Original paper

Sanitary state of surface waters in Świętokrzyskie voivodeship

Stan sanitarny wód powierzchniowych w województwie świętokrzyskim

Wioletta Adamus-Białek, Monika Wawszczak, Aneta Filipiak, Aleksandra Woźniak, Paulina Jasek, Stanisław Głuszek

Department of Surgery and Surgical Nursery with Genetics Laboratory, Faculty of Medicine and Health Sciences, Jan Kochanowski University, Kielce, Poland
Head of the Department: Prof. Stanisław Głuszek MD, PhD

Medical Studies/Studia Medyczne 2019; 35 (1): 10–15
DOI: https://doi.org/10.5114/ms.2019.84046

Key words: surface waters, epidemiological risk, microbiological indicators.

Słowa kluczowe: wody powierzchniowe, ryzyko epidemiologiczne, wskaźniki mikrobiologiczne.

Abstract

Introduction: Water is a convenient environment for the spread of pathogenic bacteria. Their presence may result from contact with water through soil, air, and animal and human activity. The identification of microorganisms allows us to determine the biological properties, the possible epidemiological risk, and the sanitary condition of the surface water.
Aim of the research: To analyse the presence of microbiological indicators in selected water samples collected in autumn, winter, and spring.
Material and methods: The research sites included three rivers and two water reservoirs located in Świętokrzyskie voivodeship. The water samples were tested based on the presence of coliform, mesophilic, and psychrophilic bacteria.
Results: The presence of E. coli was confirmed in all water reservoirs, but values above the standard limit were confirmed in just three cases (Wisła, Bernatka, and Emerald Lake). The results show that the changing microbiological parameters of the analysed water samples can result from different factors. The large number of bacteria in water can come from other local streams and groundwater, human and animal activity, and especially from influx of domestic wastewater. The sanitary state of surface water in Świętokrzyskie is consistent with data from other Polish voivodeships published in the literature.
Conclusions: Microbiological pollution of surface water in Poland is a constant and underestimated problem. It can be a serious epidemiological threat, especially in mild winters and warmer summers.

Streszczenie

Wprowadzenie: Woda jest dogodnym środowiskiem do rozprzestrzeniania się bakterii chorobotwórczych. Ich obecność może wynikać z kontaktu wody z glebą, powietrzem, zwierzętami i z działalności człowieka. Identyfikacja mikroorganizmów pozwala określić właściwości biologiczne, ryzyko epidemiologiczne i stan sanitarny wód powierzchniowych.
Cel pracy: Analiza obecności wybranych wskaźników mikrobiologicznych (ogólna liczba bakterii mezofilnych i psychrofilnych, obecność *Escherichia coli*, miano *coli*) w próbkach wody pobranych jesienią, zimą i wiosną z pięciu różnych zbiorników.
Materiał i metody: Badaniami objęto 3 rzeki i 2 zbiorniki wodne położone w województwie świętokrzyskim. Próbki wody testowano na obecność bakterii *coli* oraz ogólnej liczby bakterii mezofilnych i psychrofilnych.
Wyniki: Obecność *E. coli* została potwierdzona we wszystkich zbiornikach wodnych, ale normy były przekroczone w trzech przypadkach (Wisła, Bernatka i Szmaragdowe Jezioro). Wyniki pokazują, że zmiana parametrów mikrobiologicznych analizowanych wód może wynikać z różnych przyczyn. Duża liczba bakterii w wodach może pochodzić z innych, lokalnych strumieni i wód gruntowych, aktywności zwierząt i ludzi, zwłaszcza z napływu domowych ścieków. Stan sanitarny wód powierzchniowych w województwie świętokrzyskim jest zgodny z danymi z innych polskich województw opublikowanymi w piśmiennictwie.
Wnioski: Zanieczyszczenie mikrobiologiczne wód powierzchniowych w Polsce jest problemem stałym i bagatelizowanym. Może stanowić poważne zagrożenie epidemiologiczne, zwłaszcza w czasie łagodnych zim i coraz cieplejszych okresów letnich.

Introduction

Many bacterial species from different biotopes are often identified in surface water. It is due to contact with soil, air, rainfall, and natural microflora of animals and people. Contaminants get into the water most often through human activities like microbiological pollution via industrial waste and wastewater from food companies and households. Microorganisms occurring in water determine their biological properties and the sanitary condition. In densely populated and industrial areas, polluted air plays a very important role, e.g. bacterial plankton suspended in the air in the form of bioaerosols or bacterial fluid that penetrates via rainfall to the surface water. A characteristic feature of these bacteria is their virulence, by which they can infect specific tissues of humans and animals. Typical pathogenic bacteria in water are usually motile, like cylindrical, spiral, or comma-shaped bacterium. Staphylococci, streptococci, enterobacteria *Enterobacteriaceae*, *Helicobacter pylori*, *Enterobacter* sp. are also very common in contaminated waters. Water polluted by excrement increases the risk of spread of pathogenic microorganisms like *Salmonella* spp., *Shigella* sp., *Campylobacter* sp., *Vibrio* sp., *Legionella* sp., and *Escherichia* sp. [1–5]. *Escherichia coli* is the most important indicator of faecal pollution due to a representative role in intestinal microflora, but they can also be dangerous [6]. These bacteria survive in drinking water for between four and 12 weeks, depending on environmental conditions (temperature, microflora, etc.). Pathogenic *E. coli* strains mostly cause intestinal diseases, but extraintestinal infection are also common, like uropathogenic *Escherichia coli* strains, which cause urinary tract infections. One of the most dangerous are Shiga toxin-producing *E. coli* strains (STEC) [7]. They may lead to life-threatening diseases, including haemolytic uremic syndrome (HUS), especially in young children and the elderly. The waterborne transmission of STEC has been reported, both from contaminated drinking-water and from recreational waters [8]. The problem of insufficient water quality is present not only in African countries but also a lot of European countries have polluted water. This issue is reflected in many government programs and the promotion of environmental protection in the interests of public health. However, public awareness of how to protect health against environmental infectious agents is still poor.

Aim of the research

The aim of the study was to analyse the presence of basic microbiological indicators in the selected water samples collected in autumn, winter, and spring. In our study we present the evidence of microbiological pollution of surface water in Świętokrzyskie voivodeship during the years 2009–2011. The water samples were tested for the presence of coliform, mesophilic, and psychrophilic bacteria.

Material and methods

Study sites and sample collection

The study sites included three rivers (Nida, Bernatka, Wisła) and two water reservoirs (Emerald Lake, Pińczów) located in Świętokrzyskie voivodeship (Table 1). The samples of water were collected in sterile bottles from a depth of 30 cm from the surface of the water during autumn, winter, and spring in 2009–2011. The reservoirs were selected based on occasional contact with the local community and the use of reservoirs for recreational purposes.

Bacterial identification

The 100 µl water samples were plated by spread plate method on selective media: Yeast agar (Biomed) for the total number of mesophilic and psychrophilic bacteria. Plates were incubated at 37°C (mesophilic bacteria) and 22°C (psychrophilic bacteria) for 24–48 h, and the number of bacteria in water was estimated based on the number of grown CFUs (colony-forming units) on the plates. Additionally, the number of coliforms was determined by fermentation tube method. Samples of water were aliquoted to a broth containing lactose and bromocresol purple (LPB, Ejkman broth). The set of five tubes with Durham tubules were supplemented with 10 ml of broth, and one flask was supplemented by 50 ml of broth. All tubes and flask were filled with the same volume of water sample. The cultures were incubated at 37°C for 24 h. A positive result was a change of broth colour from purple to yellow and the presence of gas bubbles in Durham tubes. In the case of negative results, the cultures were incubated for another 24 h.

Table 1. The characteristics of research locations

Reservoir/river	Spillway weir coordinates	Date of collection			Number of stands
		Autumn	Winter	Spring	
Nida	N 50°31’07.5” E 20°30’32.5”	27.10.2010	4.01.2011	3.06.2010	1
Pińczów	N 50°31’10.1” E 20°30’50.9”	27.10.2010	4.01.2011	3.06.2010	1
Wisła	N 50°49’36.1” E 21°51’16.6”	19.11.2009, 3.12.2009	14.01.2010	Not tested	3
Bernatka	N 51°06’19.1” E 20°50’59.0”	30.10.2010	2.03.2011	1.06.2010	3
Emerald Lake	N 50°51’27.8” E 20°38’31.7”	24.10.2010	9.01.2011	Not tested	2

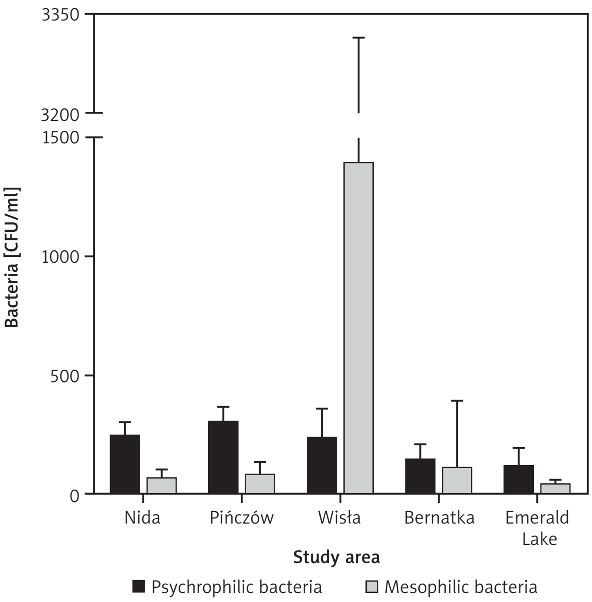

Figure 1. The total number of mesophilic and psychrophilic bacteria in water of studied reservoirs

The most probable number (MPN) of coliform bacteria present was estimated from the number of positive tubes obtained in the confirmatory test, using specially devised statistical tables. Also, the 100 µl water samples were incubated on MacConkey agar to confirm the presence of coliform. All large, red colonies surrounded by turbid zone were cultured on Endo agar to identify the *E.coli* strains [9]. Analyses were performed according to the Polish Committee of Standardisation Norms PN-EN ISO 9308-3 and PN-EN ISO 9308-1. The norms are included in the Regulations of the Minister of Health of 17 January 2019 on the supervision of the quality of bathing water and places occasionally used for bathing.

Results and discussion

The research of the selected water reservoirs and rivers included the analysis of the presence of different bacterial indicators in the water. There are many standardised methods designed for sanitary analysis of water [10–13]; however, we applied only two basic analyses: the total number of mesophilic and psychrophilic bacteria and the total number of coliforms. The results represent a general sanitary condition to indicate the potential epidemiological risk to public health. Samples were collected from five study areas of four different locations in Świętokrzyskie voivodeship (Table 1). The Nida and Pińczów reservoirs were located in the southwest, in the valley of Nida, close to hills. These attractive surroundings are often visited by locals and tourists in the summertime. The river Wisła is located in the eastern border of the voivodeship, near to Zawichost – the small village, located on a flat area. There is also a ferry across the Wisła, which may have an impact on the river environment. It is also visited mainly by tourists in summertime. Bernatka is located in Skarżysko-Kamiennain in the northeast of the Świętokrzyskie voivodeship. This river from which the samples were taken is a mountain river with a rapidly changing water level during heavy rain. The local streams that flow into the river also have an influence on the changing river parameters. The last water reservoir – Emerald Lake – is located in Kielce, the capital of świętokrzyskie. The reservoir is located in a nature reserve, which includes the hill of a sealed mine Wietrznia and the adjacent Międzygórze, being an extension of the Kielce Kadzielnia ridge. It is visited often by inhabitants throughout the year [14]. Summarising, all of the studied water reservoirs are recreational places and therefore they should be subjected to routine sanitary monitoring.

The average total number of mesophilic and psychrophilic bacteria in water samples is shown in Figure 1. The psychrophilic and mesophilic bacteria were present in all cases; however, there were more psychrophilic bacteria, and their number remained constant throughout the year. This is quite because of the low annual temperature of water in Poland. The number of mesophilic bacteria varied widely among the researched places. The most variable results were observed in the Wisła, where the highest value of mesophilic bacteria was 4500 CFU/ml. The lowest value was observed in Emerald Lake and Nida river. Similar studies of water reservoirs in Świętokrzyskie voivodeship were presented in our previous study [15]. The five different reservoirs were investigated based on the presence of mesophilic and psychrophilic bacteria. The number of mesophilic bacteria was in the same range (20–600 CFU/ml), whereas the number of psychrophilic bacteria was much higher, and in some cases they were 20,000 CFU/ml. This value may prove the extensive water eutrophication. The number of psychrophilic and mesophilic bacteria is not a standard determinate for surface water, but it could be used as a further indicator of organic matter contamination and as a source of microorganisms with potentially high adaptive properties [16].

The main indicator of faecal contamination is the number of *E. coli* CFU per 100 ml [17]. The occurrence of *E. coli* was analysed on the chromogenic agar by counting specific colonies, and the total number of bacteria was estimated in 100 ml of water. We also analysed the presence of coliforms on the Ejkman broth via the lactose fermentation and released gas. The obtained results (Table 2) were compared to each other and, referenced to the Regulation of the Minister of Health (2019), the limit value of the tested water for recreational purposes is less than 1000 CFU (or MPN) of *E. coli* per 100 ml of water and less than 400 CFU (or MPN) of *Enterocuccus* sp. per 100 ml. The faecal contamination of water was detected in all cases; how-

Table 2. The average number of *E. coli* based on typical colony-forming units (CFUs) and the most probable number (MPN) of coliform in 100 ml of tested water. Bold – value over the norm according to the Regulation of the Minister of Health [17]

Place of sampling	*E. coli* [CFU/100 ml]			MPN of coliform [MPN/100 ml]		
	Autumn	Winter	Spring	Autumn	Winter	Spring
Nida	Not tested	Not tested	Not tested	3	13	16
Pińczów	0	600	0	6	16	9
Wisła	**30,300**	0	Not tested		Not tested	
Bernatka	**1200**	600	700	4	4	11
Emerald Lake	**6950**	**5950**	Not tested	15	11	Not tested

ever, exceeding the norm according to the regulation was observed in Wisła, Bernatka, and Emerald Lake, especially during autumn. The presented results were varied in terms of place and time of measurement. Amounts of bacteria above the stabdard limit could be caused by the presence of sewage from households in the area. Additionally, the number of microorganisms in autumn could be caused by the high temperature, which favours the persistence of mesophilic bacteria in water. In the case of Emerald Lake, the high number could be explained by the specific parameters of the reservoir. Emerald Lake is characterised by low water flow, which may have an influence on the accumulation of pollutants. Secondly, it is located in a nature reserve, where wild animals are present, and the natural microflora may contaminate the water. The microbiological condition of Pińczów and Nida seems to be most stable based on the estimated number of *E. coli*; on the other hand, the MPN of coliforms was highest in these waters. The applied methods, despite the fact that they detect similar microbiological indicators, gave varied results. This may be due to the rapidly changing microbiological parameters of the tested waters and the varied sensitivity of the microbiological growth mediums and the lack of analysis at the same time. The observed contamination could be due to inflow of groundwater, which may have contact with adjoining farmlands fertilised with natural products. In all cases apart from Emerald Lake, the surrounding households are not equipped with sewage systems, which has a major influence on the high number of *E. coli* and others faecal bacteria. Faecal contamination of water is a potential source of intestinal pathogens such as *Salmonella* and *Shigella* species, which may cause epidemic and serious infections. It is extremely relevant because of the serious threat of contaminated water entering the gastrointestinal tract, e.g. after swallowing by people swimming in the examined water reservoirs and rivers [18]. Our previous results [15] also included the analysis of the total number of *E. coli*. The number of *E. coli* in one reservoir was high in spring (average 40 CFU/ml). In the present case, the number of coliforms was significantly high in all reservoirs. The widespread and continuous presence of coliform bacteria in water reservoirs indicates continuous contamination with faeces. The water reservoirs are monitored by sanitary stations; however, this issue seems not to be very attractive among the scientific community in Poland. The study by Wolny-Koładka (2016) included, besides *E. coli* and coliforms, also *E. faecalis*, *C. perfringens*, *Staphylococcus* spp., and *Salmonella* spp. The presence of the analysed bacteria was correlated with the air and water temperature, as well as with the recreational use of water during the holidays. Because of the high prevalence of the microbiological indicators, poor water quality was specified, and the author concluded that it is reasonable to include the Nowohucki Reservoir into a constant sanitary monitoring programme [19]. Augustyn *et al.* (2016) also included a wider range of microbiological analyses: coliforms, thermo-tolerant coliform, faecal enterococci, and *Salmonella* spp., in the Wisłoka river. They concluded that human-origin contamination had the main impact on the sanitary condition of the studied waters. However, their study was dedicated to estimating the water parameters and factors influencing them in the context of drinking water [20]. Most of the published research of sanitary analysis of water from various sources comes from countries with poor hygiene standards and highly contaminated water, and with insufficient resources [19, 21–27]. All authors emphasise that the quality and quantity of water in general all over the world is insufficient for people. The absence or presence of indicators in water does not always have to correlate with presence or absence of pathogenic microorganisms, and their presence does not always pose a public health risk [28]. Nevertheless, we cannot forget about the existing threat, and we should consider ways to improve the sanitary quality of water in Poland [11, 29]. Poland is among the countries with insufficient water resources, which are characterised by significant seasonal variation and uneven territorial distribution [30]. Among the 139 lakes analysed in 2010 only 4% had a very good ecological status (class I). Lakes with moderate ecological status of water (quality class III) accounted for 50% of all monitored lakes. Lakes with poor ecological status class V accounted for 9% of the examined lakes. Wastewater discharge to surface water is still noted due to the lack of a sewage system. In 2012, it was noted that sewage treatment plants support only 69% of the population in a country that mainly inhabits urban areas [30]. The sanitary infrastructure in Poland is improving every year; however, the activities of the Ministry of Health and the Environment and the State Sanitary Inspectorate seems to be insufficient to control the spread of pathogens in water. The basic and often the only analysis applied in sanitary and epidemiological stations is the assessment of the occurrence of faecal microorganisms, mainly *Escherichia coli* and *Enterococcus* sp. These analyses included the routine monitoring only of water reservoirs classified as recreational by the European Union.

Summarising, there can be many sources of water contamination, starting from contact with the natural microflora of animals and people, and ending with insufficient municipal waste management. In many small villages in Poland there is no sewage system, and the contaminants enter the soil next to the ground water and surface water. It is a potential source of pathogens, which may cause serious gastrointestinal tract diseases. The presence of pathogenic bacteria in surface waters that are used for recreational purposes could be dangerous, especially for children and adults with immunodeficiency. The most common pathogens are, e.g. *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, and *Escherichia coli*. In recent years an increase in antibiotic resistance to bacteria isolated from the environment has been observed. It is obvious that this may cause complications in the treatment of waterborne infections [31]. Other bacterial pathogens such as *Legionella* sp., *Aeromonas* sp., *Pseudomonas aeruginosa*, and *Mycobacterium avium* are indigenous aquatic organisms that can both survive and proliferate in water [32]. It is important to educate people about the risk of waterborne infection, as well as wastewater management. Increased financing for the development of sewage systems in small towns and increased supervision of the sanitary condition of water in reservoirs used for recreational purposes have an important influence on the protection of the environment and public health.

Conclusions

Taking into account our preliminary and general research, it can already be said that the estimated sanitary condition of the studied water reservoirs was contaminated by faecal pollution and can be dangerous for human health. The studied places are often visited by people, so these places should be monitored or marked as biohazards.

Acknowledgments

We would like to thank students Joanna Niedziela-Wąsacz, Katarzyna Wąsowska, Remigiusz Ziewiecki, and Monika Żurawska for their assistance in the experimental work.

This work was supported by the program of the Minister of Science and Higher Education under the name "Regional Excellence Initiative in 2019-2022", project number: 024/RID/2018/19, financing amount: 11,999,000.00 PLN

Conflict of interest

The authors declare no conflict of interest.

References

1. Gromiec M, Sadurski A, Zalewski M, Rowiński P. Zagrożenia związane z jakością wody. Nauka 2014; 1: 99-122.
2. Lenaker PL, Corsi SR, Borchardt MA, Spencer SK, Baldwin AK, Lutz MA. Hydrologic, land cover, and seasonal patterns of waterborne pathogens in Great Lakes tributaries. Water Res 2017; 113: 11-21.
3. Lee GC, Jheong WH, Kim MJ, Choi DH, Baik KH. A 5-year survey (2007-2011) of enteric viruses in Korean aquatic environments and the use of coliforms as viral indicators. Microbiol Immunol 2013; 57: 46-53.
4. Stypułkowska-Misiurewicz H, Czerwiński M. Legionellosis in Poland in 2016. Przegl Epidemiol 2018; 72: 143-147.
5. Lemaitre J, Pasetto D, Perez-Saez J, Sciarra C, Wamala JF, Rinaldo A. Rainfall as a driver of epidemic cholera: comparative model assessments of the effect of intra-seasonal precipitation events. Acta Tropica 2019; 190: 235-243.
6. Martin NH, Trmcic A, Hsieh TH, Boor KJ, Wiedmann M. The evolving role of coliforms as indicators of unhygienic processing conditions in dairy foods. Front Microbiol 2016; 7: 1549.
7. Edberg SC, Rice EW, Karlin RJ, Allen MJ. Escherichia coli: the best biological drinking water indicator for public health protection. Symp Ser Soc Appl Microbiol 2000; 29: 106S-116S.
8. Gruber JS, Ercumen A, Colford JM. Coliform bacteria as indicators of diarrheal risk in household drinking water: systematic review and meta-analysis. PLoS One 2014; 9: e107429.
9. Mwanamoki PM, Devarajan N, Thevenon F, Atibu EK, Tshibanda JB, Ngelinkoto P, Mpiana PT, Mubedi JI, Kabele CG, Wildi W, Pote-Wembonyama J. Assessment of pathogenic bacteria in water and sediment from a water reservoir under tropical conditions (Lake Ma Vallée), Kinshasa Democratic Republic of Congo. Environ Monit Assess 2014; 186: 6821-6830.
10. Bartram J, Rees G. Monitoring Bathing Waters – A Practical Guide to the Design and Implementation of Assessments and Monitoring Programmes. 2000.
11. Jones T, Gill CO, McMullen L. The behaviour of log phase Escherichia coli at temperatures below the minimum for sustained growth. Food Microbiol 2002; 19: 83-90.
12. Pickup RW, Rhodes G, Hermon-Taylor J. Monitoring bacterial pathogens in the environment: advantages of a multilayered approach. Curr Opin Biotechnol 2003; 14: 319-325.
13. Saxena G, Bharagava RN, Kaithwas G, Raj A. Microbial indicators, pathogens and methods for their monitoring in water environment. J Water Health 2015; 13: 319-339.

14. Klatka T, Mojski JE, Rlihle E. Zarys chronostratygrafii. 1980; 569: 689-710.
15. Adamus-Bialek W, Karwacka K, Bak L. Microflora of the selected water reservoirs in Swietokrzyskie Voivodship. Acta Biochim Polonica 2013; 60: 689-693.
16. Nedwell DD. Effect of low temperature on microbial growth: lowered affinity for substrates limits growth at low temperature. FEMS Microbiol Ecol 1999; 30: 101-111.
17. Rozporządzenie Ministra Zdrowia w sprawie prowadzenia nadzoru nad jakością wody w kąpielisku i miejscu wykorzystywanym do kąpieli. Dz. U. z 2011 r. Nr 86 poz. 478.
18. Sivaraja R, Nagarajan K. Levels of indicator microorganisms (total and fecal coliforms) in surface waters of rivers Cauvery and Bhavani for circuitously predicting the pollution load and pathogenic risks. Int J Pharm Tech Res 2014; 6: 455-461.
19. Wolny-Koładka K. Assessment of microbiological quality of water in the Nowohucki Reservoir with particular regard to microorganisms potentially dangerous to humans. Environ Med 2016; 19: 19-26.
20. Augustyn Ł, Babula A, Joniec J, Stanek-Tarkowska J, Hajduk E, Kaniuczak J. Microbiological indicators of the quality of river water, used for drinking water supply. Pol J Environ Stud 2016; 25: 511-519.
21. Miah MB, Majumder AK, Latifa GA. Evaluation of microbial quality of the surface water of Hatirjheel in Dhaka City. Stamford J Microbiol 2017; 6: 30-33.
22. Onyango AE, Okoth MW, Kunyanga CN, Aliwa BO. Microbiological quality and contamination level of water sources in Isiolo County in Kenya. J Environ Public Health 2018; 2018: 2139867.
23. Kanyerere T, Levy J, Xu Y, Saka J. Assessment of microbial contamination of groundwater in upper Limphasa River catchment, located in a rural area of northern Malawi. Water SA 2012; 38: 581-596.
24. Bouchalová AM, Wennberg A, Tryland I. Impact of rainfall on bathing water quality – a case study of Fiskevollbukta, Inner Oslofjord, Norway. Vann 2013; 4: 491-498.
25. Pitkänen T, Karinen P, Miettinen IT, Lettojärvi H, Heikkilä A, Maunula R, Aula V, Kuronen H, Vepsäläinen A, Nousiainen LL, Pelkonen S, Heinonen-Tanski H. Microbial contamination of groundwater at small community water supplies in Finland. Ambio 2011; 40: 377-390.
26. Zamxaka M, Pironcheva G, Muyima NYO. Microbiological and physico-chemical assessment of the quality of domestic water sources in selected rural communities of the Eastern Cape Province, South Africa. Water SA 2004; 30: 333-340.
27. Tornevi A, Bergstedt O, Forsberg B. Precipitation effects on microbial pollution in a river: Lag structures and seasonal effect modification. PLoS One 2014; 9: e98546.
28. Wu J, Long SC, Das D, Dorner SM. Are microbial indicators and pathogens correlated? A statistical analysis of 40 years of research. J Water Health 2011; 9: 265-278.
29. ECDC. Reporting on 2011 Surveillance Data and 2012 Epidemic Intelligence Data. 2013.
30. Central Statistical Office. Concise Statistical Yearbook of Poland 2013.
31. Wellington EMH, Boxall ABA, Cross P, Feil EJ, Gaze WH, Hawkey PM, Johnson-Rollings AS, Jones DL, Lee NM, Otten W, Thomas CM, Williams AP. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. Lancet Infect Dis 2013; 13: 155-165.
32. Leclerc H, Schwartzbrod L, Dei-Cas E. Microbial agents associated with waterborne diseases. Crit Rev Microbiol 2002; 28: 371-409.

Address for correspondence:

Wioletta Adamus-Białek MD, PhD
Department of Surgery and Surgical Nursery
with Genetics Laboratory
Faculty of Medicine and Health Sciences
Jan Kochanowski University
Kielce, Poland
E-mail: aloiv2002@wp.pl