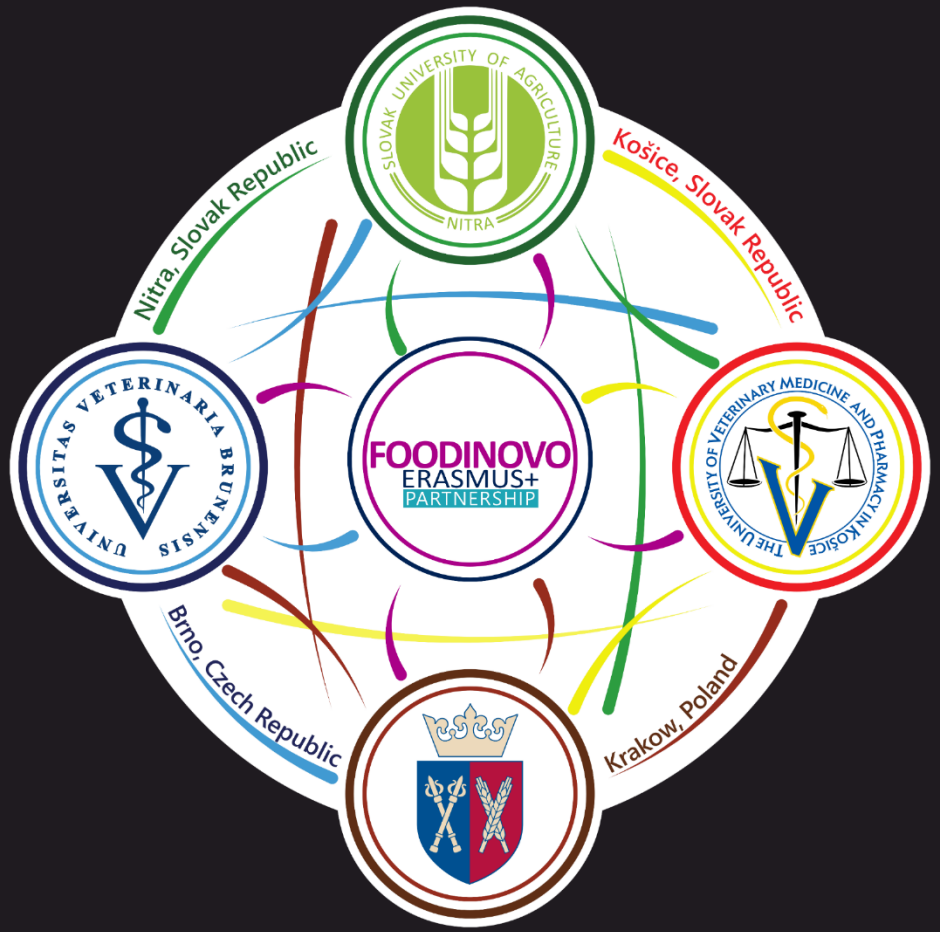
Microbial quality of drinking water

General information and legislation



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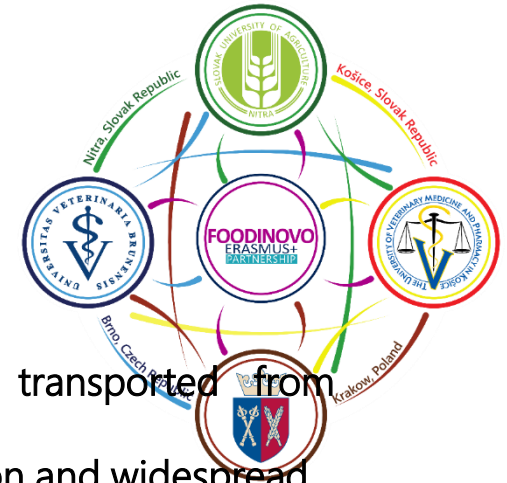
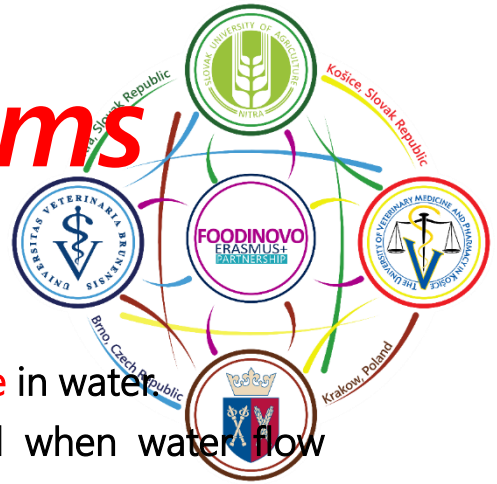
Microorganisms in water



- Water microbiology is concerned with the microorganisms that live in water, or can be transported from one environment to another by water.
- Infectious diseases caused by pathogenic bacteria, viruses, protozoa and helminths are the most common and widespread health risk associated with drinking-water.
- The presence of other disease causing microbes in water is unhealthy and even life threatening. For example, bacteria that live in the intestinal tracts of humans and other warm blooded animals, such as *Escherichia coli, Salmonella, Shigella*, and *Vibrio*, can contaminate water if feces enters the water.
- Testing for microbes that cause disease can be expensive and, if the bacteria are present in low numbers, they may escape detection. Instead, other more numerous bacteria provide an indication of fecal contamination of the water. *Escherichia coli* has been used as an indicator of fecal pollution for decades. The bacterium is present in the intestinal tract in huge numbers, and is more numerous than the disease-causing bacteria and viruses. The chances of detecting *Escherichia coli* is better than detecting the actual disease causing microorganisms. *Escherichia coli* also had the advantage of not being capable of growing and reproducing in the water (except in the warm and food-laden waters of tropical countries). Thus, the presence of the bacterium in water is indicative of recent fecal pollution. Finally, *Escherichia coli* can be detected easily and inexpensively.
- According to the WHO, the mortality of water associated diseases exceeds 5 million people per year. From these, more that 50% are microbial intestinal infections, with cholera standing out in the first place.
- In general terms, the greatest microbial risks are associated with ingestion of water that is contaminated with human or animal feces. Wastewater discharges in fresh waters and costal seawaters are the major source of fecal microorganisms, including pathogens.

Persistence and growth microorganisms in water



- While typical waterborne pathogens are able to persist in drinking-water, most do not grow or proliferate in water.
- Microorganisms like *E. coli* and *Campylobacter* sp. can accumulate in sediments and are mobilized when water flow increases.
- After leaving the body of their host, most pathogens gradually lose viability and the ability to infect. The rate of decay is usually exponential, and a pathogen will become undetectable after a certain period.
- Pathogens with low persistence must rapidly find new hosts and are more likely to be spread by person-to-person contact or poor personal hygiene than by drinking-water.
- Persistence is affected by several factors, of which temperature is the most important. Decay is usually faster at higher temperatures and may be mediated by the lethal effects of UV radiation in sunlight acting near the water surface.
- The most common waterborne pathogens and parasites are those that have high infectivity and either can proliferate in water or possess high resistance to decay outside the body.
- Viruses are unable to multiply in water. Conversely, relatively high amounts of biodegradable organic carbon, together with warm temperatures and low residual concentrations of chlorine, can permit growth of *Legionella sp., Vibrio cholerae*, *Naegleria fowleri*, *Acanthamoeba sp.* in some surface waters and during water distribution .
- Microbial water quality may vary rapidly and widely. Short-term peaks in pathogen concentration may increase disease risks considerably and may also trigger outbreaks of waterborne disease. Results of water quality testing for microbes are not normally available in time to inform management action and prevent the supply of unsafe water.

Drinking water

Water that does not cause the user health problems even after long-term use.

- **does not contain any micro-organisms, parasites or substances which, in certain quantities or concentrations, present a risk to human health from acute, chronic or late exposure and whose sensory properties do not prevent its consumption or use,It must comply with STN ISO 17994 Water Quality and the Decree of the Ministry of Health of the Slovak Republic 247/2017 and Decree of the Ministry of Health of the Slovak Republic 91/2023 on drinking water requirements and drinking water quality control.**

The requirements of Directive 98/83/EC are implemented in Slovakia in Act No. 355/2007 Coll. protection, support and development of public health and on the amendment and addition of certain laws as amended and in the Decree of the Ministry of Health of the Slovak Republic no. 91/2023 Coll., which establishes indicators of drinking water quality and hot water quality, the procedure for monitoring drinking water, risk management of the drinking water supply system and risk management of domestic distribution systems.

International Organization for Standardization (ISO) standards for detection and enumeration of faecal indicator bacteria in water

ISO standard	Title (water quality)
6461-1:1986	Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia)—Part 1: Method by enrichment in a liquid medium
6461-2:1986	Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia)—Part 2: Method by membrane filtration
7704:1985	Evaluation of membrane filters used for microbiological analyses
9308-1:2000	Detection and enumeration of *Escherichia coli* and coliform bacteria—Part 1: Membrane filtration method
9308-2:1990	Detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive *Escherichia coli*—Part 2: Multiple tube (most probable number) method
9308-3:1998	Detection and enumeration of *Escherichia coli* and coliform bacteria—Part 3: Miniaturized method (most probable number) for the detection and enumeration of *E. coli* in surface and waste water
10705-1:1995	Detection and enumeration of bacteriophages—Part 1: Enumeration of F-specific RNA bacteriophages
10705-2:2000	Detection and enumeration of bacteriophages—Part 2: Enumeration of somatic coliphages
10705-3:2003	Detection and enumeration of bacteriophages—Part 3: Validation of methods for concentration of bacteriophages from water
10705-4:2001	Detection and enumeration of bacteriophages—Part 4: Enumeration of bacteriophages infecting *Bacteroides fragilis*

Water pollution according to the presence of microorganisms

General contamination

Fecal contamination

Indicators of hygienic significance

1 . General (organic) pollution indicators:

- cultivable microorganisms at 22 °C (psychrophilic),
- cultivable microorganisms at 36 °C (mesophilic),
- coliform bacteria.

2. Fecal pollution indicators:

- Enterobacteriaceae: only *Escherichia coli,*
- Enterococci (faecal streptococci).

3. Hygienically significant and pathogenic:

- *Enterobacteriaceae: Salmonella, Shigella, Klebsiella, Proteus, Yersinia, Hafnia, Enterobacter, Escherichia, Campylobacter,*
- *Pseudomonadaceae: Alcaligenes, Pseudomonas,*
- *Vibrionaceae: Aeromonas, Haemophilus, Pasteurella, Vibrio,*
- *Streptococcaceae: Streptococcus,*
- *Micrococcaceae: Staphylococcus,*
- *Mycobacteriaceae: Mycobacterium,*
- *Legionella, Leptospira,*
- *Candida,*
- *Clostridium perfringens.*

Family representatives	Charakteristic
Escherichia	(P) C
Shigella	P
Salmonella	P
Citrobacter	(P) C
Klebsiella	(P) C
Enterobacter	C
Erwinia	
Serratia	C
Hafnia	
Edwardsiella	(P)
Proteus	(P)
Providencia	(P)
Morganella	(P)
Yersinia	P

P- pathogenic
(P) - conditionally pathogenic
C - coliform

General water contamination

1. **Psychrophilic - culturable bacteria at 22 °C** - their increased numbers signal the penetration of environmental pollution or water treatment or disinfection disorders.

2. Mesophilic bacteria - cultivable at 36 °C - their occurrence above a specified limit indicates organic pollution in drinking water. A taxonomically inhomogeneous group of organotrophic (able to grow only in the presence of org. C and N) non-sporulating and sporulating bacteria. They are able to form colonies from individual cells, their pairs, short chains or clusters of cells. These are mainly species of the genera *Pseudomonas, Bacillus, Micrococcus, Flavobacterium*.

In polluted water, rod-shaped bacteria predominate, and cocci are characteristic of clean water. An increased number of mesophiles signals pollution of the water source from the external environment, either directly by cells of microorganisms or organic substances. The determination of these groups is only relevant for the ecological assessment of water quality.

3. Coliform bacteria other than *E. coli* family Enterobacteriaceae oxidase negative and catalase positive this includes selected species of the genera: *Escherichia, Citrobacter, Enterobacter, Klebsielladoes* not belong to the species: *Escherichia fergusonii, E. vulneris, E. hermanii, Enterobacter taylorae, Klebsiella ozaenae* (lactose negative)they never occur in faeces, but they are coliform bacteria and can also be present in pure water: *Serratia fonticola, Rahnella aquatilis, Buttiauxella agrestis.*

Coliforms bacteria

- They are no longer an indicator of fecal pollution!
- "Coliforms" are coliforms that have similar growth characteristics to *Escherichia coli.*

- G^-sticks that do not form spores and are able to grow under aerobic and anaerobic conditions (optional anaerobic) on a culture medium containing bile salts or other surfactants with similar growth inhibitory properties.

- They have the ability to ferment lactose (also the mannitol-isomer of sorbitol) at a temperature of 35-37 °C with the simultaneous production of acid, gas and aldehyde for 24 hours to 48 hours. The reaction to the cytochrome oxidase assay is negative and the activity for β-D-galactosidase as well as β-D-galactoside permease is positive.

Fecal contamination

- *Escherichia coli*
- enterococci

Their presence also indicates the possible presence of obligate pathogens.

Escherichia coli

Escherichia coli, formerly Bacterium coli, was identified in 1885 German pediatrician Escherich.

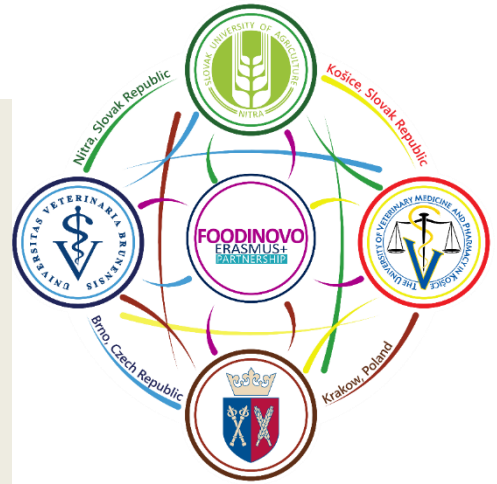
In 1892, the use of *E. coli* was proposed as an indicator of fecal contamination.

This was based on the finding that *E. coli* is present in human and animal faeces.

***E. coli* can be easily detected due to its ability to ferment glucose (later converted to lactose), its isolation was easier than the isolation of other gastrointestinal pathogens.**

The presence of *E. coli* in water indicates both fresh and older faecal contamination and indicates the possible presence of other pathogens.

Pathogenic *Escherichia coli*



1. In 1951, Buehler and his colleagues discovered that E. coli (apart from Escherichia coli O157: H7) produced the enzyme beta-D-glucuronidase, which is able to hydrolyze specific substrates (beta-D-glucuronide and its derivatives) and thus obtain an energy source.
2. For other bacteria, the occurrence is rather sporadic (except for *Shigella* and *Salmonella* strains and a few strains of *Yersinia, Citrobacter, Edwardia, Hafnia, Flavobacterium* and *Bacteroides*).
3. Various compounds have been used to determine beta-D-glucuronidase enzyme production, which have been formed by a chemical bond between a beta-D-glucuronid substrate and some chromogenic and later fluorogenic compounds.

E. coli virotypes based on the presence of certain virulence factors (interaction with host cells and tissues and toxin production):

1. *Enterotoxigenne E. coli (ETEC)*
2. *Enteropatogenne E. coli (EPEC)*
3. *Enterohemorhagické E. coli (EHEC), Shiga-like toxigenní E. coli (STEC)*
4. *Enteroinvazivne E. coli (EIEC)*
5. *Enteroagragativne E. coli (EAEC)*
6. *Diffuse adherent E. coli (DAEC).*

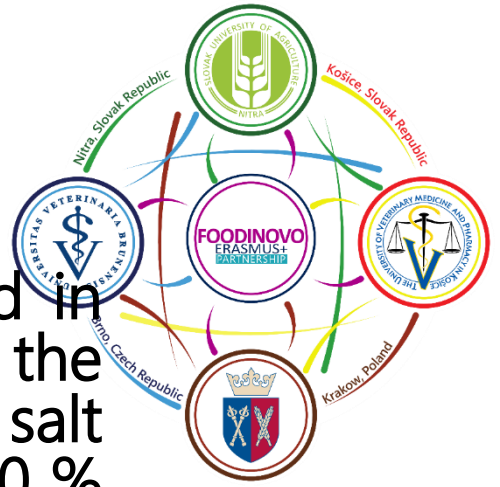
Escherichia coli O157:H7

Enterohaemorrhagic E. coli (EHEC), also known as shiga-like toxigenic E. coli (STEC) or verotoxigenic E. coli (VTEC), cause bloody diarrhea and hemolytic-uremic syndrome (ladin damage). 50-100 cells are enough and death can occur.

The most common serogroups include O4, O5, O16, O26, O55, O111ab, O113, O117, O157 and O172. The best known serotype is O157: H7.

It does not produce the enzyme *ß-D-glucuronidase* and does not ferment mannitol.

Enterococci

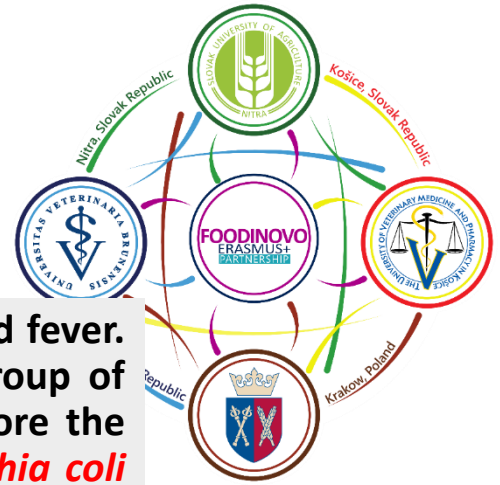


- gram-positive cocci, the cause of lactic fermentation, mostly arranged in chains with negative catalase. They have the ability to reproduce in the temperature range 10-65 °C, can grow even at relatively high salt concentrations (6.5% sodium chloride) and pH 9.6 and tolerate up to 40 % bile in the environment.

- They are present in human and animal faeces. All contain group D antigen. These include species of the genera *Enterococcus* and *Streptococcus*, namely *Enterococcus avium, E. casseliflavius, E. cecorum, E. faecalis, E. faecium, E. gallinarum, E. hirae, E. malodoratus, E. mundtii* and *E. solitarius* (WHO, 1993) and according to Švec and Sedláček (1998) they are also *E. durans, E. raffinosus, E. pseudo-avium, E. columbae, E. saccharolyticus, E. dispar, E. sulfureusa, E. flavescens.*

- An indicator of fresh faecal contamination is rarely multiplied in the water.

Pathogenic bacteria



- **Coliform bacteria cause intestinal problems and colic, but they can also cause serious diseases such as typhoid fever. We kill coliform bacteria by heating water to a temperature higher than 50 ° C. The occurrence of this group of bacteria may indicate that it has been freshly contaminated with faecal waste, soil, plant litter and therefore the severity of the pollution must be confirmed by the presence of so - called presumptive (presumed) *Escherichia coli* that has entered the soil from faeces and may be an indicator of faecal and more dangerous human contamination.**
- **Enterococci are present in water, soil and plants. These bacteria are resistant to high temperatures and high pH values. They cause urinary and biliary infections and gynecological inflammation. The presence of enterococci in the water confirms faecal contamination. However, unlike coliform bacteria, a significantly higher dose of chlorine is required to kill them.**
- ***Legionella pneumophila* is very dangerous for humans. It often settles on the inner sides and corners of water pipes, its occurrence and spread is contributed to by the low temperature of the outflow paths (best reproduced at temperatures from 30 °C), low water pressure, insufficient maintenance, distribution care and many other factors.**
- ***Pseudomonas aeruginosa*, causing dangerous infections ending in death. Due to the fact that it can easily adapt to the environment, it is very widespread and resistant, even to antibiotics such as penicillin.**
- ***Vibrio cholerae* are small, curved-shaped Gram-negative rods, with a single polar flagellum. Vibrios are facultative anaerobes capable of both fermentative and respiratory metabolism. Sodium stimulates growth of all species and is an absolute requirement for most. Most species are oxidase-positive and reduce nitrate to nitrite. Cells of certain species (*V. cholerae, V. parahaemolyticus* and *V. vulnificus*) have pili (fimbriae), structures composed of TcpA protein. TcpA formation is co-regulated with cholera toxin expression and is a key determinant of *in vivo* colonization.**

Shigella sp.

- **Typically an inhabitant of the intestinal tract of humans and other primates. It is typically spread by fecal-contaminated drinking water or food, or by direct contact with an infected person. In water, shigellae can survive for at least six months at room temperature, and this high survival favors transmission through water.**

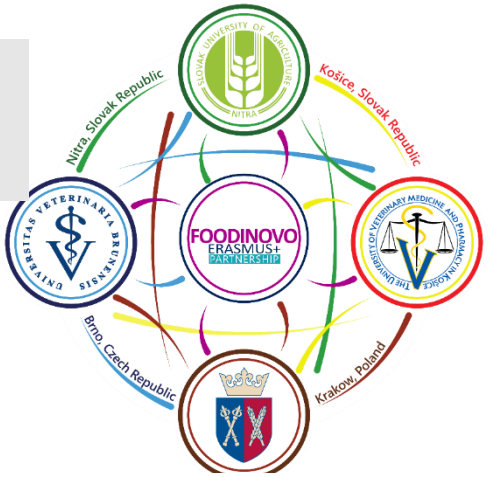
- **Gram-negative, non-sporeforming, non-motile, straight rod-like members of the family *Enterobacteriaceae*. Cells ferment sugars without gas production. Salicin, adonitol and myo-inositol are not fermented. Cells do not utilize citrate, malonate and acetate as sole carbon source and do not produce H_2S. Lysine is not decarboxylated. Cells are oxidase-negative and catalase-positive. Members of the genus have a complex antigenic pattern, and taxonomy is based on their somatic O antigens.**

- ***S. dysenteriae* serotype 1 produces hTgh levels of a cytotoxic Shiga toxin. *S. sonnei* and *S. flexneri* produce much lower amounts of this toxin. Shiga toxin binds to Galotl-4Galp (galabiose) glycolipid receptors and inhibits mammalian protein synthesis by cleaving the N-glycosidic bond at adenine 4324 in 28S rRNA. The toxic mechanism is identical to that of the plant toxin ricin, produced by *Ricinus communis*. Shigella also release a LPS endotoxin (O antigens), that cause an inflammatory response .**

Salmonella sp.

- gram-negative, mostly motile, aerogenic, lactose-negative rods, have a negative cytochrome oxidase test, reduce nitrates, use carbon from citrates, form water and do not form indole. They are the cause of serious, often epidemic diseases. The genus Salmonella has 11 species, the best known of which are *S. choleraesuis, S. typhi, S. paratyphi, S. typhimurium, S. enteritidis* and *S. gallinarum*. The genus *Salmonella*, a member of the family *Enterobacteriaceae*, include Gram-negative motile straight rods. Cells produced gas from D-glucose and utilize citrate as a sole carbon source. Salmonellae have several endotoxins: antigens O, H and Vi.
- Salmonellae pathogenic to humans can cause two types of salmonellosis:
  - typhoid and paratyphoid fever (do not confuse with typhus, a disease caused by a rickettsia),
  - gastroenteritis.
- Low infective doses (less than 1,000 cells) are sufficient to cause clinical symptoms. Salmonellosis of newborns and infants presents diverse clinical symptoms, from a grave typhoid-like illness with septicemia to a mild or asymptomatic infection.

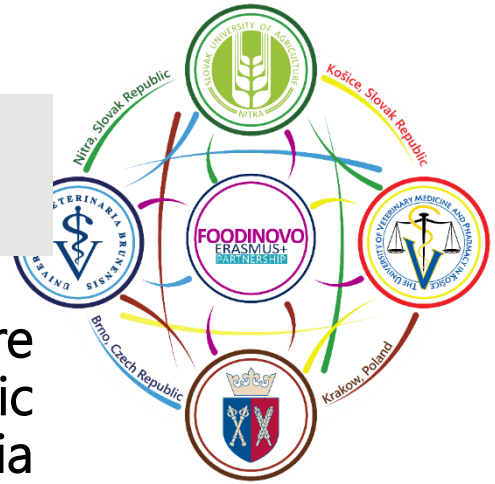
Clostridium perfringens



- **Sulphite-reducing clostridia, namely *Clostridium perfringens*, are spore-forming Gram-positive, non-motile, anaerobic, sulfite-reducing rods. *C. perfringens* is present in higher numbers in the feces of some animals, such as dogs, than in the feces of humans and less often in the feces of many other warm-blooded animals. The numbers excreted in feces are normally substantially lower than those of *E. coli.***

- ***Clostridium* spores are exceptionally resistant to unfavorable conditions in water environments, including UV irradiation, temperature and pH extremes, and disinfection processes, such as chlorination. Although clostridia probably do not growth in surface waters, the high resistance of their spores makes their presence ubiquitous in environmental waters. The presence of chlorine in water rapidly inactivates indicator bacteria such as *E. coli* and coliforms, but it leaves the most resistant pathogens almost unaffected for several hours. This creates a false sense of security by providing negative coliform and negative *E. coli* results to authorities responsible for water testing. *Giardia* cysts, *Crystosporidium* oocysts, and human enteric viruses all have higher resistance to disinfectants and constitute a major public health risk if distribution system integrity is breached. *C. perfringens* spores are less affected by the residual concentrations of chlorine.**

Pseudomonas aeruginosa



- *Pseudomonas aeruginosis*, which is a conditioned pathogen, is all the more serious because its antagonistic effect is known and its presence in the aquatic environment may preclude the detection of coliform bacteria and enterobacteria in the investigated water. *Pseudomonas aeruginosa* is a gram-negative straight or curved mallet with polar flagella is strictly aerobic, forms both catalase and oxidase.

- Due to its ability to utilize even hardly decomposable organic substances, this organism can occur in drinking water (in the absence of coliform bacteria) in cases where unsuitable structural elements have been used in water supply systems and is considered an indicator for non-compliant organic substrates. Although very often found in faeces, it cannot be considered an indicator of faecal pollution, as it multiplies very easily in the environment, even in drinking water.

Diseases caused by bacteria transmitted by contaminated water

Pathogen	Disease	Affected place in the body
Salmonella typhi	typhus	**intestinal tract**
S. enteritidis	gastroenteritis	**gastrointestinal tract**
Shigella sp.	dysentery	**gastrointestinal tract**
Vibrio cholerae	cholera	**Intenstinálny trakt**
Escherichia coli	gastroenteritis	**gastrointestinal tract**
Francisella tularensis	tularemia	**gastrointestinal tract** **respiratory system** **lymph nodes**
Leptospira icterohaemorrhagiae	leptospirosis	**blood, liver, spleen, kidneys and adrenal glands, heart muscle, brain and eye tissue**
Mycobacterium tuberculosis	tuberculosis	**lung and other organs**
Legionella pneumophila	legionnaires' disease	**lungs**
Campylobacter fetus	gastroenteritis	**gastrointestinal tract**

Drinking water - microbial quality in Slovakia

According to available data, the most common contaminants of our own wells are microbiological and chemical indicators of drinking water quality.

Of the microbiological indicators, the value is most often exceeded:

1. **Cultivable bacteria at 22 °C**
2. **Coliform bacteria**
3. **Enterococci**
4. ***Escherichia coli***

References:

Guidelines for Drinking-water Quality. World Health organization. ISBN 9788241549950.

Cabral, J.P.S. 2010. Water Microbiology. Bacterial Pathogens and Water. Int. J. Environ. Res. Public Health 2010, 7, 3657-3703; doi:10.3390/ijerph7103657

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