COMPARATIVE STUDY OF METHODS FOR ASSESSING *ESCHERICHIA COLI* SPECIES IN DRINKING WATER

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Abstract: *The study on drinking water quality is a priority for the health of consumers, the absence of pathogenic microorganisms is a prerequisite for compliance with EU Directive. Coliforms, fecal coliforms and Escherichia coli are used as indicators of fecal contamination of water supplies and recreational waters.The most important bacteriological condition for drinking water is the total absence of pathogenic microorganisms. The measurement of bacteria of the coliform group has been used extensively as an indicator of water quality.Given the relatively laborious methods of recognizing their presence and the inconsistent nature of that presence in water, the Escherichia coli level is analysed. The purpose of this study is to analyze comparatively two methods for determining the Escherichia coli species in drinking water of the drinking water supply network of Suceava city and that of own resources within Suceava county area. The analysis results of water samples studied by the two methods show that the membrane filtration method favours a specific growth and selective Escherichia coli species, compared with the multiple-tube method. Membrane filtration method is recommended for effective monitoring of drinking water contamination in terms of Escherichia coli species. Multiple-tube method is a method applicable to all types of water, and equipment is relatively inexpensive and less specialized.*

Keywords: *contamination, prevention, environment, monitoring*

1. Introduction

Drinking water microbiological quality is primarily determined by using "indicator organisms", whose presence indicates faecal contamination. The presence of the indicators is often a key in assessing potential public health risks due to pathogens and is used in drinking water quality regulations and guidelines in many countries [2, 9].

Escherichia coli is the dominant flora of the large intestine having an important role in maintaining its normal physiology and in the synthesis of proteins of group B and K. If eliminated in the external environment with faeces, it gets into water, soil, food contamination etc. Constant presence of *Escherichia coli* strains in human and animal intestines and faeces have turned these bacteria into an indicator of faecal pollution of the environment, especially water [4]. The presence of *Escherichia coli* species in water indicates recent faecal contamination.

Escherichia coli is a negative gram bacillus, which grows on simple medium in which glucose is the only organic constituent. It is an aerobic, facultative anaerobic that may have both respiratory and fermentative metabolism. On solid media it grows in the form of „S"-type colonies, and in liquid environment it causes uniform disturbances and slip ring on the tube wall [3, 7].

Escherichia coli is a bacterium that resides in high numbers in the intestines of warmblooded animals and has proven its

value to detect fecal contamination in water [5].

From biochemical point of view *Escherichia coli* ferments glucose and other carbohydrates producing acid and gas. Most strains are negative oxidase, able to reduce nitrites to nitrates. There is no urease production, proteins are not broken down leading to formation of H_2S, and citrate is not used as sole carbon source. Lactose is broken down with acid release, and proteins lead to formation of indole with a positive methyl-red reaction [8].

Contamination source is man and animal, material support of transmission being represented by water, food, hand, hospital items, teys, flies etc. The gateway varies: the digestive tract, respiratory, urinary, genital etc. The *Escherichia coli* pathotypes responsible for extraintestinal infections are uropathogenic E. coli and meningitis- associated E. coli. *Escherichia coli* from these pathotypes can cause hemolytic uremic syndrome, urinary tract infection, newborn meningitis and sepsis. The intestinal pathogenic *Escherichia coli* strains belong to the pathotypes enterotoxigenic *Escherichia coli*, enteropathogenic *Escherichia coli*, enteroinvasive *Escherichia coli*, enterohemorrhagic *Escherichia coli*, enteroaggregative *Escherichia coli* and diffusely adherent *Escherichia coli*[1, 5]. These pathotypes have been associated with cases of mild and severe diarrhea in adults and children, mostly in developing countries and it subsequentially determines the type of contamination that is installed. Prevention of contamination with *Escherichia coli* requires compliance with collective, personal and food hygiene. Thus, the most important bacteriological condition for drinking water is the total absence of pathogenic microorganisms. Given the relatively laborious methods of recognizing their presence and the inconsistent nature of that presence in water, the *Escherichia coli* level is analysed [6].

The paper presents the comparative analysis of two methods for determining the *Escherichia coli* species in drinking water of the drinking water supply network of Suceava city and that of own resources within Suceava county area.

2. Experimental

The study used 30 water samples from Suceava city's drinking water network and around 10 water samples from own sources of drinking water within Suceava county area for 6 months (January-June 2010). Water sampling was carried out as follows: 250 cm^3water samples to test the network and 500 cm^3 fountain water samples for multiple tubes method, respectively 250 cm^3 samples for membrane filter method.

Water sampling for microbiological diagnosis was made in sterile containers prepared in the laboratory, aiming to avoid contamination with the external environment. Transport of samples was performed in insulated containers and was kept in a refrigerator at 4°C, max.24 hours.

The analysis methods used complied with the requirements of SR EN ISO 8199/2008, SR EN ISO 9308-1/2004; AC-2009.

The study determined the estimated number of *Escherichia coli*/100 cm^3 sample by a membrane filtration method (method 1) and by multiple tube method (method 2).

For membrane filtration method we used commercially available culture media: agar tergitol -7 with 2,3,5-triphenyltetrazolium chloride (TTC), tryptophan broth, Kovacs reagent, oxidase reagent.

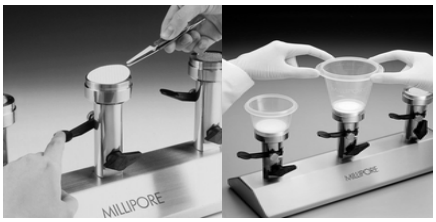


Fig. 1. Work equipment for membrane filtration method

Membrane filter was examined and we counted as lactose-positive bacteria all characteristic colonies (regardless of their size) which develop a yellow colour in the membrane medium. *Escherichia coli* suspicious colonies were counted after undergoing the procedure for confirmation: subculturing suspicious colonies on agars medium for oxidases test and on tryptophan broth medium for indole test, incubation effected at 36°C respectively 44°C for 24 h; then the confirmatory tests were performed (oxidase, indole). Finally, *Escherichia coli* colonies were counted (only those colonies that give a positive indole test (fig. 2) and negative oxidase test (fig. 3).

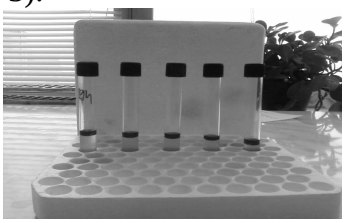
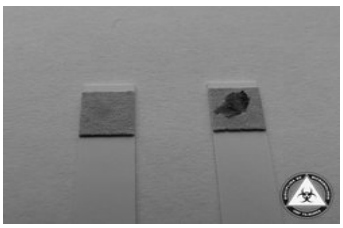


Fig 2. Positive indole test



a b

Fig.3. Negative oxidase test (a – oxidazo-negative bacteria; b - oxidazo-positive bacteria)

Confirmed colonies were counted and the results of determinations were calculated according to SR EN ISO 8199/2008. The results were interpreted in accordance with Law no. 458/2002 and Law no.311/2004.

For multiple-tube method we used: sterile buffered water, lauryl broth seeding medium (simple), concentrate lauryl sulphate broth seeding medium and McConkey confirmation medium.

After incubation at 37°C for some time, the formation of acid and gas from lactose fermentation is investigated. To confirm the presence of coliforms group further tests must be carried out using another selective culture medium.

To specify whether the microorganisms that fermented the lactose are coliforms and not other microbial species equipped with the same properties (lactobacilli, fungi), each vial or test-tube found positive in McConkey test can be substituted with lauryl sulphate broth.

The presence of *Escherichia coli* is confirmed when characteristic colonies have developed: flat colonies of blue-violet metallic luster, or raised, opaque, metallic luster mucous in the middle, or having a pink or violet-blue centre.

Determining the number of coliform bacteria in 100 cm^3 sample was carried out with tables „Calculating the most probable number per 100 cm^3 sample" laid down by ISO 8199/1998 (E).

3. Results and Discussion

The results of measurements performed in water samples taken from drinking water network of the city of Suceava are summarized in Table 1.

Table 1.
Results of determinations of *Escherichia coli* species in drinking water samples from Suceava city network

Crt. No.	Month 2010	No. *Escherichia coli*/100 cm^3 sample	
		Membrane filter method	Multiple-tubes method
1.	January	25 6	16 <1
2.	February	16 16 30 16 16 38	9 9 16 10 9 >18

3.	March	16 16	15 12
4.	April	16 9 2	15 8 <1
5.	May	25	>18
6.	June	42 10 26 8	16 4 12 2

In table 1, one can notice that by using the membrane filter method (method 1), the same samples, and the number of *Escherichia coli* is higher in comparison with multiple-tube method (method 2).

This is explained by the fact that the membrane filter method favours a specific growth and selective *Escherichia coli* species, compared with the multiple-tube method. It can be seen that the highest microbial contamination with *Escherichia coli* species was in February 2010 – 20% of the samples and the lowest contamination, in May 2010 to 3.33% of the samples (fig. 4).

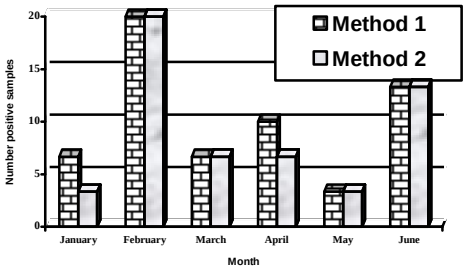


Fig. 4. Share of total number of positive samples of drinking water samples taken from Suceava city network during January-June 2010

Table 2.
Results of determinations of *Escherichia coli* species from own drinking water samples (Suceava county)

Crt. No.	Month 2010	No. *Escherichia coli*/100 cm^3 sample	
		Membrane filter method	Multiple-tubes method
1.	January	56 7	26 9
2.	February	26 64 45	>18 46 22
3.	March	24 24	17 14
4.	April	12	7
5.	May	42 8 6 2	26 2 2 <1
6.	June	42 8 6 2 12	26 2 2 <1 9

The results of measurements performed in water samples taken from own sources of drinking water in Suceava county area during the six months (January-June 2010) are shown in Table no. 2.

By analyzing the data shown in Table 2, we noticed a higher contamination with *Escherichia coli* species from samples taken from own sources of drinking water compared with samples from Suceava city network. It is obvious that such water does not comply with current legislation for drinking water, its consumption possibly affecting consumer health. In rural Romania, organized health services are almost inexistent, the transportation to storage facilities being carried out individually by consumers. Uncontrolled waste, especially from livestock (manure) contributes to microbiological and chemical pollution of groundwater and surface water in these areas.

The highest microbial contamination with *Escherichia coli* species was in June 2010 – 50% of the samples and the lowest contamination, in April 2010 – 10% of total samples analyzed (Figure 5).

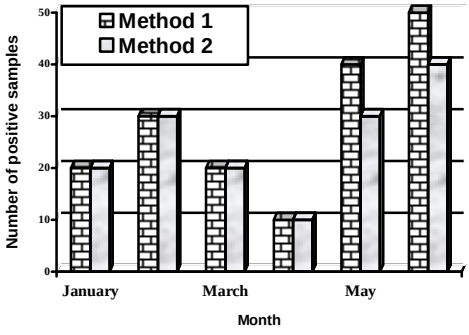


Fig. 5. Share of total number of positive samples of drinking water samples from own sources (Suceava) Suceava city network in the period analyzed

4. Conclusion

The study on drinking water quality is a priority for the health of consumers, the absence of pathogenic microorganisms is a prerequisite of compliance with EU Directive (Directive 98/83/EEC).

The monitoring of drinking water's contamination with *Escherichia coli* species was performed by two common methods: the method of membrane filtration and multiple-tube one.

The analysis results of water samples studied by the two methods show that the membrane filtration method favours a specific growth and selective *Escherichia coli* species, compared with the multiple-tube method. This is explained by the fact that the medium used for the membrane filter is optimal for increasing the *Escherichia coli* species than the medium used for multiple-tube method.

Therefore, the following conclusion can be drawn: the membrane filtration method is recommended for effective monitoring of drinking water contamination in terms of *Escherichia coli* species.

There are still cases when the membrane filtration method has limitations, especially if water samples have a high turbidity or noncoliform bacteria. For such water it is desirable to carry out parallel tests with multiple-tube method to demonstrate applicability and comparability.

Multiple-tube method is a method applicable to all types of water, and the equipment is relatively inexpensive and less specialized.

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