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DRINKING WATER MICROBIOLOGY: DESIRED AND UNDESIRED MICROBIOTA, LEGISLATION, OUTBREAKS AND ANALYSIS

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ABSTRACT

Water being a fundamental part of all living things is unique in nature, ubiquitous and needed every day for consumption so as to facilitate the metabolic process of the body, processing of food items and other domestic purposes. The taste, color and odor of water can be affected when contaminants in the form of pesticides, animal or human fecal materials is released into drinking water, making it unsafe and unfit for human consumption. According to World Health Organization (WHO), quality water going into distribution system should be devoid of coliform bacteria in 100ml of water. 95% water sample must contain no trace of *E.coli* in 100ml of the water sample throughout the year. No water sample should possess more than 10 coliforms organism in 100ml and no coliform organism should be seen in at least 100ml of at least two (2) consecutive samples. The *Escherichia coli* and coliform bacteria is used as indicators to prove if the water sample is safe and fit for consumption. Drinking water can convey some diseases which are threats to public health safety and they include cholera, typhoid fever, gastroenteritis diseases etc. Conventional and current methods employed in the assessment of drinking water are to prove the safety and quality of the water, but some limitations have been observed during the assessment. Finally, with proper regulatory and legislative measures in place to govern drinking water, the desired target of drinking water devoid of *E.coli* and coliforms as pollutants can be achieved.

1. Introduction

Potable water is a unique, ubiquitous liquid that is a fundamental part of all living things and needed as a fuel for sustaining the human life every day. Due to its unique nature and qualities water has continually been an incomplete research work for scientist. According to the National Research Council (NRC) water being a typical nutrient for life has been recommended for a daily intake of approximately 1mb/kcal of energy expended. Being a daily consumable substance, an adequate, portable and safe drinking water should be accessible due to its significant health effect. Therefore, constant improvement should be applied for quality drinking water purpose (WHO, 2008). Drinking water has been found to act as a body temperature regulator and also helps in the metabolic process of the body (Staci, 2005). Exclusively, water has not only been used for drinking purposes, but also suitable for washing, irrigation, preparation and production of food items, both domestic and industrial purposes. The need for water ranges based on different factors which include human body and animal metabolism, diet, change in climate and different choices of clothing. Water, being a good solvent, can readily take up impurities and continues to interest scientists for further research, owing to the uniqueness of its physical,

chemical, and biological properties in comparison to a set of standards. The wide use of water for the development of humans, animals, plants and industries has created a significant scientific approach to understanding its properties (Naveen, 2007).

Contaminants are chemicals or materials that, when discharged into drinking water, render it dangerous to consume. The flavor, color, and turbidity of the water can help identify some of them. Drinking water should be colorless, tasteless, and odorless, according to WHO (WHO, 1996) physical characteristics. According to other scientific literature, a high pH value leads humans to have an unpleasant taste, and a low pH value promotes corrosion (Chan *et al.*, 2007). Chemical pollution is one of the most common complaints regarding drinking water in rural areas. This is prevalent in areas where agrochemical usage results in a high level of pesticide deposit in the environment, which may migrate into nitrate from fertilizer use, or when water is pumped via led pipes, which can result in a high amount of lead (pb) in drinking water. Furthermore, exposure to increased levels of fluoride causes mottling of teeth and may lead to skeletal fluorosis and crippling. The biological properties of drinking water suggest that drinking water should be devoid of pathogenic or disease causing organism. Any bacteria found in drinking water that is indicative of fecal pollution is harmful and such water is unsafe for consumption.

2.Accepted Level of Microbiological Load in Potable Water

The usage of water for drinking has been linked to a variety of health advantages, including appetite stabilization, improved metabolism, increased energy levels, and lowering of blood pressure. Furthermore, water helps to stablize the body's homeostatic environment, control internal body temperature, and maintain bodily fluid balance (Uzma et al., 2015).

Microorganisms' inherent participation in water is responsible for a range of waterborne infections, as well as nutrient recycling in both marine and fresh water (Willey et al., 2008). The microbiological load of drinking water varies from nation to country, but the World Health Organization has established two reference criteria (WHO, 2011). They distinguished between a piped water supply system (which is defined as a communal public system by the EPA) and a single or small community supply. They also made a distinction between water that had left the treatment plant and water that had entered the distribution system.

The World Health Organization (WHO) recommended a standard for treated and disinfected water supplies, as well as a requirement that water entering the distribution system include no coliform bacteria in 100 mL of water. The standards for disinfected water are as follows: "(1) Throughout any year, 95 percent of samples should be devoid of *E. coli* in 100 ml; (2) No water sample should contain more than 10 coliform organisms per 100 ml; and, (3) Coliform organisms should not be seen in 100 ml of at least two consecutive samples." (9)The number of coliforms measured in non-piped systems should not exceed 10/100 ml.

Table 1. Guideline of microbial quality for drinking water (WHO, 1997)

Organism	Microbial critical limits
All water directly intended for drinking *E. coli* or thermotolerant coliform bacteria	No detection in any 100ml sample
Treated water entering the distribution system. *E.coli* or thermotolerant coliform bacteria	No detection in any 100ml sample
Treated water in the distribution system *E.coli* or thermotolerant coliform bacteria	No detection in any 100ml sample

2.1.Classification and Sources of Pathogens Involved in Drinking Water (Coliform bacteria, *E.coli* and other Pathogens)

Microorganisms in consumed water have formerly been used to signify that the water is unpleasant to drink. Indicator microbes may be followed by pathogens, but they do not cause sickness in and of themselves; their presence in drinking water, however, is a sign of pollution. This pollution can arise as a result of feces contamination from humans or other animals. Two important indicator microorganisms typically discovered in drinking water are *Escherichia coli* and coliform bacteria.

Some of the emerging drinking water pathogens include the *Microsporidia* include bacteria like *Mycobacterium avium intracellulare*, *Helicobacter pylori*, *Tsukamurella*, and *Cystoisospora* belli, as well as viruses including adenoviruses, parvoviruses, coronaviruses (SARS), and polyomaviruses (Woolhouse, 2006). Extensive investigation into these species has revealed that the majority of them have some level of chlorine resistance. Microsporidia, *Enterocytozoon bienusi*, *Encephalitozoon hellem*, and *E. intestinales* are all good examples. *M. avium* and certain viruses have been shown to contaminate several disinfectants used in drinking water, as well as being inactivated by UV radiation and heat pathogens (Nwachcuku and Gerba, 2004). *Salmonella* sp., *Shigella* sp., *Vibrio cholerae*, *Leptospira* sp., *Yersinia enterocolitica*, *Francisella tularensis*, *tularemia*; *Escherichia coli* (particular enteropathogenic strains); and *Pseudomonas aeruginosa* are some of the bacteria that have been discovered as drinking water pathogens.

Pathogens, on the other hand, are not easy to differentiate and recognize than indicator organisms, necessitating the use of specialized media and techniques. Other than pathogens, indicator organisms are commonly employed to grade water quality because they are easily identified using basic laboratory procedures, are more accurate, and take less time. When comparing infections with indicator organisms, diseases are more frequently detected in small quantities than indicator organisms, making them less likely to be separated.

The genus *Escherichia* is well defined and outstanding from different Enterobacteriaceae mixed-acid fermenters in general due to their capacity to ferment sugar, motility, production of indole from tryptophan, loss of urease, incapability to utilize citrate as the only carbon supply, and inhibition of growth by potassium cyanide. Despite this, the "coliform group" is not adequately explained. The "coliform group," according to the American Public Health Association's Standard Methods, is made up of all gram-negative, "aerobic and facultative anaerobic, non-spore-forming, rod-shaped bacteria that can digest lactose with gas production in 48 hours at 35 °C." *E.coli* is a member of this group, which also contains *Klebsiella pneumoniae* and *Enterobacter aerogenes*.

Coliform community is utilized as a fecal contamination indicator in drinking water; it's important to remember that coliform activity in water is quicker than diseases like *Salmonellae* and *Shigellae*. They are easily repressed by an increase in the number of various species, especially in untreated groundwater or when there is no free residual chlorine (Allen and Geldreich, 1975).

3.Diseases Associated With Drinking Unsafe Water/Examples of Outbreaks

Water has been seen as a vehicle for disease transmission because wastewater, which is a primary carrier of *E.coli*, coliforms, and other pathogens discharged into freshwaters and coastal seawaters, pollutes drinking water with human and animal feces, creating a significant health risk (Fenwick, 2006).

People suffering from severe microbial diarrhea in impoverished nations face significant public health issues, which may be caused by poor sanitation, a lack of hygienic facilities, or a lack of financial resources. This is seen in a large number of young children from Asian, African, and poor nations throughout the world (Seas *et al*., 2000). Microbial waterborne infections have an impact on advanced nations

across the world, according to the findings. According to scientific evidence, over 560,000 individuals in the United States suffer from severe waterborne diseases each year, while approximately 7.1 million people are infected with minor to slight infections, ensuing in an estimated 12, 000 fatalities each year (Medema *et al*., 2003).

Table 2. Major diseases transmitted via contaminated water and their causative agents (João, 2010).

Disease	Causative agent
Cholera	*Vibrio cholera, serovarieties* O1 and O139
Gastroenteritis caused by *vibrios*	*Vibrio parahaemolyticus*
Typhoid fever and other acute salmonellosis	*Salmonella enterica* subsp. *enterica* serovar *Paratyphi* *Salmonella enterica* subsp. *enterica* serovar *Typhi* *Salmonella enterica* subsp. *enterica* serovar *Typhimurium*
Bacillary dysentery or shigellosis	*Shigella dysenteriae* *Shigella flexneri* *Shigella boydii* *Shigella sonnei*
Severe diarrhea and gastroenterititis	*Escherichia coli*, particularly serotypes such as O148, O157 and O124

Cholera, salmonellosis, and shigellosis are some of the most common gastrointestinal disorders caused by bacteria spread by water. Contaminated water that has been polluted with human and animal feces is the major vehicles that convey these infections. As a result, drinking water can get contaminated with *E.coli*, coliforms, and other harmful bacteria, posing a serious health problem. The colony of harmful bacteria in potable water on the other hand, is unusual and unstable, with a low number and inconsistent isolation and culture of these germs. As a result, microbiological testing of drinking water entails more than just looking for dangerous microorganisms. Healthy drinking water therefore, requires the absence of any harmful germs (George and Servais, 2002).

Some of the diseases associated with drinking contaminated water and their causative agents are displayed in Table 2.

3.1.Cholera

*Vibrio*s are Gram-negative rods that have one polar flagellum and a tiny, curved appearance. They are facultative anaerobes, meaning that they have both fermentative and respiratory metabolism. Extensive research has shown that *Vibrio cholerae* is therefore, the most significant of the vibrio species. *Vibrios* are mostly aquatic bacteria, and their circulation is heavily influenced by salt content and water temperature. *Vibrios* are typically found in marine and estuarine habitats, where they live in the open or on the surfaces of marine animals' intestinal walls. Their cells can grow at 40 °C with a pH of 9–10, and sodium chloride is usually present to help them grow (Farmer and Hickam-Brenner, 2003).

The toxin produced by *Vibrio cholerae* is an exotoxin that acts directly on its target cells. The toxin binds to a particular receptor (ganglioside Gl) on the cell membrane of intestinal cells, causing the enzyme adenylate cyclase to be activated. This causes the breakdown of internal ATP to continue indefinitely, resulting in the generation of cAMP and inorganic phosphate. Diarrhea is caused by an increase in the inner concentration of cAMP, which induces an outflow of water, sodium, potassium, chloride, and carbonate ions out of the cells of the mucous membrane.

Cholera has a development period of 1–3 days. Acute and very severe diarrhea characterizes the condition, which can reach a

rate of at least a liter per hour. Thirst for water, muscle cramps, and overall weakness are some of the signs of cholera, as are oliguria, hypovolemia, hemoconcentration, accompanied by anuria, lower potassium levels in the blood, patients feeling dull, and lastly, blood distribution quits and dehydration with cyanosis (Farmer and Hickam-Brenner, 2003).

Since the 19th century, cholera has been a well-known illness. Seven major pandemics were identified in the nineteenth and twentieth centuries. The first six pandemics all began in Asia, spread over Europe, and eventually reached South America. The seventh pandemic, which is still ongoing, began in Asia's Celebes Islands in 1961. The illness first spread through Asia in the 1960s, then to the Middle East and Africa in the 1970s, and finally to South America in 1991. (Sack *et al.*, 2004).

3.2.Salmonellosis

The genus *Salmonella* is a Gram-negative motile straight rod, belonging to the family of *Enterobacteriaceae*. Its Cells are catalase-positive and oxidase-negative, and they release gas from D-glucose using citrate as a primary carbon supply. *Salmonellae* contains different endotoxins viz antigens O, H and Vi (Popoff and Le Minor, 2005).

Infections due by *Salmonellae* can be divided into two types of salmonellosis which include (1) typhoid and paratyphoid fever and (2) the gastroenteritis. Infective doses enough to cause clinical symptoms is less than 1,000 cells. Newborns and toddlers infected with salmonellosis display certain signs involving a deadly typhoid-like illness with septicemia to a lowered or asymptomatic infection. This transmission usually occurs in pediatric wards via the hands of staff handling the children (Popoff, and Le Minor, 2005).

Salmonella is found mostly in the guts of humans and animals, but it is also gotten from environmental samples because it is expelled by humans, pets, farm animals, and wild animals (Le Minor, 2003). Communal sewage, farm pollution, and storm water runoff are the major sources of pathogenic bacteria and coliforms in drinking water (Arvanitidou *et al.*, 2005).

Between 1994 and 2004, Tunisia was hit by major epidemics of salmonellosis, including outbreaks in 1997, 1999, 2002, and 2004. In 1997, serovar Mbandaka produced a salmonellosis outbreak, and in 1999, three *salmonella* outbreaks were reported from hospitals across the country. Mbandaka, Livingstone, and Typhi Vi+ serotypes were used to distinguish each epidemic. *S. enterica* subsp. *enterica* serovar *S. enterica* serovar S. *enterica* serovar S. *enterica* serovar *S. enterica* serovar The Livingstone infection was detected in a separate ward of the same hospital that had previously reported an epidemic caused by serovar Typhi Vi+ in 1999. In Tunisia, the Livingstone serovar rose to first place in human infection in the same year. Furthermore, in 2004, a second major epidemic of serovar Typhi Vi+ occurred, with the pathogen's source being a fermented juice obtained locally from palm trees (Ben *et al.*, 2007).

3.3.Shigellosis or Bacillary Dysentery

Shigella are Gram-negative, non-spore producing, non-motile straight rod-like bacteria belonging to the *Enterobacteriaceae* family. Its cells are capable of fermenting carbohydrates without producing gas. Salicin, adonitol, and myo-inositol, on the other hand, are not fermented. Shigella cells do not create H_2S because they do not utilise citrate, malonate, or acetate as their sole source of carbon. Also lysine is not decarboxylated. They are catalase-positive and oxidase-negative, and members of the genus have a diverse antigenic pattern, with classification based on somatic O-antigens (Strockbine and Maurelli, 2005).

The incubation period for bacillary dysentery is 1–4 days, during which time early indications such as fever, anorexia, lethargy, and malaise appear. Carriers have a constant stream of tiny bloody feces (sometimes excessively purulent) and stomach discomfort. Diarrhea advances to dysentery, bloody mucus, and pus in stools that reduces in volume (no more than 30

mL of fluid per kg per day) around 12-36 hours later (Germani and Sansonetti, 2003).

Blood, inflaming components, and mucus flow into the intestinal lumen due to epithelial breakdown and eventual ulceration of the intestinal mucosa. The colon's ability to absorb water is hampered, and the amount of stool produced is determined by ileocecal flow. Therefore, the patient's feces will be constant, sparse, and dysenteric (Todar, 2009). *Shigella* is mostly located around the intestines of humans and other primates (Tetteh and Beuchat, 2003). It is most commonly transmitted by feces-contaminated drinking water or food, or through direct contact with an infected individual. *Shigellosis* may survive in water for up to six months at ambient temperature, making it ideal for transmission through water.

4.Conventional and Current Techniques of Assessing Water Potability

To retain records of water quality impairments, meet with regulatory criteria to assure continual safe drinking water, monitor sources of drinking water pollution, and eventually decrease and investigate illness outbreaks caused by drinking water, drinking water potability must be assessed.

4.1.Conventional Method

Many scientists used cultural methodologies for measuring the standard of drinking water in the previous century to preserve public health. Because of its ease of use, broth culture was quickly adopted for the isolation of specific groups of microorganisms, and it became the standard approach for isolating and identifying *Escherichia coli* and other coliform bacteria. To test microorganisms in drinking water, the MacConkey broth was introduced utilizing the Most Probable Number (MPN) (Anon, 1934). Percy and Grace Frankland used Koch's solid gelatin media technique to detect the total bacteria present in drinking water sources and slow sand filters in 1880, according to scholarly literature (Bulloch, 1979). Although these approaches were effective for detecting coliforms and *Escherichia coli* in drinking water, the procedure was found to be time demanding, taking around 48 hours to complete, and lacking in specificity.

4.2.Current Methods of Assessing Water Potability

Table 3 has an abstract for existing methodologies for investigating the microbiological profile and microbial activity in drinking water. Several approaches have been utilized to determine microbial density and structure (including certain opportunistic pathogens), as well as microbiological functions, in order to reliably monitor and describe the drinking water microbiology.

Table 3. An abstract for current methods used to investigate microbiological profile and microbial activities in drinking water (Ya Zhang and Wen-Tso, 2019)

Microbial density	**Community structure and composition**	**Microbial activities**
Cultivation methods (HPC, selective and differential media)	Community fingerprint (DGGE, T-RFLP, PCR-ALH, SSCP)	Adenosine triphosphate assay
Cell counting (microscopic counts, FCM)	16S Rrna gene amplicon analysis (clone library and sanger sequencing, NGS)	Enzymatic activity tests
Molecular methods (qPCR, viable qPCR, ddPCR)	16S rRNA gene hybridization (DNA microarray) Spatial distribution (FISH, SEM)	Assimilable organic carbon assay

According to scientific studies, no one approach can offer all of the necessary information about microorganisms and their activity in potable water. To increase the perspective of microorganisms in the drinking water utilized for study, the present strategy combines many ways. Furthermore, all of the approaches have been shown to have known biases during analysis, therefore caution should be used throughout monitoring (Sartory and Walkins, 1999). (1) Measuring microbial density, (2) evaluating microbial composition, and (3) quantifying microbial activity are some of the recent approaches used to analyze the standard of water.

4.2.1.Measurement of Microbial Density

The cultivation of bacteria is still commonly used today to determine the density of microorganisms in drinking water. HPC and bacterial indicators (total coliforms and *E. coli*) have been utilized as pollution signals for determining water quality and as a standard for density measurement (Bartram *et al.*, 2004). Isolation and precise identification of disease-causing bacteria are major problems in water evaluation, hence selective and differential media approaches are employed to culture distinct pathogenic bacteria in drinking water.

Cell counting is another approach for measuring microbial density that entails counting the cells in drinking water samples using microscopy or fluorescent dyes and then placing them under an epifluorescence microscope or flow cytometry (FCM) as a total cell count to be measured. The ability to distinguish temporal bacterial dynamics requires a high frequency of fully automated online FCM (Besmer *et al.*, 2016). Flow cytometry is currently limited to structures without residual disinfectants in drinking water microbial density (DSs). Due to low cell population and blockage of bacterium-like particles, membrane filtering to pretreat water with residual disinfectants is required to concentrate bacteria at a suitable density (Van Nevel *et al.*, 2017).

4.2.2.Measuring Microbial Composition

The first step in grasping the problems of microbial presence in drinking water structures is to characterize microbial populations. There are two types of PCR amplification procedures that can be used. The first category of PCR-based approaches is known as "Community Fingerprint." It examines the amplified 16rRNA genes and produces a sample-based network structure description, which is usually represented by a nucleic acid fragment banding fashion resolved through gel electrophoresis. In detail, these network fingerprinting methodologies permits researchers to quickly evaluate microbial heterogeneity inside a microbial environment, as well as compare and contrast the microbial communities formed by different ecosystems (Liu and Stahl, 2007).

The second molecular approach is to extract 16S rRNA cistron progression from the retrieved grouped genomic deoxyribonucleic acid and create a dataset. This was originally accomplished by creating a clone library of 16S rRNA cistron chain. Nowadays, next-generation sequencing (NGS) has been used to determine the amount of 16S rRNA cistron sequences in microbial samples. Each method describes the microbiological framework based on the number of distinct 16S rRNA sequences and hence the population of each 16S rRNA sequence. The 16S rRNA sequences can also be compared to all 16S rRNA arrangement in a publicly available database. This enables researchers to determine the evolution association of individual 16S rRNA sequences and confirm whether the sequences are unique or similar with known species, demonstrating sequence similarity. (Zhang and WenTso, 2019).

Finally, evaluating the spatial structure of microbial population in-situ may be used to estimate microbial composition. The most often utilized techniques to expose the spatial structure and organization of microbial ecology are fluorescent in-situ hybridization (FISH) and scanning electron microscopy (SEM) (Fischer *et al.*, 2005).

4.2.3.Measuring Microbial Activities Technology

The composition and activity of microbial communities present throughout treatment, storage in tanks, and distribution to consumers influence the standard of drinking water (Gray, 2008). Based on several scientific studies, the bacterial population present in drinking water ranges from 1,000 to 100,000 cells per millimeter (Proctor and Hammes, 2015). As a result, some opportunistic pathogenic bacteria represent a public health risk, induce corrosion in drinking water pipes, and eventually generate metabolites that alter the taste, odor, and color of drinking water (Srinivasan and Sorial, 2011). The requirement to measure the composition of microorganisms and their activity in drinking water is of highest relevance to the public and regulatory agencies in order to avoid the repercussions of eating infections associated with unclean water. Some of the currently available methods for measuring microbial activity include the use of an ATP assay, protein activity assays, and assimilable organic carbon (AOC) testing (Lautenschlager *et al.*, 2014). The total quantity of ATP determined using bioluminescence test is used to describe all active bacteria physiologically (Stutz *et al.*, 1986). Protein activity assays measure the increase in visible radiation intensities or absorbance as a function of time due to the breakdown of substrates by specific enzymatic activities such as polysaccharide degrading enzymes (a- and b-glucosidase, cellobiohydrolase, xylosidase, chitinase) (Lautenschlager *et al.*, 2014). The highest level of growth of two (2) microorganism isolates (*Pseudomonas fluorescens* P-17 and *Spirillum* sp. strain NOX) in a drinking water sample is measured by determining the portion of dissolved organic carbon that can readily support microbial growth and is estimated by determining the highest rate of growth of two (2) microorganism isolates (*Pseudomonas fluorescens* P-17 and *Spirillum* sp (Vanderkooij et al., 1982). However, due to time constraints and labor intensiveness, microbial activity assessment has not been routinely used in water microbiome investigations.

5.Limitations of Assessing Water Potability

The cultivation of microbes, which is known to be time-consuming, low sensitivity, and low in recovery of microorganisms during culture, are all limitations that impact the evaluation of drinking water (Hammes *et al.*, 2008). Furthermore, despite their widespread usage in HPC and *E. coli* testing, culture-based enumeration approaches fail to capture disinfectant-injured or genetically engineered bacteria with injected antibiotic resistance genes (ARGs) (Li *et al.*, 2017). These pathogens are often located in the drinking water habitat, but they are often missed since they can reactivate under the correct test situation and present a health risk to consumers. As a result, most current research is dependent on molecular equipment to get deeper into the microbiome evaluation of drinking water.

5.1.Regulatory and Legislative Measures of Drinking Water

The availability of sufficient regulation, standards, and norms is perfect for effective programs to control drinking-water high quality. One of the tasks of the basic rules is to define the characteristics, authority, and obligations of the water-supply and monitoring businesses. Standards and rules should outline the standard of water to be provided to consumers, as well as the methods to be utilized in choosing and creating water assets, treatment processes, distribution structures, and strategies for approving water systems in terms of water quality. The method of the regulation in a particular country will be determined by national constitution, and other factors (WHO, 1997).

As a result, water-quality criteria should be carefully considered so that acceptance by the public health or environmental health regulatory bodies complies with health-protection regulations. Typically, such law provides for the continuation and modification of drinking-water safety rules and recommendations, as well as

regulations for the enhancement of drinking-water sources, as well as the production, continuous regulation, and dissemination of safe drinking water. It also establishes the legal capabilities and importance of the water-delivery agency, stating unequivocally that, as a corporation that sells and/or delivers water to consumers; this company has a legal obligation to provide safe and healthy water that aligns with legally established water-quality standards (WHO, 1997).

The new EU Directive on Drinking Water, which came into effect in 1998, lays forth rules for the importance of drinking water, water sold in bottles or cans, and water used in food production. Mandatory and non-mandatory parameter values are suggested by the Directive. Mandatory standards for mains water, which comprise twenty-eight microbiological and chemical characteristics, are vital for human health and the environment, and must be satisfied on specific dates. For monitoring reasons, non-mandatory indicator values are necessary, in addition to microbiological, chemical, and physical characteristics. Any violation of an indicator value must be watched, but corrective action is best conducted when there is a public health danger (Council Directive, 1998).

When water fulfills the requisite microbiological load and defined requirements established by regulatory agencies for quality water and healthy living, it is considered to be wholesome. Microbiological, chemical, and physical recommendations all have standard concentrations or values. In addition to the Directive's provisions, national legislation has a few additional norms and obligations. All water that is covered by the laws should be free of microorganisms. Microbiological parameter concentrations and values are based on an already established indicator species such as coliform bacteria, *E. coli*, *Enterococci*, *Clostridium perfringens*, and colony counts. In order to achieve quality, water must be free of any microorganism (aside from a parameter) or parasite that might pose a health risk to humans at a specific level (Council Directive, 1998).

Because this strategy captures the hazard from the water sources to the consumer's tap, the WHO suggest water safety plans as the sole option for constantly making sure of the protection of drinking water supply (Bartram *et al.*, 2009). Approach to the water safety plan is entirely anchored on the hazard analysis and critical control point system, which is often utilized in the food sector to manage food quality. Under appropriate law, the Department of the Environment, Food and Rural Affairs regulates the quality of bottle water and packaging containers in the United Kingdom (Statutory Instrument, 1999). The EU Directives (Council Directive, 1996) on the misuse and advertising of natural mineral waters are implemented by these regulations, which also integrate the legislation on other types of bottled water. All water backed by these laws must be bacteriologically sound, as determined by indicator organisms once again.

6.Conclusions

Microbial load evaluation in drinking water should be a standard worldwide since potable water has become a major global concern in the twenty-first century. Extensive microbiological research should be conducted for novel techniques of monitoring drinking water, with the goal of using biosensors to overcome the limits of time-consuming analysis and poor sensitivity. Apart from monitoring *E.coli* and coliform bacteria, which are considered as important indicators of drinking water pollution, more study should be focused on monitoring other pathogens in drinking water in order to prevent disease transmission through drinking water. Finally, the government and drinking water regulatory organizations should verify that enterprises that produce potable water follow stringent guidelines for creating safe and natural mineral water.

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