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ESCHERICHIA COLI AND OTHER GROUPS OF OPPORTUNISTICALLY PATHOGENIC AND PATHOGENIC BACTERIA IN THE OFFSHORE WATER OF THE LAKE WIGRY

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ESCHERICHIA COLI A WYBRANE GRUPY BAKTERII OPORTUNISTYCZNIE PATOGENNYCH I PATOGENNYCH W WODZIE KĄPIELISK JEZIORA WIGRY

W sezonie letnim (od czerwca do września) 1995–1999 prowadzono badania składu ilościowego i jakościowego bakterii z rodziny *Enterobacteriaceae* (w tym *Escherichia coli* i *Salmonella* sp.) oraz bakterii potencjalnie chorobotwórczych *Aeromonas hydrophila*, *Pseudomonas aeruginosa* i *Staphylococcus* sp. w wodzie 8 wybranych kąpielisk jeziora Wigry. Bakterie *Aeromonas hydrophila* występowały w wodzie wszystkich kąpielisk, najliczniej zaś w wodzie pobieranej z miejsc o zwiększonej trofii. Bakterie *Pseudomonas aeruginosa* izolowano rzadko, niezależnie od czasu i miejsca poboru prób. Bakterie z rodzaju *Salmonella*, gatunku *Escherichia coli* i *Staphylococcus aureus* stwierdzano odpowiednio w 37 (22,6%), 68 (42,4%) i 90 (55%) na ogólną liczbę 160 przebadanych prób wody kąpielisk jeziora Wigry. Stwierdzane w wodzie badanych kąpielisk (zwłaszcza w miejscowościach Stary Folwark, Leszczewek, i Rosochaty Róg) bakterie *Salmonella* sp., *Staphylococcus aureus* i *Aeromonas hydrophila* (nawet przy braku bakterii z gatunku *Escherichia coli*) sugerują, iż ocena przydatności wód jeziora Wigry dla celów kąpielowych na podstawie liczebności bakterii grupy coli typu kałowego może nie odzwierciedlać w pełni jej bezpieczeństwa dla kąpiących się. Wyniki przeprowadzonych badań sugerują konieczność uwzględnienia w ocenie przydatności wód powierzchniowych jeziora Wigry dla celów rekreacji (zwłaszcza kąpieli) badań na obecność *Salmonella* sp., *Staphylococcus aureus*, *Aeromonas hydrophila* i *Pseudomonas aeruginosa*. Ostatnie 3 gatunki niezwiązane bezpośrednio z zanieczyszczeniami kałowymi bywają, bowiem przyczyną różnorodnych schorzeń skórnych, jamy nosowo-gardzielowej, oczu, ucha środkowego i szeregu innych spotykanych wśród kąpiących się w zanieczyszczonej wodzie.

Summary

Studies were carried out in the summer seasons of 1995–1999 (from June to September) on the quantitative and qualitative composition of *Enterobacteriaceae* bacteria (including *Escherichia coli* and *Salmonella* sp.), and potentially pathogenic bacteria *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Staphylococcus* sp. in the water of 8 bathing sites of the Lake Wigry. *Aeromonas hydrophila* occurred in the all samples of studied water and was the most numerous in water sampled from sites of increased trophic levels. Irrespective of the site and time of sampling *Pseudomonas aeruginosa* was rarely isolated.

In the total of 160 samples of water analysed *Salmonella* sp., *Escherichia coli* and *Staphyloccocus aureus* were determined in 32 (22.6%), 68 (42.4%) and 90 (55%) samples, respectively. Pathogenic bacteria of the genus *Salmonella* sp. and potentially pathogenic *Staphylococcus aureus* and *Aeromonas hydrophila* identified in the analysed offshore waters (also when *Escherichia coli* were absent) suggest that the use of the Lake Wigry waters for swimming, falling into account faecal bacterial counts, may not be sufficient to fully reflect safety conditions for bathers. The results of the research suggest that the evaluation of the Lake Wigry surface waters for recreational use should include the frequency of the occurrence of *Salmonella* sp., *Staphylococcus aureus*, *Aeromonas hydrophila* and *Pseudomonas aeruginosa*. These three species, which are not directly linked to faecal contamination, can cause various diseases of the skin, nasal and oral cavities, eyes, internal ear and other problems in people swimming in contaminated water.

INTRODUCTION

A variety of potentially pathogenic bacteria can contaminate surface waters together with faecal bacteria, excreted to the environment by ill people, animals or by carriers. The main source of contamination of surface waters is constituted by faecal sewage. A series of diseases in people drinking potable water or bathing in rivers, lakes, ponds or seas as well as in swimming pools is attributed to the presence of such potentially pathogenic and pathogenic bacteria as those in the genera *Pseudomonas*, *Aeromonas*, *Staphylococcus*, *Salmonella* [18, 22], bacteria of the species *Helicobacter pylori* [17, 34], *Campylobacter jejuni*, *Vibrio cholerae*, *Yersinia enterocolitica*, genus *Shigella*, as well as enteroviruses, reoviruses, rotaviruses and protozoa *Entamoeba histolytica*, *Cryptosporidium* and *Giardia lamblia* [14]. Mariño [20] detected a relationship between skin inflammations in bathers and the occurrence of pathogenic microorganisms in water as *Pseudomonas aeruginosa*, *Candida albicans* and *Aeromonas hydrophila*. There are ample cases quoted in the literature regarding the effect of pathogenic organisms in water reservoirs on humans and animals. According to Clark *et al.* [8], high counts of *Salmonella typhimurium* in a water reservoir in Gideon, Missouri, caused food poisoning in 600 persons; 4 fatal cases were recorded. *Shigella* caused 41 cases of dysentery among people swimming in the Mississippi river near Dubuque, Iowa [29]. Apart from food poisoning, many microorganisms acquired from swimming in water can cause skin problems, illnesses of upper respiratory tract, genitourinary tract and, sporadically, meningitis or sepsis [13].

Assessment of bacteriological quality of surface waters using classic sanitary tests (number of *Escherichia coli*, faecal *Escherichia coli* and faecal *Enterococci*) can be insufficient to reflect the actual risk of using the water for washing, drinking or other domestic activities [23, 30]. Therefore, it seems advisable to analyze the occurrence of pathogenic microorganisms in addition to the sanitary and bacteriological monitoring of the water reservoir. No relationship among bacteria of the genera *Salmonella*, *Yersinia*, *Campylobacter* or *Aeromonas* and the bacteria used as indices of faecal contamination has been confirmed by analyses of the waters of the Foglia and Metauro rivers in Italy [25].

MATERIALS AND METHODS

AREA STUDY

Lake Wigry lies in the Suwalsko-Augustowskie Lake District in the middle of the Wigierski National Park (Fig. 1). It is a tunnel-valley moraine lake, covering 2118.3 ha, and

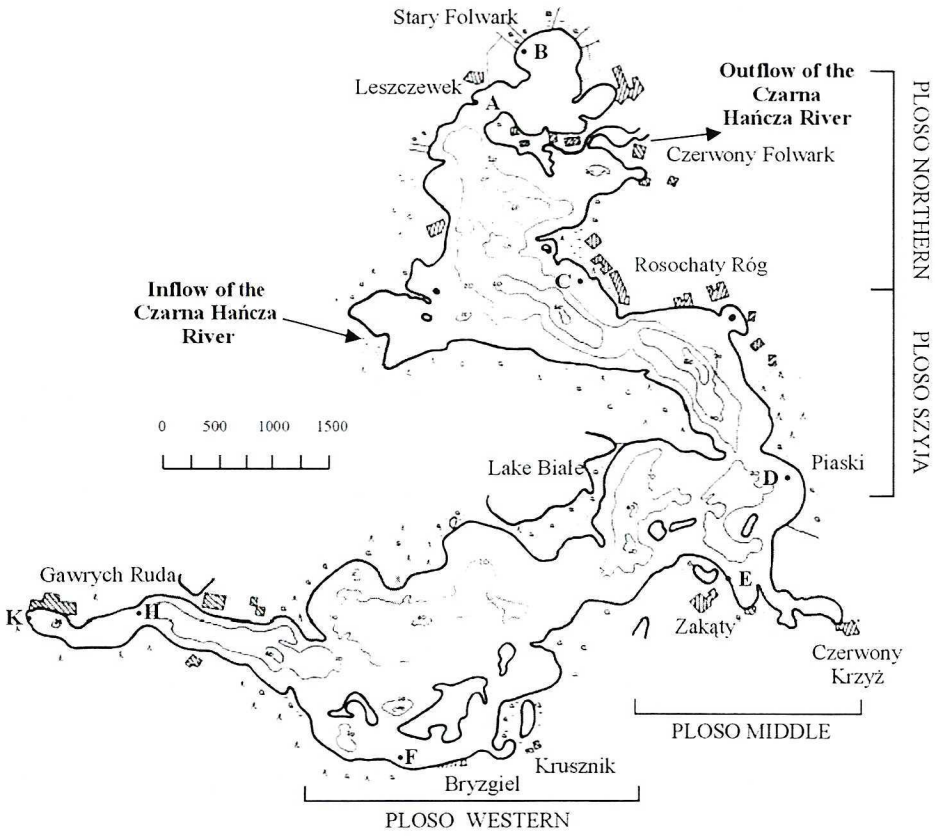

Fig. 1. Location sketch of the Wigry Lake. A, B,..., K – water sampling sites

reaching the maximum depth of 73 m. During the summer season the waters of the lake have typical thermal layers (epi-, meta- and hypolimnion). Considerable oxygen deficits appear in the meta- and hypolimnion of this lake. At the same time water transparency deteriorates and the lake is more enriched in phosphorus and other macroelements. The Czarna Hańcza River seems responsible for such a state since the amount of sewage discharged to the river from the Suwałki sewage treatment plant with tertiary treatment is about 16 to 18 thousand m^3 per day [4, 36]. To the east, north and south of the lake there are a few farmyards and summer houses.

COLLECTING OF WATER SAMPLES

Microbiological analyses conducted at monthly intervals in the summer season from June to September, in the years 1995–1999, included the waters near the shores of eight most popular bathing sites on Wigry Lake, in the vicinity of summer resorts, sailing harbour and camping sites. A short description of the sampling sites can be found in Table 1. Water samples for microbiological analyses were collected at the depth of 0.3 m directly to 250 cm^3 capacity bottles with adjusted corks. In total, 20 samples were taken at each sampling site in

the course of the study. All water samples were transported to the laboratory in containers with ice at the temperature of 4–6°C and immediately analysed. The time between the sampling to analyses never exceeded 12 hours.

Table 1. Location of sites for collecting water samples in the area of the Wigry Lake

Part of the lake	Site	Number of samples	Depth of sampling (m)
Ploso Northern	A. Bathing site Leszczewek Zadworze Bay	20	0.3
	B. Sailing harbour next to a camping site Stary Folwark Zadworze Bay	20	0.3
	C. Anchorage for fishery/angler's boats Rosochaty Róg	20	0.3
Ploso Szyja	D. Camping/ bathing site Piaski	20	0.3
Ploso Middle	E. Camping/ bathing site Zakąty	20	0.3
Ploso Western	F. Bathing site Bryzgiel	20	0.3
	H. Sailing harbour Gawrych Ruda	20	0.3
	K. Bathing site Gawrych Ruda – Uklei Bay	20	0.3

MICROBIOLOGICAL ANALYSES

Microbiological analyses involved determination of the following:

1. a number of *Aeromonas hydrophila* bacteria in 10 and 50 cm^3 of water on mA agar [27] after 24 hour incubation at 37°C;
2. a number of *Pseudomonas aeruginosa* bacteria in 100 cm^3 of water on an agar medium with cetrimide (Merck) and on King A medium [6] after 24, 48 and 72 hour incubation at 37°C and 42°C respectively;
3. a number of bacteria of the family *Enterobacteriaceae* in 1, 10 and 50 cm^3 of water on Endo [2] and Chromocult media (Merck) after 24 and 48 hour incubation at 37°C;
4. the presence of *Salmonella* sp. bacteria in 100 cm^3 of water on Rambach agar media (Merck) [26] and SS agar (Difco) after 24 hour incubation at 37°C;
5. a number of *Staphylococcus* sp. bacteria in 50 and 100 cm^3 of water on Chapman's medium [6] after 24 and 48 hours of incubation at 37°C.

The presence of *Salmonella* sp. bacteria in the afore-mentioned media was determined after previous culturing of 100 cm^3 water in bullion with tetrathionate according to Müller-Kauffman (Merck) for 24 hours at 37°C [10].

For other groups of bacteria sterile Millipore membrane filters with pores of 0.22 μm in diameter were used to condense the bacteria from water samples; next, the filters were placed on the surface of the media in Petri plates and incubated at the specified temperature for the desired period of time. After incubation typical colonies were counted.

To determine the count of *Aeromonas hydrophila* bacteria, yellow coloured colonies were counted. In order to confirm the presence of *Aeromonas hydrophila*, each colony was additionally subjected cytochrome oxidasis test.

The occurrence of *Pseudomonas aeruginosa* was verified under the light of a Wood UV lamp; colonies which produced pyocyanin were counted.

Typical *Enterobacteriaceae* colonies grown on Endo and Chromocult media, *Salmonella* sp. on Rambach-agar and SS agar media and *Staphylococcus* sp. on Chapman's medium were inoculated onto the agar-bullion medium with 2% glucose and 5% sheep blood added [6] in order to multiply the bacteria and detect haemolysins.

Additionally, bacteria of the family *Enterobacteriaceae* were analysed for the production of β-D-glucuronidase on Fluorocult medium (VRB agar – Merck), catalase (using 3% hydrogen peroxide solution) and cytochrome oxidase (using 1% tetramethylo-p-phenylduoamine solution); *Salmonella* sp. bacteria were analysed for their capability to produce flagellar antigen using agglutinating serum for HM antigen (Biomed) [6]. They were finally identified with API 20E tests (bioMerieux).

In addition to testing for haemolysins, *Staphylococcus* sp. were also checked for their capability of producing coagulase enzyme using lyophilized rabbit plasma with EDTA. Final identification was carried out with API STAPH tests (bioMerieux).

All the determinations were preceded by determination of motility of bacteria and their response to staining by the complex Gram's method.

RESULTS AND DISCUSSION

Bacteria of the family *Enterobacteriaceae*, and *Salmonella* sp. in particular, as well as bacteria of the genera *Staphylococcus*, *Pseudomonas aeruginosa* and *Aeromonas hydrophila* are an important measure of the assessment of the sanitary status of waters used for recreational purposes, especially for swimming [1, 15]. This paper reports fluctuations in the contamination of water with those microorganisms depending on the location of sampling sites and time of sample collection. The range of fluctuations in the count of *Aeromonas hydrophila* (from 100 cfu in 100 cm^3 of water at site K in July to 1.5 thousand cfu in 100 cm^3 of water at site A in September 1999), and the mean number of those bacteria in the analysed waters did not diverge from the figures quoted in the literature concerning waters in some rivers and lakes in various climatic zones. Kersters *et al.* [16] determined between 10 thousand to 1 million cfu in 1 dm^3 of surface waters in Belgium, with maximum counts found in summer, when the temperature of water was above 15°C. As a rule, the count of *Aeromonas* sp. bacteria in the waters of the Wigry Lake reached the highest values in August and September (Table 2), especially at sites A (near the village Leszczewek), B (a camping site at Stary Folwark) and C (near the village Rosochaty Róg) located on Ploso Northern, at the mouth of the Czarna Hańcza river. *Aeromonas* commonly found in water reservoirs [33] were most frequent around the sites of sewage discharge [5, 25], where the amount of nutrients was highest, although their faecal origin is disputable [3]. The count of *Aeromonas* bacteria in offshore waters of lakes can also be influenced by the presence of waterfowl. Levesque *et al.* [19], who investigated lake waters at bathing sites in Quebec (Canada), found from 6×10^6 to 1.2×10^7 cfu of *Aeromonas hydrophila* in 1 g of excrements of seagulls. Some authors claim that *Aeromonas hydrophila* is a bacterium that shows the trophic state of water

Table 2. The number (cfu/100 cm^3 of water) of *Pseudomonas aeruginosa* and *Aeromonas hydrophila* in the bathing sites water of the Lake Wigry in the years 1995–1999

Sites	*Ps. aeruginosa*																									*A. hydrophila*				
	1995					1996					1997					1998					1999					1999				
	VI	VII	VIII	IX	mean	VI	VII	VIII	IX	mean	VI	VII	VIII	IX	mean	VI	VII	VIII	IX	mean	VI	VII	VIII	IX	mean	VI	VII	VIII	IX	mean
A	2	22	2	2	7	46	0	2	0	12	67	0	0	0	17	0	0	0	0	0	0	4	0	0	1	274	320	410	1540	636
B	2	4	3	2	3	12	20	28	0	15	81	0	0	0	20	0	0	0	2	1	0	2	21	0	6	194	350	530	1340	604
C	2	6	4	2	4	11	16	26	0	13	36	0	0	0	9	0	0	0	0	0	3	0	0	10	3	344	320	690	900	664
D	0	2	2	0	1	8	12	6	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	290	280	370	840	445
E	0	4	3	0	2	6	12	4	0	6	90	0	0	0	23	0	0	0	0	0	2	2	0	0	1	164	350	670	1200	596
F	0	4	3	0	2	10	14	6	0	8	0	0	0	0	0	0	0	0	0	0	4	0	0	0	1	220	570	450	450	423
H	2	13	4	3	6	20	16	4	0	10	0	0	0	0	0	0	0	0	0	0	12	0	0	0	3	216	220	450	480	342
K	2	3	3	2	3	20	2	2	0	6	2	2	0	4	2	0	0	0	0	0	0	2	0	0	0	296	230	790	470	447
Mean from sites A-K	2	5	3	2	3	17	12	10	0	10	35	0	0	1	9	0	0	0	0	0	5	1	3	1	3	250	330	420	887	472

Table 3. The number (cfu/100 cm³ of water) of bacteria from family *Enterobacteriaceae* in the bathing sites water of the Lake Wigry in the years 1995–1999

Sites	Years																								
	1995					1996					1997					1998					1999				
	VI	VII	VIII	IX	mean	VI	VII	VIII	IX	mean	VI	VII	VIII	IX	mean	VI	VII	VIII	IX	mean	VI	VII	VIII	IX	mean
A	1350	1600	2700	5100	2688	2470	70	796	266	900	830	2350	35800	10100	12770	5045	47100	70300	397700	130036	1500	2500	4500	4600	3275
B	230	364	10800	570	2991	1530	290	422	272	629	1600	2700	72200	3000	19875	1425	73500	174700	117900	91881	1600	6200	10900	4000	5675
C	2420	3280	12600	5300	5900	3100	460	3280	1700	2135	2350	14000	8200	7100	7913	2125	4000	450000	40800	124231	4300	13500	9000	3700	7625
D	248	680	1230	980	785	1250	180	1700	390	880	118	5320	2400	3600	2860	460	7700	15000	6900	7515	4700	8300	5400	1600	5000
E	1320	1540	11200	2100	4040	2870	270	3420	976	1884	3020	8360	490	1700	3393	955	53900	8400	2900	16539	2500	5700	7500	2800	4625
F	1100	1420	4540	1940	2250	1280	115	1980	542	979	1290	1380	2200	2500	1843	3200	52100	24100	20100	24875	1200	2300	4800	1400	2425
H	3400	3790	14000	5400	6648	456	70	1650	380	639	500	20	3860	2700	1770	2305	4400	6900	6900	5126	4300	4800	17900	3100	7525
K	400	408	3720	1590	1530	5000	130	950	190	1568	1140	1400	1100	6100	2435	22500	6500	6000	21100	14025	6000	2600	10900	6800	6575
Mean from sites A-K	1309	1635	7599	2873	3354	2245	198	1775	590	1202	1356	4441	15783	4600	6545	4752	31150	94425	76788	51779	3611	8878	10567	31200	13564

reservoirs, which again is questionable [28].

Small numbers of *Pseudomonas aeruginosa* bacteria were detected in the offshore waters of the Wigry Lake throughout the whole period of analyses, regardless of the time and site of sampling, but the count never exceeded 90 cfu in 100 cm^3 of water at site E (near the village Zakąty) in June 1997. Failure to determine their occurrence in as much as 250 cm^3 of water was not uncommon (Table 2). *Pseudomonas aeruginosa* are highly susceptible to UV rays of sunlight [9], as a consequence of which even if they were present in the shallow offshore waters of the Wigry Lake, they could be eliminated from water.

The count of bacteria of the family *Enterobacteriaeace* in the waters of the Wigry Lake ranged from 20 cfu in 100 cm^3 of water in July 1997 at site H (near the village Gawrych Ruda) to 450 thousand cfu in September 1998 at site C (near the village Rosochaty Róg). During observations, and especially in 1998, the most severe contamination with these bacteria was determined in August and September (Table 3) at sites A (near Leszczewek), B (the Stary Folwark camping site) and C (near the village Rosochaty Róg). This is due to the location of the three sites (on Ploso Northern, polluted with the waters of the Czarna Hańcza River). *Enterobacter cloacae*, *Enterobacter agglomerans*, *Escherichia coli* and *Citobacter*

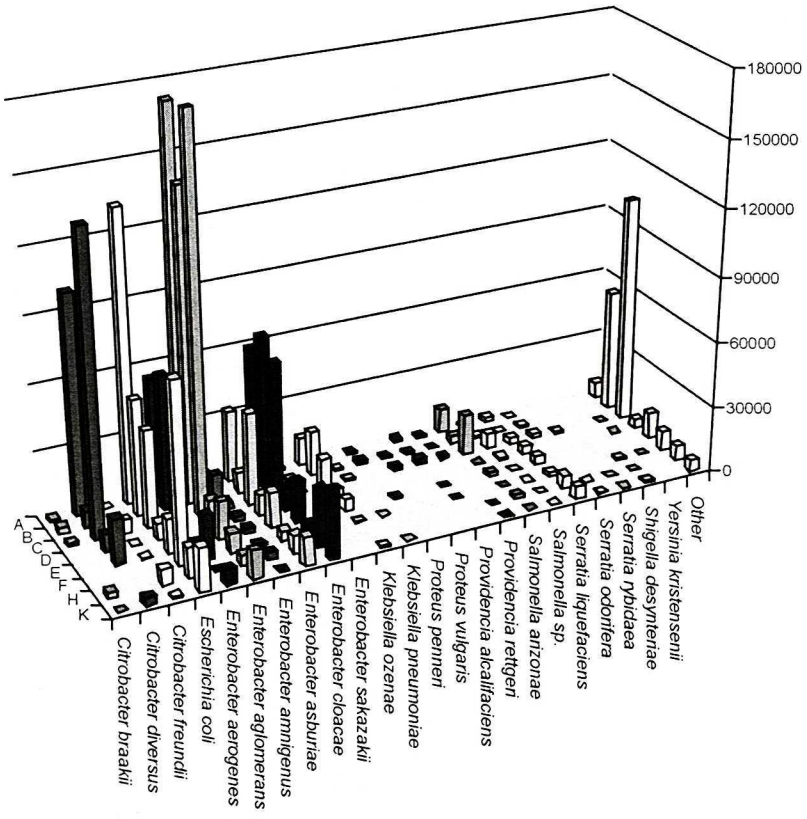

Fig. 2. Number and qualitative composition of *Enterobacteriaceae* bacteria in the water of bathing sites (A,...,K) on Lake Wigry (mean in 100 cm^3 of water in the years 1995–1999)

Table 4. Number (a) and percent (b) of different species from family *Enterobactericeae* bacteria in the bathing sites water of the Lake Wigry in the years 1995–1999

Species	Years					
	1995	1996	1997	1998	1999	Mean 1995–1999
Citrobacter braakii	1932[a] (1.8)[b]	576 (1.5)	–	–	9280 (1.9)	2358 (0.5)
Citrobacter diversus	–	–	–	277740 (16.9)	–	55548 (11.2)
Citrobacter freundii	9550 (8.9)	1000 (2.6)	4398 (2.1)	–	2930 (0.6)	3576 (0.7)
Escherichia coli	19740 (18.4)	4920 (12.8)	34300 (16.4)	294170 (17.9)	17100 (3.5)	74046 (14.9)
Enterobacter aerogenes	4400 (4.1)	2420 (6.3)	17380 (8.3)	149550 (9.1)	5870 (1.2)	35924 (7.2)
Enterobacter agglomerans	31015 (28.9)	10152 (26.4)	45864 (21.9)	463440 (28.2)	–	110094 (22.1)
Enterobacter amnigenus	3220 (3.0)	1500 (3.9)	2304 (1.1)	19720 (1.2)	3400 (0.7)	6029 (1.2)
Enterobacter asburiae	4615 (4.3)	4384 (11.4)	20524 (9.8)	118326 (7.2)	57650 (11.8)	41100 (8.3)
Enterobacter cloacae	15240 (14.2)	5150 (13.4)	44190 (21.1)	157770 (9.6)	335500 (68.7)	111570 (22.4)
Enterobacter sakazakii	215 (0.2)	230 (0.6)	438 (0.2)	–	500 (0.1)	277 (0.1)
Klebsiella ozenae	2040 (1.9)	808 (2.1)	18850 (9.0)	35155 (2.2)	–	11370 (2.3)
Klebsiella pneumoniae	536 (0.5)	77 (0.2)	210 (0.1)	1660 (0.1)	–	497 (0.1)
Proteus penneri	644 (0.6)	270 (0.7)	–	1650 (0.1)	490 (0.1)	611 (0.1)
Proteus vulgaris	–	38 (0.1)	208 (0.1)	3.284 (0.2)	–	706 (0.1)
Providencia alcalifaciens	536 (0.5)	460 (1.2)	1050 (0.5)	3290 (0.2)	–	1067 (0.2)
Providencia rettgeri	107 (0.1)	308 (0.8)	420 (0.2)	1644 (0.1)	–	496 (0.1)
Salmonella arizonae	1930 (1.8)	615 (1.6)	212 (0.1)	31224 (1.9)	3900 (0.8)	7576 (1.5)
Salmonella sp.	214 (0.2)	423 (1.1)	–	–	9800 (2.0)	2087 (0.4)
Serratia liquefaciens	6010 (5.6)	2346 (6.1)	–	–	26370 (5.4)	6945 (1.4)
Serratia odorifera	106 (0.1)	78 (0.2)	208 (0.1)	3.286 (0.2)	480 (0.1)	832 (0.2)
Serratia rybidaea	104 (0.1)	38 (0.1)	–	–	484 (0.1)	125 (0.1)
Shigella desynteriae	966 (0.9)	440 (1.1)	–	–	–	281 (0.1)
Yersinia kristensenii	105 (0.1)	77 (0.2)	210 (0.1)	–	–	78 (0.1)
Other	4095 (3.8)	2145 (5.6)	18662 (8.9)	81506 (4.9)	14546 (3.0)	24190 (4.8)

diversus were the most numerous bacteria of the family *Enterobacteriaceae* (Fig. 2, Table 4). Respectively, they amounted to 22.4, 22.1, 14.9 and 11.2% of the total number of *Enterobacteriaceae*. *Escherichia coli* occurred in 42.4% of the samples, whereas *Salmonella* sp. bacteria were found in 22.6% of the samples. *Salmonella* sp. bacteria, as well as bacteria of family *Enterobacteriaceae*, were most numerous at sites A, B and C (on Ploso Northern) and at site F (near the village Bryzgiel). The occurrence of *Salmonella* sp. bacteria in the waters of the Wigry Lake could have been caused not only by a great number of people swimming in the lake every summer, but also by water birds living near the shores of the lake. Levesque *et al.* [19] reported that excrements of seagulls inhabiting lakes near Quebec (Canada) contained from 1.1 x 10^6 to 2.4 x 10^7 *Salmonella* cfu per 1 g, with *S. brandenburg*, *S. agona*, *S. hadar* and *S. typhimurium* being the most numerous serotypes. Niemela and Niemi [23] investigated waters of rivers and small lakes in Finland whose catchments were not inhabited by humans, unused by agriculture or polluted by industries, and found out that *Serratia fonticola* (26%), *Escherichia coli* (23%) and *Enterobacter cloacae* (13%) were the most frequent representatives of the family *Enterobacteriaceae*, whereas *Citrobacter freundii* and species of the genus *Klebsilla* were found only in waters contaminated by domestic and industrial wastewaters. Krćmery [18] compared the qualitative composition of bacteria of the family *Enterobacteriaceae* in swimming waters of small lakes in Bratislava and in the water of the Danube to find out that 30% of the bacteria in water contaminated with human excrements belonged to the species *Klebsiella*. In the summer season the count of bacteria of the family *Enterobacteriaceae* varied from 10^5 to 10^6 cfu in 1000 cm^3, with *Enterobacter cloacae* and *Citrobacter freundii* being the dominant species. Outside the season *Escherichia coli* and *Enterobacter cloacae* dominated. The author claimed that the presence of bacteria of the genus *Citrobacter* was indicative of some contamination in more distant past.

The count of bacteria of the genus *Staphylococcus* in the offshore waters of the Wigry Lake reached the maximum of 1.1 thousand cfu in July 1999 at site C (near the village Rosochaty Róg). Those microorganisms were also frequent at sites A (near Leszczewek), B (Stary Folwark camping site) and H (the sailing harbour at Gawrych Ruda), especially in July (Table 5). Increased contamination with those bacteria noticed in the summer could have resulted from more intense use of the lake waters by bathers. Charoenca and Fujioka [7] as well as Šolić and Krstulović [32] determined a relationship between the number of people bathing in a lake and increase in the number of *Staphylococcus aureus* in water. Those bacteria are more tolerant to UV sunrays, salinity and water temperature compared with *Escherichia coli*. Taking into consideration frequency of bacteria of the genus *Staphylococcus* in reactant waters, Favero *et al.* [11] suggested that the upper limit on the count of those bacteria should be established for waters used for swimming at the level of 100 cfu in 100 cm^3. Over 70% of samples collected from Wigry Lake failed to meet this requirement. As regards the qualitative composition of *Staphylococcus* bacteria, the following species prevailed: *Staphylococcus capitis*, *Staphylococcus cohnii*, *Staphylococcus lentus* and *Staphylococcus xylosus* (Fig. 3, Table 6). *Staphylococcus aureus* was determined in approximately 55% of the water samples analysed. This percentage was even higher in 1995 and 1998. Similar frequency of *Staphylococcus aureus* (52.5%) was found by Šolić and Krstulović [32] in the Adriatic Sea waters near Split. In the offshore waters of the Wigry Lake, this species constituted 6.4% of the total population of genus *Staphylococcus* (Table 6). More numerous were *Staphylococcus capitis* and *Staphylococcus*

Table 5. The number (cfu/100 cm^3 of water) of bacteria from *Staphylococcus* genus in the bathing sites water of the Lake Wigry in the years 1995–1999

Sites	Years																								
	1995					1996					1997					1998					1999				
	VI	VII	VIII	IX	mean	VI	VII	VIII	IX	mean	VI	VII	VIII	IX	mean	VI	VII	VIII	IX	mean	VI	VII	VIII	IX	mean
A	310	586	576	4	369	82	374	16	78	138	198	272	478	454	351	340	350	210	434	334	714	380	384	282	440
B	257	446	552	1	314	348	330	26	64	192	178	238	268	428	278	438	332	282	306	340	672	400	244	330	412
C	110	432	570	12	281	160	208	139	88	149	60	208	150	518	234	274	474	1098	78	481	168	1140	860	220	597
D	98	171	169	2	110	110	212	27	92	110	18	138	52	378	147	76	140	78	54	87	290	422	174	120	251
E	144	430	322	28	231	98	164	42	76	95	78	154	36	328	149	172	396	22	32	156	840	660	328	138	492
F	280	416	384	11	273	128	271	29	98	132	292	196	202	188	220	422	392	60	72	237	368	508	344	138	340
H	345	406	322	22	274	113	206	36	98	113	308	242	908	136	399	690	632	78	114	379	1000	540	296	192	507
K	350	290	132	62	209	430	134	46	112	181	62	156	106	110	109	104	180	128	58	118	440	434	182	136	298
Mean from sites A-K	237	397	378	18	258	184	237	45	88	176	149	201	275	318	236	315	362	245	144	266	618	548	339	220	414

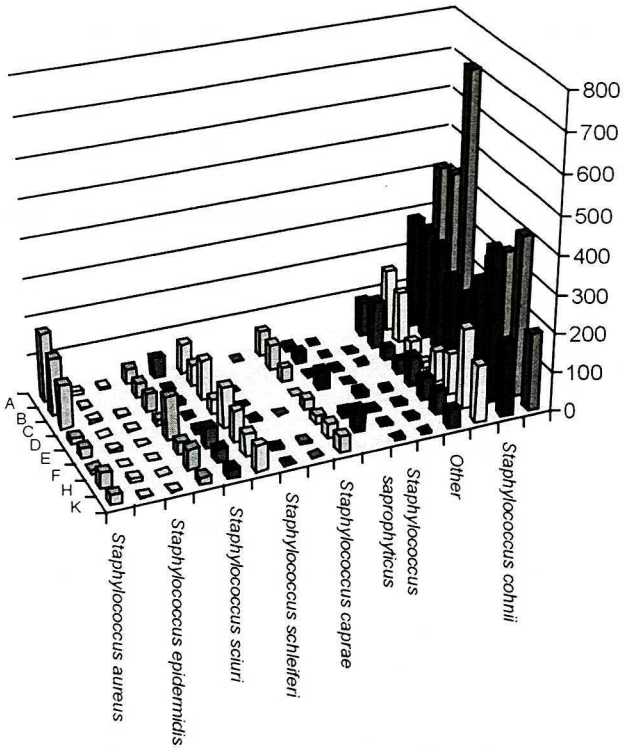

Fig. 3. Number and qualitative composition of bacteria from *Staphylococcus* genus in the water of bathing sites (A,..., K) on Lake Wigry (mean in 100 cm^3 of water in the years 1995–1999)

cohnii (35.5% and 25.1% respectively); *Staphylococcus lentus* and *Staphylococcus xylosus* made up 10.8 and 6.9%, respectively, of the population of the genus *Staphylococcus*. Charoneca and Fujioka [7] found that *Staphylococcus hominis* (23%), *Staphylococcus warneri* (23%) and *Staphylococcus aureus* (13%) prevailed among bacteria of the genus *Staphylococcus* in the sea baths waters. However, when analysing waters of ditches, streams and small lakes in Finland, Ahtiainen *et al.* [1] isolated mainly *Staphylococcus aureus* (18%), *Staphylococcus warneri* (14%), *Staphylococcus hominis* (13%) and *Staphylococcus epidermis* (13%). For practical purposes, frequency of isolation of *Staphylococcus aureus* versus frequency of occurrence of *Escherichia coli* is important. In the bathing waters of the Lake Wigry *Staphylococcus aureus* was isolated in 10% of the samples at the absence of faecal coliforms in 100 cm^3 of water. For comparison, Favero [12], Seyfried *et al.* [31] and Yoshpe-Purer and Golderman [35] were able to state that about 8% of samples contained *Staphylococcus aureus* without any bacteria of faecal *Escherichia coli* in 100 cm^3 of water. The relevant percentage was 13% in the study reported by Mates and Schaffer [21]. It is then recommendable to make tests on the presence of *Staphylococcus aureus* in addition to analyses on the count of *Escherichia coli*, faecal enterococci and *Clostridium perfringens*. The latter faecal bacteria do not produce complete picture of the sanitary status of offshore waters and threats connected with their recreational use.

The presence of potentially pathogenic bacteria in water at sites A, B and C (situated on Ploso Northern) can be attributed to great numbers of people using the bathing sites,

Table 6. Number (a) and percent (b) of different species of bacteria from *Staphylococcus* genus in the bathing sites water of the Lake Wigry in the years 1995–1999

Species	Years					
	1995	1996	1997	1998	1999	Mean 1995–1999
Staphylococcus aureus	305[a] (3.7)[b]	97 (2.2)	1 358 (18.0)	810 (9.5)	222 (1.5)	2792 (6.4)
Staphylococcus cohnii	2660 (32.3)	1826 (41.2)	4901 (65.0)	878 (10.3)	704 (4.7)	10969 (25.1)
Staphylococcus hemoliticus	–	6 (0.1)	94 (1.3)	34 (0.4)	–	134 (0.3)
Staphylococcus epidermidis	–	34 (0.8)	10 (0.1)	26 (0.3)	–	70 (0.2)
Staphylococcus capitis	2752 (33.5)	1092 (24.6)	680 (9.0)	6162 (72.4)	4822 (32.3)	15508 (35.5)
Staphylococcus warneri	488 (5.8)	79 (1.8)	254 (3.4)	24 (0.3)	980 (6.6)	1825 (4.2)
Staphylococcus sciuri	626 (7.6)	247 (5.6)	22 (0.3)	6 (0.1)	68 (0.4)	969 (2.2)
Staphylococcus xylosus	–	382 (8.6)	13 (0.2)	208 (2.4)	2426 (16.3)	3029 (6.9)
Staphylococcus schleiferi	–	32 (0.7)	18 (0.2)	–	–	50 (0.1)
Staphylococcus hominis	8 (0.1)	22 (0.5)	2 (0.1)	6 (0.1)	26 (0.2)	64 (0.1)
Staphylococcus lentus	1268 (15.4)	182 (4.1)	36 (0.5)	–	3244 (21.8)	4730 (10.8)
Staphylococcus caprae	–	205 (4.6)	50 (0.7)	244 (2.9)	1334 (8.9)	1833 (4.2)
Staphylococcus simulans	–	124 (2.8)	–	–	800 (5.4)	924 (2.1)
Staphylococcus saprophyticus	–	60 (1.4)	–	34 (0.4)	126 (0.8)	220 (0.5)
Staphylococcus chromogenes	124 (1.5)	22 (0.5)	–	–	10 (0.1)	156 (0.4)
Other	9 (0.1)	25 (0.5)	100 (1.2)	84 (0.9)	144 (1.0)	362 (1.0)

camping sites or sailing harbours situated near the three villages and to pollutants flowing to Ploso Northern of the Lake Wigry from the Czarna Hańcza River. According to the data quoted by Niewolak and Opieka [24], waters of the Czarna Hańcza analysed at the river mouth in 1996 contained up to ten thousand cells in 100 cm^3 of *Aeromonas hydrophila* and *Staphylococcus* sp., although *Psedomonas aeruginosa* appeared in low numbers.

About 2000 people may have been using the baths at sties A and B every summer, with another 700 bathers at site C ("Report on accommodation at the Wigierski National Park and its utilisation", Wigierski National Park, private information). Relatively high contamination of water at site F (near Bryzgiel locality) could have been caused by sewage leaking from cesspools in the village Bryzgiel, which is situated higher than the water table of the Lake Wigry.

CONCLUSIONS

1. Higher bacteriological contamination of the offshore waters of the Lake Wigry at Stary Folwark (A), Leszczewek (B) and Rosochaty Róg (C) is related to intensive use of those waters for recreational purposes and to increased bacteriological contamination of open waters of Ploso Northern near the Hańczańska, Zadworze and Wapiennica bays.
2. *Salmonella* sp., *Staphylococcus aureus* and *Aeromonas hydrophila* determined in the analysed offshore waters (also when *Escherichia coli* were absent) suggest that the evaluation of the Lake Wigry waters for swimming taking into consideration the count of faecal bacteria may not be sufficient to fully reflect safety conditions for bathers.
3. The results of the research suggest that when evaluating of the Lake Wigry surface waters for recreational purposes it is necessary to take into account the frequency of *Salmonella* sp., *Staphylococcus aureus*, *Aeromonas hydrophila* and *Pseudomonas aeruginosa*.

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