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**Drinking Water Microbiology**

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**Introduction**

Access to clean drinking water is required for healthy, thriving communities. It is estimated that more than 5 billion people (71% of the global population) used what the WHO has defined as a safely managed drinking water service (Fig. 1); that is, one located on premises, available when needed and free from contamination. Unfortunately, in 2015, 29% of the global population (2.1 billion people) lacked safely managed drinking water services—defined by the World Health Organization (WHO) as water at home, available, and safe. There were 844 million people in the world who lacked a basic drinking water service, which is defined as drinking water from an improved source, provided that the collection time is not more than 30 min for a round trip, including the time standing in line waiting. Fig. 2 shows the regional differences in access to different types of water across the world.

Concern over the lack of access to safe drinking water led the WHO/UNICEF Joint Monitoring Program to establish the goal to “Ensure Availability and Sustainable Management of Water and Sanitation for All” as its Sustainable Development Goal (SDG) 6, with a target of achieving “universal and equitable access to safe and affordable drinking water for all” by 2030. Further, they have defined safe drinking water as water that “has a risk level equal to or less than a 1 in 10,000 annual probability of infection or 1 in 1 million disability-adjusted life years” (DALY).

**Significance of Waterborne Disease**

On a global basis, every year, 1.5 million people die from waterborne disease. The majority of these individuals are young children who die of dehydration caused by diarrhea. According to the World Health Organization, there are almost 1.7 billion cases of diarrhea in young children annually, and diarrhea is the second leading cause of death for children under 5 year of age.

In the United States and other countries in which drinking water treatment is routinely practiced, people typically assume that all drinking water is treated, however, that is not the case. For example, in the United States, the Environmental Protection Agency (EPA) estimates that 15% of the population obtains their drinking water from untreated private wells or other untreated sources. Based on statistics compiled by the Centers for Disease Control and Prevention, most of the drinking water outbreaks in the United States are associated with untreated or inadequately treated ground water, and distribution system deficiencies. For example, in the latest update on waterborne outbreaks in the United States (2013–14), the CDC reported 1006 cases of illness, 130 of which were caused by *Legionella*, and 86% of which originated from outbreaks in which acute gastrointestinal illness was the predominant illness. Epidemiological investigations have estimated that between 12 million and 19.5 million waterborne illnesses occur annually in the United States, only a small fraction of which are recognized and/or reported.

**Waterborne Pathogens**

Worldwide, it is believed that the most common route of infection with a waterborne pathogen is the “fecal-oral” route: infection occurs as a result of exposure to water or food improperly treated sewage or directly contaminated with untreated human or animal feces. Such microorganisms are referred to as enteric pathogens.

Although other microorganisms (e.g., *Legionella*, *Naegleria*) can cause waterborne disease, most of the concern and focus on a global basis has been on microorganisms of enteric origin. Much of the reason for this is that many of these microorganisms can be readily handled through the implementation of relatively simple interventions such as handwashing and other sanitation measures.

The fecal material of infected individuals is the major source of pathogenic microorganisms in domestic wastewater: hundreds of different types of microorganisms may be present in the fecal material of infected humans and animals. In addition, urine may be a source of certain pathogenic microorganisms, such as *Leptospira* and polyomaviruses. Numerous factors affect the concentrations and types of pathogens found in domestic wastewater at any given over time; these include the disease incidence in the population producing the wastewater, season of the year, climate factors (such as rainfall, temperature), the amount of water being used, the economic status of the population, and quality of the drinking water. If fecal material or wastewater contaminated with fecal material enters a water body that is used as a source of potable water, there is a potential for disease transmission. The major groups of pathogenic microorganisms transmitted by water and food contaminated with feces include bacteria, viruses, and parasites.

As stated previously, other, nonfecal microorganisms also cause waterborne disease from the consumption or use of potable water; these are typically microorganisms indigenous to the water or sediment. For example, *Legionella*, a bacterium that is transmitted via aerosols, is a frequent cause of drinking-water associated disease. Indeed, in 2001, the CDC began including reports of *Legionella* outbreaks with the drinking water surveillance data, and *Legionella* was the most frequently-identified causative agent of waterborne disease in reported outbreaks in the United States in 2013–14. Notably, it was responsible for all 13 of the deaths...
associated with waterborne disease in that time period. *Naegleria fowleri*, an amoeba that naturally occurs in the sediments of certain warm water bodies, has also been reported as the cause of several waterborne disease outbreaks. Infection by this microorganism typically results in death. A series of fact sheets that describe the human health effects, source and occurrence, routes of exposure, and significance in drinking water for many waterborne pathogens has been compiled by the World Health Organization.

**Bacteria**

Bacteria are naturally present in the gastrointestinal tracts of humans and animals; in fact, they are necessary for the proper functioning of the digestive system. The vast majority of these bacteria are harmless. However, when an individual is infected by an enteric bacterial pathogen, these microorganisms grow and reproduce in the gastrointestinal tract, and they are shed in the fecal material. Because the enteric bacteria are well-adapted to the conditions present in the gastrointestinal tract, which include high organic carbon concentrations, low pH, and a relatively high temperature (~37°C), they tend to have a relatively difficult time growing in the environment. This is because the conditions in soil, water, etc. are generally very different from those in the gastrointestinal tract: nutrients are generally present in low concentrations and/or in difficult-to-metabolize forms, the temperature is not optimal for growth, the moisture content is lower, etc. As a result, the enteric bacteria typically do not compete well with the indigenous soil or water bacteria for the available nutrients. This adversely affects their ability to reproduce, or even survive without reproducing, in the environment. Thus, their longevity in the external environment is typically limited to a few days to weeks, depending on the environmental conditions.

Historically, the bacterial pathogens have been the focus of waterborne disease, mainly because they were among the first microorganisms recognized. Bacteria cause many diseases, such as cholera, typhoid, and dysentery that have been responsible for
large epidemics (and, in the case of cholera, pandemics) all over the world. In general, many of the bacteria pathogens are relatively easy to remove through wastewater and drinking water treatment processes, and, therefore, are of less concern than the other groups of pathogens in many industrialized countries. However, these pathogens are still of great concern in many of the less-industrialized countries, due to the lack of adequate sanitary treatment processes in those countries. According to the WHO/UNICEF, in 2015 only 39% of the global population (2.9 billion people) used a safely managed sanitation service; that is, excreta safely disposed of in situ or treated off-site. More than 2 billion people lack basic sanitation, and almost 1 billion people still practice open defecation.

Some of the most common bacterial pathogens found in human and animal waste that are transmitted by contaminated water and food are listed in Table 1. Some relevant characteristics, such as infectivity, survival in water, resistance to chlorine, and health impacts are also provided.

**Viruses**

Although fecal material always contains high concentrations of nonpathogenic bacteria because they are part of the normal flora of the gastrointestinal tract, like pathogenic bacteria pathogenic viruses are typically only present in fecal material if the individual is infected. Enteric viruses are very different from enteric bacteria in a number of ways. Some of the most important differences in this context are:

- Viruses are nonliving. They can only reproduce inside of susceptible host cells, as they use the host’s biochemical mechanisms to reproduce themselves.
- Viruses are relatively more host-specific than bacteria. Thus, many viruses are only able to infect a single species or a few species. This can make it easier to identify the source of contamination. For example, if a human-specific virus is found in water, it is clear that the water has been contaminated by human fecal material.
- Because viruses are metabolically inert when outside of a host cell, they are not affected as much as bacteria by the presence of nutrients in the external environment. As they cannot reproduce in the environment, their numbers can only decrease over time.
- Viruses are typically able to survive for longer periods in the environment, compared to bacteria. Typically, viruses can survive for weeks to months; survival has even been reported for 2 years in a cold groundwater.
- Viruses are much smaller than bacteria. The typical enteric virus is 25–80 nm in size, while the typical size of an enteric bacterium is 1–2 mm. This affects their ability to be transported through the soil and into the groundwater.

More than 100 types of enteric viruses can be present in domestic wastewater. The types of symptoms that result from enteric virus infections range widely—from asymptomatic infections to relatively mild, self-limiting diarrhea, to more severe illnesses, including respiratory illness, infectious hepatitis, paralysis, encephalitis, and myocarditis. Several of the pathogenic human enteric viruses, along with some relevant characteristics, such as infectivity, survival in water, resistance to chlorine, and health impacts, are listed in Table 1.

**Parasites**

Another group of pathogenic microorganisms that can be transmitted through contaminated drinking water is the parasites. This is an extremely diverse group of organisms, the common feature of which is that they live on or in a host organism and obtain their food from or at the expense of the host. The enteric parasites that are pathogenic to humans can be classified into two groups: the protozoa (sometimes referred to as the protists) and the helminths, which include the intestinal worms. Protozoa are single-celled microorganisms whose life cycles include at least one vegetative as well as at least one resting stage. The infective stage of the protozoa is different for different parasites. In the case of Cryptosporidium, the resting oocyst form must be ingested to initiate infection; for others, the vegetative form must be ingested for infection to occur. The resting stage of the organism (cyst, ovum) is generally relatively resistant to inactivation from environmental stresses and during conventional drinking and wastewater treatment processes. Thus, those parasites whose resting stage is the infective stage may be of greater concern than those whose infective form is the vegetative stage, even for consumers of (inadequately) treated water.

Many of the intestinal protozoa are transmitted by fecally-contaminated water, food, or other materials. Some of the most common protozoan pathogens found in human and animal waste that are transmitted by contaminated water and food are listed in Table 1. Some relevant characteristics, such as infectivity, survival in water, resistance to chlorine, and health impacts are also listed.

**Indicators of Fecal Contamination of Water**

As stated previously, bacteria are always present in the gastrointestinal tract of humans and other animals, and therefore are present in fecal material. On the other hand, pathogenic enteric microorganisms are typically only present when an individual is infected. Therefore, if one wants to assess the safety of water for human consumption, the water would have to be continuously monitored for the presence of pathogens in order to be certain of their presence or absence. Additionally, there are hundreds of different pathogens that may be present in feces, so the water would need to be monitored for all of these microorganisms to assure the safety for consumption.
Table 1  Selected pathogens that can be transmitted by water

| Organism                        | Disease                                                                 | Incubation Period | Duration of Illness | Health Significance\(^a\) | Resistance to Chlorine\(^b\) |
|---------------------------------|------------------------------------------------------------------------|-------------------|---------------------|---------------------------|----------------------------|
| **Bacteria**                    |                                                                        |                   |                     |                           |                            |
| Burkholderia pseudomallei       | Pneumonia                                                             | 3–5 days          | 1–4 days            | High                      | Low                        |
| Campylobacter                   | Gastroenteritis                                                       | 5–66 h            | 2–7 days            | High                      | Low                        |
| Legionella                      | Pneumonia                                                             | 2–14 days         | Weeks to months     | High                      | Low                        |
| Legionella                      | Pontiac fever                                                         |                   |                     |                           |                            |
| Leptospira                      | Weil’s disease (headache, chillls, fever, nausea, neck or joint pain) | 2–20 days         | 3 days to 3 weeks   | High                      | Low                        |
| Shiga-toxin producing E. coli   | Gastroenteritis, hemolytic uremic syndrome, kidney failure             | 12 h - 8 days     | 1 day to 3 weeks    | High                      | Low                        |
| Salmoneilla enterica serovar typhi | Typhoid fever                                                        | 7–28 days         | Weeks to months     | High                      | Low                        |
| Salmonella                      | Salmonellosis                                                         | 8–48 h            | 3–5 days            | High                      | Low                        |
| Shigella                        | Bacillary dysentery                                                   | 1–7 days          | 4–7 days            | High                      | Low                        |
| Vibriocholera 01                | Profuse, watery diarrhea, vomiting, rapid dehydration                 | 9–72 h            | 3–4 days            | High                      | Low                        |
| Vibriocholera non-01            | Watery diarrhea                                                       | 1–5 days          | 3–4 days            | High                      | Low                        |
| Yersinia enterocolitica         | Gastroenteritis                                                       | 2–7 days          | 1–21 days           | Moderate                  | Low                        |
| Protozoa                        |                                                                        |                   |                     |                           |                            |
| Cryptosporidium parvum          | Diarrhea                                                              | 1–2 weeks         | 4–21 days           | High                      | High                       |
| Cyclospora cayatanensis         | Watery diarrhea alternating with constipation                          | 2–11 days         | days                | High                      | High                       |
| Entamoeba histolytica           | Amoebic dysentery                                                     | 2–4 weeks         | weeks to months     | High                      | High                       |
| Giardia intestinalis            | Diarrhea, malabsorption                                               | 5–25 days         | weeks to months     | High                      | High                       |
| Microsporidium                  | Chronic diarrhea, weight loss                                         |                   |                     |                           |                            |
| Naegleria fowleri               | Primary amoebic meningoencephalitis                                   | Minutes to hours  |                     |                           |                            |
| Toxoplasma gondii               |                                                                        |                   |                     |                           |                            |
| Adenovirus                      | Respiratory illness, conjunctivitis, vomiting, diarrhea               | 1–4 days          | 2–3 days            | Moderate                  | Moderate                   |
| Astrovirus                      | Vomiting, diarrhea                                                    | 3–4 days          | 2–7 days            | Moderate                  | Moderate                   |
| Calicivirus                     | Vomiting, diarrhea                                                    |                   |                     |                           |                            |
| Coronavirus                     | Vomiting, diarrhea                                                    |                   |                     |                           |                            |
| Enterovirus:                    | Paralysis, meningitis, fever                                           | 3–14 days         | variable            | High                      | Moderate                   |
| Poliovirus                      | Meningitis, fever, herpangina, respiratory illness, paralysis         |                   |                     | High                      | Moderate                   |
| Coxsackie A                     | Myocarditis, congenital heart anomalies, rash, fever, meningitis,     |                   |                     | High                      | Moderate                   |
| Coxsackie B                     | respiratory illness, pleurodynia                                       |                   |                     | High                      | Moderate                   |
| Echovirus                       | Meningitis, encephalitis, respiratory illness, rash, diarrhea, fever,  |                   |                     | High                      | Moderate                   |
| Enterovirus 68–71               | Myocarditis, endocarditis                                             |                   |                     | High                      | Moderate                   |
| Hepatitis A virus               | Infectious hepatitis                                                  | 15–50 days        | 1 week to several   | High                      | Moderate                   |
| Hepatitis E virus               | Hepatitis                                                             | 15–65 days        | 1 week to several   | High                      | Moderate                   |
| Norovirus                       | Epidemic vomiting and diarrhea                                         | 1–3 days          | 1–3 days            | High                      | Moderate                   |
| Reovirus                        | Respiratory illness                                                   |                   |                     | Moderate                  | Moderate                   |
| Rotavirus                       | Diarrhea, vomiting                                                    | 1–3 days          | 3–7 days            | High                      | Moderate                   |
| Sapoviruses                     | Gastroenteritis                                                       |                   |                     | High                      | Moderate                   |

\(^a\)Health significance relates to the severity of impact, including association with outbreaks.

\(^b\)When the infective stage is freely suspended in water treated at conventional doses and contact times and pH between 7 and 8. Low means 99% inactivation at 20 °C generally in <1 min, moderate 1–30 min and high >30 min. It should be noted that organisms that survive and grow in biofilms, such as Legionella and mycobacteria, will be protected from chlorination.

Modified from Yates, M. V. (editor in chief) (2016). Manual of Environmental Microbiology, 4th ed. American Society for Microbiology, Washington, DC. 1088 pp.
However, it is not technically or economically feasible to test wastewater for the presence of all different types of pathogens, nor is it feasible to continuously monitor water for pathogenic microorganisms. As a consequence, the microbiological quality of water historically has been assessed by monitoring for the presence of alternative organisms—those enteric organisms that are always present in fecal material—the so-called indicator microorganisms. The rationale for using fecal indicator microorganisms is that, if they are found in water, the water contains fecal material, and therefore may also contain pathogenic microorganisms. In contrast, if fecal indicators are absent, the water is considered not to be contaminated by fecal material, and therefore, no fecal pathogens are present. Common indicators or groups of indicator organisms used are the total coliform bacteria, the thermotolerant coliform bacteria (also called fecal coliform bacteria), the enterococci (also called fecal streptococci); examples include *Escherichia coli*, *Clostridium perfringens*, and *Bifidobacterium*.

While the initial focus of monitoring water for safety was on the potential presence of pathogenic bacteria, in industrialized countries, many of the bacterial diseases have been controlled through the use of drinking water and wastewater treatment, including disinfection. As this has occurred, more of the focus in drinking water and wastewater treatment has been on controlling the concentrations of parasitic and viral pathogens. Unfortunately, the bacterial indicators mentioned above do not always correlate well with the presence or behavior of the nonbacterial pathogens. Efforts are underway to identify more appropriate indicators, such as bacteriophages (viruses that infect fecal indicator bacteria) for these pathogens.

### Characteristics of Microbial Indicators

In 1966, Bonde developed the first systematic delineation of the attributes of an ideal indicator organism for the assessment of health risk or treatment efficiency. These criteria state that an ideal indicator should:

- Be present whenever the pathogens are present;
- Be present only when the presence of pathogens is an imminent danger; that is, they must not proliferate to any greater extent in the aqueous environment;
- Occur in much greater numbers than the pathogens;
- Be more resistant to disinfectants and to the aqueous environment than the pathogens;
- Grow readily on simple media;
- Yield characteristic and simple reactions enabling as far as possible an unambiguous identification of the group;
- Be randomly distributed in the sample to be examined, or it should be possible to obtain a uniform distribution by simple homogenization procedures; and
- Grow widely independent of other organisms present, when inoculated in artificial media; that is, the indicator bacteria should not be seriously inhibited in their growth by the presence of other bacteria.

Unfortunately, no indicator organism or group of organisms was found to have all of these attributes. The criteria that have been most difficult to meet are the first and fourth: many waterborne disease outbreaks have occurred in the absence of indicator microorganisms, which, at least in some cases is due to the fact that the pathogens are less resistant to disinfectants and environmental stressors than the indicator microorganisms. This is especially true for nonbacterial pathogens.

Over time, as new pathogens are identified, more/different applications for indicators have been found, and new microbial sampling and detection methods have been developed, Bonde’s criteria have been reevaluated. For example, a National Academies of Science committee developed a set of criteria recommended for use when assessing the appropriateness of any indicator or group of indicators. These separate the biological attributes of the indicators themselves from the attributes of the methods used to detect the indicators. The desirable biological attributes of indicators, as developed by the NRC are:

- Correlated to health risk
- Similar (or greater) survival to pathogens under environmental conditions
- Similar (or greater) transport to pathogens
- Present in greater numbers than pathogens
- Specific to a fecal source or identifiable as to source of origin

The desirable attributes of the indicator detection methods, according to the NRC, are:

- Specificity (independent of matrix effects)
- Broad applicability
- Precision
- Adequate sensitivity
- Rapidity of results
- Quantifiable
- Measures viability or infectivity
- Logistical feasibility

For most situations, there is agreement that the most important characteristic of any indicator or indicator system is its ability to indicate possible health risk. This characteristic can be shown directly through conducting epidemiological studies in which the association between the indicators and health outcomes are assessed. A different, more common, and generally less expensive way
to demonstrate the correlation between indicators and health risk is to monitor water for the presence and/or concentration of pathogen and compare those data to data on potential indicator microorganisms. While the latter approach might be simpler and less expensive, it may be more problematic. This is because most pathogen detection methods detect a single pathogen or closely related group of pathogens, and a correlation (or lack thereof) between an indicator and a single pathogen or pathogen group does not infer that a correlation exists (or does not exist) with other waterborne pathogens. To obtain complete and accurate information regarding the potential for an indicator to provide information on the microbiological safety of the water, correlations for each pathogenic microorganism or related group of microorganism would need to be examined.

Common Indicators

It is technologically, economically, and practically not possible to test water for all possible pathogenic microorganisms. As a result, the typical practice has been, and continues to be, to monitor water for one or more indicator microorganisms (or groups of indicator microorganisms) to indicate whether or not there is a health risk associated with the consumption of water. Different groups have been used for many years as indicators of the microbiological quality of drinking water and of recreational waters. For example, total coliform bacteria have been used for almost a century to assess the microbiological quality of drinking water.

Coliform bacteria

The group of indicator microorganisms in longest use for assessing the microbiological quality of drinking water and wastewater is the total coliform bacteria. More recently, other coliform bacteria, including the thermotolerant coliforms (previously termed fecal coliforms and a subgroup of the total coliforms), and _E. coli_, a specific thermotolerant coliform bacterium, considered specifically of fecal origin, have been added to the commonly used indicator organisms. The US Environmental Protection Agency requires the monitoring of all public sources of drinking water for total coliform bacteria. The use of the total coliform standard to assess the microbiological safety of water has had a dramatic effect on reducing waterborne disease outbreaks in countries that have implemented one.

Numerous studies, however, have documented that many pathogenic microorganisms, especially enteric viruses and parasites, are not as easily inactivated by water and wastewater treatment processes as are coliforms. The fact that waterborne disease outbreaks continue to occur every year in countries that have implemented coliform monitoring standards is clear evidence that better ways to assess the microbiological quality of water must be found. Although there is no perfect indicator organism, research is ongoing to find a rapid, relatively inexpensive, and accurate way to assess the microbiological quality of water.

Fecal enterococci

Like the coliform bacteria, the fecal enterococci or fecal streptococci, which include some members of the genera _Streptococcus_ and _Enterococcus_, are found in the intestinal tract of humans and many animals. Use of this group of organisms has some advantages over the coliform group for assessing the microbiological safety of water. These include the fact that they rarely multiply in environmental waters, they are more resistant to treatment processes and environmental stresses, and they generally persist for longer in the environment than do the coliform bacteria. The most common use for these organisms is to indicate the microbiological quality of recreational bathing waters. In addition, enterococci are recommended as one of the groups of fecal indicator organisms that can be used to assess the microbiological quality of groundwater by the US Environmental Protection Agency.

_Clostridium perfringens_ spores

As indicated previously, the resting stages of many protozoan parasites are especially resistant to inactivation during water and wastewater treatment. Therefore, the use of bacterial indicator organisms may not provide accurate information regarding the presence of pathogenic parasites in water. The search for a surrogate organism for parasite removal during treatment processes has led to investigations into the potential use of _Clostridium perfringens_ spores. These bacterial spores are present, typically in very low numbers, in human and animal feces. However, like the protozoans, they are relatively resistant to many forms of drinking water and wastewater treatment processes, so may be useful indicators for parasite and virus removal by treatment. _Clostridium_ spores are used in conjunction with enterococci as secondary indicators of water quality in Hawai‘i, due to problems with coliform regrowth in the warm temperatures of the tropical environment.

Heterotrophic plate count bacteria

Another group of bacteria that can be used to assess the microbiological quality of water, especially with respect to the efficacy of treatment processes, is the heterotrophic plate count (HPC) bacteria. The HPC is defined as “the number of aerobic and facultatively anaerobic bacteria that obtain their carbon and energy from organic sources.” This is a very general test of the bacteriological quality of water, and both pathogenic and nonpathogenic microorganisms are enumerated, but they are not and cannot be differentiated from one another using this method. Studies to assess the health effects of consumption of water containing high HPC have not documented adverse health effects. In addition, some studies have found that the presence of HPC bacteria in water do not indicate potential adverse human health effects.

The numbers of HPC bacteria in a specific water are typically less useful than changes in the number at a particular location, as the change may indicate a problem with the water/wastewater treatment processes, a lack of integrity of the water distribution system, or the potential for bacterial regrowth in the system.
**Bacteriophages**

The large differences between the physical properties, especially size, of bacterial indicators and waterborne viruses has caused concern over the use of bacterial indicators to evaluate the virological quality of water, especially groundwaters. This has led to investigations of the use of bacteriophages, viruses that infect bacteria, as indicators for groundwaters used as drinking water sources. The US Environmental Protection Agency allows the use of bacteriophages as an indicator of fecal contamination of groundwater in the Ground Water Rule.

**Further Reading**

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