Pathogens in (Drinking) Water?

Drinking water quality is generally good in most industrialized countries. Despite this, there have been recurrent "accidents" worldwide over the last few years, which have often lead to illness patterns of epidemic proportions. Drinking water quality is routinely monitored for microbial contamination based on cell counts of so-called "indicator" organisms. Such organisms include, for example, harmless *Enterobacteriaceae,* which are part of the normal flora of the intestinal tracts of humans and other mammals. But for some emerging pathogens that have shown up more frequently in recent years, the concept of testing only for conventional indicator organisms is inadequate. Molecular techniques, based on biochemical, genetic or immunological principles, are gaining importance. These methods allow selective detection of certain pathogens and are usually more rapid and more sensitive than traditional methods.

Microorganisms are present in any water, but the situation becomes of critical concern when viruses, bacteria or protozoa with human pathogenic potential are present in large numbers [1]. This not only applies to drinking water; the spread of microbial diseases may also occur by taking a bath or a shower with contaminated water as well as through the consumption of raw fruit or vegetables that have been watered or washed with contaminated water. It is also important that water used in industrial aquaculture of fish, shrimps or mussels, be free of any pathogens.

The Century of Drinking Water Plagues

A typical characteristic of diseases caused by pathogens in drinking water is the occurrence of acute symptoms due to replication of the pathogen in the host. In contrast, consumption of chemically contaminated drinking water typically leads to chronic disease. In the 1800s, the "century of drinking water plagues", catastrophic epidemics caused by contaminated drinking water were almost a part of daily life in central Europe. The larger cities in particular, regularly fell victim to cholera, typhoid fever, and dysentery. As many as 50% of the people contracting waterborne diseases actually died. Even today, these "classic" drinking water diseases occur sporadically in industrialized countries, although normally in very localized incidents. A few of the more recent cases are listed in Table 1.

Generally speaking, drinking water quality in Switzerland is excellent, and there is no need for concern. Even with 60% of our drinking water being distributed to households without any kind of treatment, the legal requirements for drinking water quality are fully met. There could, however, be a considerable number of unreported cases of disease caused by drinking water contaminated with pathogenic organisms. Improved epidemiological data collection, i.e., the introduction of a requirement to report all cases as is being practiced in the USA, the UK, Australia or Sweden, would also be highly desirable in Switzerland.

Legionnaire's Disease on the Rise

Industrialized countries are experiencing increasing numbers of cases caused by so-called "emerging" pathogens (Tab. 2). In most instances, the causative agents are well known, but have been observed only very rarely as pathogenic microorganisms. Legionnaire's Disease is such an example. It is caused by the bacterium *Legionella pneumophila* and has been diagnosed in increasing numbers over the past several years. This bacterium and closely related species are present in small numbers in all natural waters, can survive in amoeba and

Year	Place	Cause	Number of persons ill (dead)
2001	Pamplona, SP	*Legionella*-infection in hospital	18 (3)
2001	Paris, F	*Legionella*-infection in hospitall	12 (6)
2001	Murcia, SP	*Legionella*-infection in village	315 (2)
2000	Walkerton, CAN	Heavy downpour washes pathogenic enterohemoragic *E. coli* (EHEC) from liquid cow manure into drinking water supply	2 000
1998	La Neuveville, CH	Defective pump causes back-up of waste water and overflow into ground water; pathogens: *Shigella sonnei, Campylobacter jejuni*	1 600
1998	All of Switzerland	*Legionella*-infection	78 (8)
1993	Milwaukee, USA	Defective filters in drinking water processing plant cause spread of highly chlorine-resistant oocytes of *Cryptosporidium parvum*	403 000
1979/80	Ismaning, DE	Contamination of a drinking water source by defective sewer line causes spread of bacterial dysentery (*Shigella* and others)	2 450
1963	Zermatt, CH	Discharge of untreated waste water into Zmuttbach, a stream used as drinking water source, and simultaneous malfunction of chlorinating plant in Zermatt led to the spread of *Salmonella typhii*	437

Tab. 1: Examples of major incidents involving drinking water in industrialized countries.

Pathogen		Disease symptoms
Bacteria	Pathogenic *Escherichia coli* (EHEC)	Dyspepsis, severe diarrhea
	Pseudomonas aeruginosa	Skin and ear infections
	Legionella pneumophila	Pneumonia, "Pontiac fever"
	Aeromonas hydrophila and others	Diarrhea, wound infections
	Campylobacter jeuni and others	Intestinal infections, diarrhea
	Yersinia enterocolitica	Enteritis, inflammation of the intestines, possibly arthritis
	Chlamydia	Eye infections
Viruses	*Calici* viruses	Flu-like infections, summer influenza, sore throat
	Rota viruses	Severe diarrhea, especially in children
	Hepatitis A	Infectious jaundice
	Norwalk virus (small round virus)	Intestinal infections, particularly in children during winter
Protozoa and Parasites	*Cryptosporidium parvum*	Diarrhea, dangerous for children, the elderly and AIDS patients
	Pfisteria	Mostly fish diseases
	Giardia intestinalis	Diarrhea

Tab. 2: "New" pathogenic microorganisms and associated symptoms. For a number of these organisms, current information is limited about their occurrence, distribution pathways, effects and infectious dose.

biofilms, and are harmless to the human body if ingested in drinking water. If the bacterium reaches the lungs by inhalation of aerosols, however, it may cause severe pneumonias (Tab. 2). Aerosols may form in showers or in air conditioning systems. A risk only exists if the warm water systems are operated at too low temperature, i.e., below 55 °C, where *Legionella* can reproduce rapidly. Outbreaks of Legionnaire's Disease have caused numerous mortalities, especially in hospitals. According to the statistics of the Swiss Federal Institute of Public Health, Switzerland registers an average of 40–80 cases per year, with 10% of the infections resulting in death [1, 2]. For a number of gastrointestinal infections caused by (drinking) water, we have to assume that "new pathogens" in the form of viruses are responsible.

The Indicator Concept: Strengths and Weaknesses

Routine tests that determine the microbial quality of drinking water (as well as mineral water, swimming pool water, or process water) are not specifically looking for pathogens. Such analyses would be far too involved. Instead, the assumption is made that pathogens are excreted in human or animal feces together with harmless intestinal microorganisms and that they are subsequently distributed in water. If "indicator species" are identified, their presence suggests contamination of the water with human or animal feces. According to the World Health Organization (WHO), indicator organisms should satisfy the following conditions:

- be excreted by the host and always be present when pathogenic organisms are present,
- be present in larger numbers than the pathogens,
- be specific to feces,
- be more resistant to environmental stress and disinfection than the pathogens,
- not be pathogens themselves,
- be easily and quickly detected and enumerated by simple methods.

This rather demanding list illustrates why the "ideal" indicator organism may not exist. Worldwide, however, a number of different organisms have become the standard indicators (Tab. 3). The intestinal tract bacteria, *Escherichia coli* and enterococci, and the total number of heterotrophic bacteria (not as an indicator of pathogens but as a general measure of eutrophication) have become such generally accepted standard indicators. Additionally, other bacteria or viruses may be used as indicators in certain regions or in specific cases. The standard set for the maximum acceptable number of *E. coli* and enterococci in water in Switzerland is less than 1 cell per 100 ml for untreated, natural drinking water. That *E. coli* does not always adequately fulfill the role of an indicator organism was shown in a case in Milwaukee (Tab. 1): despite the fact that the drinking water was adequately chlorinated and fulfilled the regulatory requirements with respect to *E. coli* numbers, an outbreak of cryptosporidiosis occurred. This was due to the presence of oocytes (a permanent stage of the organism), which are extremely resistant to chlorination.

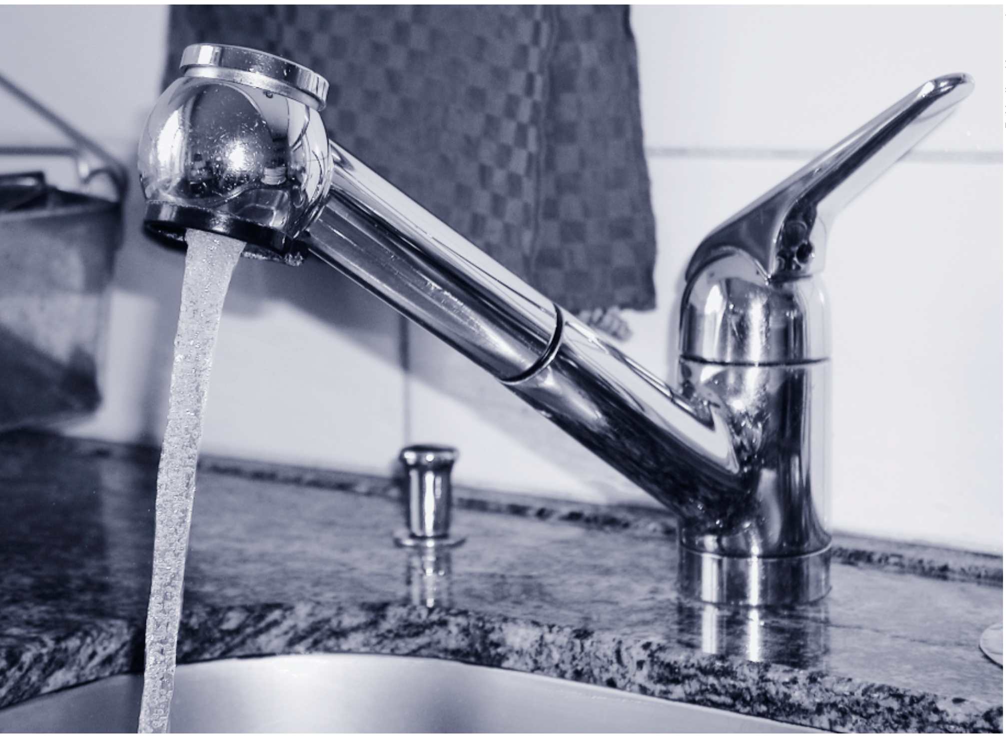
Traditional and Molecular Detection Methods

One especially difficult problem in the analysis of drinking water is that small numbers of organisms have to be detected in large volumes of water. Enrichment is, therefore, the first step in practically all methods designed to detect pathogens in drinking water. In classic culture techniques, which are easy and inexpensive to perform, individual *E. coli* and enterococci cells are transferred to agar plates of selective media where they multiply to form visible colonies. The drawback of this method is that it is rather time-consuming: it can take three working days before a result is obtained. Another disadvantage is the relatively poor selectivity of the approach.

The situation is even more challenging if one attempts to detect individual pathogens instead of indicator organisms. For many

Indicator organism	% in feces of mammals	Numbers per g feces	Advantages	Disadvantages
Escherichia coli	100	10^7–10^9	Easy to enumerate	Less resistant than some pathogens
Enterococci	100	10^5–10^6	Ubiquitous in waste water	Reservoirs in the environment
Clostridium perfringens	13–35	10^6–10^7	Resistant in the environment and towards disinfection	Difficult to culture because of anaerobic techniques
Coliphagen (F-specific)	6	10^1–10^2	Possibly models for enteroviruses	Not resistant in the environment

Tab. 3: Indicator organisms currently used to detect contamination of (drinking) water with feces and possibly by microbial pathogens.



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Safe drinking water – to be taken for granted?

pathogenic organisms, there are either no practical culturing techniques or the existing techniques are extremely time consuming, elaborate and expensive. For this reason, there is growing interest in using molecular techniques in microbial analyses of drinking water [3]. Many of these methods have been used successfully in medicine for the diagnosis of diseases caused by microorganisms. Unfortunately, they must be modified before they can be used to analyze drinking water.

For example, molecular techniques can detect fragments of nucleic acids that contain a specific sequence indicative of a certain pathogen. Even if only one cell (and so only one single fragment) with the desired nucleic acid sequence is present, the so-called PCR-method (polymerase chain reaction) can, in theory, selectively multiply this fragment until a detectable number of fragments has been produced. Other methods use antibodies; that is, they are based on the immunological detection of cell components specific for the pathogen of interest (overview in OECD report, in preparation). The coupling of antibodies or nucleic acid fragments to dyes can further simplify their detection. In terms of selectivity and time required for analysis, molecular methods clearly have an advantage over classical culturing methods. For some molecular methods, it is even conceivable that the sample processing could be fully automated. Molecular methods are likely to play a major role in basic research of the behavior of pathogenic bacteria and viruses during epidemic outbreaks; however, applications in routine analyses are still relatively uncommon. There is substantial hope that molecular methods can create enormous advantages for the detection of viruses as well. Expectations range from being able to detect pathogens by a "dip stick" method to on-line measurements employing glass fibers coated with antibodies that send light signals upon contact with pathogens, where the light signal is again picked up by optical fibers.

A Holistic Approach to Clean and Safe Drinking Water

The development and validation of molecular methods for drinking water analysis and the comparison to results obtained by classical methods is a research focus that is being pursued all over the world. A working group formed jointly by the OECD and the WHO, in which EAWAG representatives play a leading role, is currently working on a document that will present general guidelines. The document, due to be published this year, will summarize state-of-the-art concepts and methodologies for microbial drinking water analysis and present some thoughts on future developments. Experts agree that it is not necessary to create entirely new concepts; they would rather recommend a holistic approach, i.e., to work within the existing system of barriers (wastewater treatment plants, protective zones, disinfection in drinking water processing, etc.) and to incorporate and link information on water resources, climate and hydrology, monitoring of drinking water preparation, collection of epidemiological data regarding waterborne disease, risk assessments, to name a few.

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