# Microbial indicators, pathogens and methods for their monitoring in water environment

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## ABSTRACT

Water is critical for life, but many people do not have access to clean and safe drinking water and die because of waterborne diseases. The analysis of drinking water for the presence of indicator microorganisms is key to determining microbiological quality and public health safety. However, drinking water-related illness outbreaks are still occurring worldwide. Moreover, different indicator microorganisms are being used in different countries as a tool for the microbiological examination of drinking water. Therefore, it becomes very important to understand the potentials and limitations of indicator microorganisms before implementing the guidelines and regulations designed by various regulatory agencies. This review provides updated information on traditional and alternative indicator microorganisms with merits and demerits in view of their role in managing the waterborne health risks as well as conventional and molecular methods proposed for monitoring of indicator and pathogenic microorganisms in the water environment. Further, the World Health Organization (WHO) water safety plan is emphasized in order to develop the better approaches designed to meet the requirements of safe drinking water supply for all mankind, which is one of the major challenges of the 21st century.

**Key words** | drinking water quality, fecal contamination, microbial indicator, molecular techniques, waterborne disease, water safety plan

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## INTRODUCTION

Ensuring the safety of drinking water (DW) is an ongoing process. Water that looks suitable for drinking may be contaminated with pathogens that may cause serious health hazards. The microbiological examination of DW for the presence of indicator microorganisms (IMs) is key to determining microbiological quality and ensuring public health safety. The presence of IMs represents the fecal contamination of DW with pathogens and quality deterioration. The microbiological assessment of DW quality is based on the relationship between IMs and pathogens (Borrego *et al.* 2002a, 2002b; Koster *et al.* 2003; WHO 2008). However, DW illness outbreaks have occurred both in the presence and absence of IMs. This is because of either failure of treatment processes that do not completely eliminate the pathogens from DW or entry of contaminated water harboring pathogens into distribution systems through cracks/

leakage (Figueras & Borrego 2010). In spite of specific legislation, DW illness outbreaks are still occurring worldwide and the associated control measures are being carried out (Figueras & Borrego 2010).

The World Health Organization (WHO) has published several guidelines in collaboration with the International Water Association (IWA) and the Organisation for Economic Co-operation and Development (OECD) for improvement in DW quality (Dufour *et al.* 2003; EIWID 2003; WHO 2008). Water safety plans (WSPs) are the most recent document to create awareness among water quality professionals, so that they can develop the preventive strategies to protect public health (Bartram *et al.* 2009).

Many waterborne pathogens are still difficult to detect and/or quantify due to the lack of easy and reliable methods. The specific methods that are used to detect IMs have also

been reviewed in many studies (Koster *et al.* 2003; NRC 2004). Nowadays, new approaches based on the virulence factor-activity relationship (VF-AR) to detect emerging waterborne pathogens are being explored (Karanis *et al.* 2007; Cangelosi 2009). However, the routine application of these methods for the examination of pathogens is not yet the reality and is restricted to research studies or to cases of suspected outbreaks. This review article emphasizes the traditional and alternative IMs with merits and demerits in view of their role in managing waterborne health risks, as well as conventional and molecular methods proposed for monitoring of indicator and pathogenic microorganisms in water environments. In addition, the pathogens removal efficiency of DW and wastewater treatment plants (WWTPs) is discussed. Further, WSPs are given in order to develop better approaches to meet the requirement of safe DW supply for all mankind.

The presence of indicator organisms will likely continue to be used as a criterion of DW quality and it will be useful if attention is given for the development and use of optimal methods for their recovery. The existing data also indicate that there is limited utility of IMs under certain circumstances, although there is no ideal IM and research is ongoing to search for a suitable microorganism that can indicate the presence of pathogenic microorganisms more accurately than traditional indicators.

## DRINKING WATER AS A SOURCE OF DISEASES

Water is the essence of life. A clean and safe DW supply may be the norm in European and North American countries, but in developing countries the assessment of both clean and safe DW is not the rule and hence waterborne illness outbreaks are common. In Asian and African countries, children under 5 years of age are primarily affected by diarrheal diseases (DDs) transmitted through contaminated DW (Seas *et al.* 2009). It is also reported that around two and a half billion people have no access to clean and safe DW and more than 1.5 million children die each year from DDs (Fenwick 2006). This is due to the contamination of DW with different types of pathogenic microorganisms present in urban sewage, and feces of infected humans and animals (Table 1). These include enteric bacteria, viruses and

**Table 1** | Pathogens transmitted through contaminated DW and diseases caused by them

| Pathogens | Diseases |
|---|---|
| **Bacteria** | |
| *Campylobacter jejuni* | Gastroenteritis |
| Enteropathogenic *E. coli* | Gastroenteritis |
| *Legionella pneumophila* | Acute respiratory illness |
| *Salmonella* | Typhoid, paratyphoid, salmonellosis |
| *Shigella* | Bacillary dysentery |
| *Vibrio cholerae* | Cholera |
| *Yersinia enterocolitica* | Gastroenteritis |
| **Protozoa** | |
| *Cryptosporidium* | Diarrhea |
| *Entamoeba histolytica* | Amoebic dysentery |
| *Giardia lamblia* | Diarrhea |
| *Naegleria fowleri* | Meningoencephalitis |
| **Enteroviruses** | |
| *Adenoviruses* | Respiratory illness, eye infection, gastroenteritis |
| *Astroviruses* | Gastroenteritis |
| *Caliciviruses* | Gastroenteritis |
| *Coxsackievirus A* | Meningitis, respiratory illness |
| *Coxsackievirus B* | Myocarditis, meningitis, respiratory illness |
| *Echovirus* | Meningitis, diarrhea, fever, respiratory illness |
| *Hepatitis A viruses* | Infectious hepatitis |
| *Norwalk viruses* | Diarrhea, vomiting, fever |
| *Polioviruses* | Meningitis, paralysis |
| *Rotaviruses* | Diarrhea, vomiting |

Adapted from Metcalf & Eddy (1991), AWWA (1999), Ashbolt (2004), Gerba & Smith (2005), and Arnone & Walling (2007).

parasites, and can be transmitted through contaminated water and food (NRC 2004; Toranzos *et al.* 2007; WHO 2008).

In general, any practice that involves the distribution of domestic wastewater in soil has the potential to cause microbial contamination of ground water. The wastewater discharged in fresh water and costal seawaters is also a major source of pathogenic microbes (Fenwick 2006; WHO 2008). The wastewater treatment processes applied at WWTPs do not completely eliminate or inactivate the pathogenic microbes present in wastewater. The pathogen

**Table 2** │ Pathogen removal in treated sewage

| Infective dose of pathogen | Enteric viruses | Salmonella | Giardia |
|---|---|---|---|
| No. of cells or particles | 1 > 10 | $>10^3$ | 25–100 |
| Amount in feces | $10^6$–$10^{10}$/g | $10^{10}$/g | $9 \times 10^6$/g of stool |
| Concentration in raw sewage (No./L) | $10^3$ | 5,000–80,000 | 9,000–200,000 |
| % Removal of pathogens during | | | |
|    Primary treatment | 50–98.3 | 95.8–99.8 | 27–64 |
|    Number remaining | 1,700–50,000 | 160–3,360 | 72,000–146,000 |
|    Secondary treatment | 53–99.92 | 98.65–99.996 | 45–96.7 |
|    Number remaining | 85–47,500 | 3–1,075 | 6,480–109.500 |
|    Tertiary treatment | 83–99.9999998 | 99.99–999999995 | 98.5–99.99995 |
|    Number remaining | 0.0002–17 | 0.000004–7 | 0.099–2,951 |

Adapted from Yates (1998) and Toze (2006).

removal efficiency of wastewater treatment processes, as well as the concentration of pathogenic microbes that remained in digested sludge even after treatment processes, is summarized in Tables 2 and 3, respectively.

## INDICATORS OF MICROBIAL CONTAMINATION OF DRINKING WATER

The presence of enteric pathogens in DW is of great concern (Maal-Bared *et al.* 2008). Thus, the legislation in

**Table 3** │ Concentration of microorganisms in digested sludge

| Organisms | Type of stabilization (no./g of dry weight) | |
|---|---|---|
| | Anaerobic | Aerobic |
| *Enteroviruses* | 0.2–210 | 0–260 |
| *Rotaviruses* | 14–485 | ND |
| *Salmonella* | 3–$10^3$ | 3 |
| Total coliforms | $10^2$–$10^6$ | $10^5$–$10^6$ |
| Fecal coliforms | $10^2$–$10^6$ | $10^5$–$10^5$ |
| *Shigella* sp. | 20 | ND |
| *Yersinia enterocolitica* | 10 | ND |
| *Giardia* spp. | $10^2$–$10^3$ | ND |
| *Ascaris* | – | – |
| *Trichuris* | – | – |
| *Toxocara* | – | – |

Adapted from Straub *et al.* (1993) and Lepeuple *et al.* (2004).
Average of all types of digested sludge (% viable); ND: no data.

Europe, the USA and other countries requires adequate examination of IMs to determine microbiological quality. Hence, the most useful tool to detect pathogens in the water environment is the simultaneous analysis of several microorganisms classed as 'Indicator' microorganisms (Ashbolt *et al.* 2001). These IMs are being used to assess the microbiological quality of environmental water (Ashbolt *et al.* 2001). However, the criterion for an ideal IM to indicate the presence of pathogens in water environments has been discussed in many studies (Tyagi *et al.* 2006; Savichtcheva & Okabe 2006; Cabral 2010; Zheng *et al.* 2013). Generally, IMs are not themselves human pathogens (Verhille 2013); this has been the foundation upon which the protection of public health from water-borne diseases (WBDs) has been developed. To avoid ambiguity in the term 'Microbial Indicator' the following three groups are now recognized (Table 4). The most widely used IMs are coliforms (total coliforms (TCs)), fecal or thermotolerant coliforms, *Escherichia coli*, enterococci (fecal streptococci or intestinal enterococci) and bacteriophages.

### Potential applications of an IM

The microorganism used as an indicator should also be chosen on the basis of the particular application of the information. Thus, the potential application of an IM should be to indicate:

(a) the fecal pollution;

**Table 4** | Definitions for indicator and index microorganisms of public health concern

| Group | Definition |
|---|---|
| Process indicator | A group of organisms that demonstrates the efficacy of a process such as total heterotrophic bacteria or total coliforms for chlorine disinfection |
| Fecal indicator | A group of organisms that indicates the presence of contamination such as the bacterial groups, thermotolerant coliforms or *E. coli*. Hence, they only infer that pathogens may be present |
| Index and model organisms | A group/or species indicative of pathogen presence and behavior, respectively, such as *E. coli* as an index for Salmonella and F-RNA coliphages as models of human enteric viruses |

Adapted from Ashbolt *et al.* (2001).

(b) the presence of domestic sewage;

(c) the presence of pathogenic microbes;

(d) the efficiency of a particular water or wastewater treatment process;

(e) the environmental fate of a target pathogen;

(f) the movement of particles suspended in water during subsurface transport.

## Traditional indicators

The microorganisms that have been reported as an indicator of fecal contamination are summarized in Table 5, whereas the IMs that have been used to establish the performance criteria for various water uses are listed in Table 6.

## Coliforms or total coliforms

The coliforms belong to the family *Enterobacteriaceae*, which includes harmless *E. coli* and *Enterobacter*, the common intestinal organisms and occasional pathogens like *Klebsiella*, *Citrobacter*, *Kluyvera* and *Leclercia* genera and some members of the genus *Serratia* (Figueras & Borrego 2010). These bacteria are classically used as an indicator of fecal contamination in water because they are considered as the residents of intestinal tracts of homeothermic animals (Figueras *et al.* 2000; Staradumskyte & Paulauskas 2012) and, thus, are of sanitary significance. The TCs in a distribution system may provide an enhanced knowledge of water quality throughout the system as well as overall system condition. In a distribution system, the coliform bacteria act as operational indicators. Their presence indicates the deterioration in water quality, possibly via bacterial re-growth problems or post-treatment

contamination in DW that can be investigated further. The TCs are bacteria that are predominant in the natural environment. There are some limitations in the general use of coliforms as IMs, which include: (a) their ability to grow in natural water; and (b) lack of correlation between the number of coliforms and those of microbial pathogens. Furthermore, several studies have demonstrated the presence of coliforms in drinking water distribution systems (DWDS) associated with biofilm growth problems (WHO 2004; O'Reilly *et al.* 2007). Therefore, these can serve as an indicator of treatment efficiency of WWTPs because of their sensitivity to chlorine. However, some members of the TC group are no longer used as an indicator of fecal contamination because advances in the science of taxonomy have shown that they are not specific to the human intestine. Hence, these can also be found in the natural environment (Leclerc *et al.* 2001; Tallon *et al.* 2005; Verhille 2013). Thus, the presence of TC bacteria is not a definitive proof that the environment has been contaminated with fecal material.

## Fecal coliforms or 'thermotolerant coliforms'

Coliforms that are able to grow and ferment lactose with the production of acid and gas at 44.5 °C in the presence of bile salts are grouped as fecal coliforms (FCs) (Payment *et al.* 2003; WHO 2008; Staradumskyte & Paulauskas 2012). For this reason, 'thermotolerant coliforms' would be the scientifically more accurate term for this group (Fong *et al.* 2007; Figueras *et al.* 2008). Thus, the bacteria of this coliform subgroup have been found to have a positive correlation with fecal contamination of warm-blooded animals (Fenwick 2006; Fong *et al.* 2007; Toranzos *et al.* 2007). The physiological basis of the elevated temperature phenotype in FCs has

**Table 5** | Definitions of key fecal IMs

| Indicator organisms | Characteristics/features |
|---|---|
| Total coliforms | Gram-negative, non-spore-forming, oxidase/indole-negative, rod-shaped facultative anaerobic bacteria that ferment lactose (with β-galactosidase) to acid and gas within 24–48 h at $36 \pm 2\,°C$ in a medium containing bile salts and detergents. Not specific indicators of fecal pollution |
| Fecal coliforms | Thermotolerant coliforms that produce acid and gas from lactose fermentation at $44.5 \pm 0.2\,°C$ within $24 \pm 2$ h, also known as fecal coliforms due to their role as fecal indicators |
| Escherichia coli (E. coli) | Thermophilic coliforms that produce indole from tryptophan, but also defined now as coliforms able to produce β-glucuronidase (although taxonomically up to 10% of environmental *E. coli* may not). Most appropriate group of coliforms to indicate fecal pollution from warm-blooded animals |
| Fecal streptococci (FS) | Gram-positive, catalase-negative non-spore-forming cocci from selective media (e.g., azide dextrose broth (sodium azide = strong inhibitor of respiratory chain) or m-Enterococcus agar) that grow on bile aesculin agar and at 45 °C, belonging to the genera Enterococcus and Streptococcus possessing the Lancefield group D antigen. This group had been used in conjunction with fecal coliform to determine the source of recent fecal contamination (man or animals). Several strains appear to be ubiquitous and cannot be distinguished from the true fecal streptococci under usual analytical procedure, which detracts from their use as an indicator organism |
| Bacteroides | An anaerobic, non-spore-forming, Gram-negative, pleiomorphic bacillus, has been proposed as human-specific indicator |
| Enterococci | The subset of fecal streptococci that grow at pH 9.6, 10 and 45 °C and in 6.5% NaCl. Nearly all are members of the genus Enterococcus, and also fulfill the following criteria: resistance to 60 °C for 30 min and ability to reduce 0.1% methylene blue. Alternatively, enterococci can be directly identified as microorganisms capable of aerobic growth at $44 \pm 0.5\,°C$ and of hydrolyzing 4-methlumbelliferyl-β-D-glucoside (MUD, detecting β-glucosidase activity by blue florescence at 366 nm), in the presence of thallium acetate, nalidixic acid and 2, 3, 5-triphenyltetrazolium chloride (TTC, which is reduced to red formazan) in the specified medium (ISO/FDIS 7899-1 1998). The enterococci are generally found in lower numbers than other indicator organisms; however, they exhibit better survival in sea water |
| Clostridium perfringens | Gram-positive spore-forming, non-motile, strict anaerobe, sulfite-reducing bacilli, ferment lactose, sucrose and inositol with the production of gas, produce stormy clot fermentation with milk, reduce nitrate, hydrolyze gelatin and produce lecithinase and acid phosphatase. Spores most often of fecal origin and always present in sewage. These indicate the presence of Protozoa and Enteroviruses. Not all sulphite-reducing clostridia (SRC) in receiving waters are indicators of fecal pollution, hence *C. perfringens* is the appropriate indicator |
| Bifidobacteria | Gram-positive, obligate anaerobic, non-acid-fast, non-spore-forming, non-motile bacilli which are highly pleomorphic and may exhibit branching bulbs (bifids), clubs, coccoid, coryneform, Y and V forms. They are all catalase-negative and ferment lactose (except the three insect species; *B. asteroides, B. indicum* and *B. coryneforme*) and one of the most numerous groups of bacteria in the feces of warm-blooded animals |
| Bacteriophages (phages) | Bacterial viruses which are ubiquitous in the environment and resistant to disinfection. For water quality testing and to model human enteric viruses and protozoans, most interest in somatic coliphages, male-specific RNA coliphages (F-RNA coliphages) and phages infecting *B. fragilis* |

Adapted from Ashbolt *et al.* (2001).

been described as 'thermotolerant adaptation of proteins'. Therefore, their stability at temperatures found in the enteric tracts of animals is both constant and higher than the temperature in most aquatic and terrestrial environments (Clark 1990).

However, some thermotolerant coliform bacteria that conform to this definition also belong to the genus Klebsiella and have been isolated from environmental samples in the apparent absence of fecal contamination (Fenwick 2006; Toranzos *et al.* 2007; Figueras *et al.* 2008). Similarly, other members of the thermotolerant coliform group including *E. coli* have been detected in some pristine areas (Hazen & Toranzos 1990). These were also associated with the re-growth events in DWDS (O'Reilly *et al.* 2007; Collado *et al.* 2010). However, the potential for re-growth or multiplication is less than that of the TCs. Therefore, caution needs to be exercised when deciding whether the presence of IMs does indeed represent fecal contamination and thus is a

**Table 6** | Indicator organisms used to establish the performance criteria for various water uses

| Water use | Indicator organisms |
| --- | --- |
| Drinking water | Total coliforms |
| Freshwater recreation | Fecal coliforms (*E. coli*) |
| Salt water recreation | Fecal coliforms, total coliforms and Enterococci |
| Shellfish-growing areas | Total coliforms, fecal coliforms |
| Agricultural irrigation (for reclaimed water) | Total coliforms |
| Wastewater effluent | Fecal coliforms |
| Disinfection | MS2 coliphages |

Adapted from Metcalf & Eddy (2003).

threat to public health. The FCs also display a survival pattern similar to those of bacterial pathogens (Figueras *et al.* 2008). However, their usefulness as an indicator of protozoans and viral contamination is limited and hence tends to be replaced by *E. coli* in several legislations (WHO 2004).

*Escherichia coli* (mostly non-pathogenic) is the most reliable indicator of enteric pathogens. It is therefore the indicator of choice to indicate the occurrence of recent fecal contamination in DW (Leclerc *et al.* 2001; Payment *et al.* 2003; Wade *et al.* 2003; Tallon *et al.* 2005; Verhille 2013). However, only some strains of *E. coli* are capable of causing disease (Tallon *et al.* 2005; Scheutz & Strockbine 2005; Hrudey 2011), but at present *E. coli* appears to provide the best bacterial indication of fecal contamination in DW (WHO 2008). This is based on the following: (a) the prevalence of thermotolerant (fecal) coliforms in temperate environments as compared to the rare incidence of *E. coli*; (b) the prevalence of *E. coli* in human and animal feces and generally not elsewhere in the environment; and (c) the availability of affordable, fast, sensitive, specific and easier test methods to detect *E. coli*.

Therefore, *E. coli* is the best and commonest microbial indicator available to date to inform public health risks associated with the consumption of contaminated DW (Staradumskyte & Paulauskas 2012; Odonkor & Ampofo 2013). Several European and American countries included this organism in their regulations as a primary indicator of fecal contamination in DW. Further, new data have shown that *E. coli* can also survive for an extended period of time in lake sediments (Byappanahalli *et al.* 2003).

## Fecal streptococci, enterococci or intestinal enterococci

Fecal streptococci, enterococci and intestinal enterococci are the three synonyms used to describe the members of genus *Enterococcus* comprising different species of sanitary significance. However, the survival characteristics and the proportions of the species of this group are not the same in animal and human feces (Borrego *et al.* 2002b; Figueras *et al.* 2008). It is advantageous to use these microorganisms as a useful indicator of the microbiological quality of DW because: (a) they show a close relationship with the health risks due to the consumption of contaminated DW, mainly for gastrointestinal symptoms; (b) they are always present in the feces of warm-blooded animals; (c) their inability to multiply in sewage-contaminated water resources; (d) they are not ubiquitous as coliforms; and (e) their die-off rate is slower than that of coliforms in water as well as their persistence pattern being similar to that of potential waterborne bacterial pathogens (Figueras *et al.* 2008; Layton *et al.* 2010).

## Alternative IMs

The traditional microbial indicators proposed by various researchers have presented several shortcomings and they cannot be used in all water types. In this instance, other indicators named 'alternative', should be used to determine the possible threats to the public health (Figueras *et al.* 2000; WHO 2004, 2008).

## Sulfite-reducing clostridia

The genus *Clostridium* (*C. perfringens*) as an indicator of fecal contamination and sanitary quality of water is based on the following assumptions (WHO 2008; Figueras *et al.* 2008):

(a) the presence of these microorganisms in the feces of all warm-blooded animals as well as in sewage;
(b) more stability in environmental water and greater resistance to disinfection processes than most pathogens;
(c) successful use in the monitoring of sewage contamination in water (Figueras *et al.* 2000; WHO 2004, 2008).

Nevertheless, the sulfite-reducing clostridia are considered ubiquitous in aquatic sediments and their spores are highly resistant to environmental stress. This explains

their long-term persistence in environmental water compared to other fecal indicator bacteria (Horman *et al.* 2004; Zheng *et al.* 2013), although they can also be used as indicators of remote or old fecal contamination (Desmarais *et al.* 2002) or to evaluate the virus/cyst inactivation in DW by disinfection methods (Figueras *et al.* 2000; Chauret *et al.* 2001; Borrego *et al.* 2002a; Viau & Boehm 2011; Vierheilig *et al.* 2013). However, WHO (2008) does not recommend the routine monitoring of distribution systems for the presence of clostridia due to their long period of survival and because they can be detected long after as well as far from the contamination site, leading to possible false signals.

## Bifidobacterium

*Bifidobacterium* spp. is one of the most numerous and extremely variable groups of bacteria present in the feces of warm-blooded animals (Bonjoch *et al.* 2004; Wilson 2005). The genus contains 25 spp., most of which have been detected in the human gastrointestinal tract (GI-tract) (Sinton *et al.* 1998; Biavati & Mattarelli 2003; Wilson 2005).

Bifidobacteria are present in high numbers in the feces of humans and some animals. Several Bifidobacterium species are specific either for humans or for animals. *Bifidobacterium cuniculi* and *B. magnum* have only been found in rabbit fecal samples, *B. gallinarum* and *B. pullorum* only in the intestines of chickens and *B. suis* only in piglet feces. In human feces, the species composition changes with the age of the individual. In the intestines of infants, *B. breve* and *B. longum* generally predominate. In adults, *B. adolescentis*, *B. catenulatum*, *B. pseudocatenulatum* and *B. longum* are the dominant species. In both human and animal feces, bifibobacteria are always much more abundant than coliforms (Sinton *et al.* 1998; Biavati & Mattarelli 2003; Wilson 2005; Cabral 2010).

Bifidobacteria have been found in sewage and contaminated water but appear to be absent from decontaminated or pristine environments such as springs and decontaminated soils (Lamendella *et al.* 2008). This results from the fact that upon introduction into the environment, bifidobacteria decreases appreciably in numbers, probably due to their stringent growth requirements. Bifidobacteria grow poorly below 30 °C and have rigorous nutrient requirements. The reports available on the survival of bifidobacteria in environmental waters indicate that their survival is lower than that of coliforms (Biavati & Mattarelli 2003; Wilson 2005).

A study carried out in a highly contaminated stream near Bologna, Italy, revealed that *B. adolescentis*, *B. catenulatum*, *B. longum*, *B. pseudocatenulatum* and *B. thermophilum* were the most representative species, whereas *B. angulatum*, *B. animalis* subsp. *animalis* (*B. animalis*), *B. breve*, *B. choerinum*, *B. minimum*, *B. pseudolongum* subsp. *globosum* (*B. globosum*) and *B. subtile* occurred only in low numbers (Wilson 2005; Cabral 2010).

Thus, the presence of bifidobacteria spp. in the water environment is considered as an indicator of fecal contamination of water because some species are specific to humans and animals (King *et al.* 2007; Wery *et al.* 2010; Cabral 2010) as well as the alternative water quality indicators in tropical and temperate areas (Stewart *et al.* 2008; Mushi *et al.* 2010). Therefore, the identification of bifidobacterium species from contaminated water could provide information about the origin of fecal contamination (Sinton *et al.* 1998; Biavati & Mattarelli 2003; Wilson 2005).

## Bacteroides

Bacteroides are among the most oxygen-tolerant microbes of the entire anaerobic microflora found in the human GI-tract. Till now, the need to maintain anoxic conditions for cultivation, isolation and biochemical identification has limited the use of anaerobic bacteroide species as a fecal indicator. The survival of bacteroides in water environments is usually much lower than that of coliforms (Wilson 2005; Cabral 2010). However, the increasing use of recent molecular methods overcomes this problem (Kreader 1995; Balleste & Blanch 2010) as certain bacteroide species are highly host-specific and it is now possible to identify the source of fecal contamination by tracking the host-specific bacteroide species (Bernhard & Field 2000; Simpson *et al.* 2004; Bonjoch *et al.* 2005; Field & Samadpour 2007; Sauer *et al.* 2011; Zheng *et al.* 2013).

## Genetic markers from fecal *Bacteroides* distinguishing the source of fecal pollution

To distinguish the source of fecal pollution in fresh and marine waters, a new polymerase chain reaction (PCR)-based

indicator system, utilizing molecular markers from the *Bacteroides* group of fecal anaerobic bacteria, has been developed (Field *et al.* 2003; Bower *et al.* 2005; Fogarty & Voytek 2005; Sauer *et al.* 2011). This method detects the genetic marker sequences that are not only specific to fecal bacteria but are also specific to the host species that produced the feces, allowing discrimination among different potential sources of pollution. Furthermore, this method does not require isolation and growth of the indicator bacteria and it is rapid and accurate. Conversely, samples can be stored before analysis if necessary or convenient since the analysis does not require living cells. This allows for convenient and flexible field handling. In addition, scoring does not require complex pattern interpretation. The utility of this method of fecal source discrimination depends on the range of applicability of the genetic markers. Markers represent particular related groups of fecal bacteria, found in one host organism but not another. The specific primers amplify a band, which indicates the specific type of fecal pollution, or if there is no band, it indicates that the specific source of fecal pollution is absent or present at a level below the detection limit.

Recently, the identification of human- and bovine-specific *Bacteroides-Prevotella* 16S rRNA genetic markers by using length heterogeneity-PCR and terminal-restriction fragment length polymorphism has been reported, and the conclusion is that these markers could be used to detect the human or bovine origin of fecal pollution (Bernhard & Field 2000; Zheng *et al.* 2013). Owing to these advantages, PCR detection of *Bacteroides* markers has emerged as a potential tool for fecal source-tracking studies in the USA (Bernhard *et al.* 2003), France (Gourmelon *et al.* 2007), UK, Portugal, Ireland (Gawler *et al.* 2007), Belgium (Seurinck *et al.* 2006), Japan (Okabe *et al.* 2006) and Australia (Ahmed *et al.* 2008). Real-time PCR methods have been developed and used to quantify the human-specific *Bacteroides* genetic markers in environmental samples (Okabe *et al.* 2006; Seurinck *et al.* 2006).

However, *Bacteroides* spp. generally survive up to 6 days under limited oxygen while the human-specific *Bacteroides* genetic marker could persist in freshwater for up to 8 days at 23 °C under laboratory conditions (Seurinck *et al.* 2005). Further, the die-off rate of *Bacteroides* spp. could be triggered by environmental factors such as temperature and predation (Rozen & Belkin 2001). A limitation of the *Bacteroides* genetic markers is that the specificity must be assessed prior to application because horizontal transfer of fecal bacteria is possible among the species in close contact, such as humans and dogs (Dick *et al.* 2005).

## Bacteriophages

Bacteriophages have been proposed as an indicator of fecal and viral contamination and also as models to evaluate the chlorination efficiency of water treatment plants (Leclerc *et al.* 2000; Duran *et al.* 2003; Mendez *et al.* 2004). The proposed groups include somatic coliphages, F (male)-specific RNA bacteriophages (F-RNA phages) and phages of *Bacteroides fragilis* (Duran *et al.* 2003).

Somatic coliphages are specific to *E. coli* and have been commonly used as an indicator of fecal and/or sewage contamination in several water resources. These are also used as biotracers to identify the source of contamination in surface water and aquifers (Paul *et al.* 1995; Muniesa *et al.* 2012). In addition, they indicate the pathogen removal efficiency of WWTPs (Harvey 1997). Hence, on the basis of differences in origin and ecology between the enteric viruses and somatic coliphages, it is doubtful to conclude that this phage group could successfully be used in all the situations as enteric viruses (Havelaar & Pot-Hogeboom 1988). They also may not be a useful indicator of an integrity problem in a distribution system, even when the problem involves the introduction of fecal contamination (Hot *et al.* 2003).

F-RNA phages are used as an indicator of fecal contamination as well as model viruses in water hygiene because of: (a) similar size and shape to human enteric viruses; (b) direct correlation with degree of sewage contamination; and (c) inability to replicate in the water environment (Duran *et al.* 2003).

However, the low incidence of this phage group in human feces and its low specificity for its bacterial host has suggested that they would multiply in sewer systems (Cornax *et al.* 1994). Hence, the presence of F-RNA phages in water should be primarily used as an indicator of sewage contamination rather than fecal contamination (Scott *et al.* 2002; Hot *et al.* 2003). *Bacteroides fragilis*, a strict anaerobe, is found at high concentrations in the human intestinal tract and dies rapidly when discharged into the water environment. A phage of strain HSP 40 of *B. fragilis,* which

was isolated from Hospital San Pablo, Barcelona, Spain, has been reported as a specific indicator of human fecal contamination in the water environment (Duran et al. 2003) because: (a) the phages against this bacterial strain are human-specific and are not isolated from the feces of other homoeothermic animals; (b) B. fragilis HSP 40 phages are consistently isolated from the water contaminated with sewage and feces and their sediments, but not from the decontaminated samples; (c) the number of phages is related to the degree of contamination; (d) B. fragilis phages always outnumber the human enteric viruses; and (e) in model experiments, no replication of these phages has been observed under simulated environmental conditions (Leclerc et al. 2000).

Thus, the low prevalence to these phages in water with low or moderate levels of fecal contamination and complex methodology for their recovery are major drawbacks for the general use of these viruses as IMs (Cornax et al. 1991; Ashbolt et al. 2001; Miernik 2004).

## Heterotrophic plate counts

The total aerobic bacteria or heterotrophic plate count (HPC) were among the first parameters that were used in the late 1800s to assess the 'purity' of source water (Bartram et al. 2003; Maal-Bared et al. 2008). However, these are no longer used as health-related indicators (Edberg & Smith 1988; Verhille 2013). At present, they have become the general indicator of water quality within the DWDS (WHO 2004, 2008). Today, significant changes in HPC serve as an alert for possible deterioration of water quality, triggering further investigation (Bartram et al. 2003; Verhille 2013). An increase in HPC in treated water indicates the failure of the water treatment plant or a change in quality of the water source, prior to treatment (Bartram et al. 2003). If the HPC level in water leaving the treatment plant is acceptable, but it is high in the distribution system, it indicates that either the re-growth has taken place or it has been contaminated due to cracks/leakage in the distribution system (Bartram et al. 2003; Verhille 2013).

## Emerging waterborne bacterial pathogens

Emerging waterborne pathogens pose a major health hazard in both developed and developing countries. The rapid emergence of waterborne pathogens has created a serious threat to DW safety. Hence, there is an urgent need to develop strategies for the identification of these potentially emerging waterborne pathogens. There may be several reasons for the continuous emergence of waterborne pathogens such as: (a) increase in sensitive populations; (b) globalization of trade and travel; (c) development of molecular techniques (MTs) for detection and source tracking; (d) changes in DW treatment technology; (e) changes in food production and supply; (f) molecular evolution (genetic reassortment); and (g) multi-drug resistance (Sharma et al. 2003; Nwachcuku & Gerba 2004; Skovgaard 2007; Chugh 2008; Sherchand 2012; Morens & Fauci 2013). However, several microbial agents which may qualify as emerging waterborne pathogens have been reported by many authors (Sharma et al. 2003; Nwachcuku & Gerba 2004; Health Canada 2006; Petrini 2006; Cohen et al. 2007; Skovgaard 2007; Cangelosi 2009; Cabral 2010; Adrados et al. 2011; Whiley et al. 2012). These include environmental mycobacteria, aeromonads (Aeromonas sobria, A. caviae and A. hydrophila), Legionella pneumophila, Vibrio sp. (V. cholerae O139 and V. cholerae O1), pathogenic E. coli (enteroinvasive E. coli, enteropathogenic E. coli, enteroaggregative E. coli and shiga toxin producing E. coli), Yersinia enterocolitica, Helicobacter pylori, multidrug resistant Pseudomonas aeruginosa and Campylobacter jejuni. These pathogens have the potential to be spread through the supply of DW. However, their presence does not correlate with the presence of E. coli or other microbial indicators. In most cases there are no perfect microbial indicators to indicate the presence of emerging waterborne pathogens. The development of microbial source tracking (MST) has allowed the identification of sources of these waterborne pathogens (Noble et al. 2003; Giao et al. 2008; Beumer et al. 2010; Adrados et al. 2011; Furtula et al. 2012; Zheng et al. 2013). However, more studies are required to understand the behavior and ecology of these waterborne pathogens so that their true potential as emerging waterborne pathogens may be evaluated.

## Fecal sterol biomarkers as alternative indicators of fecal pollution

The detection of pathogens still depends on the development of more flexible and reliable techniques. In addition,

most of the IMs are not suitable for monitoring the level of fecal pollution in tropical and temperate environments because they could multiply or be a part of natural flora (Savichtcheva & Okabe 2006). Therefore, certain fecal organic compounds such as fecal sterols could be used as an alternative indicator of fecal contamination (Isobe *et al.* 2002, 2004; Furtula *et al.* 2012; Zheng *et al.* 2013). Coprostanol (5β-cholestan-3β-ol) is one of the major fecal sterols produced by indigenous bacteria (normal microflora) present in human and animal intestines and excreted in feces (Cabral 2010). Under aerobic conditions, coprostanol can be microbially degraded in the water column and has a half-life of less than 10 days at 20 °C (Isobe *et al.* 2002). It indicates the presence of microbes in water environments and its measurement has been proposed as a powerful tool for monitoring fresh fecal contamination in tropical regions of Japan, Malaysia and Vietnam (Isobe *et al.* 2002, 2004; Ashbolt *et al.* 2007; Derriena *et al.* 2012). However, a sensitive and simple analytical method using gas chromatography–mass spectrometry has been successfully developed for various environmental samples (Isobe *et al.* 2002; Savichtcheva & Okabe 2006).

Since coprostanol is hydrophobic, it is readily associated with sewage and water particles. Fecal sterols could, therefore, be incorporated into sediments and preserved for a long time under anoxic conditions without significant biodegradation (stable for 450 days at 15 °C) (Isobe *et al.* 2002). All these facts explain the findings that coprostanol was also found far from the possible fecal pollution source. Therefore, the presence of coprostanol mainly in sediments may indicate old or remote fecal pollution (Savichtcheva & Okabe 2006). In addition, lack of studies on application of host specificity, and desirable detection sensitivity and correlation with pathogens limit current application of fecal sterols on a large scale and indicate the need for future investigations.

### Recovery of injured bacteria

Many indicator bacteria may become damaged during the water and wastewater treatment process due to the sublethal exposure to a wide variety of chemical/physical agents (Toranzos *et al.* 2007; Cordoba *et al.* 2010; Kahlisch *et al.* 2010). These injured bacteria are unable to form colonies on selective media and ∼90% of indicator bacteria in treated DW may be damaged (McFeters 1990). As a consequence, the injured cells remain undetected in water leading to the underestimation of fecal contamination levels in treated DW in distribution systems (Bucklin *et al.* 1991). The abilities of several methods to detect the damaged bacteria were reviewed earlier by various workers (WHO 2004, 2008; Kahlisch *et al.* 2010). The detection of damaged bacteria in the distribution system may be an indication of either re-growth of damaged bacteria in the presence of high level of nutrients or contamination due to cracks/leakage in the distribution system. Thus, it may provide guidelines to diagnose the problems within the water distribution system (Toranzos *et al.* 2007).

## TECHNIQUES FOR THE DETECTION OF INDICATOR AND PATHOGENIC MICROORGANISMS IN WATER ENVIRONMENTS

A significant number of human microbial pathogens are present in urban sewage and may be considered as environmental pollutants. Although most of the pathogens can be removed during the wastewater treatment, many are discharged into wastewater and entering and receiving waters. Point-source pollution enters the environment at distinct locations, through a direct route of discharge of treated and untreated wastewater. Non-point sources of pollution are of significant concern with respect to the spread of pathogens and their indicators in water environments.

Traditional microbial indicators viz. FCs, *E. coli* and enterococci are the most commonly used IMs. These are analyzed to evaluate the level of fecal contamination in water environments and are also used to assess the pathogen removal efficiency of water treatment plants. However, whether these bacteria are suitable indicators of human pathogens has been questioned (Tree *et al.* 2003; Wery *et al.* 2008). There are several limitations in the general use of indicator bacteria as water quality indicators such as they are sensitive to inactivation during treatment processes and also by sunlight exposure (Hurst *et al.* 2002; Sinclair *et al.* 2009), short survival as compared to pathogens (McFeters *et al.* 1974), non-exclusive fecal sources (Scott *et al.* 2002; Simpson *et al.* 2002), ability to

multiply in some natural environments (Solo-Gabriele *et al.* 2000; Pote *et al.* 2009), inability to identify the source of fecal pollution (point or non-point) (Field *et al.* 2003) and bad correlation with the presence of pathogenic microorganisms (Horman *et al.* 2004; Savichtcheva & Okabe 2006). Culturing the pathogens in laboratories is also a laborious process that involves culture enrichment and selective media for the isolation of pathogens from the background organism. It is often difficult to achieve the appropriate culture enrichment, which makes the work even more tedious. Moreover, the concentrations of pathogens may be too low for the culture detection but still potent enough to cause disease. As a result, there are no ideal bacterial indicators currently used that fulfill all the established criteria for drinking or bathing water quality. Thus, the direct detection of pathogens of health concern without culturing in laboratories but with the help of MTs is considered to be a more suitable alternative (Gilbride *et al.* 2006) (Table 7).

The MTs, particularly nucleic acid amplification methods, provide sensitive, rapid and quantitative means for detecting the specific pathogens of public health and environmental concern including new emergent bacterial strains and indicators. These techniques are used to evaluate the microbiological quality of water and pathogens removal efficiency from DW and WWTPs. These techniques are together regarded as MST or bacterial source-tracking. These MTs aim to identify, and in some cases quantify, the dominant fecal contamination sources in the environment, and more particularly in water resources (Fong & Lipp 2005; Hundesa *et al.* 2006; Albinana-Gimenez *et al.* 2009). These MTs allow researchers to rapidly and specifically detect the microorganisms of public health and environmental concern, although recent improvements have allowed the simultaneous detection of several or groups of microorganisms in a single assay (Maynard *et al.* 2005; Straub *et al.* 2005; Marcelino *et al.* 2006). These developments have allowed the potential standardization and automation of some of these techniques. In some cases, these facilitate the identification, enumeration, genotyping, viability assessment and source-tracking of the fecal pollution of human or animal origin, if host-specific, most prevalent pathogens are analyzed. The MTs available today are being continuously improved in order to make them standardized and applicable to a wide variety of matrices, to increase their sensitivity, rapidity and to reduce the time, costs and steps used for the detection of pathogens and indicators. The standardization and validation of protocols is considered critical for the implementation of MTs used in the clinical or environmental area and has a potential impact on the evaluation of the data produced in many studies (Doring *et al.* 2008; Raymaekers *et al.* 2009; Harwood *et al.* 2009; Bustin 2010).

## Assessing the viability of pathogenic microorganisms

A number of pathogenic bacteria can be found in the environmental media (air, water and soil). Therefore, it is important to assess the viability status of these organisms to determine whether or not they pose a serious threat to public health. The conventional methods available for the determination of bacterial viability are based on the ability of cells to grow actively and form visible colonies on solid media. Moreover, under certain conditions the number of viable organisms may be severely underrepresented by such methods as sublethally damaged organisms (Blackburn & McCarthy 2000), fastidious unculturable bacteria (Ward *et al.* 1990) and viable cells that have lost their ability to form colonies under test conditions. These conventional methods are laborious and time consuming. Therefore, MTs have been developed as an alternative to overcome the limitations of conventional methods. These MTs offer speed, sensitivity and specificity wherein both the DNA and RNA have been analyzed using nucleic acid amplification methods such as PCR, reverse transcriptase-PCR and nucleic acid sequence-based amplification. However, due to the variable persistence of nucleic acids in the cells after death, the correlation between the presence of DNA and RNA and viability is not clear. Similarly, the choice of target and sensitivity of the methods used to assess the bacterial viability can significantly affect the validity of the viability assays. However, the accuracy of such methods is greatly dependent on both the manner of cell death and environmental conditions. Further, detailed information on assessing bacterial viability can be found in the review article entitled 'Molecular methods for the assessment of bacterial viability' (Keer & Birch 2003).

**Table 7** | Techniques for the detection of pathogens and indicators from water, wastewater and other environmental samples

| Technique | Merit | Demerit |
|---|---|---|
| Conventional techniques | | |
| Microscopy | Simple, rapid and direct observation of microbial cells | Majority of bacterial population cannot be identified |
| Culture-dependent methods | Easy to identify the individual microbes | Majority of bacteria cannot be cultured on the general purpose-basic media |
| Microbial indicator-based pathogen estimation | Easy to perform, current standard for coliform has been established | Labor intensive, time consuming and indirect estimation of pathogens rather than direct detection |
| Molecular techniques | | |
| Ribotyping | Highly reproducible; classify isolates from multiple sources | Complex, expensive; labor intensive; geographically specific; database required; variation in methodology |
| Amplified ribosomal DNA restriction analysis (ARDRA) | Culture-independent technique and suitable for analysis of a variety of microbes | Not quantitative and require DNA extraction and PCR biases |
| Ribosomal RNA intergenic spacer analysis (RISA) | Culture-independent technique, suitable for analysis of a variety of microbes and give remarkable heterogeneity in length and sequence among bacteria | Not quantitative and require DNA extraction and PCR biases |
| Pulse-field gel electrophoresis (PFGE) | Extremely reproducible and highly sensitive to point genetic difference | Long assay time, too sensitive for broadly discriminate source, limited simultaneous processing and required database |
| Denaturing-gradient gel electrophoresis (DGGE) | Culture-independent technique, suitable for analysis of a variety of microbes and use rRNA gene sequence heterogeneity | DNA extraction and PCR biases |
| Terminal-restriction fragment length polymorphism analysis (T-RFLP) | Fast, semi-quantitative, culture-independent technique and suitable for analysis of a variety of microbes | DNA extraction and PCR biases |
| Fluorescent *in situ* hybridization (FISH) | Quantitative and directly visualize the microbial cells including non-culturables | Inactive cells may not be detected |
| Quantitative PCR (qPCR) | Culture-independent technique and suitable for analysis of a variety of microbes | Expensive equipment; technically demanding |
| Repetitive DNA sequences (Rep-PCR) | Simple and rapid | Reproducibility a concern; cell culture required; large database required; variability increases as database increases |
| Length heterogeneity-PCR (LH-PCR) | Culture-independent technique | Expensive equipment; technically demanding |
| Multiplex PCR (mPCR) | Fast and simultaneous detection of several target microorganisms | Combination of primer pairs must function in a single PCR reaction |
| Nucleic acid microarrays | High throughput design with wider applications | Low sensitivity and processing complexities for environmental samples |
| Host-specific 16S rDNA | Does not require culturing or a database; indicator of recent pollution | Only tested on human and cattle markers; limited simultaneous processing; expensive equipment; technically demanding; little information about survival of *Bacteroides* spp. in environment |
| On-chip technology | Combination of PCR with nucleic acid hybridization on a single chip and less interference between parallel reactions | Integration and packaging |

Adapted from Simpson *et al.* (2002), Scott *et al.* (2002), Meays *et al.* (2004), Gilbride *et al.* (2006) and Girones *et al.* (2010).

# DRINKING WATER QUALITY STANDARDS AND GUIDELINES

At present, microbial indicators that are being used to monitor the microbiological quality of DW in developed countries are TCs, FCs and/or *E. coli*, although the reliance on IMs as the main source of information about the safety of DW is under review in many jurisdictions (Ashbolt *et al.* 2001; WHO 2008; Health Canada 2013) (Table 8).

## WHO guidelines and WSPs

The WHO has recommended WSPs for consistently ensuring the supply of safe DW. These safety plans manage the risk from the catchment or water source to the consumer's home (WHO 2004, 2008; Bartram *et al.* 2009). The risk assessment included in WSP should provide a better understanding of the risks associated with water contamination at each step along with the distribution system. Therefore, the preventive strategies should be designed (a multi-barrier protection system) in order to correctly manage these risks efficiently and effectively to protect the public health. This approach does not rely solely on end-point testing, but on the establishment of critical control points that will be subject to on-line monitoring. The parameters that can be measured on-line and in real-time are free chlorine, water pressure, dissolved oxygen and turbidity to which critical safety limits are established. In this way, any sudden anomalous changes in any of these parameters may indicate a problem within the distribution system that can be managed before the water is supplied to the consumers.

The introduction of these early warning or control parameters from source to consumer's home that can predict or alert the possible deterioration of DW quality before it is distributed to the consumers are the key elements of WSPs. Analyzing the DW microbiologically, for the enumeration and identification of IMs, is too slow (i.e., requires minimum of 24–48 h) and, therefore, is not suitable. However, they play an important role as validation tools because they verify whether the barriers work properly and the whole process is under control. In reality, many large water companies have long been adopting the principles of risk assessment and risk management, mostly in

the form of operational procedures, for the treatment processes and distribution networks. Therefore, the adoption of these approaches will not be difficult, but beneficial as already reported (Gunnarsdóttir & Gissurarson 2008). In the EU research-financed project (Healthy Water), one of the objectives was to train the water companies on the principles of WSPs, and experience has demonstrated that companies acknowledged the benefit of the new approaches; some of the largest water companies have already incorporated it.

However, small water companies will only implement the WSPs, when the approaches become a mandatory requirement under new EU legislation. In reality, the legislation is the driving force for such improvements, as outlined previously. For instance, the National Spanish Legislation on *Legionella*, promulgated after the world's largest outbreak, requires a part of the microbiological control with two other obligations:

(i)   to pass a training course for those who are responsible for handling the installations at risk of propagating the legionellosis;
(ii)  to implement the control plans based on the methodology of HACCP.

Further, according to Bartram *et al.* (2004), health care facilities should have general WSPs as an important part of their infection control strategies. Such plans may be generic (i.e., applicable to health centers in general) or specific for larger buildings (i.e., hospitals and nursing homes) and should address microbial growth in addition to control of external contamination by *P. aeruginosa* and *Legionella*, etc.

The WSPs have to be developed by a team and require:

• specific measures to protect raw water used to produce DW (i.e., fencing);
• the appropriate level of treatment in water companies as well as during the storage and distribution through pipe networks to customer's homes to guarantee the quality of DW;
• that customers are aware of their role and responsibilities for keeping the water as wholesome as possible in their properties, which include both public buildings as well as private homes.

**Table 8** | International DW standards or guidelines

| Parameters | Canada | United States | Australia | New Zealand | United Kingdom | EU Directive | WHO | India | Middle East | South Africa | South America |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Guidelines or standards | G[a] | S | G | S[b] | S | S | G | G | G | G | S[c] |
| Total coliforms | 0/100 mL in 90% | 0/100 mL[d] in 90% | 0/100 mL in 95% | | 0/100 mL (s) | 0/100 mL | | 3/100 mL[e] in 95% | 0/100 mL | | ≤3/100 mL in 95% |
| Thermotolerant coliforms or *E. coli* | | 0/100 mL | 0/100 mL in 98% | | | | 0/100 mL | 0/100 mL | | <1/ 100 mL | 0/100 mL in 95% |
| *E. coli* | | 0/100 mL | 0/100 mL[f] | 0/100 mL | 0/100 mL(t) | 0/100 mL | 0/100 mL[g] | 0/100 mL | | 0/100 mL | 0/100 mL |
| Enterococci | | | | | 0/100 mL(t) | 0/100 mL | | | | | |
| *Cryptosporidium parvum* | | 995 removal or inactivation | | | <1 Oocyte/ 10 mL | | | | | | |
| *Clostridium perfringens* with spores | | | | | 0/100 mL | (h) | | | | | |
| *Pseudomonas aeruginosa* | | | | | | 0/250 mL[i] | | | | | |
| Colony count 22 °C | | | | | No abnormal change | No abnormal change | | | | | |
| Colony count 37 °C | <500 cfu/mL[j] | | | | No abnormal change | 20/mL[i] | | | 10 to <100 cfu/mL[j] | | <500 cfu/ mL[j] |

Adapted from DWAF (1996), Tallon et al. (2005), Tyagi et al. (2006), WHO (2008) and Pinto et al. (2012).

Spaces left blank indicate parameters that are not specified. All values are CFU/100 mL (colony forming units) unless otherwise stated. EU – European Union (includes Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, Netherlands, Portugal, Spain, Sweden, United Kingdom of Great Britain and Northern Ireland. Ten countries joined the EU on 1 May 2004: Cyprus (Greek part), the Czech Republic, Estonia, Hungary, Latvia, Lithuania, Malta, Poland, Slovakia and Slovenia.) WHO – World Health Organization, G – guidelines, S – standards, HPC – heterotrophic plate count.

∗Data are based on literature published before 2004.

[a]Alberta, Quebec and British Columbia have standards.

[b]New Zealand has non-enforceable standards and has made the decision to use only *E. coli*.

[c]Standards in the following countries of South America: Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Paraguay, Peru, Uruguay and Venezuela.

[d]A coliform positive sample requires repeat sampling within 24 h. If this repeat sample is also coliform-positive then it must be tested for fecal coliforms and *E. coli*. A positive result for this test requires notification of appropriate authorities.

[e]In an occasional sample, but not in consecutive samples.

[f]Recommends that *E. coli* be retained as the primary compliance parameter for fecal contamination.

[g]Recognizes that *E. coli* is the preferred indicator of fecal contamination.

[h]Necessary only if the water originates from or is influenced by surface water.

[i]Necessary only in the case of water offered for sale in bottles or containers.

[j]HPC (35 °C for 48 h) or <200 background coliforms on a total coliform membrane filter. '<' indicates less than.

Protection of the entire catchment areas is the first step of multiple barrier protection concepts. Modeling can be used for establishing the microbial risks in DW catchments and can also be an excellent management tool in the development of WSP (Gunnarsdottir & Gissurarson 2008). There is much evidence indicating that inappropriate water handling is an important source of water contamination at the consumer's home. Considering this, the WHO has prepared a specific manual for the management of the microbiological quality of DW in a piped distribution system. Further, the information on how to implement the WSP can be found in WHO technical guidance documents including the WSP manual (Bartram *et al.* 2009) in which many specific case studies are presented. A dedicated website on the WSPs has also been developed by the 'IWA' (www.wsportal.org/ibis/water-safety-portal/eng/welcome). In addition, the WHO and the IWA have developed guiding documents to initiate such processes considering all levels of resources available, so that they can be implemented all over the world, even in poorly developed countries.

## Strategies minimizing waterborne illness outbreaks in developing countries

In developing countries, the following strategies are used as low-cost alternatives, which are effective in preventing the spread of WBDs (WHO 2007).

### Chlorination

It is a widely used method for water purification wherein chlorine (a disinfectant) is added in liquid or tablet form to kill the pathogenic microorganisms present in DW supply reservoirs. Therefore, water that has been treated with chlorine is effective in preventing the spread of WBDs.

### Boiling

It is the oldest and most commonly used effective approach to disinfect water at a household level that kills most of the microbes causing intestine-related diseases.

### Solar disinfection

It is a type of portable water purification systems (self-contained units) that is also known as point-of-use water treatment. In this method, the clear DW in disposable clear plastic bottles is exposed to sunlight for a day. Principally, it is the combined action of heat and UV light received from the sun that is used to inactivate the pathogens present in water.

### Filtration

It is used to purify water through high-quality ceramic filters with small pores, often coated with silver, which has been shown to be effective in removing many microbes and other suspended solid particles. These devices can be directly installed at water outlets in order to protect users against potentially harmful waterborne pathogens by providing a barrier to them and/or minimizing patient exposure. To be effective, filters need to be cleaned regularly to maintain the flow of water.

### Combined flocculation/disinfection systems

It is the addition of chlorine in powder or tablet form to coagulate and flocculate the sediments present in water followed by a timed release of disinfectant (chlorine). It is particularly used for the treatment of turbid water. The water is normally stirred for few minutes, strained to separate the flocculants and then allowed to stand for another half hour for the complete disinfection process.

### Safe storage

It has been shown that water that is safe at the point of collection is often subject to fecal contamination during the collection, transport and use at home, mainly by unclean hands. Many studies have also shown that vessels with narrow mouths and taps can significantly reduce such contamination and reduce the risk of DDs. Wherever possible, safe storage should also be included in the interventions to treat the water at home.

## Future implications for international guidelines and national regulations

Traditionally, the indicators have played a crucial role in implementing the guidelines and national standards. However, these are being seen as an adjunct to management controls such as sanitary surveys and there is a move away from a specified indicator level end product. In other words, indicators are being replaced by on-line analyzes (say for chlorine residual or particle sizes) at critical control points. A single indicator, or even a range of indicators, is unlikely to be appropriate for every occasion and, therefore, it is useful to tailor indicator choice to local circumstances when translating the international guidelines into national standards. In addition, with the change in the management paradigm, more indicators of process efficiency are required rather than reliance on the 'old-style' fecal indicators.

## CONCLUSIONS

(a) The availability of safe DW for all is one of the major challenges of the 21st century.

(b) The microbiological control of DW should be the norm everywhere.

(c) An adequate, safe and accessible supply of DW must be available to all.

(d) Routine basic microbiological analysis of DW should be carried out for the presence of *E. coli* by culture-dependent methods. On-line monitoring of glucuronidase activity is currently too insensitive to replace the culture-based detection of *E. coli*, but it is a valuable complementary tool for high temporal resolution monitoring. Whenever the financial resources are available, coliform determinations should be complemented with the quantification of enterococci.

(e) Financial resources should be devoted to the better understanding of sources of microbial contaminants (human versus animal), their transport, prevalence and fate in water environments. The health risks posed by these microbial contaminants also needs to be investigated.

(f) The development of alternative fecal indicators to replace or to combine with conventional ones requires additional adequate investigation and an epidemiological survey of their applications.

(g) The techniques available for direct detection of pathogens/microbial indicators require improvement in order to make them standardized and of wider application, but further research and evolution of new methods are still needed.

(h) More studies are required in order to understand the behavior and ecology of waterborne pathogens so that their true potential as emerging waterborne pathogens may be evaluated.

Thus, in conclusion we can say that there is no universal IM, but a number of microorganisms each with certain characteristics are being used as an indicator of the microbiological quality of DW. Therefore, this review article has mainly focused on elucidating the appropriate use of microbes as IMs with a view to their role in the management of waterborne microbial risks.

## ACKNOWLEDGMENTS

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