A systematic review of microorganisms as indicators of recreational water quality in natural and drinking water systems

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ABSTRACT

The purpose of this review was to highlight the most frequent biological indicators used to estimate the microbiological quality of drinking and recreational water. It was observed that the incorporation of other microbiological indicators should be considered to strengthen the decision-making process on water quality management and guarantee its safe consumption in recreational activities.

Key words | drinking water, microbiological indicators, recreational water

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HIGHLIGHTS

- The incorporation of other microbiological indicators should be considered to strengthen the decision-making process on water quality management.
- The presence and concentration of bacterial, viral, and parasitic indicators are similar in several countries with diverse environmental conditions.

INTRODUCTION

Water is one of the most valuable natural resources, an essential element for life development and human activities (Romeu-Álvarez *et al.* 2012). It is intended for different uses: human consumption, industrial processes, recreational activities of primary and secondary contact, ecological conservation, among others (Baird & Cann 2014).

Due to alterations in natural and anthropogenic processes, it is necessary to guarantee its quality so that there is no risk for humans and the environment (Samboni-Ruiz *et al.* 2007; Lugo & Lugo 2018). Water is suitable for human consumption and domestic use, including personal hygiene and recreational activities, when it has low physical and chemical contaminant concentrations (WHO 2011; Lugo-Arias *et al.* 2020). Although the use of water in recreational activities can bring health benefits, it can also generate adverse effects when it is contaminated (WHO 2000). To assess the microbiological water quality, organisms are used as single indicators since their presence reveals contamination (Silva-Iñiguez *et al.* 2007).

Microbiological indicators of fecal contamination expose pathogenic organisms. They must be in higher concentration than pathogens and have similar survival characteristics (Noble *et al.* 2003; Pulido *et al.* 2005). Fecal contamination indicators have proven to be an alternative to the difficulty of identifying and quantifying pathogens that cause water-origin diseases (Campos-Pinilla *et al.* 2008). Microbiological quality control of water for human consumption and recreational use (ACH) requires pathogenic microorganisms' analysis (Silva-Iñiguez *et al.* 2007).

These analyses are hard to execute due to the great variety of cultivable pathogenic bacteria, isolation tests, the low concentration of aggressive species, the need for specialized laboratories, high economic costs, and time-consuming. To identify the presence of pathogens in a reliable way, water

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quality monitoring should be done by searching for fecal contamination indicators approved by international and national standards.

Microorganisms must meet the following requirements: (1) be a normal constituent of the intestinal microbiota of healthy individuals; (2) be present exclusively in the feces of homeothermic animals; (3) be present where pathogenic intestinal microorganisms are; (4) appear in high numbers; (5) facilitate isolation and identification; (6) be unable to reproduce outside the intestine of homeothermic animals. Their survival time must be equal to or greater than a pathogenic bacterium, and their resistance to environmental factors must be equal to or greater than fecal pathogens (Fernández *et al.* 2001). The objective of this review is to highlight the most common microbiological indicators that are used to evaluate the microbiological quality of drinking and recreational water to identify new methods that optimize microbiological monitoring of water quality.

METHOD

The research was conducted in the Science Direct, Redalyc, Scielo, and in the Google Scholar search engine selecting peer-reviewed documents and published research articles in the last ten years, based on the following keywords: microbiological indicators, drinking water, and recreational water. Articles' selection criteria were as follows: (1) biological indicators to assess the microbiological quality of drinking and recreational water quality; (2) water bodies that were affected by outflow wastewater; (3) the impact of microbiological water quality on individuals' health; and (4) recreational water affected by bathers.

Recreational waters in artificial systems such as swimming pools were not considered as they have different environmental conditions, compared to those of interest in this work. For example, swimming pool water quality criteria are like those for the quality of drinking water, including microbiological parameters that differ from the water quality characteristics of natural bodies (Carrasquero Ferrer *et al.* 2020). Also, swimming pool water is treated with different treatment methods, for example, disinfection with chlorine, UV-C radiation, ozone, membrane processes, among others (Dudziak *et al.* 2019; Skibinski *et al.* 2019). The above is not feasible from a technical and economic point of view for natural water bodies such as seas and rivers.

Articles that did not meet the above criteria were excluded from the analysis. The guidelines were also based on microbiological quality standards for drinking and recreational water of the following organizations: the World Health Organization (WHO), the United States Environmental Protection Agency (EPA), and the standards of water quality of the European Union (EU). These served as the basis for defining the microbiological water quality criteria in Latin America and the world.

RESULTS AND DISCUSSION

The most damaging effect of contaminated water has been the transmission of diseases by microorganisms that can inhabit humans (see Table 1) (Romero *et al.* 2009).

Table 1 | Main microorganisms that transmit diseases in the water

Type of microorganism	Name of the microorganism	Disease
Bacterias	*Escherichia coli*	Diarrhea and stomach pain
	Shigella spp.	Shigellosis
	Vibrio cholerae	Cholera
	Salmonella typhi	Typhoid fever
	Salmonella spp.	Salmonellosis
	Yersinia enterocolitica	Yersiniosis
	Campylobacter jejuni	Enteritis
Viruses	Enterovirus	Various diseases: intestinal, respiratory, among others
	Rotavirus	Various diseases: intestinal, fever, among others
	Adenovirus	Various diseases: colds, conjunctivitis, among others
Protozoa	*Giardia lamblia*	Giardiasis
	Cryptosporidium parvum	Cryptosporidiosis
	Entamoeba histolytica	Dysentery
Helminths	*Ascaris lumbricoides*	Ascariasis

Source: Modified from WHO (2003).

Bioindicators serve as a complementary tool to evaluate water microbiological quality. Their application requires organism identification based on diversity indices, adjusting intervals that quantify water quality. For example, in Japan, the authorities in charge of water monitoring have illustrated guides of the organisms (bacteria, fungi, insects, amphibians, and fish) that can be found in water sources, including information on the tolerance of pollutants (heavy metals, dioxins, among others) to chemical compounds. This outlines information on the state of the water in real-time (Koschelow & Briedis 2013). An organism is considered a bioindicator when its degree of tolerance is known, since not all of them offer information due to their eating habits, life cycle, and frequency with which they are found (Miravet Sánchez *et al.* 2016).

It is found that the presence and concentration of bacterial, viral, and parasitic indicators are similar in several countries with diverse environmental conditions (Liberatore *et al.* 2015), proving their use in different types of water (Campos-Pinilla *et al.* 2008). Domestic contamination generates high health risks due to high concentrations of fecal microorganisms (Wade *et al.* 2015). Therefore, it is important to have laboratory tests, exposing bacteria, viruses, and parasite concentrations in a precise moment with low economic costs. The most common bacteria used for drinking water control, wastewater, and recreational uses are total coliforms, fecal coliforms, *Escherichia coli*, and *Enterococcus faecalis*.

Clostridium perfringens has also been proposed as an indicator of distant fecal contamination in groundwater and as an indicator of the presence of protozoan cysts (Campos-Pinilla *et al.* 2008). The presence of coliform bacteria is considered the best indicator of fecal contamination, and in the long term, it serves to monitor the effectiveness of control programs. Its use is advantageous because it gives a quick response to environmental changes such as pollution based on domestic discharges (Koschelow & Briedis 2013). Numerous epidemiological studies in different aquatic environments have shown a relationship between coliform counts and the appearance of infectious diseases in humans.

However, various studies reveal that there is no significant relationship between these indicators and diseases related to seawater bathing (Vergaray *et al.* 2007). Total coliforms and *Escherichia coli* (*E. coli*) have been suggested as reliable indicators of wastewater contamination. Recreational waters such as seawater are susceptible to fecal contamination, which can increase the health risk associated with swimming in contaminated beach water (Praveena *et al.* 2013). In water analysis, the presence of *E. coli* indicates fecal contamination, with a positive correlation between the concentration of organisms and the amount of contamination (Romero *et al.* 2009).

Also, there is a trend towards linearity between the concentrations of thermotolerant coliforms and *E. coli*, which shows that such indicators could be used as a tool for river water microbiological quality (Romeu-Álvarez *et al.* 2012).

This includes fecal enterococci (FE) as a bacteriological indicator in marine waters for recreational use due to its resistance to seawater conditions, temperature, and relationship with gastrointestinal, respiratory, and dermatological diseases (Silva-Iñiguez *et al.* 2007). Although no indicator can accurately determine water quality, enterococci have a predictive bent in marine environments, while somatic bacteriophages are useful bioindicators in seawater ecosystems (Janelidze *et al.* 2011). In contrast, a study by Atoyan *et al.* (2011) suggests that FE and fecal coliforms (FC) are not reliable indicators of human fecal contamination in the Pettaquamscutt River due to inconsistencies found in high rates of false-positive and negative tests. Furthermore, there was a considerable amount of spatial and temporal variability in the reliability of the tests.

The inclusion of chemical nucleic acid-based methods for evaluating the microbiological quality of water can improve the identification of sources of human fecal contamination in water sources (Ahmed *et al.* 2015). The United States Environmental Protection Agency requires the use of FE as an indicator of the risk of contracting a gastrointestinal illness from swimming activities in waters contaminated by FC. However, none of these indicators are definite for human fecal contamination because there is a wide variety of warm-blooded animals (Atoyan *et al.* 2011). That is why several techniques have recently emerged for monitoring human fecal contamination sources based on indicators related to human beings.

Adolescentis bifidobacterium has been suggested as a specific bacterium of the human intestinal tract that can accurately identify human fecal contamination in freshwater. It is an anaerobic Gram-negative bacillus that grows exclusively in human intestines and has been suggested as an alternative fecal contamination indicator, since it does not survive in seawater, so it cannot reproduce itself in the environment. Its evaluation is carried out using a nested polymerase chain reaction (PCR) method for the exclusive detection of *A. bifidobacterium* in human feces.

It has been used in water and sediment samples (Atoyan *et al.* 2011). On the other hand, the presence of pathogenic viruses has gained importance in recent decades due to epidemiological studies and improvements in environmental detection techniques.

However, these techniques require molecular procedures that are not within the reach of most water analysis laboratories (Moresco *et al.* 2012). As an alternative, bacteriophages such as the ones that infect *Bacteroides fragilis* have been proposed. It was observed that its behavior is like some viruses that cause diseases of hydric origin, and its detection can be done in a few hours. Depending on the type of water and environmental conditions, the use of phages is recommended (Campos-Pinilla *et al.* 2008). Monitoring coastal waters with total coliforms, FC, and *E. faecalis* phage indicators are recommended to prevent potential waterborne diseases (Janelidze *et al.* 2011).

Also, water sample testing is essential to verify the presence of indigenous microflora, such as pathogenic Vibrio species. It can be useful to predict and prevent waterborne diseases, such as cholera and Vibrio-related gastroenteritis (Janelidze *et al.* 2011). The risk of infection by enteric viruses and other pathogens in the use of recreational waters has been described in some studies. For example, the coastal water quality in Brazil was evaluated through base fecal indicators, such as coliforms or *Enterococcus* spp. (Prasad *et al.* 2015). However, bacterial contamination did not correlate with the presence of human enteric viruses (Wong *et al.* 2009).

Human viruses, such as adenoviruses (HAdV), hepatitis A (HAV), polyomaviruses (JCPyV), and human noroviruses (HuNoV), are associated with waterborne diseases. HAdV, HAV, and HuNoV can replicate inside the gastrointestinal tract and are excreted in high concentrations in feces of infected individuals (Moresco *et al.* 2012). JCPyV is found in the urine of approximately 80% of adults, and the infection is spread during childhood. These viruses are usually spread into the water by industrial or agricultural activities (Lyons *et al.* 2015; Rusiñol *et al.* 2015). Once in the water, these viruses are very stable and resistant to chemical agents such as UV radiation and chlorine.

The presence of viruses in various water environments has been revealed, highlighting the need to include them in the analysis of water quality. In a study carried out by Moresco *et al.* (2012), no correlation was observed between virus detection and FC presence. It can be assumed that these common bioindicators (FC) are not accurate in associating the risk of disease contraction with water environments (Rusiñol *et al.* 2015). In the case of parasites, the presence of helminth eggs and protozoan cysts is the main risk to human health. The parasites most studied are giardia cysts and *Cryptosporidium* oocysts. The detection and counting of these parasites are an alternative health indicator since analysis is easy to carry out in laboratories with basic equipment (Campos-Pinilla *et al.* 2008).

Recreational water quality criteria

Water quality standards have been used through microbiological parameters that measure the degree of contamination (Bonamano *et al.* 2015). Table 2 shows the microbiological quality criteria for primary contact with recreational water. Table 3 shows studies of microbiological indicators for recreational waters.

Microbiological water quality analysis must consider the sources of microbial contamination of coastal areas since it has been found that fecal indicators, coliphages, and

Table 2 | Criteria for evaluating the microbiological quality of recreational water

Fecal indicator	Allowable limit	Normative
Total coliforms (TC)	<1,000 MPN/100 ml	Decree 1594 of 1984 (Colombia)
Fecal coliforms (FC)	<200 MPN/100 ml	Decree 1594 of 1984 (Colombia)
Enterococci	40 CFU/100 ml 200 CFU/100 ml 185 CFU/100 ml	NTS-TS 001-2 WHO (2003) Directive 2006/7/EC of the European Council
Escherichia coli	500 CFU/100 ml	Directive 2006/7/EC of the European Council

Source: Campos-Pinilla *et al.* (2008).

Table 3 | Studies carried out to determine the microbiological quality of recreational water

Water type	Indicator	Determination technique	Geographic localtion	References
Seawater	Total coliforms	Membrane filtration (CFU/ 100 ml)	(Black Sea, Georgia); (Teluk Kemang Beach, Malaysia); (Kuwait Bay)	Janelidze *et al.* (2011); Praveena *et al.* (2013); Lyons *et al.* (2015)
		Most probable number (MPN)	8 beaches of Peru	Vergaray *et al.* (2007)
	Fecal coliforms	Membrane filtration (CFU/ 100 ml)	(Black Sea, Georgia); (Kuwait Bay)	Janelidze *et al.* (2011); Lyons *et al.* (2015)
		Most probable number (MPN)	(8 beaches in Peru); (2 beaches in Costa Rica)	Vergaray *et al.* (2007); Badilla-Aguilar & Mora-Alvarado (2019)
	Fecal enterococci	Membrane filtration (CFU/ 100 ml)	(5 beaches in Brazil); (Kuwait Bay)	Lamparelli *et al.* (2015); Lyons *et al.* (2015)
		Most probable number (MPN)	(Playa la Boquita-México); (Black Sea, Georgia); (8 beaches in Peru); (Adriatic Sea, Italy); (2 beaches in Costa Rica)	Silva-Iñiguez *et al.* (2007); Janelidze *et al.* (2011); Vergaray *et al.* (2007); Liberatore *et al.* (2015); Badilla-Aguilar & Mora-Alvarado (2019)
	Escherichia coli	Most probable number (MPN)	(8 beaches in Peru); (2 beaches in Costa Rica)	Vergaray *et al.* (2007); Badilla-Aguilar & Mora-Alvarado (2019)
		Membrane filtration (CFU/ 100 ml)	(Santa Catarina Island-Brazil); (5 beaches in Brazil); (Teluk Kemang Beach, Malaysia)	Moresco *et al.* (2012); Lamparelli *et al.* (2015); Praveena *et al.* (2013)
	Clostridium perfringens	Membrane filtration (CFU/ 100 ml)	Atlantic Beaches (Colombia)	Moreno *et al.* (2019)
	Vibriopathogens	Membrane filtration	Black Sea, Georgia	Janelidze *et al.* (2011)
	Coliphages	Membrane filtration	Black Sea, Georgia	Janelidze *et al.* (2011)
	Adenovirus (HAdV)	Flocculation	Santa Catarina Island-Brazil	Moresco *et al.* (2012)
	Polyomavirus (JCPyV)	Flocculation	Santa Catarina Island-Brazil	Moresco *et al.* (2012)
	Norovirus	Flocculation	Santa Catarina Island-Brazil	Moresco *et al.* (2012)
River water	Total coliforms	Membrane filtration (CFU/ 100 ml)	(Río Cúpira, Venezuela); (Danube river)	Koschelow & Briedis (2013); Kirschner *et al.* (2009)
		Most probable number (MPN)	(Río Nazas, Mexico); (Machángara and Monjas rivers, Ecuador)	Romero *et al.* (2009); Campaña *et al.* (2017)
	Fecal coliforms	Membrane filtration (CFU/ 100 ml)	(Río Cúpira, Venezuela); (Luyanó River, Cuba)	Koschelow & Briedis (2013); Romeu-Álvarez *et al.* (2012)
		Most probable number (MPN)	(Río Nazas, Mexico); (Machángara and Monjas rivers, Ecuador)	Romero *et al.* (2009); Campaña *et al.* (2017)
	Fecal enterococci	Membrane filtration (CFU/ 100 ml)	(Danube river)	Kirschner *et al.* (2009)
	Escherichia coli	Membrane filtration (CFU/ 100 ml)	(Danube river); (Río Luyanó, Cuba); (Puerto Rico); (Umgeni River Catchment, South Africa); (Rivers of Poland)	Kirschner *et al.* (2009); Romeu-Álvarez *et al.* (2012); Wade *et al.* (2015); Baker *et al.* (2015); Lenart-Boroń *et al.* (2017)
	Bacteroides HF183	PCR	(Puerto Rico)	Wade *et al.* (2015)
	A. bifidobacterium Pseudomonas aeruginosa and Salmonella	Fluorescence	(Pettaquamscutt River, USA)	Atoyan *et al.* (2011)

Source: Developed by the authors.

enteroviruses are significantly associated with precipitation, sewage discharges, and temperature (Janelidze *et al.* 2011).

Microbiological indicators of drinking water quality

Purification systems are characterized by their efficiency in the elimination of bacteria but not in viruses. Thus, the above performance does not guarantee the removal of viruses and parasites since they can be resistant to chlorine disinfection systems (Campos-Pinilla *et al.* 2008). Viruses, parasites, and bacteria that indicate fecal contamination must be considered to evaluate the microbiological quality of water sources. Various studies have associated the presence of viruses with bacterial indicators of fecal contamination (Rusiñol *et al.* 2015).

There is low confidence in populations in developing countries to drink tap water for it not complying with sanitary conditions. This is due to contamination in distribution networks (WHO 2011). For bottled water, it is essential to highlight the following origins of the bacterial flora: (1) bacteria belonging to the point of emergence are found (autochthonous microflora); (2) the bacteria 'added' to the water during the packaging process (non-native microflora) (Payares *et al.* 2013). In the absence of fecal indicators in treated water, the heterotrophic plaque count (HPC) serves to assess its overall microbiological quality (Bartram *et al.* 2004).

Although it is not an indicator of adverse effects on human health, its increase may indicate problems with raw water quality, water treatment, or the distribution system (WHO 2012). Sometimes it is high, and operational decision-makers are faced with the problem of long lists of zero-count samples. Since water distribution systems are not sterile, this reflects that the conventional approach is a limited diagnostic tool (Gillespie *et al.* 2014). One of the most promising developments in microbiological surveillance of water quality is flow cytometry (FCM) (Gillespie *et al.* 2014). Switzerland was the first country to incorporate a standardized method for FCM in the water industry to determine the count of different total cells and populations (Harry *et al.* 2016).

The number of bacteria detected in drinking water by FCM is greater than the number of HPC (Hammes *et al.* 2008). Van Nevel *et al.* (2017) exposes multiple reasons (including cost) why FCM is a suitable alternative to replace HPC. However, FCM has disadvantages such as its high investment cost due to the nature of the equipment, the procedures involved, the need for expensive dyes (used for sample staining), and the high initial capital cost of the equipment. Therefore, the use of FCM is inappropriate to be applied in field investigations (Bridgeman *et al.* 2015). Fluorescence analysis is a quick technique that requires low sample volumes. They are typically 10–1,000 times more sensitive than those of UV absorption spectroscopy with the detection of individual molecules (Henderson *et al.* 2009).

A fluorescence device with LED technology has been proposed to facilitate the monitoring of water quality (Bridgeman *et al.* 2015; Sorensen *et al.* 2018). Besides, it is essential to include such techniques to evaluate the microbiological quality of drinking water since the information obtained from the microorganisms is in real-time and it does not take long to generate the data (as in conventional techniques) (Sorensen *et al.* 2015; Bonadonna *et al.* 2019).

It must be ensured that the supplied drinking water does not pose any health risk to consumers (Bridgeman *et al.* 2015). Tables 4 and 5 show the recommended microorganisms for microbiologically evaluating the quality of drinking water.

CONCLUSIONS

The application of the most used microbiological indicators to assess the microbiological quality of drinking and recreational water has been described, highlighting reliable techniques for analyzing pathogenic microorganisms, considering technical and economic aspects. It is necessary to include other microbiological indicators to strengthen water

Table 4 | Microorganisms recommended as indicators of the quality of drinking water

Bacterias	Total coliforms Fecal coliforms *Escherichia coli* Heterotrophic microorganisms *Clostridium perfringens*
Viruses	Somatic coliphages Coliphages F specific Phages
Parasites	*Giardia lamblia* *Cryptosporidium parvum*

Source: Developed by the authors.

Table 5 | Criteria for evaluating the microbiological quality of drinking water

Fecal indicator	Allowable limit	Normative
Total coliforms	Not detectable	Resolution 2115/2007 (Colombia)
Fecal coliforms		U.S. EPA
Fecal coliforms		WHO
Escherichia coli		WHO
Giardia lamblia		Resolution 2115/2008 (Colombia)
Legionella		U.S. EPA
Enteric viruses		U.S. EPA
Legionella		U.S. EPA
Enteric viruses		U.S. EPA

Source: Developed by the authors.

quality research and guarantee the safety of its consumption and use in different recreational activities. These could be some biological agents that are not usually included in international regulations, such as viruses and parasites.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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