Research article/Raziskovalni prispevek

ESTABLISHING THE INFLUENCE OF THE IN-HOUSE WATER DISTRIBUTION SYSTEM AND SAMPLING PROCEDURES ON THE MICROBIOLOGICAL QUALITY OF DRINKING WATER

UGOTAVLJANJE VPLIVA HIŠNEGA VODOVODNEGA OMREŽJA IN METODE VZORČENJA PITNIH VOD NA MIKROBIOLOŠKO KAKOVOST PITNIH VOD

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Key words: *drinking water; first flush; in-house water distribution system; Escherichia coli; heterotrophic plate count*

Abstract – Background. *The purpose of this study was to find out how the in-house water distribution system affects the microbiological quality of drinking water and to find a suitable way to evaluate the results of microbiological tests according to the sampling locations, develop methods for establishing the influence of the in-house water distribution system on the quality of drinking water and evaluate the suitability of some procedures for sampling.*

Methods. *The study was conducted in south-eastern Slovenia between October 2003 and October 2004, thereby eliminating the influence of the seasons. Four-hundred-and-sixty-eight samples of drinking water were included in the study.*

Results. *The results of the samples included in this study suggest (p < 0.05) that the first flush samples do not contain Escherichia coli more often than the samples after flushing and the sample from the primary water distribution system. The results also show that the heterotrophic plate count is significantly higher in the first flush samples (p < 0.001).*

Conclusions. *The study demonstrated that the in-house water distribution system affects the microbiological quality of drinking water, and that certain sampling methods significantly affect the test results, and also that knowing the characteristics of the sampling locations is also of significant importance. Some results cannot be analyzed well, therefore additional research is needed.*

Ključne besede: *pitna voda; prva voda; hišno vodovodno omrežje; Escherichia coli; heterotrofne bakterije*

Izvleček – Izhodišča. *Mnogo je dokazov, da lahko interno omrežje spremeni pitno vodo. Odločili smo se, da bomo poskušali: (i) spoznati, kako interno omrežje vpliva na mikrobiološko kakovost pitne vode; (ii) ugotoviti, kako vrednotiti rezultate mikrobioloških preizkušanj glede na mesto odvzema; (iii) razviti metode za preverjanje vplivov internega omrežja na kakovost pitne vode in (iv) potrditi (oceniti) primernost metode vzorčenja, ki se izvaja po internem navodilu za vzorčenje pitne vode in vode za pripravo hrane in pijače, ki je pripravljeno skladno s standardom ISO 5667-5(E). Osrednji cilj naloge je vsekakor boljše razumevanje rezultatov mikrobioloških preizkusov vzorcev pitne vode, odvzetih na pipah uporabnikov. Poleg tega lahko postane uporabljeni model izhodišče za pripravo standardnega postopka za preverjanje vplivov internega omrežja na kakovost pitne vode.*

Metode. *Raziskava je potekala na področju jugovzhodne Slovenije v obdobju oktober 2003 do oktober 2004, s čimer je izničen vpliv letnih časov. Odvzetih je bilo 468 vzorcev pitne vode iz sedmih vodovodov, s katerimi upravlja sedem upravljavcev. Odvzemna mesta za vzorčenje pitne vode so bila določena skladno z zahtevami standarda ISO 5667. Pri vsakem vzorčenju smo odvzeli dva vzorca vode po razkuževanju zunanjosti pipe. Enega takoj po odprtju pipe (prva voda) in drugega po odtakanju vode (vzorec po odtakanju). Na ta način je omogočeno primerjanje rezultatov preskusov parnih vzorcev, pri čemer naj bi prva voda pokazala stanje vode v internem omrežju takoj po odprtju pipe in drugi vzorec naj bi odražal stanje pitne vode, kot jo pripravi upravljavec. Poleg teh dveh vzorcev so vzorčevalci pri vsakem vzorčenju odvzeli še en kontrolni vzorec pitne vode na vodovodnem omrežju, s katerim upravlja upravljavec vodovoda (vzorec iz primarnega omrežja). V vzorcih smo določali Escherichia coli, koliformne bakterije in heterotrofne bakterije, ki rastejo pri 22 °C in pri 36 °C.*

Rezultati. *Preskušenih je bilo 468 vzorcev: (i) 156 vzorcev prve vode; (ii) 156 vzorcev pitne vode po odtakanju in (iii) 156 vzorcev iz primarnega vodovodnega omrežja. Rezultati preiz-*

kusov na odvzetih vzorcih v tej raziskavi so pokazali, da ni mogoče trditi (p < 0,05), da je prva voda bolj pogosto kontaminirana z E. coli oziroma koliformnimi bakterijami kot odtočena voda in voda iz primarnega omrežja. Ugotovili smo, da je bilo povprečno število bakterij pri 22 °C v vzorcih odtočene vode nižje kot v vzorcih prve vode (p < 0,001). Ni pa bilo statistično značilnih razlik v povprečnem številu bakterij, ki rastejo pri 22 °C med vzorci odtočene vode in vzorci vode iz primarnega vodovoda. Povprečno število bakterij, ki rastejo pri 36 °C, pa je bilo (i) statistično značilno nižje v vzorcih odtočene vode v primerjavi z vzorci prve vode (p < 0,001) in (ii) statistično značilno nižje tudi v vzorcih vode iz primarnega vodovoda v primerjavi z vzorci odtočene vode (p < 0,001).

Zaključki. *Raziskava je pokazala, da interno vodovodno omrežje vpliva na mikrobiološko kakovost pitne vode, da metoda vzorčenja (in tehnika izvedbe metode) pomembno vpliva na rezultat in da je poznavanje odvzemnega mesta zelo pomembno.*

V vodi je skoraj vedno nekaj heterotrofnih bakterij, ki rastejo pri 22 °C. Na število teh bakterij statistično značilno vpliva metoda vzorčenja pitne vode. Zaradi tega lahko z vrednotenjem tega parametra sklepamo o kakovosti vzorčenja. Dobra interpretacija rezultata pa je možna le, če je odvzemno mesto dobro poznano. Eden izmed možnih načinov je uvedba kontrolnih kartonov za izbrana odvzemna mesta.

Ugotovili smo, da interno omrežje vpliva na razrast bakterij pri 22 °C in 36 °C. Vprašanje je, ali ta vpliv in posledično povečanje števila bakterij omogoča kakšno sklepanje o za zdravje pomembnih značilnostih pitne vode. Ob statistično pomembnih odstopanjih od povprečnih vrednosti teh parametrov za posamezno odvzemno mesto moramo razmišljati v dveh smereh: (i) bodisi, da se je poslabšala kakovost pitne vode ali (ii) da vzorec ni bil odvzet skladno z navodilom (kriterij za kontrolo kvalitete vzorčenja).

Praktična korist spremljanja števila teh bakterij je skladna z zgornjo dilemo: po eni strani je potrebno preveriti, ali se kaj dogaja s pitno vodo, po drugi strani pa je znak za preverjanje ustreznosti vzorčenja.

Navedeni rezultati raziskave kažejo, da se z izbrano metodo vzorčenja rezultati preskušanj na internem vodovodnem omrežju približujejo rezultatom preskušanj na primarnem vodovodnem omrežju. Zaradi statistično značilnih razlik pri bakterijah, ki rastejo pri 36 °C, ne moremo potrditi, da je metoda popolnoma ustrezna. Potrebno bo nadaljnje raziskovalno delo v dveh smereh: v izboljševanju metode vzorčenja in v preučevanju vplivov internega omrežja na heterotrofne bakterije.

Razumevanje vplivov na mikrobiološko kakovost pitne vode je pomembno tudi z vidika varovanja zdravja ljudi. Ljudi bo potrebno osveščati, da je potrebno pred uporabo vodovodno pitno vodo najprej odtočiti, zlasti če so bile pipe dalj časa zaprte (npr. že čez noč).

Introduction

When the test results of the drinking water samples taken from the in-house water distribution systems (1) do not meet health safety criteria, various questions are raised. The nature of these questions usually reflects the interest of the one who is asking them. The water supplier tries to establish whether the test results truly reflect the quality of the supplied drinking water and which specific elements caused the results. Consumers, on the other hand, are only interested in whether the water is safe and why and how it can pose a health risk to them.

These issues are founded on empirical testing and studies which suggest that drinking water can change or deteriorate in the in-house water distribution system. There is evidence that drinking water can chemically change in-house water distribution system (e.g. dissolving of lead from lead piping or mineral oils that were used during the piping assembly) (2,3). Less is known about the influences of the in-house water distribution system on the microbiological deterioration of drinking water (4). These influences are considered when the test results of the drinking water samples are higher than expected.

Therefore, we conducted a study with the following goals: (i) to establish the influences of the in-house water distribution system on the microbiological quality of drinking water; (ii) to establish how the microbiological test results can be evaluated according to the specific sampling location; (iii) to develop a suitable model to recognize the influences of the inhouse water distribution system on the drinking water quality; (iv) to verify/assess the suitability of our internal operating procedure for water sampling, which is based on the ISO 5667- 5(E) standard (5).

The overall purpose of this research is to gain additional knowledge of what microbiological test results of the samples taken from the consumers' taps mean. In addition, a model could be prepared as a starting point for developing an operating procedure for estimating the influences of the in-house water distribution system on the drinking water quality.

Methods

Waterworks and sampling locations

Seven waterworks run by seven different water suppliers (Table 1) were included in this study. They are the largest waterworks that are run by individual water suppliers. These waterworks were selected for the study based on long-term data from these waterworks to eliminate bias from specific features of the systems. These waterworks are continually minitored by our institution and are well characterized. Between 1999 and 2003 drinking water from these waterworks met the health safety criteria.

Table 1. *The water suppliers and waterworks included in this study.*

Razpr. 1. *Preskrbovalci z vodo in vodovodi, ki so obdelani v študiji.*

* – number of people consuming drinking water from individual water distribution systems

število ljudi, ki uporabljajo pitno vodo iz individualnih vodooskrbnih sistemov

Symbols: \checkmark sampling carried out / odvzem vzorca, · no sampling / brez odvzema vzorca

First, sampling locations and sampling frequency were chosen for all waterworks (Table 2). Sampling locations were selected in accordance with the ISO 5667 standards which contain guidelines for the selection of sampling locations (5). Permission was gained from the consumer (the owner) or the water supplier. All sampling sites allowed paired sampling. We wanted to collect samples throughout the year to avoid influence of the seasons (6).

A description of each sampling location was prepared. The location of the sampling point, preparation procedures (disinfection, flaming, flushing, mesh removal, etc.) and duration of flushing were included. Unless the sampling location required otherwise, the water from the pumping site and water storage tank was flushed for at least two minutes, and the water from other sampling locations was flushed for at least three minutes.

Drinking water samples

At the selected sampling locations (consumers' taps) two samples were taken: the first one immediately after opening of the tap (the first flush) preceded only by disinfection of the exterior tap surfaces to eliminate the possibility of contamination ('the first flush sample'), and the second one following the flushing ('sample after flushing'). All of these procedures were carried out in accordance with the ISO 5667-1:1980 standard (5). This enables the comparison of the test results of the paired samples, where the first flush sample indicates the condition of the water in the in-house water distribution system, while the second sample (after flushing) reflects the condition of the drinking water as it is supplied by the water supplier. Aside from these two samples, an additional sample was taken from the water distribution system, ie. water storage tank or pumping site ('the sample from the primary water distribution system'). Based on these test results, an analysis of the drinking water from the sampling locations was made. The results were used also to verify how successful the application of the ISO 5667-1:1980 standard is (it could also prove to be useful in assessing the suitability of these standards) (5). Altogether 162 first flush samples were analyzed. This is also shown in Table 2 together with the number of samples taken from each water distribution system and the sampling locations used. Only 156 samples (96.3%) out of 162 samples taken were included in the study. The other six samples were excluded because one sample of the pair was not taken. Therefore, 468 samples were included in this study: 156 first flush samples, 156 samples taken after flushing and 156 samples from the primary water system.

Test methods

Microbiological methods

The microbiological parameters and methods are shown in Table 3. They were based on the Drinking Water Act (1).

Razpr. 3. *Mikrobiološki parametri in metode testiranja.*

Statistical methods

To determine the significance of the differences t-test (Student's t distribution) (7) was used on means of the dependent paired samples (one-tailed test).

Results

In total 468 samples were analyzed: (i) 156 first flush samples; (ii) 156 samples after flushing; (iii) 156 samples from the primary water system.

Escherichia coli was found in seven samples (1.5% of all samples): in four first flush samples (2.6% of all first flush samples), in two samples after flushing (1.3% of all samples after flushing) and in one sample from the primary water system (0.6% of all such samples).

Observed differences were not significant ($p \le 0.05$), therefore we can not say that the first flush contains *E. coli* more often than water after flushing or water from the primary water system.

Coliform bacteria were found in 13 samples (2.8% of all samples): in seven first flush samples (4.5% of all first flush samples), in three samples after flushing (1.9% of all samples after flushing) and in three samples of the water taken from the primary water system (1.9% of all such samples). The conclusion here is the same as with *E. coli:* the difference is not significant ($p \le 0.05$), therefore we can not say that the first flush contains coliform bacteria more often than water after flushing or water from the primary water system.

Samples were analyzed for heterotrophic plate count (HPC) at 22 °C and 36 °C (in accordance with the regulations) (1,8). Bacteria that grow at 22 °C were found in: (i) all first flush samples (100% of all first flush samples); (ii) 151 samples after flushing (96.8% of all samples after flushing); (iii) 151 samples from the primary water distribution system (96.8% of all such samples).

The presence of these bacteria is typical for drinking water samples included in this study. The question is whether their count significantly varies from one sampling location to another and from one sampling method to another.

The comparison of HPC counts at 22 °C in the first flush samples and samples after flushing showed that the difference is significant (t-test, $p \le 0.001$). Based on these results, the assumption that there are no differences in the arithmetic mean between these types of samples was discarded ($p \le 0.001$). The conclusion was that the mean HPC count at 22 °C was lower in samples after flushing than in first flush samples.

The differences in the mean HPC count at 22 °C in the samples after flushing and samples from the primary water distribution system were not significant (p < 0.05).

Bacteria that grow at 36 °C were found in: (i) 155 first flush samples (99.4% of all first flush samples); (ii) 152 samples after flushing (97.4% of all samples after flushing); (iii) 150 samples from the primary water distribution system (96.2% of all such samples).

Statistical tests showed that the differences in HPC counts at 36 °C between sample types were significant. We found a significant difference between the first flush samples and samples after flushing (p < 0.001) and between samples after flushing and samples from the primary water distribution system $(p < 0.001)$. Therefore, we can claim that the mean HPC count at 36 °C: (i) was significantly lower in samples after flushing compared to first flush samples; (ii) was significantly lower in samples from the primary water distribution system compared to samples after flushing.

Discussion

The study was conducted over a large area in south-eastern Slovenia over a period of more than a year. In this way, influences of the specific features of municipal water systems and seasons were eliminated.

We believe analytical procedures starting with sampling can introduce considerable bias when assessing water quality.

Therefore, there is a need for research that would analyze the microbiological quality of water also through the uncertainty of the methodology. Other published paper that compare microbiological quality of water from supplier to consumer do not clearly state the methods of sampling. Hence, this discussion focuses only on data gathered in this research (5,9)

E. coli **and Coliform bacteria**

Based results of this study, we can say that when *E. coli* and/ or coliform bacteria are found in the samples from the internal water distribution system, fecal contamination originating from fecal contamination of the primary water distribution system is the cause. Therefore, when drinking water is contaminated with *E. coli* and/or coliform bacteria, an inappropriate processing and supply of the water should be the first thing to be considered and adequate measures are to be taken.

This applies to carefully chosen and examined sampling locations. In other cases (e.g. new sampling locations), typical features of the sampling location should be analyzed first.

HPC counts at 22 °C

The results confirm that drinking water almost always contains some heterotrophic bacteria growing at 22 °C (they were absent only in 2.1% of 468 samples). The results also indicate that the concentration of these bacteria is significantly affected by the sampling method. Therefore, the purpose of the sampling is of great significance.

A proper interpretation of the HPC counts at 22 °C is only possible if the sampling location is well known. A good way to achieve this is to introduce control charts for the sampling locations (or even for the entire waterworks if the typical interval can be established). An example is shown in Figure 1. Therefore it is important to assess each result descriptively (e.g. if it meets the health criteria) when comparing various waterworks or even different water testing locations of the same waterworks.

Figure 1. *Control chart for the evaluation of the results for HPC count at 22 °C. Values under the red line are expected – they represent 97.6% of all values assuming normal distribution. Higher values should be assessed in terms of the validity of the sampling procedure and water safety.*

Sl. 1. *Kontrolni karton za vrednotenje rezultata poskusa na število bakterij, ki rastejo pri 22 °C. Vrednosti do rdeče linije so normalne – zajemajo 97,6% vseh vrednosti pri normalni porazdelitvi. Višje vrednosti je treba oceniti, ali gre za nepravilno vzorčenje ali pa je vzorec pitne vode zdravstveno neustrezen.*

The study demonstrated that there are no significant differences in the HPC counts at 22 °C in the samples after flushing and samples from the primary water distribution system.

Therefore, this parameter can be used to assess the quality of sampling from the internal distribution system.

HPC counts at 36 °C

This study indicates that significant differences in these counts are associated directly with the sampling method as well as the sampling location. There is a significant difference between the HPC counts at 36 °C of the samples after flushing and the samples from the primary water distribution system. There are two explanations for this: (a) the sampling method where the duration of the flushing depends on the stabilization of the flushed water temperature is unsuitable, and (b) the internal water distribution system affects the bacteria concentration. Results of the samples after flushing can be evaluated with the help of a control chart shown in Figure 1 or another one, based on differences in HPC counts between samples after flushing and samples from the primary water distribution system.

Control of tap use

One of the largest biases in this study was the uncontrolled use of taps before sampling, since the use of taps before sampling was not recorded. In further research the use of taps before sampling should be well defined. Taps should to be opened until the temperature stabilizes, after that the tap should be closed and opened after a pre-determined amount of time (e.g. after six hours or as is described elsewhere (e.g. 4).

This bias does not diminish the importance of the conclusions reached, as it reflects real life, where people use water when they need it. This bias only hinders the repeatability of the study.

In case of unexpected test results

We have shown that the internal water distribution system affects the HPC counts at 22 °C and 36 °C. The question remains whether conclusions about the health safety of drinking water can be made based on this observed influence. When results of the HPC parameters appear, which are significantly different compared to the average values, the following should to be considered: (i) whether the quality of drinking water has deteriorated; (ii) whether the sampling complied with the procedure (quality control).

Therefore, implications of unexpected HPC counts are twofold: the drinking water has to be re-tested to determine safety and the sampling procedure also has to be reviewed.

Assessment of the sampling procedure

The sampling method chosen (our internal operating procedure for sampling) is used to provide samples for assessment of quality of drinking water supplied to the consumer by the water supplier. This method is used for sampling drinking water at taps of final consumers as well as for sampling at locations within the water distribution system. Therefore, the results of this study will become a valuable tool in assessing the suitability of our sampling method.

There were no significant differences ($p < 0.05$) in HPC counts at 22 °C between the results of the samples taken at the consumers' tabs after flushing and the results of the samples from the primary distribution water system. However, HPC counts at 36 °C were significantly higher in samples taken from the consumers' tabs after flushing compared to samples taken from the primary water distribution system. Differences in *E. coli* and coliform counts were not significant (all three sample groups, $p \le 0.05$) and therefore cannot be used for evaluation of the sampling procedure.

All HPC counts were lower in the samples after flushing compared to first flush samples ($p \le 0.05$).

In summary, the study indicates that the test results of the samples from the internal water distribution system obtained following our internal operating procedure may approximate the results of the samples from the primary water distribution system. However, there are significant differences in HPC at 36 °C between those samples, which show that these samples may not be truly equivalent. Additional research should to be carried out to improve the sampling method and establish the influences of the internal water distribution system on HPC.

Conclusions

In summary, the study results have confirmed that the in-house water distribution system affects the microbiological quality of drinking water. The sampling method has a significant effect on the test results and good knowledge of sampling locations is also of great importance. Simple control charts for at least key sampling locations are a useful tool for the assessment of the results of microbiological tests.

Understanding the influences on the microbiological quality of drinking water is of great importance for health safety reasons. The general population should be instructed to flush tap water before consumption, especially if taps have been shut for a long period of time (e.g. overnight).

References

- 1. Pravilnik o pitni vodi, Ur. l. RS 19/04 (01.03.2004) in 35/04 (09.04.2004) (Available at: http://zakonodaja.gov.si/rpsi/r03/predpis_PRAV3713.html).
- 2. Svetovna zdravstvena organizacija. Guidelines for drinking water quality. 2nd ed., vol 2: Health criteria and other supporting information. Ženeva: WHO, 1996.
- 3. Conway JB. Water quality management. In: Wallace RB, Doebbeling BN eds. Maxcy-Rosenau-Last – Public health and preventive medicine. 14th ed. Stamford: Appleton & Lange, 1998: 737–63.
- Pepper IL, Rusin P, Quintanar DR, Haney C, Josephson KL, Gerba CP. Tracking the concentration of heterotrophic plate count bacteria from the source to the consumer's tap. Int J Food Microbiol 2004; 92: 289–95. (Available at: doi:10.1016/j.ijfoodmicro.2003.08.021).
- 5. International organization for standardization. ISO 5667 series Water quality – Sampling.
- Bartram J, Cotruvo J, Exner M, Fricker C, Glasmacher A. Heterotrophic plate count measurement in drinking water safety management. Report of an expert meeting Geneva, 24-25 april 2002. Int J Food Microbiol 2004, 92: 241–7 (Available at: doi:10.1016/j.ijfoodmicro.2003.08.005).
- 7. Košmelj B, Rovan J. Statistično sklepanje. Ljubljana: Ekonomska fakulteta, 2000; 222–4.
- 8. Medicinska fakulteta Univerze v Ljubljani, Inštitut za slovenski jezik Frana Ramovša Znanstvenoraziskovalnega centra SAZU, Zdravniška zbornica Slovenije. Slovenski medicinski slovar. Ljubljana: Medicinska fakulteta, 2002.
- Sartory DP. Heterotrophic plate count monitoring of treated drinking water in the UK: a useful operational tool. Int J Food Microbiol 2004, 92: 297-306 (Available at: doi:10.1016/j.ijfoodmicro.2003.08.006).