

Antibiotic resistance of neustonic and planktonic fecal coliform bacteria isolated from two water basins differing in the level of pollution

Resistencia a antibióticos de bacterias coliformes fecales, nesutónicas y planctónicas, aisladas de dos cuerpos de agua con distinto nivel de contaminación

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ABSTRACT

Antibiotic resistance of fecal coliform bacteria isolated from the surface microlayer and the subsurface layer of a polluted pond and a non-polluted lake was studied. On the basis of the conducted studies it was stated that the isolated fecal coliform bacteria from both aquatic basins showed various resistance to tested antibiotics. Enteric bacteria were the most resistant to ampicillin, clindamycin, novobiocin, penicillin and the most sensitive to amikacin, gentamycin, neomycin and oxytetracycline. Fecal coliform bacteria inhabiting the pond showed similar resistance to the majority of tested antibiotics than coliform bacteria isolated from the lake. The multiple antibiotic resistance (MAR) index value for fecal coliform bacteria was higher in the pond than the lake. Only in a downtown pond fecal coliform bacteria isolated from the subsurface water were more resistant to tested antibiotics than those inhabiting the surface microlayer. The antibiotic resistance level of the bacteria depended on the chemical structure of antibiotics.

Key words: Antibiotic resistance, bacteria, fecal coliform, pollution.

RESUMEN

Se estudió la resistencia a antibióticos de bacterias coliformes fecales aisladas de la microcapa superficial y la capa subsuperficial de un estanque contaminado y de un lago no contaminado. Los resultados sobre la resistencia a los antimicrobianos indicaron que en el depósito de agua estudiado el nivel de bacterias coliformes fecales varió con diversos antibióticos probados. Las bacterias entéricas fueron las más resistentes a la ampicilina, clindamicina, novobiocina, penicilina y presentaron mayor sensibilidad a amikacina, gentamicina, neomicina y oxitetraciclina. Las bacterias coliformes fecales habitantes de estanques fueron más resistentes a casi todos los antibióticos probados comparados con las bacterias coliformes del lago. El índice de la resistencia múltiple a antibióticos (MAR, siglas en inglés) mostró que los valores de bacterias coliformes fecales fueron mayores en el estanque que en el lago. Por regla general, las bacterias coliformes fecales aisladas tanto en cuencas hidrográficas, como de agua del subsuelo fueron más resistentes a los antibióticos probados que las de la microcapa superficial del mismo cuerpo de agua en el que habitan. El nivel de resistencia de las bacterias a los antibióticos dependió de la estructura química de los antibióticos.

Palabras clave: Coliformes fecales, bacterias, resistencia a los antibióticos, contaminación.

INTRODUCTION

One of the current tasks of ecology is to assess the pollution of water ecosystems different with pharmaceutical substances mainly antibiotics (Lobova *et al.*, 2002; Brown *et al.*, 2006). Antibiotics are discharged in various amounts into the aquatic environments as a result of the indiscriminate use of those organic compounds in medical, veterinary, agriculture, animal husbandry and aquaculture practices (Lin *et al.*, 2004; Toroglu *et al.*, 2005; Alpay-Karaoglu *et al.*, 2007). According to Le and Munekage (2004) and Tamtam *et al.* (2008) antibiotic concentration in water basins ranged from 0.01 to 544 ng dm⁻³. Antibiotics were shown to be quite resistant to biodegradation in water and sediments and these organic compounds showed direct toxic effect to aquatic organisms (Kümmerer *et al.*, 2000; Tamtam *et al.*, 2008).

Bacteria are often served as good indicators for the presence of pollutants and thus, used to monitor the state of the aquatic environment (Lobova *et al.*, 2008). One of the properties of bacteria used to assess the anthropogenic impact on water ecosystems is their level of antibiotic resistance (Mary *et al.*, 2000; Lobova *et al.*, 2002; Mudryk, 2005). Two different sources of the spread of antibiotic resistance growth can be observed in the aquatic environment. One of them includes some of autochthonous bacterioplankton strains showing the natural resistance to many antibiotic groups. The second source constitutes fecal coliform bacteria getting into the water basins with pollutants. Antibiotic resistance growth increased due to the extensive use of antibiotics by people (Meirelles-Pereira *et al.*, 2002; Miranda & Zemelman, 2002). Due to this reason, during recent years in different parts of the world most investigations (Reinthaler *et al.*, 2003; Ahmed *et al.*, 2008) on antibiotic resistance in aquatic habitat have concerned bacteria of fecal origin because they are used as pollution indicators or may be associated with infectious diseases. Fecal coliform bacteria mainly *Escherichia coli* have been generally accepted as the predominant vehicle for the dissemination of resistance genes and vectors due to its abundance in water ecosystems (Alpay-Karaoglu *et al.*, 2007).

Grabow and Prozesky (1973) have shown that about 30% of all fecal coliform bacteria have transferable antibiotic resistance. The rapid increase in antibiotic resistance of fecal coliform is partially due to an ability of those bacteria in horizontal transfer of antibiotic resistant genes among the bacterial population by cell to cell contact (Reinthaler *et al.*, 2003; Harakeh *et al.*, 2006). The agents responsible for horizontal transfer of resistance is an extrachromosomal genetic element termed an R - factor or R - plasmid. Bacteria containing R - plasmids have been isolated from humans and animals at a high frequency of occurrence and this problem is well documented (Alpay-Karaoglu *et al.*, 2007).

More and more research concerning antibiotic resistance growth of bacteria has been carried in natural environment. However, only sporadically such research is carried in the contact

zone between atmosphere and water called surface membrane. This is a layer of totally unique physical and chemical properties, inhabited by bacteria population called bacterianeuston (Norkans, 1980).

Bacterianeuston plays a decisive role in the exchange of matter between water and its surface. Bacteria can migrate from the surface membrane into the subsurface layer and the other way round in the specific diel cycle connected with the activity of adverse environmental factors (De Souza Lima & Chretiennot-Dinet, 1984). When the activity of the stressful factor is most intense in surface microlayer, a part of bacteria migrates into the water column. When there is a great accumulation of nutrients in the surface membrane, the number of bacteria grows rapidly as well. Transport of bacteria to the surface membrane is possible due to several phenomena. "Upwelling" occurs more often and includes rising up from beneath the water surfaces. Bacteria are risen up to the surface of a water basin due to floatation with gas bubbles and precipitation (Maki, 1993).

Due to this fact, surface membrane is thought to mediate in the matter and energy exchange processes occurring between the water basin and atmosphere. The surface membrane also constitutes a specific buffer zone for anthropogenic pollution from the atmosphere i.e. pesticides, hydrocarbons, heavy metal compounds, etc. (Maki & Herwig, 1991). In the surface membrane the accumulation of Coli bacteria is observed (Skórczewski & Mudryk, 2009) originating from the sewage and brought with bioaerosol from the land. The bacteria constitute the specific type of antibiotic resistant genes that are spread in aquatic environment. Thus, the aim of the research was to compare antibiotic resistance of fecal bacteria occupying the surface microlayer and the subsurface layer of two water basins differing in the level of pollution.

MATERIAL AND METHODS

The study was carried out in two water basins that are characterized by various level of anthropogenic impact on the environment; the downtown pond Łabędzi and the coastal lake Dolgie Wielkie. The pond Łabędzi was situated in the centre of a city occupied with 100,000 inhabitants. The surface is about 1.5 ha and the average depth is about 2 meters. The studied pond is connected by a channel with the polluted Stupia river which large amounts of river water abundantly penetrate into the pond. As a result, the studied pond is characterized by high levels of pollution (Table 1). The pond is dominated by fecal type pollution and all other pollution that is washed out from the streets and that flows into the pond along with pollution from storm drainage

The coastal Lake Dołgie Wielkie is situated in the World Biosphere Reserve - Słowiński National Park (Poland). The lake is separated from the Baltic Sea by a 2 km wide belt of dunes. The lake area is about 156 ha and it is very shallow (1.4 m average

Table 1. Contamination of fecal indicators and the concentration of nitrogen, phosphorus and copper ions in the studied water basins (Skórczewski, 2008).

Parameters		Lake Dołgie	Pond Łabędzi
Total Coli (cell · 100 cm ⁻³)	Average	397	806
	Min	3	3
	Max	2400	4300
Fecal Coli (cell · 100 cm ⁻³)	Average	6	85
	Min	3	3
	Max	16	460
Fecal Streptococci (cell · 100 cm ⁻³)	Average	34	570
	Min	3	23
	Max	430	2400
N-NO ₃ (mg · dm ⁻³)	Average	177	140
	Min	40	16
	Max	695	503
P-PO ₄ (mg · dm ⁻³)	Average	89	113
	Min	5	6
	Max	406	504
Cu (µg · dm ⁻³)	Average	2.21	3.84
	Min	0.12	0.57
	Max	7.69	7.81

Min = minimum; Max = maximum.

depth). The shallow depth as well as the lack of shielding winds enables a complete mixing of water in both vertical and horizontal profiles. Lake Dołgie Wielkie, which is located in the National Park, is rarely visited by people so this water basin is not polluted (Antonowicz & Trojanowski, 2002/2003). In the said water basin, the number of fecal bacteria was maintained at the significantly lower level than in the pond (Table 1) and the pollution was mainly of epizootic origin.

Water samples from the studied pond and the lake were taken from the site located in the littoral zone. Samples were taken from the surface microlayer and the subsurface of water. The surface microlayer (SL) samples (thickness of $240 \pm 40 \mu\text{m}$) were collected with a 40x50 cm polyethylene Garrett net (24 mesh net of 2.54 cm length) (Garrett, 1965). Polyethylene net was rinsed with ethyl alcohol prior to sampling. About 50 cm³ of water was collected at one time and poured into sterile glass bottles. Water from the subsurface layer (SUB) was taken with sterile glass pipettes at the depth of about 10-15 cm. The water samples were poured into sterile glass bottles and stored in an ice-box where the temperature did not exceed +7 °C, and immediately transported to the laboratory; the analysis commenced within 2-3 hours.

In order to form fecal coliform bacterial strains, the collected water samples were filtrated through a 0.45 µm pore size

membrane filter. The filters were aseptically transferred to a dish containing agar Endo (BIOCORP) which is a medium specific for the isolation of fecal coliform. The filters were incubated for 24 h at 44 °C. Colonies with metal shine (Lac+) were identified as fecal coliform organisms. Back up was carried with the use of API 20 e tests. 44 strains were isolated from each water layer in both water basins and their antibiotic resistance was determined.

Antibiotic resistance of fecal coliform bacteria was determined by the single disc diffusion method according to the Bauer-Kirby (Arvanitidou *et al.*, 1997). In order to determine the antibiotic resistance, bacteria were multiplied on H-M agar slants. After 72 h they were washed off the slants with sterile buffered water and the standard optical density of suspension was defined (Walczak & Donderski, 2004). Subsequently the bacterial suspension prepared in this way was introduced into dissolved M-H agar cooled to 40-45 °C. Paper discs impregnated with an antibiotic were applied to the surface of the seeded medium with the automatic disk dispenser. The blotting paper discs (φ13 mm) were manufactured by Oxoid Company. The dishes were kept at 4 °C for 1 h in order to allow antibiotic diffusion from the discs into the agar medium and then incubated at 26 °C for 48 h. The degree of resistance or sensitivity of the strains was determined on the basis of the measurements of lightened zones (in mm) around the disc and were compared with the data given by the manufacturer instructions.

Susceptibility tests followed NCCLS (2000) breakpoints. Strains showing resistance or intermediate behavior were subsumed under the category resistance. All others strains were classified as sensitive. The following eighteen antibiotics used widely in the clinical practice (their concentration given in parentheses) were tested in antibiograms: amikacin (AK, 30 µg), amoxicillin (AX, 25 µg), amoxicillin/clavulanic acid (AXC, 20/10 µg), ampicillin (AM, 10 µg), cefaclor (CEC, 30 µg), cefuroxime (CXM, 30 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg) clarithromycin (CLR, 15 µg), clindamycin (CA, 2 µg), doxycycline (DO, 30 µg), erythromycin (E, 15 µg), gentamycin (GN, 10 µg), neomycin (N, 30 µg), novobiocin (NV, 30 µg), oxytetracycline (OT, 30 µg), penicillin (P, 10 µg), rifampicin (RA, 5 µg). The results were used to calculate the Antibiotic Resistance Index (ARI) and Multiple Antibiotic Resistance (MAR) index for bacteria according to Matyar *et al.* (2007).

All tested antibiotics according to their chemical structure were divided into eight groups: aminoglycosides (amikacin, gentamycin, neomycin), macrolides (erythromycin, clarithromycin), β -lactams (amoxicillin, amoxicillin/clavulanic acid, ampicillin, cefaclor, cefuroxime, penicillin), rifampicins (rifampicin), lincosamides (clindamycin), quinolones (ciprofloxacin), tetracyclines (doxycycline, oxytetracycline), other (chloramphenicol, novobiocin).

Statistical analysis. The antibiotic resistance is expressed as a percentage. All statistical analyses were calculated using Statistica 9.0 software. The normal distribution of the data was checked by using the Shapiro-Wilk test before the statistical

analysis. Statistical differences between non-normally distributed data was analyzed by using the Mann-Whitney U test. A value of $p < 0.05$ was defined as indicating a significant difference.

RESULTS

The data presented in figure 1 shows that fecal coliform bacteria isolated from the pond Łabędzi and lake Dolgie Wielkie is characterized by insignificant differences in the level of resistance to studied antibiotics. In both studied water basins among all the tested fecal coliform bacteria strains the higher percentage of bacteria (82-100%) was resistant to ampicillin, clindamycin, novobiocin and penicillin. On the other hand, less than 25% of the fecal coliform bacteria strains were resistant to amikacin, gentamycin, neomycin and oxytetracycline. The data shows that there were differences between fecal coliform bacteria inhabiting polluted and non-polluted water basins in terms of their resistance to the antibiotics used in this study. Enteric bacteria inhabiting the polluted downtown pond were more resistant (ARI=0.55) to nearly all tested antibiotics (with the exception of amikacin, amoxicillin/clavulanic acid, cefaclor, gentamycin and novobiocin) than fecal coliform bacteria (ARI = 0.46) isolated from the non-polluted lake. However, statistical significance of those differences was not showed ($p < 0.05$).

The collection of fecal coliform bacteria isolated from the surface microlayer and the subsurface water of both studied wa-

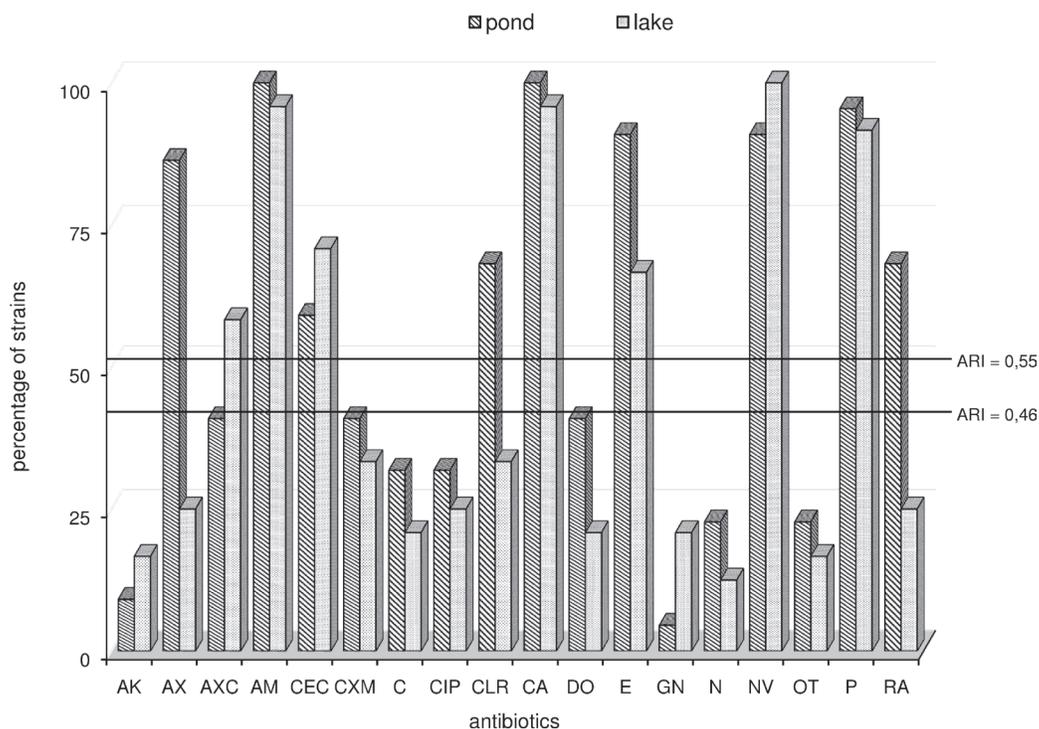


Figure 1. Percentage of antibiotic resistant fecal coliform strains inhabiting pond Łabędzi and coastal lake Dolgie Wielkie.

ter basins was analyzed for multiple antibiotic resistance (MAR) index (Fig. 2). It is of great interest that the MAR index for fecal coliform bacteria isolated from the polluted pond was higher than the index proven among enteric bacteria inhabiting the non-polluted lake. It has been determined that above 60% of fecal coliform bacteria isolated from the microlayer of the pond showed a 0.6 MAR (i.e. resistance to 11-12 of the 18 antibiotics tested) and 45% of fecal coliform bacteria inhabiting the subsurface water showed resistance to eight antibiotics (MAR = 0.4). In lake Dołgie Wielkie the higher percentage (45-63%) of bacterial fecal coliform strains isolated from the surface microlayer and subsurface water show a 0.3 MAR (resistance to 6-7 of the 18 antibiotics tested).

Data on antibiotic resistance of fecal coliform bacteria inhabiting the surface microlayer and the subsurface water in both studied waters basin is given in table 2. The data shows that the statistically significant differences ($p < 0.05$) in the antibiotic resistance occurred only between the isolated bacteria from both pond surfaces. In the said water basin bacteria inhabiting the surface layer are characterized by significantly lower resistance (ARI = 0.44) than bacteria from the subsurface layer (ARI = 0.67). Such regularity was not stated in the lake where the difference in bacteria resistance isolated from both water layers (ARI = 0.42-0.49) was not statistically significant.

In the pond all fecal coliform bacteria isolated from the surface microlayer were susceptible to amikacin, gentamycin and neomycin.

Table 3 shows data on bacterial strain resistance with the relation of different classes of antibiotics. The majority of fecal coliform bacteria (96-100%) isolated from the studied pond and

the lake was the most resistant to the lincosamide antibiotics, while 83-88% of fecal coliform strains were the most susceptible to aminoglycosides ones. This regularity was observed among fecal coliform bacteria isolated from the surface and the subsurface layer in both studied water basins.

DISCUSSION

The results of the present study showed that fecal coliform bacteria isolated from the studied pond and the lake differed a little in the resistance level to tested antibiotics. Among all the isolated enteric bacteria the highest percentage of strains was resistant to ampicillin, clindamycin, novobiocin and penicillin. The resistance to ampicillin and penicillin of fecal coliform bacteria has been previously demonstrated in different aquatic environments such sewage, sludge, irrigation water rivers and drinking water in range between 70-100% (Reinthal *et al.*, 2003; Toroglu *et al.*, 2005; Alpay-Karaoglu *et al.*, 2007). The resistance to β -lactam antibiotics such ampicillin and penicillin among *Enterobacteriaceae* family is increasing in the United States and Europe. Due to the chemically unstable β -lactam ring in structure of β -lactam antibiotics, they are readily susceptible to bacterial hydrolysis by β -lactamases and are easily eliminated (Hirsch *et al.*, 1999). In the present study high levels of antibiotic resistance of many fecal coliform bacteria isolated from the pond and the lake against penicillin and ampicillin indicated that the β -lactamase gene may be widely present in the gene pool of microbes in this studied aquatic environments (Lin *et al.*, 2004).

Bacteria occurring in many water basins show multiple antibiotic resistance (MAR) as it was reported by Qureshi and Quershi

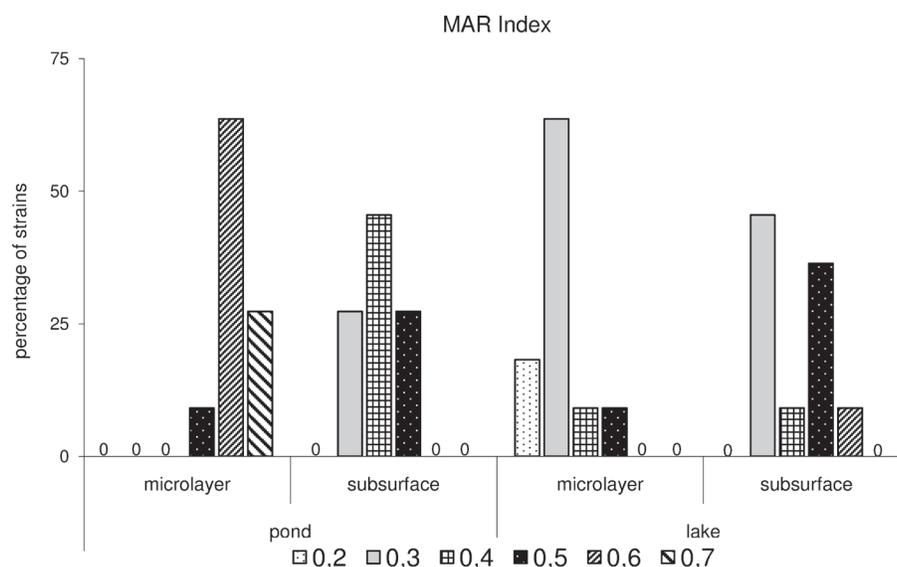


Figure 2. Multiple antibiotic resistance (MAR) index fecal coliform strains in surface microlayer and subsurface water inhabiting pond Łabędzi and coastal lake Dołgie Wielkie. Zero means that no index of such strains was detected.

Table 2. Percentage of antibiotic resistant coliform bacteria in surface microlayer and subsurface water layers.

Antibiotics	(µg)	Lake Dołgie		Pond Łabędzi	
		SL	SUB	SL	SUB
Amikacin	30	8.3	25.0	0.0	18.2
Amoxicillin	25	16.7	33.3	81.8	90.9
Amoxicillin/clavulanic Acid	30	58.3	58.3	45.5	36.4
Ampicillin	10	100.0	91.7	100.0	100.0
Cefaclor	30	75.0	66.7	36.4	81.8
Cefuroxime	30	33.3	33.3	27.3	54.5
Chloramphenicol	30	8.3	33.3	9.1	54.5
Ciprofloksacin	5	25.0	25.0	9.1	54.5
Clarithromycin	5	25.0	41.7	36.4	100.0
Clindamycin	2	91.7	100.0	100.0	100.0
Doxycycline	30	8.3	33.3	18.2	63.6
Erythromycin	15	66.7	66.7	100.0	81.8
Gentamycin	10	8.3	33.3	0.0	9.1
Neomycin	30	16.7	8.3	0.0	45.5
Novobiocin	30	100.0	100.0	81.8	100.0
Oxytetracycline	30	16.7	16.7	18.2	27.3
Penicillin	10	91.7	91.7	90.9	100.0
Rifampicin	30	16.7	33.3	45.5	90.9
ARI		0.42	0.49	0.44	0.67

(1992), Mary *et al.* (2000), Miranda and Zemelman (2002), Mudryk and Skórczewski (2009). According to Meirelles-Pereira *et al.*, (2002) multiple resistance may be coded on plasmids, mutational events or on even smaller and mobile genetic elements called transposons. The present study also showed multiple antibiotic resistance of fecal coliform bacterial strains inhabiting both studied water basins. The majority of enteric bacteria in the pond was resistant to 11-12 antibiotics and in the lake 6-7 antibiotics of 18 antibiotics used in this study. That means that coliforms are perfectly capable of detoxicating those pharmaceutical organic compounds. The calculated MAR values were higher than those reported by Miranda and Zemelman (2002). Adaptive responses of bacterial communities to several antibiotics observed in the present study may have possible implications for the public health (Quershí & Quershí, 1992) and may reflect the history of antibiotic application.

Recent studies (Lin *et al.*, 2004; Lobo *et al.*, 2008) demonstrated positive correlations between the level of water basin pollution and antibiotic resistance of bacteria. Our studies show similar results for fecal coliform bacteria. Enteric bacteria inhabiting the polluted pond showed higher multiple antibiotic resistance than fecal coliform bacteria isolated from the non-polluted coastal lake.

The results of research carried out in the Słupia river in northern Poland show that fecal bacteria accumulate in the surface

microlayer (Skórczewski & Mudryk, 2009). The studies carried out by Hermansson *et al.* (1987) along the Swedish west coast, Jones *et al.* (1991) in lake Michigan and Walczak and Donderski (2004) in Jeziorak Mały lake showed that autochthonic neustonic bacteria were much more resistant to antibiotics than planktonic ones. By contrast, the results obtained in the present study do show that coliform bacteria isolated from the subsurface water were more resistant to tested antibiotics than those inhabiting the surface microlayer. The lower level of antibiotic resistance of enteric bacteria inhabiting the surface microlayer can be explained by the occurrence in surface microlayers many stressful fluctuations of environmental factors mainly the UV radiation, temperature, pH and relatively high accumulation of heavy metals and pesticides (Maki, 1993), which cannot only lower the level of antibiotic resistance of allochthonous fecal coliform bacteria, but also limit their growth and the survival rate in this extreme environment.

Most classes of antibiotics are approved for human, animal and agriculture use. Therefore, the resistance to multiple classes of antibiotics is not uncommon in fecal coliforms bacteria isolated from aquatic environments (Harwood *et al.*, 2000; Lin *et al.*, 2004). The present data demonstrates that fecal coliforms bacteria isolated from water of the pond and the lake were most resistant to lincosamide antibiotics. The high resistance of the studied fecal bacteria to these antibiotics is because lincosamides effi-

Table 3. The resistance of bacteria with respect to their chemical structure (in percentage).

Water basin	Layer	aminoglycosides	macrolides	β -lactams	rifampicins	tetracyclines	quinolones	lincosamides	others
Lake Dołgie	SL	11.1	45.8	62.5	16.7	12.5	25.0	91.7	54.2
	SUB	22.2	54.2	62.5	33.3	25.0	25.0	100.0	66.7
	average	16.7	50.0	62.5	25.0	18.8	25.0	95.8	60.4
Pond Łabędzi	SL	0.0	68.2	63.6	45.5	18.2	9.1	100.0	45.5
	SUB	24.2	90.9	77.3	90.9	45.5	54.6	100.0	77.3
	average	12.1	79.6	70.5	68.2	31.8	31.8	100.0	61.4

SL = surface microlayer; SUB = subsurface water layers.

ciently inhibit the growth of gram-positive bacteria mainly staphylococcal and streptococcal forms, but have low activity against many gram-negative bacteria, such as fecal bacteria (Lüthje & Schwarz, 2007). Lincosamides inhibit protein synthesis by binding ribosomes (Menninger & Coleman, 1993). Bacterial resistance to lincosamide antibiotics occurs by one of three mechanisms: target site modification, active efflux mechanisms and enzymatic inactivation of the drug (Lüthje & Schwarz, 2007).

Fecal coliform bacteria isolated from the water of the studied pond and the lake were most susceptible to aminoglycoside antibiotics. It means that those tested enteric bacteria are not actively capable of detoxifying those antimicrobial agents. According to Zembower *et al.* (1998) bacterial susceptibility intensively increased mainly due to *Enterobacteriaceae* to aminoglycosides, which have a very broad antibacterial spectrum in different part of the world. Aminoglycosides are a large and diverse class of antibiotics which have bactericidal activity against some gram-positive and many gram-negative organisms (Ryu & Rando, 2001). Aminoglycoside inhibition of bacterial cell growth occurred by inhibition of one or more of biochemical steps involved in translation on the ribosome and disrupt the integrity of the bacterial cell membrane (Zembower *et al.*, 1998). The binding of aminoglycosides to sensitive ribosomes and decoding the region constructs occurs in the μM range (Ryu & Rando, 2002). Aminoglycosides bind to the 30S subunit of the ribosome at its interface with the 50S subunit which leads to interruption of protein synthesis (Zembower *et al.*, 1998; Boehr *et al.*, 2003). Bacterial resistance to aminoglycosides occurs by one of four mechanisms: reduced uptake, mutational modification of 16S rRNA, enzymatic modification of 16SrRNA and modification and inactivation of antibiotics by three extracellular enzymes: phosphotransferase (APH), nucleotidyltransferase (ANT), acetyltransferase (AAC) (Boehr *et al.*, 2003).

The carried research did not confirm the occurrence of the significant differences in the antibiotic resistance of coliform bacteria originating from the environments exposed to and not exposed to strong anthropogenic impact. It may prove the significant

spread of antibiotic resistance genes in natural environment. The presence of the high number of antibiotic resistant fecal coliform bacteria in waters may have ecological and public health implications. This emphasizes the need for further studies, especially in relation to the genes encoding resistance in different pathogens bacterial species, as well as, the possibility of the returning of resistance genes to the human population through the water usage. We think that the occurrence of antibiotic resistant fecal coliform bacteria in water basins could possibly be used as an alternative indicator for fecal contamination.

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