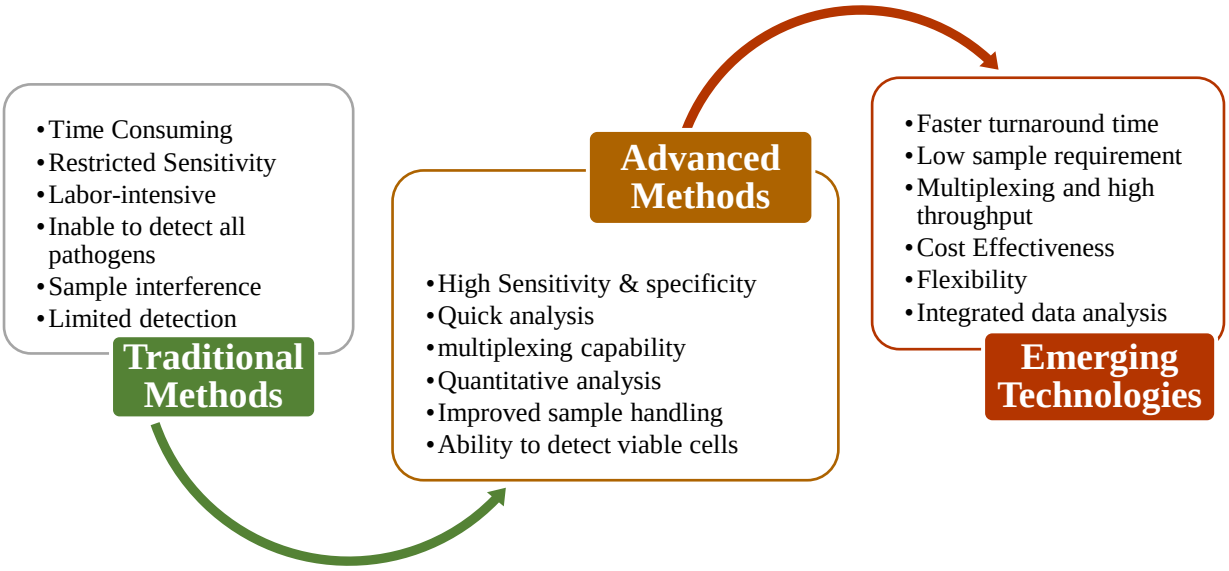

Figure 5: Comparison of traditional, advanced and emerging technologies.

Many nanosensors have very low detection limits. The ultra-small size of a nanosensor-based detection system ensures that biological elements such as viruses, bacteria, and antigen-antibody pairs are effectively identified. Sample preparation, such as concentration and purification, may be required for better detection after the usage of nanosensors [67].

6.2 Microfluidics: a quick analysis method

The precise manipulation of very tiny fluid volumes via the use of micro-scale devices is known as microfluidics [68]. Microfluidic devices are the main tool used to regulate fluids in micro-fabricated channels and chamber structures. Additionally, microfluidics may be utilized in combination with a range of detection techniques, including mass spectroscopy, fluorescence spectroscopy, PCR, and LAMP, enabling on-chip or after-chip analytic detection [69]. There are several advantages to microfluidics over conventional laboratory-scale investigations. The ratio of surface area to volume scaled inversely in terms of characteristic length may lead to an improvement in heat and mass transmission into and out of a chip as the device's dimensions are reduced. It may also be possible to use additional physicochemical interfacial phenomena that are not usually observable at macroscopic scales. Furthermore, separation may be completed more rapidly and successfully at smaller sizes. Additionally, the integration capabilities of microfluidics enable the automation and integration of all benchtop laboratory protocols, including sample handling, reaction, separation, and detection, on a single chip in a manner akin to the unit operations of a chemical plant. Microfluidics has the ability to provide a multitude of new opportunities for cell manipulation and research due to its tiny size [70].

6.3 Wireless Sensors Network

Android smartphones and most Bluetooth transceivers may be used with our wireless sensor system. With an Android application, users may now adjust the frequency-sweeping step size and start/end frequencies in impedance analysis. The phone's "Connect" button allows the sensor and Bluetooth to link. Bluetooth communication offers two benefits: efficient power consumption of less than 10 mW and standardization for desktops and cellphones. The Arduino microprocessor board then asks the AD 5933 chip to deliver sinusoidal impulses to the bacteria sensor. Based on the input parameters from the smartphone, the necessary instructions are generated by the Arduino microprocessor board. The Bluetooth shield and Arduino board return the relevant signals that the AD 5933 chip receives to the smartphone, contingent on the concentration of bacteria [71]. The smartphone software graphs the impedance value in proportion to frequency on the screen when this procedure is finished. Smartphone users may utilize this technology to find out how many *E. coli* germs are present in as little as 10 milliliters of water [72].

6.4 Machine Learning and Artificial Intelligence

Machine learning is a concept in artificial intelligence. It is used to build models or systems that use historical facts to forecast future events. To observe data, machine learning systems have the ability to interpret and modify their structures. Diabetes, coronary artery disease, COVID-19, and waterborne infections are among the ailments that machine learning (ML) is used to identify and forecast, much like other AI techniques. Using machine learning methods, several researchers have predicted waterborne infections as a means of preventing sickness. Aquatic disease outbreaks of varying sizes and durations are simulated using a machine learning model [73]. Predictive models estimate densities of bacterial indicators, such as *E. coli*. The state standard will be exceeded by using readily or

quickly observable environmental and water quality factors. The use of predictive algorithms allowed the evaluation of recreational water quality in near real-time. Using these techniques, *E. coli* concentrations in recreational rivers are also predicted [74].

6.5 CRISPR-Cas system

Prokaryotes have the CRISPR family of DNA sequences. It was first found in the 1980s as a method for genome editing. Different CRISPR-Cas systems, including CRISPR-Cas9, CRISPR-Cas12, CRISPR-Cas13, CRISPR-Cas14, and CRISPR-Cas3, employ distinct mechanisms to detect and differentiate biological activities and nucleotide sequences. The CRISPR-Cas system is developed to identify nucleic acids and biomarkers from infections, which will aid in the development of low-cost, sensitive, and tailored diagnostics for infectious illnesses. The flexibility of CRISPR-based diagnostic tools stems from their capacity to target any nucleic acid sequence via modification of the guide RNA sequence. Typically, CRISPR/Cas systems are integrated with PCR, LAMP, and transcriptional technologies to enhance diagnostic effectiveness. Data can be detected using qPCR or a gel electrophoresis readout [75].

7. Conclusions:

Detecting waterborne pathogens is crucial for safeguarding public health as contaminated water lead to various diseases. Traditional methods like culture-based methods, PCR, ELISA, LFIA, and LAMP have played a fundamental role in identifying and monitoring waterborne pathogens. However, these methods often suffer from limitations in terms of sensitivity, specificity, labor-intensive, cost effective, and turnaround time. Additionally, they may not be well-equipped to detect emerging pathogens. Despite these limitations, traditional methods provide baseline for comparison with advanced techniques and can still be useful in certain context where sophisticated equipment may not be readily available. However, limitations of traditional methods underscore the need for the adoption of advanced and emerging technologies such as DNA microarray, next generation sequencing, metagenomics, and biosensors. These approaches offer enhanced sensitivity and rapid detection compatibilities. With the increasing complexity of water contamination, researchers are developing innovative technologies like microfluidics, nanosensors, wireless sensors, artificial-intelligence, machine-learning, and CRISPR-Cas system. These emerging technologies revolutionized the ability to identify and monitor broader range of pathogens including emerging contaminants. Combination of traditional, advanced, and emerging technologies provide a comprehensive toolkit for addressing the challenges of pathogen detection. Continued research in these technologies is essential to safeguard public health and ensuring the provision of safe drinking water for all.

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