MOLECULAR AND EXPERIMENTAL BIOLOGY IN MEDICINE

IS OUR DRINKING WATER MICROBIOLOGICALLY SAFE?

Ana Kovacic

Abstract:

The purpose of microbiological analysis of drinking water is to ensure that it is free of pathogenic microorganisms, but also to control the sanitary maintenance of water supply systems. In Croatia, there are several ways in which water for human consumption is regularly controlled. The analyses can be carried out only in laboratories that are authorized by the Croatian Ministry of Health. The Council Directive on the quality of water intended for human consumption has been implemented in the Croatian legislation. Therefore, the maximum permissible concentrations for microbiological parameters in Croatia are the same as in the rest of the EU. Currently used microbiological parameters ensure public health protection from microbiologically unsafe drinking water, but it is necessary to implement modern, molecular-based techniques in routine microbiological analysis of water in order to gain faster and more precise results.

Public Health Institute of Split and Dalmatia County, Split, Croatia

Corresponding author:
Ana Kovacic
Public Health Institute of Split and Dalmatia County,
Vukovarska 46, 21000 Split, Croatia
Tel: +385 21 401 109
e-mail: ana.kovacic@nzjz-split.hr

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INTRODUCTION

Microbiological analysis of water differs from the analysis of food or human samples. Microorganisms in water deal with both a severe lack of nutrients and unfavorable temperatures for their growth. Therefore, in both chlorinated and fresh-water samples, they can often be found in a viable but nonculturable state (VBNC), which makes microbiological analysis complicated.[1, 2 ,3] There are two basic methods for the detection and enumeration of microorganisms in water. The most probable number (MPN) method is a statistically-based method which includes the use of several portions of broth inoculated with different volumes of a water sample.[4] Although this method enables the resuscitation of sublethally injured microorganisms, it cannot give the exact number of examined microorganisms and is time-consuming since it requires additional confirmation of presumptive results. The MPN method is usually applied for monitoring of raw (untreated) fresh water. In order to get the exact number of tested microorganisms, the membrane filtration method has been developed. It is based on the filtration of a desired volume of water sample through the sterile membrane filter with a pore size of 0.45 or 0.22 μm, with subsequent incubation on a selective medium. Membrane filtration is usually the method of choice for testing of water for human consumption (drinking water), i.e. water with low bacterial background flora.[5]

The purpose of microbiological analysis of drinking water is to ensure that it is free of pathogenic microorganisms, but also to control the sanitary maintenance of water supply systems. It is achieved by the measuring of the so-called microbiological indicators. The use of bacteria as indicators of the sanitary quality of water dates far back to 1880 when some microorganisms were considered to be "characteristic of human feces".[6] Today, there are two groups of indicators that are routinely used in the microbiological analysis of water: general (process)

microbial indicators and fecal indicators. The first group, also designated as indicator parameters, are not of direct health significance, but they are important for the monitoring of sanitation treatment of water supply systems. These parameters include: colony count at 22°C and 36°C (total number of heterotrophic microorganisms); coliform bacteria and *P. aeruginosa*. The second group, designated as wholesome microbiological indicators, consists of *E. coli*, enterococci and *C. perfringens*, and they are used as indicators of fecal contamination.[7]

Microbiological analysis of water in Croatia

The Council Directive on the quality of water intended for human consumption has been implemented in the Croatian legislation with the National Regulation.[8, 9] The maximum permissible concentration requirements for microbiological parameters in Croatia are listed in Table 1.

There are several ways in which water for human consumption is controlled on a regular basis. The first level of control is obtained by the water supply companies, through HACCP and Water safety plans (WSPs)[9], which are designed to ensure the control of drinking water at all steps, from catchment to consumers. The second level of control is provided by the laboratories that are authorized by the Croatian Ministry of Health. Such analyses include water samples from all buildings that are newly built or renovated. This certificate of microbiological conformity of water from the new building pipes is issued to the owners in order to prove that water supply system in the building meets the Regulation requirements. Microbiological analysis of water must be also regularly carried out in all catering facilities (such as restaurant, fast-food objects, café bars), educational and health-care institutions. The third level of control is provided by the Ministry of Health itself, both by Sanitary inspection control and Counties that are bound by law to finance a program of monitoring of drinking water. In these two cases, only laboratories from the County Public Health Institutes are authorized to carry out the analysis. The number of tested samples is calculated in relation to the annual amount of water consumption in each County.[9]

What happens if a water sample is not in compliance with the Regulation?

The answer depends on the type of water sample and the analysis. In the case of positive microbiological results of the samples from new buildings, the water supply system needs to be washed out and disinfected until wholesome water is provided. If samples are analyzed at the request of Sanitary inspection, upon information about their non-compliancy, they supervise the implementation of measures that need to be conducted either by the water supply company or by the owner of the facility. Results of positive samples gained through the County monitoring program are immediately announced both to the Ministry of Health and the water supply company that is responsible for the distribution of such water. Their action depends on the type of microorganisms found in drinking water. If the number of indicator microorganisms is found to be above the MWR value, hazard assessment has to be carried out and, depending on the conclusion, necessary remedial actions are taken by the water supply company. Failure to meet the MWR values relating to fecal microorganisms is automatically considered to be a potential danger to human health, and such water is prohibited or restricted for human consumption until the cause is detected and eliminated.

Table 1. Maximum permissible concentration for microbiological parameters for water for human consumption.[9]

Parameter	Method	Unit	MPC
Colony count (36°C)	HRNEN ISO 6222:2000	cfu/1 ml	100
Colony count (22°C)	HRNEN ISO 6222:2000	cfu/1 ml	100
Coliform bacteria	HRNEN ISO 9308-1:2014	cfu/100 ml	0
E. coli	HRNEN ISO 9308-1:2014	cfu/100 ml	0
Enterococci	HRNEN ISO 7899-2:2000	cfu/100 ml	0
P. aeruginosa	HRNEN ISO 16266:2008	cfu/100 ml	0
C. perfringens	HRNEN ISO 14189:2016	cfu/100 ml	0
enteroviruses	rtPCR	number/5 L	0

Legend: MPC – maximum permissible concentration

DISCUSSION AND CONSLUSION

Water samples are controlled on a daily basis, and for all non-complying results remedial actions are taken immediately. One of the most serious objections to the microbiological analysis of water is the time required to obtain results. Standard microbiological methods usually entail the need for the isolation of pure cultures and several confirmatory tests. Therefore, there have been some improvements made both in the modification of classical methods and in the invention of new, faster and more reliable ones. One such improvement is the use of chromogenic compounds in growth media. The medium changes color (or fluorescence) after being modified by the enzymatic activity of bacteria. In this way, the period of analysis is shortened by at least 24 hours.[6] Immunomagnetic separation (IMS) is a method which enables the concentration and identification of microorganisms in a relatively short period of time.[10] The principle of this method is to concentrate specific microorganisms using immunomagnetic beads that are attached by antigen-antibody bound. When IMS is combined with PCR, the results are even more reliable.[11] The most precise and fastest method for detection and enumeration of microorganisms is real-time PCR. It is nowadays routinely used for the identification of viruses in water samples.[12, 13] Gene sequence-based methods are also used very often in various cases of outbreaks when it is of crucial importance to identify the source and the way of transmission of a pathogenic microorganism.[14]
The parameters and their values that are currently examined in drinking water are generally based on the World Health Organization Guidelines for drinking water (WHO, 2017).[15] They assure the public health protection from using microbiologically unsafe drinking water. Nevertheless, there is a constant need for refining requirements in order to avoid any health risk. Implementation of new parameters and the introduction of modern, molecular-based techniques in routine analyses would provide faster and more precise results, especially when viral and protozoal pathogens are concerned.[16, 17]

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