



## Evaluation of Microbiological Pathogen Detection Methods as Per Indian Standards in Drinking Water

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### Abstract

Safe drinking water is essential for public health, yet microbial contamination remains a significant challenge, particularly in developing countries such as India. This review critically evaluates microbial pathogen detection methods used to assess drinking water quality in the context of Indian standards and global regulatory frameworks. Key indicator organisms and pathogens, including *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, *Vibrio cholerae*, and *Pseudomonas aeruginosa*, are discussed in relation to their health impacts and role in water quality monitoring. The review compares conventional culture-based techniques such as membrane filtration, multiple tube fermentation, and heterotrophic plate count with modern rapid detection methods including polymerase chain reaction (PCR), real-time PCR (qPCR), loop-mediated isothermal amplification (LAMP), enzyme-linked immunosorbent assay (ELISA), biosensors, microfluidics, and next-generation sequencing (NGS). Conventional methods remain widely used due to their reliability and cost-effectiveness but are limited by long processing times and inability to detect viable but non-culturable organisms. In contrast, rapid methods offer high sensitivity, specificity, and faster results, though their application is constrained by cost, technical complexity, and infrastructure requirements. The review also examines the regulatory roles of the Bureau of Indian Standards (BIS) and the Food Safety and Standards Authority of India (FSSAI), highlighting alignment with World Health Organization (WHO) and ISO guidelines. It concludes that integrating conventional and modern techniques provides a more robust and efficient approach to microbial detection. Emerging technologies, including portable diagnostic tools and real-time monitoring systems, are expected to further enhance water quality surveillance and public health protection.

**Keywords:** Microbial Detection; Drinking Water Quality; BIS Standards; FSSAI Regulations; ISO/IEC 17025

### Introduction

Global public health requires access to clean drinking water, especially in developing nations where infrastructure for water treatment and distribution may be inadequate. Nearly three billion people lack proper sanitary facilities, and over one billion people do not have access to safe drinking water, according to the World Health Organization. As a result, waterborne illnesses cause over

1.7 million fatalities per year [46]. These illnesses primarily affect immunocompromised populations, children, and the elderly. India's varied water sources, fast urbanization, and unpredictable climate all pose problems for the country's water quality. Over 200,000 places were found to have poor water quality under the Eleventh Five-Year Plan because of high levels of iron, fluoride, salinity, nitrates, and arsenic [60]. Human health can be seriously impacted

by waterborne pathogens, which include bacteria and viruses. These pathogens can cause everything from gastrointestinal infections to serious illnesses like cholera and hepatitis [3]. Under IS 10500:2012, the Bureau of Indian Standards (BIS) set drinking water quality standards that outline acceptable limits for physical, chemical, and microbiological characteristics [11]. Even stricter microbiological requirements are placed on bottled drinking water by the Food Safety and Standards Authority of India (FSSAI), which mandates that it be free of pathogens like *Salmonella spp.* and *Escherichia coli* [19]. This concerning increase emphasizes how important it is to have efficient microbial monitoring and detection systems [35].

### Importance of safe drinking water for public health

Preventing waterborne illnesses and preserving public health depend on safe drinking water. Human health is directly impacted by the quality of drinking water since contaminated water can spread bacteria, viruses, and protozoa, among other infections. Water safety is crucial in India, because a large section of the population depends on surface and groundwater sources [18]. Hepatitis, cholera, typhoid fever, acute gastroenteritis, and other dangerous conditions can result from drinking tainted water. In India, waterborne illnesses have a significant financial impact on millions of families each year due to medical expenses and missed productivity. Improved water quality can dramatically lower the prevalence of diarrheal illnesses, which continue to be a major cause of death for children under five, according to studies [54].

### Issues of microbial contamination in water distribution systems

Despite advancements in water treatment technologies, microbial contamination in water distribution systems continues to be a problem. Because of leaks, cross-connections, and biofilm formation, the distribution network itself may become a source of contamination [7]. The water quality between the treatment plant and the consumer's tap deteriorates in many Indian towns due to outdated infrastructure and poor maintenance. Even purified water can become contaminated during distribution, according to studies done in different locations of India [18]. Microorganisms in distribution pipes have a protected environment thanks to biofilm formation, which makes them resistant to treatment. Heterotrophic bacteria are further supported in their growth by the water's organic materials and nutrients. Few studies has shown

that heterotrophic bacteria with antibiotic resistance profiles may be present in bottled drinking water sold in Indian marketplaces, potentially endangering the health of immunocompromised people. To avoid contamination, distribution systems must be properly maintained and regularly monitored [46].

### Rise in consumption of packaged drinking water

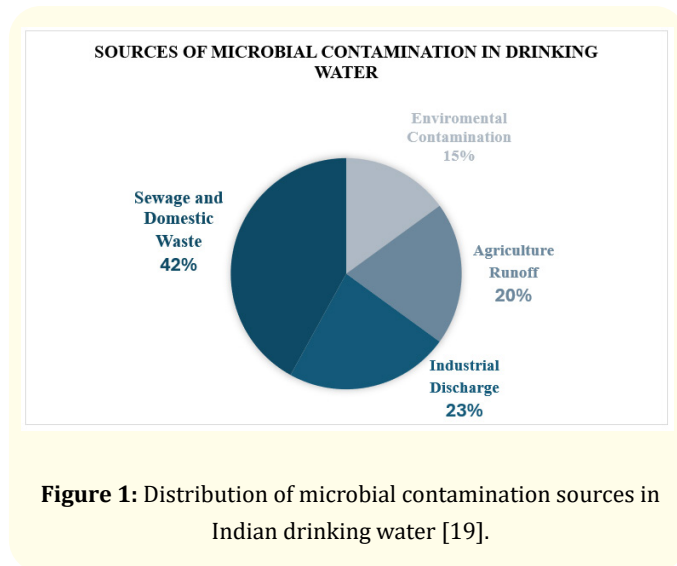
Over the past 20 years, India packaged drinking water market has grown at an exponential rate. The market for bottled water has grown dramatically due to factors like growing urbanization, growing health consciousness, and worries about the quality of tap water. Between 2025 and 2035, the bottled water market in India is expected to expand at a compound annual growth rate of 6.5%. This growth is driven by the belief that bottled water is safer than tap water [55]. But the packaged water industry's meteoric growth has also sparked worries about regulatory compliance and quality control. The nation thousands of bottling facilities make it difficult to maintain constant quality. According to recent studies, pesticide residues, fluoride, and high levels of total dissolved solids were found in over two-thirds of the water samples that were collected. This emphasizes the necessity of strict quality control and regulatory standard enforcement in the bottled drinking water industry [12].

### Need for effective microbial detection methods

Finding microbiological pathogens in drinking water is essential to maintaining public health and safety. Even if they are dependable, traditional culture-based techniques take time and might miss some living things. Rapid detection methods have revolutionized water quality monitoring by making it possible to identify pathogens more quickly and sensitively. However, a number of variables, such as sensitivity, specificity, cost, and the level of technical know-how needed, influence the choice of suitable detection techniques [22]. Detection techniques that are appropriate for various geographic and climatic circumstances are required in the Indian context. Techniques that can be implemented in environments with low resources are especially crucial for rural areas where laboratory infrastructure could be insufficient. A thorough approach to water quality monitoring can be achieved by combining the speed and sensitivity of molecular approaches with the dependability of culture-based techniques [4].

### Sources and types of microbial contamination

Creating efficient methods for monitoring water quality requires an understanding of the types and origins of microbiological contamination. Sewage discharge, industrial effluents, agricultural runoff, and environmental contamination are some of the sources of microbial contamination of drinking water. Environmental factors and the source of contamination determine the kind of microorganisms found in water [59].



### Sources of microbial contamination

#### Sewage and domestic waste

One of the main causes of microbiological contamination in drinking water is sewage and household wastewater. Surface water and groundwater sources may get contaminated due to inadequate sanitation facilities and inappropriate human waste disposal. Untreated wastewater is dumped into water bodies in many Indian cities due to inadequate or nonexistent sewage facilities. This contaminates the water supply with a variety of harmful microbes, such as bacteria, viruses, and protozoa [24]. *Escherichia coli* and *enterococci* are examples of fecal indicator bacteria that show recent fecal contamination and the possible presence of enteric pathogens in water. Study has carried out demonstrated that a variety of pathogens, including as *Salmonella spp.*, *Giardia lamblia*, and *Entamoeba histolytica* can contaminate urban water systems. The degree of pollution in urban water sources were highlighted by the multiplex Polymerase Chain Reaction (PCR) which showed that municipal water samples tested positive for these [26].

### Industrial discharge

Water sources may become microbiologically contaminated by industrial effluents, especially if they contain organic debris that encourages bacterial growth. Wastewater harboring pathogenic bacteria may be released by food processing companies, pharmaceutical production facilities, and other industrial establishments [20]. The quality of water used for drinking can be harmed by the release of untreated or insufficiently treated industrial effluents into rivers and lakes. Membrane filtration techniques are an efficient way to clean industrial wastewater. According to the study, pre-treatment, ultrafiltration, and reverse osmosis can reduce pollutants by more than 88%, resulting in water quality that can be used again in industrial processes. This emphasizes how crucial it is to properly cleanse industrial effluents before releasing those [53].

### Agricultural runoff

Animal waste discharge and the use of tainted irrigation water are two ways that agricultural operations can lead to microbial contamination of water. Untreated sewage is frequently used for irrigation in India's peri-urban areas, which can transfer viruses into agricultural fields and then into groundwater. Studies have reported contamination in Indian sources [26].

### Environmental contamination

Water pollution by microorganisms can also result from natural environmental sources. Numerous microorganisms found in soil and sediment may find their way into water sources through runoff and erosion. Flooding can transfer pollutants from the environment into water sources during the monsoon season, increasing microbial burdens [35]. Climate warming is making this issue worse by increasing the frequency and intensity of rainstorm events that contaminate water. Extreme precipitation and flooding events have been shown to significantly increase microbial contamination, including *E. coli* and total coliforms, by transporting pathogens through runoff and disrupting water systems.

### Types of microorganisms in contaminated water

#### Bacteria

The most frequently found microbes in tainted water are bacteria. *Escherichia coli* (especially enteropathogenic and enterohemorrhagic strains), *Salmonella spp.*, *Shigella spp.*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, and *Campylobacter spp.*

are among the pathogenic bacteria of concern in drinking water. From minor gastroenteritis to serious, perhaps fatal infections, these bacteria can cause a variety of ailments [36]. Indicator organisms like fecal coliforms and total coliforms indicate the possibility of enteric pathogens and recent fecal contamination. *Pseudomonas aeruginosa* and other opportunistic bacteria have been linked to outbreaks in healthcare settings and can infect immunocompromised people. *P. aeruginosa* is especially difficult to handle because it can create biofilms in plumbing systems. Study has demonstrated that despite disinfection efforts, this bacterium can persist in water distribution systems, underscoring the necessity of routine monitoring and enhanced control measure in healthcare water system [20].

### Viruses

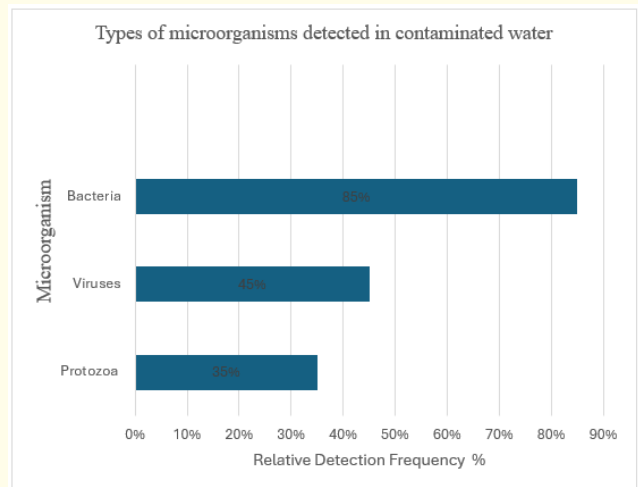
Contaminated water can spread enteric viruses such as rotavirus, norovirus, hepatitis A virus, and adenovirus. Viruses are a major concern for water safety because they are typically more resistant to treatment than bacteria. The virological requirements of the IS 10500:2012 standard demand that water samples be virus-free. Testing for MS2 phage as a sign of viral contamination is required by the standard [10,11].

### Protozoa

Important waterborne pathogens include protozoan parasites including *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium spp.* These organisms are particularly difficult to control because they produce cysts or oocysts that are extremely resistant to disinfection. Conventional water treatment methods may not always eliminate the cysts, which can persist in the environment for long periods of time. These parasites have been found in urban water sources in India, according to studies, underscoring the need for suitable detection techniques [2].

### Factors influencing microbial growth in water

The development and survival of microorganisms in water are influenced by a number of factors. A key factor is temperature, with higher temperatures typically encouraging bacterial growth. Due to rising temperatures and runoff contaminating water sources, India's monsoon season is linked to a higher prevalence of waterborne illnesses [36]. Microbial survival is also impacted by pH, the availability of nutrients, and the presence of disinfectants. In water distribution systems, biofilms give bacteria a safe haven



**Figure 2:** Types of microorganisms detected in contaminated water [20].

where they can thrive and proliferate even in the presence of disinfectants. Studies have demonstrated that biofilms can contain significant numbers of heterotrophic bacteria, which may include opportunistic diseases. Developing successful solutions for managing water quality requires an understanding of these issues [35].

### Indicator organisms and major waterborne pathogens

Because of the variety of microorganisms and the limitations of detection techniques, it is not feasible to identify every potential pathogen in water. In order to evaluate the microbiological quality of water and the possible presence of diseases, indicator organisms are employed. The main indicator organisms and waterborne pathogens of concern in the Indian context are covered in this section [14].

### Indicator organisms

#### Total coliforms

A kind of bacteria known as total coliforms is frequently employed as a water quality indicator. Both the environment and warm-blooded animals excrement contain them. Coliforms in treated water are a sign of either insufficient treatment or post-treatment contamination, even if not all of them are fecal in origin. According to the IS 10500:2012 standard, every 250 mL sample of drinking water must be free of coliform organisms [11]. Total

coliforms in water are frequently found using the membrane filtering method and the multiple tube fermentation approach. For regulatory purposes, these techniques are generally recognized and standardized. However, because some coliform species are found naturally in the environment, the presence of total coliforms alone does not always imply fecal contamination. As a result, more testing is needed to verify fecal contamination [20].

### Fecal coliforms

A subgroup of total coliforms that are particularly linked to fecal contamination are called fecal coliforms. The most significant member of this group and the main sign of fecal contamination is *Escherichia coli*. Fecal coliforms in water are a sign of recent fecal material pollution and possible enteric pathogen presence. Therefore, the detection of fecal coliforms is more important for public health than the detection of total coliforms [27].

### *Escherichia coli*

The most used indication of fecal contamination in water is *Escherichia coli*. Its presence suggests that there may be intestinal infections and that the water has been tainted by feces. Because *E. coli* is only found in feces and has well-established detection techniques, it is chosen as an indication. According to the IS 10500:2012 standard, every 250 mL sample of drinking water must be free of *E. coli*. *E. coli* O157:H7 is one of the pathogenic strains of the bacteria that can cause serious sickness. Contaminated water has been linked to outbreaks of *E. coli* O157:H7 infection. PCR that targets particular virulence genes is one of the specialist techniques needed to detect pathogenic *E. coli*. [39].

### Fecal Streptococci (Enterococci)

Additional markers of fecal contamination are fecal streptococci, especially enterococci. They can endure longer in water and are more resilient to environmental stress than *E. coli*. Because of this, they are helpful markers in cases where pathogens may still exist but *E. coli* may have died off. Methods for identifying intestinal enterococci in water are provided under the IS 15186:2002 standard. Gastroenteritis epidemics linked to recreational water consumption have been linked to enterococci. Enterococci levels have been found to be strongly correlated with swimmers risk of gastroenteritis. Enterococci have been linked to nosocomial infections in healthcare settings, especially in neonatal critical care units [16].

### Major waterborne pathogens

#### *Salmonella spp.*

Worldwide, *salmonella* is a leading cause of foodborne and waterborne sickness. An estimated 93 million infections and 155,000 fatalities are caused by non-typhoidal *salmonella* each year. Typhoid fever is caused by *Salmonella typhi*, which is thought to produce 11–21 million illnesses and 128,000–161,000 fatalities annually. Typhoid is still a major public health issue in India. Water tainted with feces can spread *salmonella* [22]. *Salmonella spp.* were found in 17.72% of municipal water samples examined using the multiplex PCR, indicating the pervasiveness of infection. *Salmonella* in water must be detected by enrichment, culture on selective media, or molecular techniques like PCR that targets the *invA* gene [5].

#### *Shigella spp*

An estimated 165 million cases of *Shigella*-caused diarrhea are recorded globally each year, mostly in underdeveloped nations. As few as 10–100 cells can cause infection, making the infectious dosage extremely low. Because of this, *Shigella* is especially harmful when water is contaminated. 18% of pupils at a rural Chinese school were impacted by a *Shigella flexneri* outbreak, underscoring the possibility of rapid transmission in unsanitary environments [25]. *Shigella* is difficult to find in water because of its small population and the presence of other microbes. After isolation, biochemical validation is performed using selective media such XLD (xylose lysine deoxycholate) agar and SS (*salmonella shigella*) agar. More sensitive detection can be achieved by molecular techniques that target particular genes [32].

#### *Vibrio cholerae*

The cause of cholera, a severe diarrheal illness that, if left untreated, can cause fast dehydration and death, is *Vibrio cholerae*. Hundreds of thousands of individuals have been impacted by recent outbreaks, indicating that cholera is still a threat. In many parts of India, especially those with inadequate sanitation, cholera is still endemic. Finding possible infection sources and putting control measures in place depend on the identification of *V. cholerae* in water. PCR targeting particular genes, like *ctxA* (cholera toxin subunit A), can confirm the existence of toxic strains, and TCBS (thiosulphate- citrate-bile salts sucrose) agar is frequently employed for isolation [36].

### *Pseudomonas aeruginosa*

An opportunistic pathogen that can infect people with weakened immune systems is *Pseudomonas aeruginosa*. Because it has been linked to outbreaks in neonatal critical care units, it is especially worrisome in healthcare settings [2]. The pathogen is challenging to control because it can develop biofilms in plumbing systems and is inherently resistant to many disinfectants. *P. aeruginosa* must not be present in 250 milliliters of water, according to the IS 14543 standard for packaged drinking water. Usually, detection is carried out using selective media like milk agar, which enables identification based on casein hydrolysis and pyocyanin pigment formation [42].

### *Staphylococcus aureus*

Although *Staphylococcus aureus* is mostly linked to foodborne illnesses, human contact can potentially contaminate water. Food poisoning can result from the pathogen's production of heat-stable enterotoxins. 61 elementary school pupils in Hebei, China were impacted by a recent *S. aureus* outbreak after consuming tainted food. *S. aureus* in water is a sign of poor cleanliness and possible health hazards [59].

### Health impacts and disease outbreaks

In India, waterborne illnesses still represent a serious threat to public health. Nearly 70% of all illness outbreaks recorded by the Ministry of Health and Family Welfare are waterborne, according to the integrated illness Surveillance Programme. These illnesses are becoming more common, and climate change is making the problem worse by increasing flooding and contaminating water sources [48].

### Indian regulatory standards for drinking water

To guarantee the safety of drinking water, India has put in place a thorough regulatory structure. Setting and implementing drinking water quality standards is a major responsibility of the Food Safety and Standards Authority of India (FSSAI) and the Bureau of Indian Standards (BIS). The development, specifications, and application of Indian standards for drinking water microbiological safety are covered in this section [11,19].

### Role of Bureau of Indian Standards (BIS)

India's national standards authority, the Bureau of Indian Standards (BIS), is in charge of creating and enforcing standards

in a number of industries. The Indian Standards Institution (ISI), which was founded in 1947, was replaced by the BIS by the BIS Act, 1986. On October 12, 2017, the BIS Act, 2016 went into force, strengthening BIS statutory power and requiring certification for a number of items, including bottled drinking water [12].

### Historical evolution of BIS

Dr. Lal C. Verman was the first head of the Indian Standards Institution, which was founded on January 6, 1947. In order to demonstrate adherence to quality standards, the ISI Certification Marks Scheme was established in 1952 [60]. Enhanced legislative powers brought about by the 1986 switch to BIS allow for more efficient standard enforcement. In order to guarantee consumer safety, the BIS Act of 2016 further reinforced the regulatory framework by requiring BIS certification for bottled drinking water [12].

### IS 10500: Drinking water specification

IS 10500 is the primary Indian standard for drinking water quality, applicable to both municipal supply and packaged drinking water. The standard was originally published in 1983 and has undergone two major revisions, with the current version being IS 10500:2012. The 2012 revision introduced significant changes including a dual limit system with Acceptable Limit and Permissible Limit in the Absence of Alternate Source, alignment with EU Directives and USEPA standards, and enhanced microbiological requirements [11]. According to IS 10500:2012 bacteriological standards, all samples collected from the distribution system including customer premises should ideally be free of coliform germs. *E. coli* and total coliforms must not be found in any 100 mL sample when tested in compliance with IS 1622, according to the standard [60].

### IS 14543: Packaged drinking water

Compared to normal drinking water standards, IS 14543 has stricter requirements for packaged drinking water (apart from natural mineral water). The standard was first published in 1998. It was first revised in 2004 and then again in 2016. The 2016 revision included seven major modifications and revisions, such as updated heavy metal limits, updated definitions, and harmonization with the Food Safety and Standards Act of 2006 [12]. Packaged drinking water must meet IS 14543 microbiological criteria for *E. coli*,

*coliforms, fecal streptococci, Staphylococcus aureus, Pseudomonas aeruginosa, yeast and mold, Salmonella, Shigella, and Vibrio* species. According to IS 5402, the total viable colony count should not be more than 250 CFU/ml at 20–22 °C in 72 hours and 20/ml at 37 °C in 24 hours [2].

**Role of Food Safety and Standards Authority of India (FSSAI)**

The Ministry of Health and Family Welfare’s highest food safety regulating organization is the Food Safety and Standards Authority of India (FSSAI). FSSAI was created in accordance with the Food Safety and Standards Act of 2006 and is in charge of establishing science-based standards for food items as well as controlling their production, importation, distribution, storage, and sale. In order to guarantee adherence to quality and safety regulations for bottled drinking water, FSSAI collaborates with BIS [19,46].

**Evolution of food safety regulation in India**

The Prevention of Food Adulteration Act of 1954, the Fruit Products Order of 1955, and the Meat Food Products Order of 1973 were among the regulations that governed food safety in India prior to 2006. These laws were combined into a single regulatory framework with the passage of the Food Safety and Standards Act, 2006. To give scientific assistance on food safety standards, the FSSAI was founded with a Scientific Committee and 21 Scientific Panels made up of more than 200 specialists from organizations like Indian Council of Medical Study (ICMR) and Council of Scientific and Industrial Study (CSIR) [18,50].

**FSSAI regulations for packaged drinking water**

Through the Food Safety and Standards (Food Products Standards and Food Additives) standards, 2011, as periodically updated, FSSAI has published detailed standards for packaged drinking water. The rules outline microbiological requirements that mandate the absence of *Salmonella, Shigella, Vibrio species, E. coli,* and coliforms. The limits for aerobic microbial counts are 20 CFU/ml at 37 °C and 100 CFU/ml at 20–22 °C [19]. *E. coli, coliform, fecal streptococci, Staphylococcus aureus, Pseudomonas aeruginosa, yeast and mold, Salmonella, Shigella, Vibrio cholerae, and V. paraahaemolyticus* are among the microbiological characteristics for which this program requires monthly testing. Pesticide residues need to be checked every six months, but chemical and physical parameters need to be examined every three months [14].

**Permissible microbial limits**

The permissible limits for microbial parameters in drinking water are specified in IS 10500:2012 and IS 14543:2016. These standards establish maximum acceptable levels for various indicator organisms and pathogens to ensure the microbiological safety of drinking water. The following table summarizes the key microbiological requirements:

Parameter	IS 10500:2012	IS 14543:2016	Test Method
<i>E. coli</i>	Absent/100mL	Absent/250mL	IS 1622
Total Coliforms	Absent/100mL	Absent/250mL	IS 1622
<i>Fecal Streptococci</i>	Not specified	Absent/250mL	IS 15186
<i>S. aureus</i>	Not specified	Absent/250mL	IS 5887
<i>P. aeruginosa</i>	Not specified	Absent/250mL	IS 5887
<i>Salmonella spp.</i>	Not specified	Absent/250mL	IS 5887
<i>Shigella spp.</i>	Not specified	Absent/250mL	IS 5887
<i>Vibrio cholerae</i>	Not specified	Absent/250mL	IS 5887
Aerobic Microbial Count	Not specified	100 CFU/mL (20-22C)	IS 5402
Yeast and Mould	Not specified	Absent/250mL	IS 5403

**Table 1:** Permissible limits of pathogen [11,12].

**Monitoring and compliance requirements**

Effective monitoring and compliance are essential for ensuring that drinking water meets regulatory standards. FSSAI Scheme of Testing mandates specific testing frequencies for different parameters. Microbiological testing must be conducted monthly, chemical and physical parameters quarterly, and pesticide residues six-monthly. All testing must be conducted at FSSAI-notified NABL accredited laboratories that are ISO/IEC 17025 certified [42]. Manufacturers are required to take prompt corrective action, including a thorough assessment of the causes of contamination, in the event of microbiological non-compliance. Only after five consecutive batches of compliance and satisfactory corrective

actions may production resume. For regulatory review, records of all testing and remedial measures must be kept for at least five years. Before being used, the source water must be tested, and before production starts, new sources must undergo thorough testing [3].

### Global guidelines and standards

International standards and recommendations are essential for coordinating national water quality regulations and promoting international trade. National standards are based on extensive guidelines created by the International Organization for Standardization (ISO) and the World Health Organization (WHO). The alignment of international recommendations with Indian norms is covered in this section.

### World Health Organization guidelines

A thorough foundation for guaranteeing the safety of drinking water around the world is provided by the WHO Guidelines for Drinking-water Quality. Published in 2022, the fourth edition of the guidelines takes into account the most recent scientific findings regarding the health concerns connected to contaminated drinking water. Many nations have accepted the recommendations as the foundation for their national standards, and they are widely acknowledged as the international standard for drinking water quality [58]. The creation of Water Safety Plans (WSPs) that identify and manage risks throughout the water supply chain is advised by the WHO recommendations, which place an emphasis on a risk-based approach to water safety. According to the standards, no 250 mL sample of drinkable water should contain *E. coli* or thermotolerant coliforms. The guidelines also offer recommendations for the selection of suitable treatment methods and the management of particular diseases [14].

### International Organization for Standardization

A set of guidelines for laboratory certification and water quality testing has been created by ISO. The general standards for testing and calibration laboratories' competency are outlined in ISO/IEC 17025:2017. This widely accepted standard serves as the foundation for laboratory certification systems across the globe. In compliance with ISO/IEC 17025:2017, laboratories in India are accredited by the National Accreditation Board for Testing and Calibration Laboratories (NABL) [28]. The evolving requirements of laboratory accreditation are reflected in the evolution of ISO/IEC 17025. The standard has undergone multiple changes since it

was first created in 1978 as ISO Guide 25. Significant modifications were made to the current edition, ISO/IEC 17025:2017, including a risk-based approach, a redesigned clause structure, and an increased emphasis on impartiality and secrecy. General requirements, structural requirements, resource requirements, process requirements, and management system requirements are all covered by the standard [58].

### Comparison between Indian and Global Standards

Indian drinking water quality standards are generally in accordance with worldwide best practices and WHO recommendations. Many of the WHO recommended values for chemical and microbiological criteria are included in IS 10500:2012. The goal of the 2012 IS 10500 modification was to align Indian standards with USEPA and EU directives. Nonetheless, there are some variations in the criteria for implementation and monitoring [11]. IS 14543:2016 sets guidelines for packaged drinking water that are similar to international standards like the Codex Alimentarius Commission's requirements for bottled and packaged drinking water. International food safety standards are in line with the FSSAI's microbiological parameter requirements [19]. India's regulatory structure is now more in line with international practices, where product safety is assured through testing and surveillance rather than forced certification, thanks to the recent elimination of mandatory BIS certification while keeping strict testing requirements [23].

### Conventional microbiological detection methods

#### Membrane filtration technique

Coliform bacteria, *E. coli*, and fecal streptococci are frequently counted in water using the membrane filtration method. A membrane filter with a pore size of 0.45 micrometers is used to filter a known volume of water. After that, the filter is put on a selective agar medium and let to incubate at the proper temperature. After incubation, the colonies that develop on the filter are counted and expressed as colony-forming units (CFU) per 250 mL of water [23]. One of the many benefits of the membrane filtering method is its capacity to examine high sample volumes, which raises detection sensitivity. The technique is specified in IS 15186 for enterococci detection and IS 1622 for coliform detection. Depending on the target organism, various selective media may be employed. Chromogenic Coliform Agar (CCA), where *E. coli* colonies look blue and other coliforms appear pink or red, is frequently used to detect *E. coli* [31].

**Multiple tube fermentation (Most Probable Number - MPN)**

The multiple tube fermentation method, also known as the Most Probable Number method, is a statistical technique for estimating the number of coliform bacteria in water. The method involves inoculating multiple tubes of liquid medium with different volumes of water sample. After incubation, the tubes are examined for growth and gas production, which indicates the presence of coliforms [58]. The combination of positive tubes is used to estimate the bacterial concentration using MPN tables. For water samples with high turbidity that could obstruct membrane filtration, the MPN technique is especially helpful. Brilliant green lactose bile broth is used for proven testing, and lauryl tryptose broth is used for presumptive testing. The findings are given as MPN per 250 ml of water. The method has been widely utilized for regulatory purposes and is still legal under Indian norms, despite the fact that it merely yields a statistical estimate rather than an actual count [50].

**Heterotrophic plate count (HPC)**

The heterotrophic plate count, also known as total plate count, measures the total number of viable bacteria in water that can grow on a nutrient-rich medium. The method involves plating a known volume of water sample on nutrient agar or R2A (reasoner’s 2A) agar and incubating at a specified temperature for a defined period. The colonies that develop are counted and expressed as CFU/mL. IS 5402 specifies the method for aerobic microbial count in packaged drinking water [31]. The HPC provides a general indication of water quality and treatment efficacy. High HPC values may indicate inadequate treatment, post-treatment contamination, or bacterial regrowth in the distribution system [49]. However, the HPC includes both pathogenic and non-pathogenic bacteria, and the count does not directly correlate with health risk. The Bureau of Indian Standards prescribes an HPC limit of 100 CFU/mL at 22°C in 72 hours on nutrient agar for packaged drinking water. Study has shown that bottled drinking water in Indian markets may contain heterotrophic bacteria with antibiotic resistance, highlighting the need for monitoring [33].

**Selective and differential media**

To isolate and identify particular pathogens in water, selective and differential media are crucial instruments. Ingredients in selective media prevent non-target organisms from growing while promoting the growth of the target organism. Indicators included

in differential media enable the visual differentiation of target organisms according to their biochemical traits. Typical selective mediums for pathogen detection are summarized in the following table.

Medium	Target Organism	Key Characteristics
Chromogenic Coliform Agar	<i>E. coli</i> /Coliforms	<i>E. coli</i> : blue; Coliforms: pink/red
Xylose Lysine Deoxycholate	<i>Salmonella/Shigella</i>	<i>Salmonella</i> : red with black; <i>Shigella</i> : colorless
Thiosulphate-Citrate-Bile Salts sucrose Agar	<i>Vibrio cholerae</i>	<i>V. cholerae</i> : yellow; <i>V. parahaemolyticus</i> : green
Baird-Parker Agar	<i>S. aureus</i>	Black colonies with white margins
Milk Agar	<i>P. aeruginosa</i>	Pyocyanin pigment, casein hydrolysis
Slanetz-Bartley Medium	Fecal Streptococci	Red/maroon colonies

**Table 2:** Different Media used in pathogen detection [28].

Biochemical tests are used to confirm the identity of isolated organisms. Standard tests include the oxidase test for differentiating oxidase-positive organisms from *Enterobacteriaceae*, the catalase test for distinguishing *Staphylococcus* from *Streptococcus*, and the coagulase test for confirming *S. aureus*. Commercial identification kits such as API strips and automated systems like VITEK have simplified biochemical identification [15].

**Modern rapid detection methods**

Rapid detection technologies that can yield results in hours instead of days have been developed as a result of the limitations of traditional culture-based approaches. These contemporary techniques are useful instruments for evaluating water quality because they provide increased sensitivity, specificity, and speed. The concepts, uses, and benefits of several fast detection techniques are covered in this section [46].

**Polymerase chain reaction**

Target microorganisms can be found even at low concentrations thanks to a molecular technique called polymerase chain reaction, which amplifies particular DNA sequences. The technique produces

exponential amplification of the target sequence by repeating cycles of denaturation, primer annealing, and DNA synthesis. By focusing on distinct genetic markers, PCR can identify particular infections [58].

### Multiplex PCR

When compared to individual assays, multiplex PCR saves time and money by enabling the simultaneous identification of several pathogens in a single reaction. The method employs several primer sets that target various pathogens within a single PCR mixture. Study has shown that multiplex PCR assays may concurrently identify *Vibrio cholerae*, *Shigella spp.*, and *Salmonella spp.* in water samples. This method is especially useful for thorough evaluations of water quality when there may be several diseases [31].

### Real-Time PCR (qPCR)

Target organisms can be simultaneously detected and quantified using real-time PCR, sometimes referred to as quantitative PCR (qPCR). The technique makes use of fluorescent dyes or probes that produce signals proportionate to the quantity of amplified DNA [15]. Assays based on TaqMan probes have been designed to identify *Vibrio cholerae*, *Salmonella species*, and *E. coli* in water samples. *Salmonella* can be easily counted without enrichment, according to a study that used molecular beacon real-time PCR for *Salmonella* identification in surface and drinkable water from northern India [1]. High sensitivity, specificity, and the capacity to measure target organisms are among qPCR benefits. The technique has a high probability of detecting as few as 10 genomic equivalents per PCR cycle. However, qPCR use in environments with low resources is constrained by the need for costly equipment and skilled workers. Another drawback that has been addressed by the application of viability PCR techniques is the inability to differentiate between viable and dead cells [8].

### Loop-mediated isothermal amplification (LAMP)

The isothermal nucleic acid amplification method known as loop-mediated isothermal amplification (LAMP) has a number of benefits over traditional PCR. The technique offers great specificity by using several primers that identify different parts of the target DNA [43]. Because LAMP doesn't require thermal cycling, amplification can be done using basic tools like a heating block or water bath. Large volumes of DNA are produced by the reaction, allowing for visual detection by turbidity or color change [20].

Study has proved the application of LAMP for detecting waterborne infections. A study on *Vibrio parahaemolyticus* detection in seafood samples indicated that LAMP may detect as little as 1 CFU per response, with sensitivity comparable to real-time PCR. The experiment could be finished in 30 to 60 minutes and was much more sensitive than traditional PCR. LAMP is appropriate for field applications and point-of-care testing due to its speed and ease of use [29].

### Enzyme-Linked immunosorbent assay (ELISA)

An immunological technique called ELISA employs antigen-antibody interactions to identify pathogens. The technique can detect specific pathogens based on their surface antigens [1]. For the purpose of identifying *E. coli* in aquatic environments, indirect ELISA techniques with high sensitivity and specificity have been developed. Lateral flow immunoassays are appropriate for field screening because they offer quick detection without requiring laboratory equipment [20].

### Biosensors

By combining biological recognition components with transducers to generate quantifiable signals, biosensors constitute a quickly developing field in disease detection. A variety of biosensors, such as electrochemical, optical, and piezoelectric biosensors, have been created for the detection of waterborne pathogens. By focusing on the heat-stable toxin gene, study has created an electrochemical biosensor that can identify *E. coli* in 20 minutes. The sensor detects using electrochemical impedance spectroscopy and nano composite materials [3].

### Electrochemical biosensors

Changes in electrical characteristics upon pathogen binding are detected using electrochemical biosensors. Within 30 minutes, gold nanorod-based substrates were able to detect *E. coli* at a limit of 8.4 CFU/mL. *E. coli* and *Salmonella typhimurium* can be distinguished with great specificity using impedimetric sensors that use indium tin oxide electrodes with immobilized antibodies. Portable gadgets that can be used in the field for quick screening are among the most recent innovation [40].

### Optical biosensors

Surface plasmon resonance and other optical phenomena are used by optical biosensors to identify pathogens. *Vibrio cholerae*,

*E. coli*, and *Enterococcus faecalis* can all be detected by biosensors that use gold-coated photonic crystal fibers. These sensors provide real-time monitoring and label-free detection. Gold nanoparticle-based colorimetric biosensors enable detection without the need for specialist equipment by producing noticeable color changes upon target identification [33].

### Microfluidics and lab-on-chip technologies

Microfluidic platforms combine DNA amplification, detection, and sample processing into compact automated devices. With little assistance from the user, these lab-on-a-chip devices can carry out a whole analysis from sample to result. Centrifugal microfluidic platforms have been developed for automated microbial lysis, DNA extraction, and analysis [40]. Miniaturization allows for portable point-of-care testing and lowers reagent use. Study has shown that pathogens including *Salmonella*, *Listeria*, and *E. coli* can be identified using microfluidic devices that include immunological capture, chemical lysis, isothermal amplification, and acoustic detection. Compared to traditional approaches, these systems may attain detection levels of 1–5 CFU per 25 mL in 4.5 hours, which is a five-fold reduction in analysis time. For the quick colorimetric identification of *E. coli* in environmental water samples, microfluidic paper-based analytical devices (muPAD) have been developed [5,57].

### Next-Generation sequencing (NGS)

Comprehensive characterization of water microbial communities is made possible by next-generation sequencing technology. All microorganisms found in a sample, even unidentified or unculturable species, can be identified using metagenomic sequencing. NGS makes it easier to follow the evolution of pathogens and monitor antibiotic resistance genes. Whole-genome sequencing offers comprehensive details regarding the traits and virulence factors of strains [44]. Although technology is still in its infancy, the use of NGS in water quality monitoring holds great promise for thorough microbial profiling. The technology can monitor the spread of antibiotic resistance, evaluate microbial diversity, and find new diseases. However, widespread use of NGS in water quality monitoring is still limited by its high cost, complexity, and need for bioinformatics knowledge. NGS is anticipated to become more significant in the evaluation of water safety as costs come down and analytical pipelines become more user-friendly [20].

### Comparative evaluation of detection methods

The selection of appropriate detection techniques is influenced by several factors, including sensitivity, specificity, time restrictions, cost, and technological know-how. This section provides a comparative examination of classic and modern detection techniques to aid in method selection for different applications [9].

#### Sensitivity and specificity

In general, molecular approaches are more sensitive than those based on culture. Unlike culture methods, which usually require 10–100 CFU for effective detection, PCR and qPCR can detect as few as 1–10 target organisms per response. When compared to traditional techniques, the multiplex PCR assay created at AIIMS showed 100% sensitivity and specificity, identifying infections in 56.32% of samples as opposed to 6.96% by culture. LAMP is easier to use and has sensitivity that is comparable to qPCR. In molecular techniques, specialized primers or probes are used to achieve specificity, as are selective media in culture techniques [9]. By necessitating the recognition of six different sections of the target DNA, the multiple primer design of LAMP offers great specificity. Nucleic acid hybridization or antibody-antigen binding are two ways that biosensors attain specificity. For immunological techniques, cross-reactivity with non-target species is still an issue and needs to be assessed during method validation [21,29].

#### Time required for detection

When monitoring water quality, time to result is crucial, especially when investigating outbreaks. Results from culture-based procedures take 24 to 72 hours, and certain diseases require considerably longer incubation times. While qPCR cuts this down to 2–3 hours, PCR procedures can yield results in 4–6 hours. With findings available in 30 to 60 minutes, LAMP provides the fastest amplification. Biosensors are appropriate for quick screening because they can detect in 15 to 60 minutes [41].

#### Cost-Effectiveness

With their low equipment and reagent requirements, culture-based approaches continue to be the most economical choice for routine monitoring. Thermal cyclers and certain reagents are needed for PCR techniques, which raises expenses. Because qPCR necessitates costly real-time detection devices, its availability is restricted in environments with limited resources. With only basic heating equipment needed, LAMP provides an affordable

substitute for PCR. The cost of biosensors varies according on the technology; electrochemical sensors are often less expensive than optical devices [17].

**Technical expertise required**

Although culture-based techniques require knowledge of microbiology, skilled personnel with rudimentary laboratory abilities can carry them out. Training in molecular biology is necessary for PCR techniques, as is close attention to avoid contamination. Additional knowledge of data analysis and interpretation is required for qPCR. Because LAMP is easier to use and understand, labs with less experience in molecular biology can use it more easily. Because of its user-friendly design, biosensors may be operated with little training [41].

**Field applicability vs laboratory methods**

Field adaptability is crucial for point-of-care testing and quick screening. Laboratory facilities with regulated incubation conditions are necessary for culture procedures. To avoid contamination, heat cyclers and hygienic laboratory conditions are necessary for PCR [47]. LAMP is appropriate for field deployment because to its isothermal nature and low equipment requirements. For on-site detection, portable biosensors have been created; some devices can deliver results in a matter of minutes. Field applicability is further improved by the creation of smartphone-based detecting platforms [1].

Method	Time	Sensitivity	Specificity	Cost	Field Use
Culture	48-96h	High	High	Low	Limited
PCR	4-6h	High	High	Medium	Limited
qPCR	2-3h	Very High	Very High	High	Limited
LAMP	30-60min	High	High	Low-Med	Good
Biosensors	15-60min	Medium-High	High	Medium	Excellent
ELISA	2-4h	Medium	High	Medium	Moderate

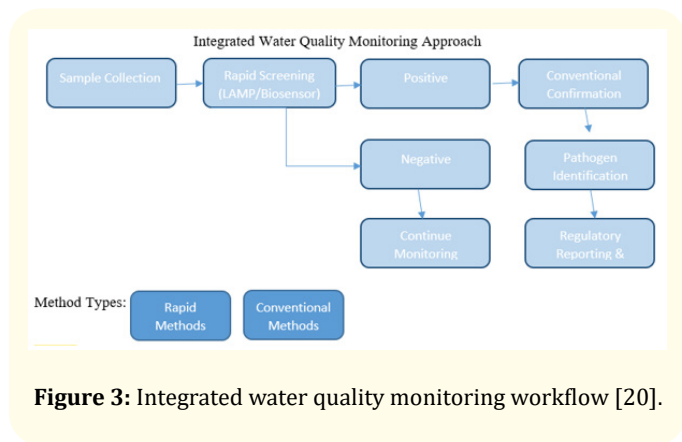
**Table 3:** Comparative time detection [41].

**Integration of conventional and modern methods**

By combining the speed and sensitivity of molecular approaches with the dependability of culture-based techniques, the integration of traditional and contemporary detection methods provides a comprehensive approach to water quality monitoring. The benefits of combining methods and real-world applications in water monitoring systems are covered in this section [44].

**Advantages of combined approaches**

There are several advantages to monitoring water quality by combining traditional and contemporary techniques. While molecular techniques offer quick answers and increased sensitivity for epidemic study, culture-based techniques identify live organisms and are crucial for regulatory compliance. When both methods are used, data can be confirmed and a more comprehensive picture of water quality is produced. Culture-based confirmation guarantees the data regulatory acceptance when quick screening techniques identify possible contamination [47]. Better risk assessment is also made possible by the integration. Molecular techniques can identify certain infections and virulence genes, whereas culture approaches show the presence of indicator organisms. Determining the level of contamination and putting in place suitable control measures are made easier with the use of this information. A more precise evaluation of health risk is obtained by combining quantitative data from qPCR with viability data from culture techniques [45].



**Figure 3:** Integrated water quality monitoring workflow [20].

### Practical applications

In real-world applications, quick techniques like biosensors or LAMP can be utilized for preliminary water sample screening. Following a positive test, samples can undergo additional characterization and confirmation based on culture. This tiered method preserves the dependability of results while cutting down on the time and expense involved in testing a large number of samples [23]. The strategy has been effectively used in outbreak investigations and surveillance programs. The benefits of combination techniques have been shown by study conducted in India. The multiplex PCR assay demonstrated 100% sensitivity and specificity when tested against traditional microscopy and culture techniques for the detection of *Salmonella*, *G. lamblia*, and *E. histolytica*. For molecular approaches to be accepted by regulators, this validation is crucial. In a similar vein, the performance features of the LAMP assay for *V. parahaemolyticus* were established by comparing it with both conventional and real-time PCR [37].

### Improved accuracy and rapid response

When approaches are integrated, water quality monitoring becomes more accurate and responsive. While culture confirmation guarantees that the right organisms are identified for source tracking and control measures, rapid approaches allow for prompt action when contamination is confirmed. This is especially crucial in times of epidemic, when prompt action can stop the spread of illness [37]. The significance of thorough monitoring is reflected in the FSSAI Scheme of Testing for Packaged Drinking Water. The program requires monthly testing for microbiological parameters using defined techniques, and non-compliance requires prompt remedial action. Public health is best protected when rapid screening techniques are combined with regular monitoring [23].

### Challenges in microbial detection

There are still a number of obstacles in the way of guaranteeing the microbiological safety of drinking water, despite advancements in detection technologies. The identification of viable but non-culturable organisms, infrastructural constraints, problems with cost and accessibility, and the need for standardization are some of these difficulties. Improving water quality monitoring in India requires addressing these issues [32].

### Detection of viable but non-culturable (VBNC) organisms

A viable but non-culturable state can be reached by many pathogenic bacteria in stressful situations including hunger,

chlorination, or temperature fluctuations. Organisms are still viable and potentially contagious in this stage, but culture-based techniques are unable to identify them. Because current approaches may underestimate the true microbial load, this presents a substantial difficulty for water quality monitoring [47]. Under the right circumstances, VBNC pathogens can revive and perhaps cause illness. Molecular techniques can identify VBNC organisms using DNA-based techniques, but they are unable to differentiate between living and dead cells. To overcome this restriction, methods like viability PCR which employ dyes to remove DNA from dead cells have been developed. These techniques aren't currently commonly used for regular monitoring, though, and they need more confirmation. Methods for accurately identifying live infections in all physiological stages are still being studied [34].

### Infrastructure limitations in developing regions

Sufficient laboratory facilities are necessary for efficient monitoring of water quality. Access to well-equipped laboratories with qualified staff is lacking in many parts of India. Due to a lack of resources and infrastructure, water testing is particularly difficult in rural locations. Although testing capacity has increased with the introduction of NABL-accredited laboratories, there are still gaps in testing capabilities and geographic coverage [42]. By modernizing food testing facilities nationwide, the FSSAI's Strengthening of Food Testing Laboratories program seeks to eliminate infrastructural gaps. To guarantee that all areas have access to high-quality testing services, however, consistent investment is required. By allowing testing at the point of collection, portable and field-deployable detection techniques can assist in overcoming infrastructure constraints [6].

### Cost and accessibility of advanced technologies

Advanced detection technologies such as qPCR and next-generation sequencing require expensive equipment and reagents that may not be affordable for all laboratories. The cost of testing can be a barrier to routine monitoring, particularly for small water systems and rural areas. While LAMP and some biosensor technologies offer more affordable alternatives, their adoption requires investment in training and validation [13]. Government support through funding programs and subsidies can help improve access to advanced technologies. The development of low-cost, indigenous detection platforms is also important for making advanced methods more accessible. Collaboration between

study institutions, industry, and government can accelerate the development and deployment of affordable detection solutions [52].

### Standardization issues

For results from different laboratories to be comparable, quick detection techniques must be standardized. Many quick methods lack approved reference materials and standardized procedures, whereas conventional methods have established protocols and reference standards. For novel techniques to be accepted by regulators, they must be validated against recognized reference methods [42]. A framework for laboratory accreditation is provided by ISO/IEC 17025:2017, which contains specifications for quality control and technique validation. NABL accreditation guarantees that laboratories uphold quality control and adhere to standard operating procedures. However, method developers, regulatory agencies, and accreditation bodies must work together to produce standardized standards for developing technologies [13,23].

### Future prospective and innovation

The field of microbial detection is rapidly advancing with new technologies that enhance water quality monitoring. Emerging innovations are improving speed, accuracy, and accessibility of pathogen detection [43].

### Portable detection kits

These portable, easy-to-use kits are perfect for situations with limited resources since they allow for on-site water testing without the need for laboratory equipment. Rapid and accurate results are produced by technologies like paper-based devices, smartphone-linked biosensors, and LAMP-based assays. Accessibility has been improved by the development of affordable biosensors for *E. coli* detection by Indian institutions [30].

### AI and automation

By enabling precise analysis of complex data often surpassing 90% accuracy artificial intelligence and machine learning are revolutionizing pathogen detection. Automation boosts productivity and lowers human error. AI and IoT sensors enable real-time water quality monitoring and prediction, assisting in the prevention of pollution incidents [51].

### Real-time monitoring systems

Water quality data and early contamination alerts are continuously provided by real-time systems that use automated analyzers and biosensors. While there are still issues with sensor longevity, performance and dependability are being improved by developments in materials and sensor networks [56].

### Policy improvements

In order to accommodate new technologies, regulatory frameworks must change. The focus of recent FSSAI modifications is on risk-based regulation. To guarantee safety, innovation, and successful implementation, stakeholders must work together and harmonize with international standards [38].

### Conclusion

Ensuring the microbiological safety of drinking water is fundamental to protecting public health, particularly in regions facing rapid urbanization and infrastructure challenges such as India. This review highlights that conventional microbiological methods, including membrane filtration, multiple tube fermentation, and heterotrophic plate count, continue to play a vital role in regulatory monitoring due to their standardization, reliability, and cost-effectiveness. However, their limitations especially long detection times and inability to identify viable but non-culturable organisms reduce their effectiveness in situations requiring rapid response. Modern detection techniques, such as PCR, qPCR, LAMP, biosensors, and next-generation sequencing, have significantly improved the speed, sensitivity, and specificity of pathogen detection. Among these, LAMP and biosensor-based methods show considerable promise for field applications due to their rapid turnaround time and minimal equipment requirements. Despite these advantages, widespread adoption is limited by high costs, technical expertise requirements, and lack of standardized protocols for routine use. The analysis indicates that no single detection method is sufficient for comprehensive water quality assessment. An integrated approach combining rapid screening with conventional confirmatory methods offers the most practical and reliable solution. This strategy enables timely detection, accurate identification, and regulatory compliance. Key challenges remain, including infrastructure limitations, accessibility of advanced technologies, and detection of complex microbial states. Strengthening laboratory capacity, promoting cost-effective

innovations, and improving standardization are essential. Future advancements in portable technologies, automation, and artificial intelligence-driven monitoring systems are expected to transform water quality management and support sustainable access to safe drinking water.

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### Conflicts of Interest

We declare that there are no conflicts of interest.

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