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Microbiological Research of Mineral and Mountain Spring Waters in Bulgaria

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

In Bulgaria is observed a great variety of spring waters. They are mineral and mountain spring waters. According to their temperature they can be cold (up to 37 °C), warm (from 37°C to 60 °C) and hot (over 60 °C). This is Bulgarian standard for the qualification of waters according to their temperature.

The mountain spring waters are cold with temperature up to 25 °C.

By their chemical composition they fall into three categories – low mineralized (up to 2 g/L), moderately mineralized (2 to 15 g/L) and highly mineralized (15 – 30 g/L). According to their chemical composition the mineral waters are divided into sulphate, sulfide, hydrogen carbonate, chloride and carbonic. With regards to their gas composition they are nitrogen, sea and carbonic waters.

The waters that correspond to the requirements of Decree No. 14 regarding the resort resources, resort localities and resorts, have balneological parameters.

The main objective of the current review is to show springs, which have been examined by microbiological parameters and are in compliance with Ordinance No. 9 / 2001, Official State Gazette, issue 30, and decree No. 178 / 23.07.2004 regarding the quality of water intended for drinking and household purposes, and Decree № 14 regarding the resort resources, resort localities and resorts.

Keywords: Mineral waters; mountain spring waters; microbiological parameters.

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1. INTRODUCTION

In the current study mineral waters and mountain spring waters from mountain regions of Bulgaria have been studied. It is well known that in the mountain areas of Bulgaria live the most of long-living people and centenarians. The studies are conducted by microbiological laboratory of Trakia University, Stara Zagora headed by Nedyalka Valcheva, accredited laboratory Eurotest Control, and the laboratory of Scientific Research Center of Medical Biophysics. Analyses of the following bacteria were performed – *Total Coliforms*, *Escherichia coli*, *Sulphite reducing anaerobic bacteria (Clostridium perfringens)*, *Pseudomonas aeruginosa*.

We analyze springs, which were examined in respect of microbiological composition and correspond to Ordinance No. 9 / 2001, Official State Gazette, issue 30, and decree No. 178 / 23.07.2004 regarding the quality of water intended for drinking and household purposes, and Decree No. 14 regarding the resort resources, resort localities and resorts.

2. MATERIALS AND METHODS

In the research was performed microbiological tests of 91 mountain and mineral springs in the following regions – Plovdiv, Haskovo, Stara Zagora, Sliven, Burgas, Varna, Sofia, Yambol, Pazardzhik, Lovech, Pleven. The basic studies were made from Nedyalka Valcheva.

2.1 Nutrient Media

1. Nutrient agar (MPA) with contents (in %) – meat water, peptone – 1%, agar – agar – 2%. Endo's Medium (for defining of *Escherichia coli* and *coliform* bacteria) with contents (g/dm³) – peptone– 5,0; triptone– 5,0 ; lactose – 10,0 ; Na₂SO₃ – 1,4 ; K₂HPO₄– 3,0 ; fuchsine– 0,14 ; agar – agar– 12,0 pH 7,5 – 7,7.
2. Nutrient gelatine (MPD) (for defining of *Pseudomonas aeruginosa*) with contents (in%) – Peptic digest of animal tissue; 25 % gelatin ; pH = 7, 0 – 7, 2.
3. Medium for defining of enterococci (esculin – bile agar).
4. Medium for defining of sulphite reducing bacteria (Iron Sulfite Modified Agar).
5. Wilson-Blair medium (for defining of sulphite reducing spore anaerobes (*Clostridium perfringens*) with contents (g/dm³) – 3% Nutrient agar; 100 cm³20%

solution Na₂SO₃ ; 50 cm³ 20% glucose solution; 10 cm³ 8% solution of Fe₂SO₄.

2.2 Methods for Determination of Microbiological Indicators

1. Methods for evaluation of microbiological indicators according to Ordinance No. 9 / 2001, Official State Gazette, issue 30, and decree No. 178 / 23.07.2004 about the quality of water, intended for drinking purposes.
2. Method for determination of *Escherichia coli* and coliform bacteria – BDS EN ISO 9308 – 1: 2004;
3. Method for determination of enterococci – BDS EN ISO 7899 – 2;
4. Method for determination of sulphite reducing spore anaerobes – BDS EN 26461 – 2 : 2004;
5. Method for determination of total number of aerobic and facultative anaerobic bacteria – BDS EN ISO 6222 : 2002;
6. Method for determination of *Pseudomonas aeruginosa* – BDS EN ISO 16266 : 2008.
7. Determination of coli – titer by fermentation method – Ginchev's method
1. Determination of coli – bacteria over Endo's medium – membrane method.
8. Determination of sulphite reducing anaerobic bacteria (*Clostridium perfringens*) – membrane method.

2.3 Media for Research of *Escherichia coli*, *Total Coliforms*, *Enterobacter aerogenes* and *Clostridium perfringens*

The presence of Total Coliforms and *Escherichia coli* is determined by the membrane method (membrane filtration) and according to Ginchev's method (fermentation method). In both methods the results are equally positive – presence of large number of *Total Coliforms* and *Escherichia coli*.

Ginchev medium includes (g/dm³): peptone – 10,0 ; lactose – 20,0 ; NaCl – 4, 0 ; Na₂HPO₄·12H₂O – 0,50 ; (NH₃)₂SO₄ – 1,0 ; K₂SO₄ – 2,0 ; MgSO₄ – 0,5 ; 3-bromylpyrrolidine-2,5-dione C₄H₄ BrNO₂ – 0,04, pH 7,2 -7,4 [1,2,3,4,5].

Endo medium is with Suspend 41 g in 1 litre of distilled water. Add 4 ml of 10% w/v alcoholic solution (96% ethyl alcohol) of basic fuchsin (Cat. No. 47860). Bring to boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes.

Mix well before pouring. The assessment of ammonia-induced cell envelope injury in *E. coli* and *Enterobacter aerogenes* [6,7]

Endo medium contents (g/dm³) – peptone– 5,0; triptone– 5,0 ; lactose – 10,0 ; Na₂SO₃ – 1,4 ; K₂HPO₄– 3,0 ; fuchsine– 0,14 ; agar – agar– 12,0 pH 7,5 – 7,7.

Wilson-Blair medium (for defining of sulphite reducing spore anaerobes (*Clostridium perfringens*) is with contents (g/dm³) – 3% Nutrient agar; 100 cm³20% solution Na₂SO₃; 50 cm³20% glucose solution; 10 cm³8% solution of Fe₂SO₄ [8].

3. RESULTS AND DISCUSSION

Microbiological research is conducted of mineral and mountain springs in Northern and Southern Bulgaria.

Table 1. shows bacteria during study and microbiological parameters

Type of bacteria	Norm	Limit value
<i>Escherichia coli</i>	100	cfu/cm ³
Total Coliforms	100	cfu/cm ³
<i>Clostridium perfringens</i>	100	cfu/cm ³
<i>Pseudomonas aeruginosa</i>	100	cfu/cm ³

Table 2. Shows the springs by regions, which correspond to Ordinance № 9 / 2001, Official State Gazette, issue 30, and decree № 178 / 23.07.2004

Region	Spring
Sliven	Sliven Mineral baths; Hadji Dimitar, Shivachevo; Banya; Gunchov Spring, Karandila locality, Sliven; Nova Zagora
Varna	Drilling No P83-St.St. Konstantin and Elena; P-1x-Aquarium; P-106 x Dom Mladost; P-161xPrimorski;
Burgas	Burgas Mineral Baths; Shivarovo; Polyanovo; Drilling No B73-Medovo; Drilling No B73-Kamenar;
Yambol	Karavelovo; Stefan Karadzho;v;
Haskovo	Drilling No. 2VP, Drilling No. 3VP, KEI No. 5
Stara Zagora	Drilling No. K-3, Ovoshtnik, Drilling No. SZ-37, Yagoda; Trakia, St. Nikolay; Holly Mother of God; Center Maglij; Kazanlak; Kran-Enina; Ayazmo; Trite Chuchura; Pavel Banya - Drilling No. SZ-7; Drilling No. SZ-8; Drilling No. X-19; Drilling No. 3;
Plovdiv	Drilling No. 16-Lenovo; Drilling No. 1-Asenovgrad; Badjova voda. Hisar-Key Momina Banya; Kei Momina Salza; KEI Stublata; KEI Toplitsata; Svejest; Bistritsa; Bancheto Miromir; Choban Chesma; Chair Banya; Drilling No. 1; Staro Zhelezare Drilling No. 2; Staro Zhelezare Drilling No. 4; Drilling No. 3; Drilling No. 5; Drilling No. 6; Drilling No. 7; Parilkite; Bulgarian Rose; Narechenski bani -KEI Banski Kaptaj; Soleno izvorche. Banya – KEI Tsentralen Kaptaj; Jensko Banche; Drilling No. 1- Kokalche; Drilling No. 8 - Dragoyново; Drilling No. 8
Pazrdzhik	No. 2 -Vetren dol; Strelcha Velingrad – Drilling No. 5 Syarna banya; Drilling No. 3 Mizinka; Drilling No. 7 – Veliova banya; Varvara - Drilling No. 3 - Varvara; Drilling No. 5 - Varvara; Drilling No. 6 =Varvara. Banya – KEI No. 1 - Bancheto; KEI No. 2-Vetren dol; KEI No. 1 - Bancheto;
Sofia	Drilling No. 1- Ivanyane; Drilling No. 3- Gorna Banya; KEI Pchelinski bani; Sofia - Center
Pleven	Dolni Dabnik - Gradina
Lovech	Teteven - Dolnata cheshma; Gornata cheshma; Sondata; Klindiovo; Babintsi; Gechovoto. Golyam izvor - Tulyushovets; Krivina Troyan – Shipkovo; Chiflik. Letnitsa – Krushuna

Table 3. Shows microbiological results from waters in the region of Sliven

Indicators	Measuring unit	Thermal mineral spring Sliven Mineral baths, t=48°C	Mountain spring "Hadji Dimitar" Shivachevo, t=22,5°C	Thermal mineral spring Banya village, t=37°C	Mountain spring "Gunchov Spring", Karandila locality, Sliven, t=21.5 °C
Coliforms	cfu/cm ³	0/100	0/100	0/100	4/100
<i>Escherichia coli</i>	cfu/cm ³	0/100	0/100	0/100	4/100
Enterococci	cfu/cm ³	0/100	8/100	0/100	0/100
Sulphite reducing anaerobic bacteria (<i>Clostridium perfringens</i>)	cfu/cm ³	0/100	0/100	0/100	0/100
<i>Pseudomonas aeruginosa</i>	cfu/cm ³	0/250	0/250	0/250	0/250

In Southern Bulgaria are examined springs in the regions of Plovdiv [9,10], Haskovo [11,12], Stara Zagora [13], Sliven [14], Burgas [15], Blagoevgrad [16].

In Northern Bulgaria are examined mineral springs in the regions of Varna [17,18], Lovech [19] and Pleven. In Northern Bulgaria there is a great variety of mountain spring waters. The highest number of springs tested is in municipalities of Teteven [20], Yablanitsa [21] and Ugarchin [22], Lovech region.

The properties of mountain spring water are owed to its purity from snow and ice melting [23]. One of its unique features is the availability of additional energy among the hydrogen bonds in their transition from a solid to a liquid phase [24]. Examined water with such properties is without any presence of pathogenic micro-organisms. One of the secrets of longevity is the microbiological purity of the water and the availability of the following minerals – Calcium (Ca), Magnesium (Mg), Zinc (Zn) and Manganese (Mn) [25,26].

In many areas the long-livers and centenarians consume mineral water rich in Potassium (K) and Sodium (Na) [27,28]. The six indicated minerals support the balance in the human body and metabolism, and antioxidant effects occur [29,30].

Based on the conducted physicochemical and microbiological evaluations it is established that from the four examined springs at the territory of Sliven district, Bulgaria [7] only non-thermal spring "Hadji Dimitar" locality "Hot water" town Shivachevo corresponds to all controlled parameters according to Ordinance No. 9 / 2001, Official State Gazette, issue 30, and decree No. 178 / 23.07.2004 about the quality of water,

intended for drinking purposes. With regards to microbiological parameters thermal healing water Sliven Mineral baths, Banya mineral source and non - thermal spring "Hadji Dimitar" are in compliance with the requirements for drinking water.

4. CONCLUSION

In the present article is presented analysis of studies with regards to microbiological parameters of mineral and mountain spring waters in Bulgaria, conducted by Laboratory in microbiology, Trakia University, Bulgaria with a leader Nedyalka Valcheva.

The following bacteria, which are including in Ordinance No. 9 / 2001, Official State Gazette, issue 30, and decree No. 178 / 23.07.2004 were tested - *Total Coliforms*, *Escherichia coli*, *Sulphite reducing anaerobic bacteria (Clostridium perfringens)*, *Pseudomonas aeruginosa*.

In Southern Bulgaria are examined springs in the regions of Plovdiv, Haskovo, Stara Zagora, Sliven, Burgas, Yambol. Pazardzhik, Blagoevgrad.

Performed are specific microbiological studies of springs from Southern Bulgaria.

In Northern Bulgaria are examined mineral springs in the regions of Varna, Lovech and Pleven. In Northern Bulgaria there is a great variety of mountain spring waters. The highest number of springs tested is in municipalities of Teteven, Yablanitsa and Ugarchin, Lovech region.

The purity of stated springs complies with Ordinance No. 9/2001, Official State Gazette,

issue 30, and decree No.178 /23.07.2004 regarding the quality of water intended for drinking and household purposes, and Decree № 14 regarding the resort resources, resort localities and resorts.

The waters that correspond to Ordinance No. 9 / 2001 are suitable for drinking consumption.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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