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# CHLORINE-RESISTANT MICROBIAL CONTAMINANTS IN DRINKING WATER

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**Abstract.** The current situation of drinking water treatment in Estonia is characterized. The samples of water were analysed for lactose-positive coliforms, enterococci, lecithinase-positive staphylococci, *Pseudomonas aeruginosa*, pathogenic enterobacteria, and enteroviruses. These chlorine-resistant strains of microorganisms were determined as indicators of sanitary risk and for surveillance of drinking water quality to evaluate the water treatment process. Proposals were made to implement more effective, supplementary methods of disinfection.

**Key words:** drinking water, water standards, enteroviruses, disinfection.

## INTRODUCTION

Water is one of the most important natural resources in Estonia. The annual flow rate of rivers makes up over 4 km$^3$, annual groundwater resources amount to more than 0.5 km$^3$. The annual water consumption was 2082 km$^3$ in 1993, of which surface water made up 79%, ground water 17%, and sea water 4%. Of the total 79% was used as cooling water (mainly in power plants), 6% for domestic usage and as drinking water, 4% in industry, 1% in agriculture, 9% in fishery, and 1% in other spheres (Narusk, 1994). Reduced industrial production in recent years has been accompanied with a decrease in water extraction and water pollution loads.

The quality of drinking water has been steadily worsening in Estonia for the last 20—30 years, particularly, if one considers results of chemical analyses (increasing content of nitrates). However, the results of monitoring by bacteriological standards show some improvement. Summarized data are given the Table.

Our analysis revealed big territorial differences, which indicates a certain dependence of water quality on peculiarities of water sources. The quality of drinking water depends on a wide range of factors: natural composition of water protective properties of the ground, contamination of the catchment area and application of raw water protection zones, state and safety of drinking water pipelines and sewerage, etc. Community water supply systems in many towns are obsolete, they often break down. Losses of water in passing through pipelines reach 30—35%.

Drinking water monitoring is presently the responsibility of two systems in Estonia:

1. The Health Protection Department, including the Estonian Sanitary-Quarantine Office, carries out the surveillance of drinking water for the fishing fleet and merchant ships. Over a long time, the prevailing activity

of the Health Protection Department has been antecedent and current surveillance of drinking water, waste water, and water from other sources. Its bacteriological laboratories analysed a total of 23 542 samples of drinking water in 1993.

2. Drinking water producers have their own water quality control systems — either their own laboratories or several other laboratories perform the needed analyses on order.

The results of drinking water analyses from Health Protection Offices in towns and counties are submitted to the National Board for Health Protection for generalization, drawing conclusions, and submission in the annual report to the State Statistical Office. The National Board for Health Protection also collects and generalizes the results of analyses made by producers of drinking water. These data are included in the annual report for submission to the State Statistical Office.

The Ministry of the Environment collects and generalizes data obtained by four subordinate control laboratories. There are also other institutions, such as the Estonian Geological Survey, Tallinn Technical University, Institute of Preventive Medicine, which monitor drinking water quality. Unfortunately, there is no constructive collaboration and exchange of information between the National Board for Health Protection, the Ministry of the Environment, and other institutions.

The principal risk to human health associated with the consumption of drinking water in Estonia is microbiological in its nature. In order to evaluate the sanitary microbiological standards of water, a study of the raw and treated drinking water was undertaken. In general, the presence of faecal coliforms indicates recent and potentially dangerous contamination of the water. The fact that there have been cases of contamination of water sources with microorganisms such as pathogenic enterobacteria and viruses, or chlorine-resistant microflora, has created a need for continuous monitoring.

The purpose of this study was to estimate the microbial pollution of the drinking water for the evaluation of the existing water treatment technology, considering hygienical aspects.

Our study consists of several parts with the following main tasks:

— determination of the bacterial and viral pollution in raw water of Tallinn Water Treatment Plant;
— estimation of the occurrence of chlorine-resistant bacteria and enteroviruses in treated drinking water of Tallinn;
— evaluation of the removal efficiencies of microorganisms in the drinking water treatment processes.

**Suitability of samples (made by the National Board for Health Protection) to water quality standards (GOST)**

| Years | Chemical characteristics | | Bacteriological characteristics | |
|---|---|---|---|---|
| | Average number of analyses per year | Percentage of unsuitable analyses | Average number of analyses per year | Percentage of unsuitable analyses |
| 1980—84 | 9 842 | 13.3 | 29 150 | 15.5 |
| 1985—89 | 10 234 | 13.6 | 28 928 | 13.5 |
| 1990—93 | 9 702 | 14.9 | 23 441 | 14.2 |

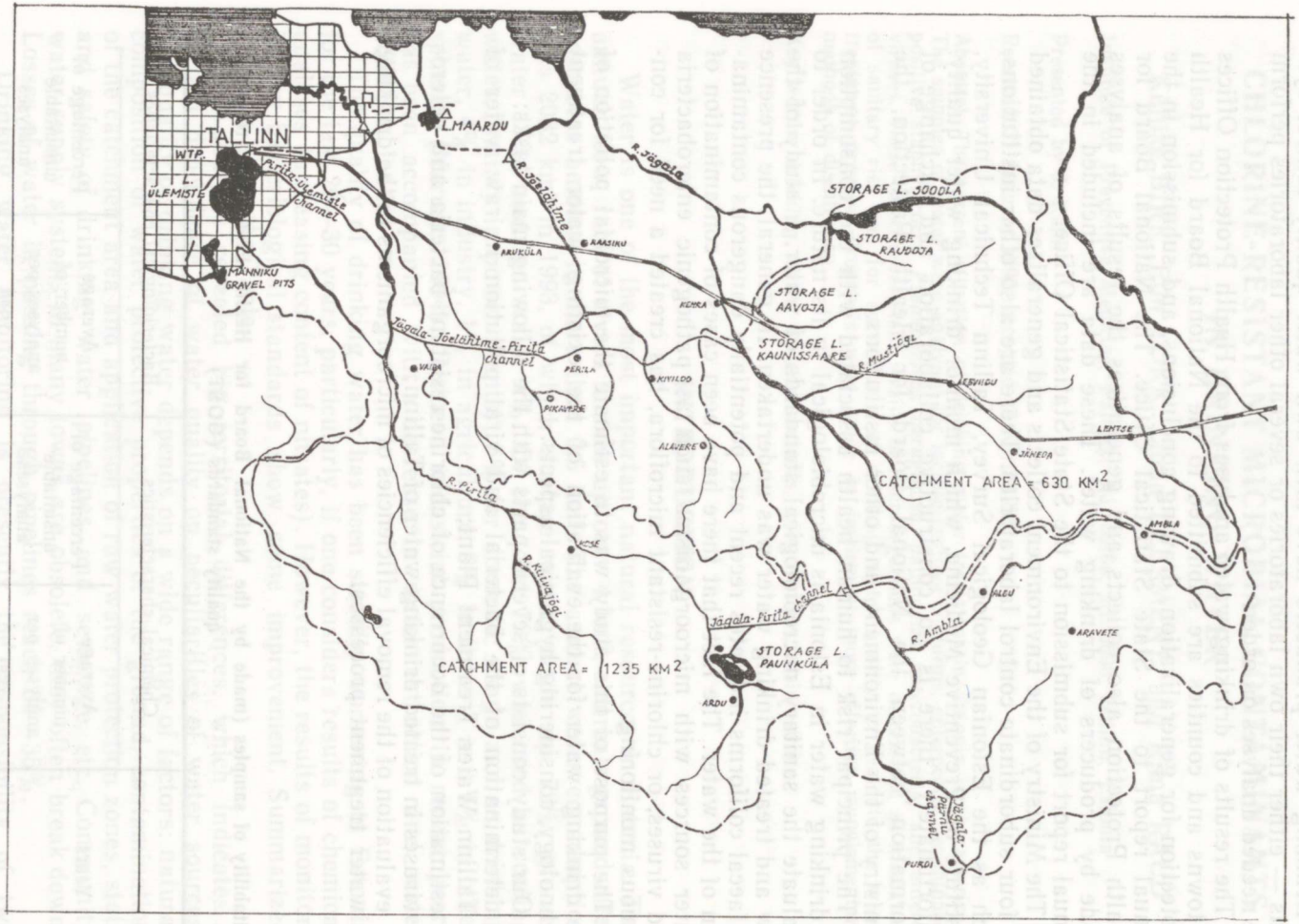


Fig. 1. Map of water sources.

## MATERIAL AND METHODS

**Site description.** About 80% of the water consumption of Tallinn is covered by the Water Treatment Plant. The waterworks is based on Lake Ülemiste (Fig. 1). The water is collected into the lake from small rivers, impounding reservoirs, and channels. The catchment area is approximately 2000 km$^2$. In this area the major sources of pollution are the runoffs from agricultural land and the water routes that pass through settlements. Pollution results in the deterioration of the water quality. The content of mineral compounds, nutrients, microorganisms, and phytoplankton increases significantly. The purification and disinfection of such water demands great amounts of the reagents.

The diurnal output of purified drinking water of the Tallinn Water Treatment Plant is $220 \cdot 10^3$ m$^3$. Some information on the technical details of the process is given in Fig. 2. The propeller pumps force the water through 10 microfilters (efficiency varies from 25 to 75%). In the contact reservoir the water receives the predosage of chlorine (from 2 to 8 mg $\cdot$ dm$^{-3}$), lime CaO in dosages from 10 to 40 mg $\cdot$ dm$^{-3}$, and the coagulant $Al_2O_3$ is added in concentrations from 12 to 25 mg $\cdot$ dm$^{-3}$. From

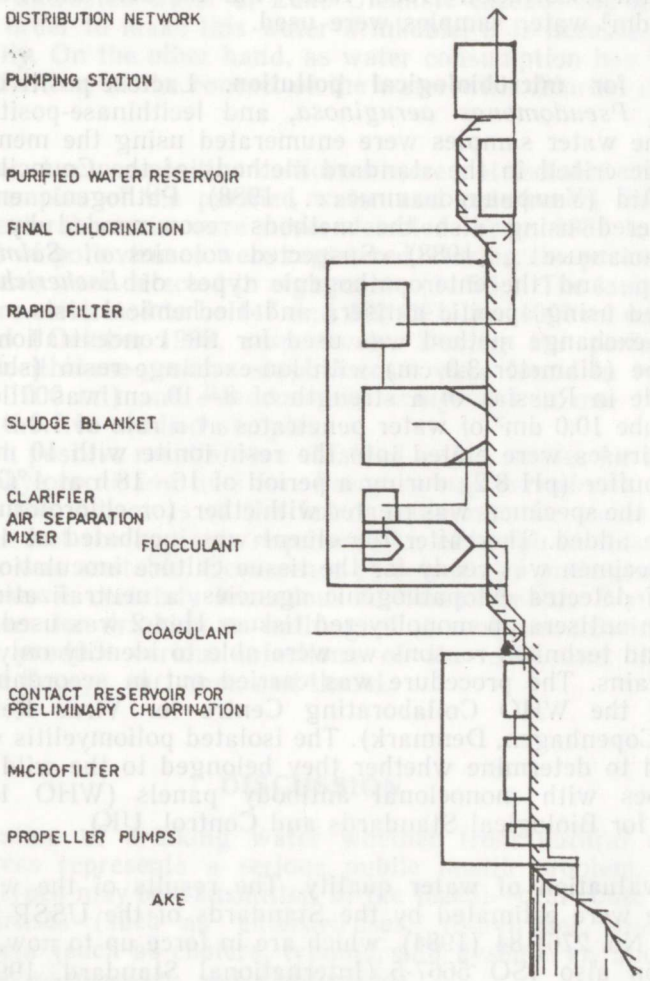


Fig. 2. Technological cycle of the Tallinn Water Treatment Plant.

the contact reservoir the water is pumped into the mixer to discharge gases. As the water flows out of the contact reservoir, polyacryl amid flocculant is added (from 0.15 to 0.35 mg · $dm^{-3}$). From the mixer the water flows into the sludge blanket clarifier with distribution, working, and protective zones. The rising speed of water in the working zone is 1.3 mm · $s^{-1}$, in the protective zone 0.8 mm · $s^{-1}$. Thickened sediment is removed from the clarifier once in 24 h. Clarified water is fed on rapid filters with a filtration speed of 9 m · $s^{-1}$. The working cycle of the rapid filters is 16 to 32 h. Filtered water receives the postdosage of chlorine (from 0.2 to 1.0 mg · $dm^{-3}$). The filters are composed of layers of sand (grain diameter from 0.5 to 1.2 mm, layer thickness 1.5 m) and activated carbon AG3 (layer thickness 0.5 m). Filtered water is pumped into purified water reservoirs. The second stage pumping station feeds water into the city water distribution network.

**Sample collection.** The water samples were collected simultaneously from the influent and effluent of the Tallinn Water Treatment Plant from 12 January 1987 to 22 February 1993 and from 15 March 1994 to 14 June 1994. For the bacteriological analyses water samples were collected into sterilized bottles according to the Standard Methods of the American Public Health Association (1985, 1989). For the determination of enteroviruses 10 $dm^3$ water samples were used.

**Monitoring for microbiological pollution.** Lactose-positive coliforms, enterococci, *Pseudomonas aeruginosa*, and lecithinase-positive staphylococci in the water samples were enumerated using the membrane filter procedure described in the standard methods of the Council for Mutual Economic Aid (Унифицированные..., 1988). Pathogenic enterobacteria were recovered using also the methods recommended by the CMEA (Унифицированные..., 1988). Suspected colonies of *Salmonella* spp., *Shigella* spp., and the enteropathogenic types of *Escherichia coli* were characterized using specific antisera and biochemical tests.

The ion-exchange method was used for the concentration of viruses. A glass tube (diameter 3.0 cm) with ion-exchange resin (slurry) (ionite AB-17, made in Russia) of a strength of 8—10 cm was filled. Through the glass tube 10.0 $dm^3$ of water penetrates at a rate of 1.0—1.2 $dm^3 \cdot h^{-1}$. Adsorbed viruses were eluted into the resin-ionite with 10 ml of 0.05 M phosphate buffer (pH 8.2) during a period of 16—18 h at 4 °C. To destroy the bacteria the specimen was treated with ether (or chloroform) and antibiotics were added. Thereafter the eluent was incubated at 4 °C for 24 h. Now the specimen was ready for the tissue culture inoculation. For identification of detected cytopathogenic agencies, a neutralization test with type-specific antisera on monolayered tissues Hep-2 was used. Because of economic and technical reasons we were able to identify only part of the isolated strains. The procedure was carried out in accordance with the methods of the WHO Collaborating Centre for Virus Reference and Research (Copenhagen, Denmark). The isolated poliomyelitis viruses were investigated to determine whether they belonged to the wild or vaccine-related types with monoclonal antibody panels (WHO International Laboratory for Biological Standards and Control, UK).

**Hygienic evaluation of water quality.** The results of the water quality surveillance were estimated by the Standards of the USSR No. 2874-82 (1982) and No. 2761-84 (1984), which are in force up to now, taking into consideration also ISO 5667-5 (International Standard, 1991) and the guidelines of the World Health Organisation (1993). Currently the new National Drinking Water Standard is being worked out.

## RESULTS

**Microbiological pollution of the raw water of the Treatment Plant.** The water quality of the rivers running into Lake Ülemiste and the impounding reservoirs is constantly worsening. High levels of faecal pollution by lactose-positive coliforms and enterococci have been detected systematically in the raw water of the Tallinn Water Treatment Plant, but pathogenic enterobacteria have been isolated only sporadically. From 1987 to 1989, 16.6% of the samples contained enteroviruses, including the polioviruses' vaccine strains types 1 and 2, ECHO 18, 22, and *Coxsackie* B5 types. The results of the later investigations (1990—93) showed also that the water of Lake Ülemiste was continuously contaminated with enteroviruses (39.1%), though in this period polioviruses were not detected. Positive results for lecithinase-positive staphylococci were observed in 28.3% of the 184 samples in 1988—93 and in 66% in 1994; 65.1% of the samples contained *Pseudomonas aeruginosa* in the first period of investigation and 44% did in 1994. In 8.3% of the samples, values higher than 1000 CFU per 1000 ml were obtained for *Pseudomonas aeruginosa.*

The Tallinn Water Treatment Plant is able to purify polluted water to the I or the II level (by Standard USSR 2761-84), but the bacterial pollution of the untreated water of Lake Ülemiste exceeds the limits of the III level. In order to make this water drinkable, it is necessary to chlorinate it heavily. On the other hand, as water consumption has been growing rapidly the time of the contact of the water with chlorine is becoming shorter.

**Treated water.** Lactose-positive coliforms were not isolated from 96.67% of the 183 samples of the purified water and enterococci from 98.04%. Pathogenic enterobacteria were never detected. From 1987 to 1989, five cases (8.3%) of polioviruses were registered. During the period of 1990—93 enteroviruses were detected in eight cases (14.5%). The lactose-positive coliforms were estimated to be 147 and 180 CFU per 1000 ml on 7 September 1988 and 4 October 1988, respectively. During the period between these dates lecithinase-positive staphylococci were found in two (36 and 63 CFU per 1000 ml) and *Pseudomonas aeruginosa* in one (6 CFU per 1000 ml) of the five studied samples.

In general, positive results were obtained in 11 samples for lecithinase-positive staphylococci and in 9 for *Pseudomonas aeruginosa.* Therefore, according to the indicators of the faecal pollution, the water quality of some samples met the hygienic requirements, although in this period the water quality was unstable. Occurrence of potentially pathogenic bacteria or enteroviruses is certainly hazardous to human health. Water used by such consumers for drinking or bathing, if it contains chlorine-resistant bacteria, can produce various infections of the skin and the mucous membranes of the eye, ear, nose, and throat.

## DISCUSSION

Contamination of drinking water whether from natural or anthropogenic sources represents a serious public health problem. Infectious agents of all types may be transmitted by the faecal—oral route via water, including viruses (such as enteroviruses, rotaviruses, and Norwalk agent), bacteria (such as cholera, typhoid, and dysentery), and parasites (*Giardia*, *Cryptosporidium*, and *Entamoeba*).

Acute intestinal infections continue to pose serious problems for public health authorities worldwide. Between 1971 and 1985, a specific diagnosis

was reached for only 245 or 50% of the 485 waterborne outbreaks which were recorded in the United States. Ninety outbreaks (19%) were caused by parasites, 59 (12%) by bacteria, 40 (8%) by viruses, and 51 (11%) by chemicals (Tauxe, 1990). For the next 2-year period (1991—92), 17 states and territories reported 34 outbreaks associated with water intended for drinking. The outbreaks caused an estimated 17 464 persons to become ill. A protozoal parasite (*Giardia lamblia* or *Cryptosporidium*) was identified as the etiologic agent for seven of the eleven outbreaks for which an agent was determined. Five (71%) of the outbreaks caused by protozoa were associated with a surface influenced groundwater source. One outbreak of cryptosporidiosis was associated with filtered and chlorinated surface water. *Shigella sonnei* and hepatitis A virus were implicated in one outbreak each; both were linked to consumption of contaminated well water. Two outbreaks were due to acute chemical poisoning. No etiology was established for 23 (68%) of the 34 outbreaks (Moore et al., 1993). In Sweden 32 waterborne outbreaks were reported between 1975 and 1984 (Anderson & Stenström, 1987). In Finland, 17 waterborne disease outbreaks were registered during 1975—87 (Hirn, 1988).

The principal risk to human health associated with the consumption of drinking water in Estonia is microbiological in nature. Between 1945—93 a total of 164 waterborne outbreaks causing illnesses in about 8000 people were reported in Estonia (Fig. 3). *Shigella* was the agent most commonly associated with drinking water, causing 84 outbreaks with 5548 clinical cases. Enteropathogenic *Escherichia coli* and hepatitis A virus caused 5 and 31 outbreaks respectively. In 23 out of 37 typhoid outbreaks the contamination of individual well water was responsible (Pool & Jõgiste, 1993). In samples of water collected in the settlement of Tamsalu during a waterborne outbreak of typhoid fever, *Salmonella typhi* phagotype $A_1$ was found in three samples of water from drilled wells. There was an insufficient protection zone around water sources in many cases, and specified requirements were not met (Birk & Lokk, 1992).

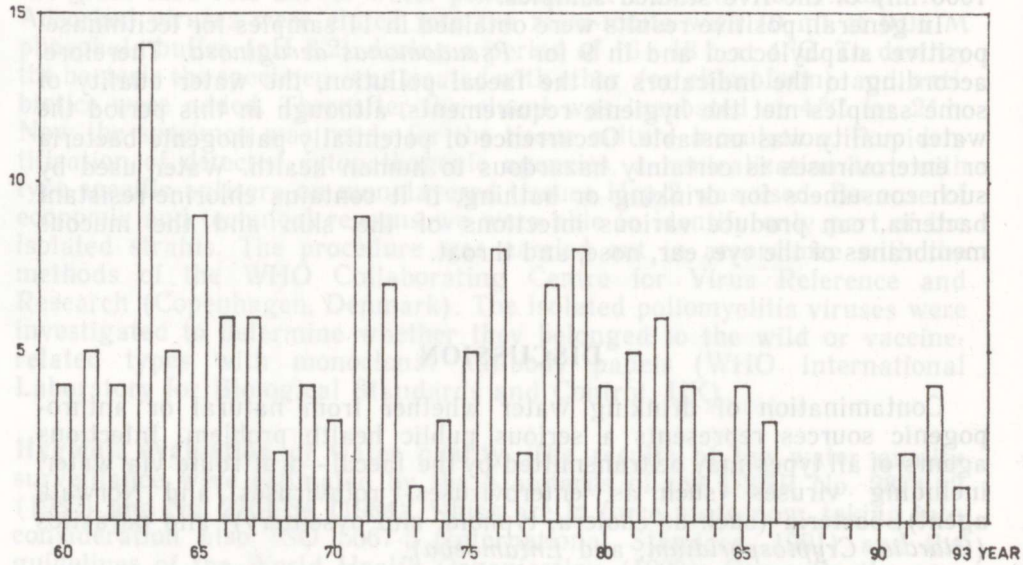


Fig. 3. Waterborne disease outbreaks in Estonia in 1960—93.

Even the smallest deviation in the quality of drinking water can cause outbreaks of diseases among water consumers. So waterborne disease outbreaks began increasing in 1991. The largest outbreak of hepatitis A with 575 affected persons in autumn 1993 was attributed to direct cross-connection between sewage effluent and drinking water at Sõmeru near Rakvere (Kerde, 1994).

Faecal pollution of drinking water may be sporadic and the degree of faecal contamination may be low or fluctuate widely. In communities where contamination levels are low supplies may not carry life-threatening risk, and the population may have used the same source for generations. However, where contamination levels are high, consumers (especially visitors, the very young, the old, and those suffering from some immunodeficiency-related disease, for instance through malnutrition) may be at a significant risk of infection.

Thus the relative importance of drinking water quality to the maintenance of public health may vary due to a number of geographical, social, seasonal, and microbiological factors. What is becoming increasingly clear, however, is that all factors relating to the quality and availability of drinking water are potentially important and must be taken into consideration. In this context it is worth emphasizing that one of the few general conclusions that may be drawn about drinking water quality is that if faecally-derived pathogens are not present, no endemic or epidemic waterborne disease will occur.

Opportunistic pathogens are naturally present in the environment and are not formally regarded as pathogens. They are able to cause disease in people with impaired local or general immuno-defences, such as the elderly or the very young (small children), patients with burns or extensive wounds, those undergoing immunosuppressive therapy. Water used by such patients for drinking or bathing, if it contains these organisms, can produce various infections of the skin and the mucous membranes of the eye, ear, nose, and throat. Examples of opportunistic pathogens of this type include *Pseudomonas aeruginosa* and certain species of *Klebsiella*, *Acinetobacter*, *Aeromonas*, etc.

Kolmos et al. (1993) demonstrated that five patients with extensive deep burns developed septicaemia due to *Pseudomonas aeruginosa* shortly after they had been admitted to hospital and four other burned patients became colonized with the same strains. The source of infection was contaminated tap water used for irrigation of the burns as part of the first-aid treatment which the patients received when entering the hospital. Contamination was restricted to showers and tubing that were permanently connected to the taps, and the outbreaks stopped after these had been disinfected.

However, there have been many outbreaks of disease coinciding with detectable levels of indicator bacteria in drinking water. In addition there have been several incidents where, due to inadequate treatment, disease has occurred even in the absence of observed indicator bacteria. Although such cases are rare, they do indicate the need for vigilance, the undesirability of relaxing the present guideline values (World Health Organisation, 1993) and minimum treatment requirements.

The microbiological quality of tap water and that of water from 50 coolers were comparatively studied in Quebec. Aerobic and facultative anaerobic heterotrophic bacteria, total coliforms, and two indicators for faecal contamination (faecal coliforms and faecal streptococci) as well as three types of pathogenic bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa*, and *Aeromonas* spp.) were enumerated. It was found that they were unable to discern the dominant factors responsible for the contamination of water coolers, but cleaning the water dispenser every two

months seemed to limit the extent of contamination (Levesque et al., 1994).

Drinking water must essentially be free of human enteroviruses to ensure negligible risk of transmitting viral infection. Any drinking water supply subject to faecal contamination presents a risk of viral disease to consumers. Two approaches can be used to ensure that the risk of viral infection is kept to a minimum: providing drinking water from a source verified free of faecal contamination, or adequately treating faecally contaminated water to reduce enteroviruses to a negligible level.

Virological studies have shown that drinking water treatment can considerably reduce the levels of viruses but may not eliminate them completely from water. Virological, epidemiological, and risk analyses are providing important information, although it is still insufficient for deriving quantitative and direct virological criteria. Such criteria cannot be recommended for routine use because of the cost, complexity, and lengthy nature of virological analyses, and the fact that they cannot detect the most relevant viruses. Still, it is necessary to carry out regular virological monitoring of the drinking water and conduct special investigations in the event of accidental contamination.

The most important factor to be taken into account is that in most communities the principal risk to human health derives from faecal contamination. The minimum level of analysis by guidelines of the World Health Organisation (1993) should include testing for indicators of faecal pollution (thermotolerant faecal coliforms), turbidity, chlorine residual, and pH (if disinfection with chlorine is practised).

However, other microbiological indicators which are not associated with faecal pollution may also be urgently needed for the assessment of drinking water quality and especially for the evaluation of the efficiency of water treatment systems. The primary task for the future is to introduce the determination of lecithinase-positive staphylococci and *Pseudomonas aeruginosa* into the water quality surveillance system. These studies are of great importance, as they will allow the determination of potential health hazards to humans, which may be related to drinking water contaminated with chlorine-resistant microorganisms, and, therefore, prevention of these types of waterborne outbreaks.

## CONCLUSIONS

Our data indicate the need for effective water protection measures aimed at minimizing the water pollution problems and the potential health risk associated with various pathogenic and conditionally pathogenic chlorine-resistant microorganisms in raw drinking water.

It was established that the available water disinfection technology used cannot guarantee the epidemiological safety of the drinking water. It is therefore necessary to put into practice supplementary and more effective methods of disinfection, such as ozonization.

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