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FREQUENCY OF ANTIBIOTIC RESISTANCE IN BACTERIA INHABITING WATER OF DOWNTOWN POND

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Abstract

Antibiotic resistance of heterotrophic bacteria isolated from the surface microlayer and subsurface water of downtown pond was determined. The levels of resistance of bacteria to various antibiotics differed considerably. It follows from the results that bacteria were most resistant to penicillin and sensitive to gentamycin, neomycin and oxytetracycline. Majority of bacterial strains were characterised by resistance to 6-8 of 18 antibiotics tested. As a rule, there were no significant differences in antibiotic resistance between neustonic and planktonic bacteria. Pigmented bacteria were more antibiotic resistant than non-pigmented ones.

Key words: pond, water, bacteria, antibiotic resistance

INTRODUCTION

Many antibiotics have been used in the last several decades in medical, veterinary, agriculture and aquaculture practices (Chelossi et al. 2003, Costanzo at al. 2005, Alpay-Karaoglu et al. 2007). Recently, there has been a growing interest in the presence of different pharmaceutical substances, mainly antibiotics in the aquatic environment (Gòmes-Lus 1998, Hirsch et al. 1999, Schwartz et al. 2003). Antibiotics released into the aquatic environment are of concern for the following reason: (i) contamination of raw, treated and recycled water used for drinking, irrigation and recreation; (ii) potential to accelerate widespread bacterial resistance to antibiotics and (iii) negative effect on important ecosystem bacteria through death or inhibition (Colignon 1999, Daughton and Ternes 1999, Costanzo et al. 2005). The wide application of antibiotics by humans has led to large-scale dissemination of bacteria resistant to antibiotics in water basins (Schwartz et al. 2003, Dang et al. 2006). The presence and persistence of antibiotic resistant bacteria in aquatic ecosystems is a growing public health concern (Reinthaler et al. 2003). Antibiotic resistance of bacteria in the water basins has been reported in the literature, particularly in regions prone to pollution containing antibiotics (Smith et al. 1994, Huys et al. 2000, Che-

lossi et al. 2003), though resistance has been observed in unpolluted sites (Jones et al. 1986, Boon 1992, Boon and Cattanach 1999). For antibiotic resistance to develop, it is necessary that two key elements combine: the presence of an antibiotic capable of inhabiting the majority of bacteria present in a colony and heterogeneous colony of bacteria where at least one of these bacterium carries the genetic determinant capable of expressing resistance to the antibiotic (Levy and Marshall 2004). Resistance to antibiotics can be natural or acquired and can be transmitted horizontally or vertically. Whereas the natural form of antibiotic resistance is caused by a spontaneous gene mutation in the lack of selective pressure due to presence of antibiotics and is far much less common then the acquired one, it can also play a role in the development of resistance (Alanis 2005). Antibiotic resistance in bacteria can be determined both by chromosome genes and by extrachromosome genetic elements for example R-plasmids which can be transferred between bacteria (Baya et al. 1986, Gòmes-Lus 1998). According to Costanzo et al. (2005) plasmid borne resistance genes are the most prolific mechanisms of resistance transfer between bacteria more so in eutrophic water basins due to elevated carbon concentration and therefore, increased bacterial activity.

Most of the studies on development of antibiotic resistant bacteria have mainly focused on lakes (Lobova et al. 2002, Walczak and Donderski 2004), rivers (Mutanda et al. 1983, Tamtam et al. 2008), estuarine, coastal and marine waters (Hermansson et al. 1987, Nair et al. 1992, Mudryk 2002, 2005) however, there are few (Mutanda et al. 1983, Tendencia and de la Peňa 2001) published studies covering an antibiotic resistant bacteria in pond. This is why the aim of the present study was to determine antibiotic resistance of neustonic and planktonic bacteria inhabiting downtown pond.

MATERIAL AND METHODS

Description of the study area

The studies were carried out in downtown pond called “the of Łabędzi Pond” (the Swan’s Pond) situated in centre of Słupsk. The pond area is about 1.5 ha and it is very shallow (1.4 m average depth). The shallow depth as well as the lack of shielding winds enables a full mixing of water in both vertical and horizontal profiles. As a result, the pond can be regarded as a polymictic basin in which no thermal or oxygen stratification is observed. Studied pond is connected via channel with of the polluted river Słupia, whose large volumes of river water abundantly penetrate into the pond. The main species of macrophytes inhabiting the Łabędzi Pond are: *Phragnites cannus*, *Elodea canadesis* and *Ceratophyllum demerscum*.

Sampling

In order to form the neustonic and planktonic bacterial collection, water samples were taken in November 2006 from one site located in littoral zone of the Łabędzi

Pond. Water samples were collected from two layers. Surface microlayer (SL) samples (thickness of 240 ± 40 μm) 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. Water from subsurface layer (SUB) was taken with sterile glass pipettes at the depth of about 10-15 cm. The water samples were collected 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 has started within 2-3 h.

Bacterial strains isolation

Plate techniques were used in order to isolate neustonic (SL) and planktonic (SUB) bacteria. Water samples were vortex mixed, and then serial tenfold dilutions were prepared with sterile buffered water to reach final concentrations ranging from 10^{-1} to 10^{-4}. Diluted samples were inoculated by the spread method in three parallel replicates on plate count agar (BICORP). Incubation was carried out at 20^{o}C for 10 days. Afterwards, *ca.* 50 individual bacterial colonies per each water layer were picked out from the whole surface of the plates or from selected sectors and transferred to a Mueller-Hinton (M-H) agar (Oxoid). Cultures maintained on this medium after purity control were kept at 4°C and used for the analysis of their antibiotic resistance.

Antibiotic resistance determined

Antibiotic resistance of neustonic (SL) and planktonic (SUB) bacteria was determined by the single disc diffusion method according to Bauer et al. (1966) using M-H agar. The following eighteen antibiotics used widely in the clinical practice (with their concentrations 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 antibiotic impregnated discs (Warsaw Serum and Vaccine Production Company and the Becton-Dickinson Company) were than applied to the surface of the seeded medium at distances not shorter than 2 cm. The degree of resistance or sensitivity of the bacterial strains was determined on the basis of the measurements of lightened zones (in mm) around the disc and their comparison with data given by the manufacturer instructions. Strains showing resistant or intermediate behavior were subsumed under the category resistant. All others strains were classified as sensitive.

RESULTS

The data presented in figure 1 show that bacteria isolated from water of the Łabędzi Pond are characterised by large differences in the level of resistance to studied antibiotics. Among all the strains tested the highest percentage (88%) of bacteria were resistant to penicillin. About 60-70% of bacterial strains studied were resistant to amoxicillin/clavulanic acid, ampicillin, clindamycin and erythromycin, while less than 20% of the strains were resistant to gentamycin, neomycin and oxytetracycline.
The collection of neustonic and planktonic bacterial strains was analysed for multiple antibiotic resistance (MAR) (Fig. 2). About 30% of neustonic bacteria showed a 6 and 8 MAR pattern (i.e resistance to 6 and 8 of the 18 antibiotics tested). No bacteria inhabiting surface microlayer resistant to all studied antibiotics were determined. About 30% of planktonic bacteria showed a 6-8 MAR and about 10% planktonic bacteria were resistant to all tested antibiotics.
Data on antibiotic resistance of bacterial strains inhabiting surface microlayer and subsurface water of the Łabędzi Pond are given in figure 3. Those data show that besides amoxicillin/ clavulanic acid, ampicillin, cefuroxim and doxycycline there were no differences between the neustonic and planktonic bacteria in their resistance to the antibiotics used in this study. The higher percentage (60-70%) of neustonic bacteria was resistant to cefuroxime, erythromycin and penicillin, while about 80% of bacterial strains isolated from surface microlayer were sensitive to gentamycin, neomycin and oxytetracycline. Among planktonic bacteria 70-80% of these organisms were resistant to amoxicillin/clavulanic acid, ampicillin, clindamycin and penicillin while about 80% of the planktonic organisms similarly to neustonic bacteria were sensitive to gentamycin, neomycin and oxytetracycline.
Figure 4 presents the results of the studies of antibiotic resistance in pigmented and non-pigmented bacteria isolated from water of the Łabędzi Pond. Pigmented bacteria were more antibiotic resistant than non-pigmented ones to 14 out of the 18 antibiotic tested. About 30% of chromogenic bacterial strains where resistant to ampicillin, cefaclor, clindamycin and penicillin while the lowest percentage of pigmented bacterial strains were resistant to gentamycin and oxytetracycline. Among bacterial strains that are characterised by the ability to synthesise carotenoides pigments above 20% organisms was resistant to clindamycin, erythromycin and penicillin while 90% achromogenic bacterial strains were sensitive to amikacin, doxycycline and neomycin.

DISCUSSION

In the present study it has displayed that bacteria isolated from water of the Łabędzi Pond are characterised by large differences in level of resistance to particular tested antibiotics. Among all the isolated strains about 90% bacteria were resistant to penicillin which is an inhibitor of the bacterial cell wall synthesis (Foster 1983). In other water bodies also a high level of bacterial resistance to penicillin was noted. Mudryk and Skórczewski (1998) in the Gdańsk Deep, Mudryk (2002) in estuarine lake

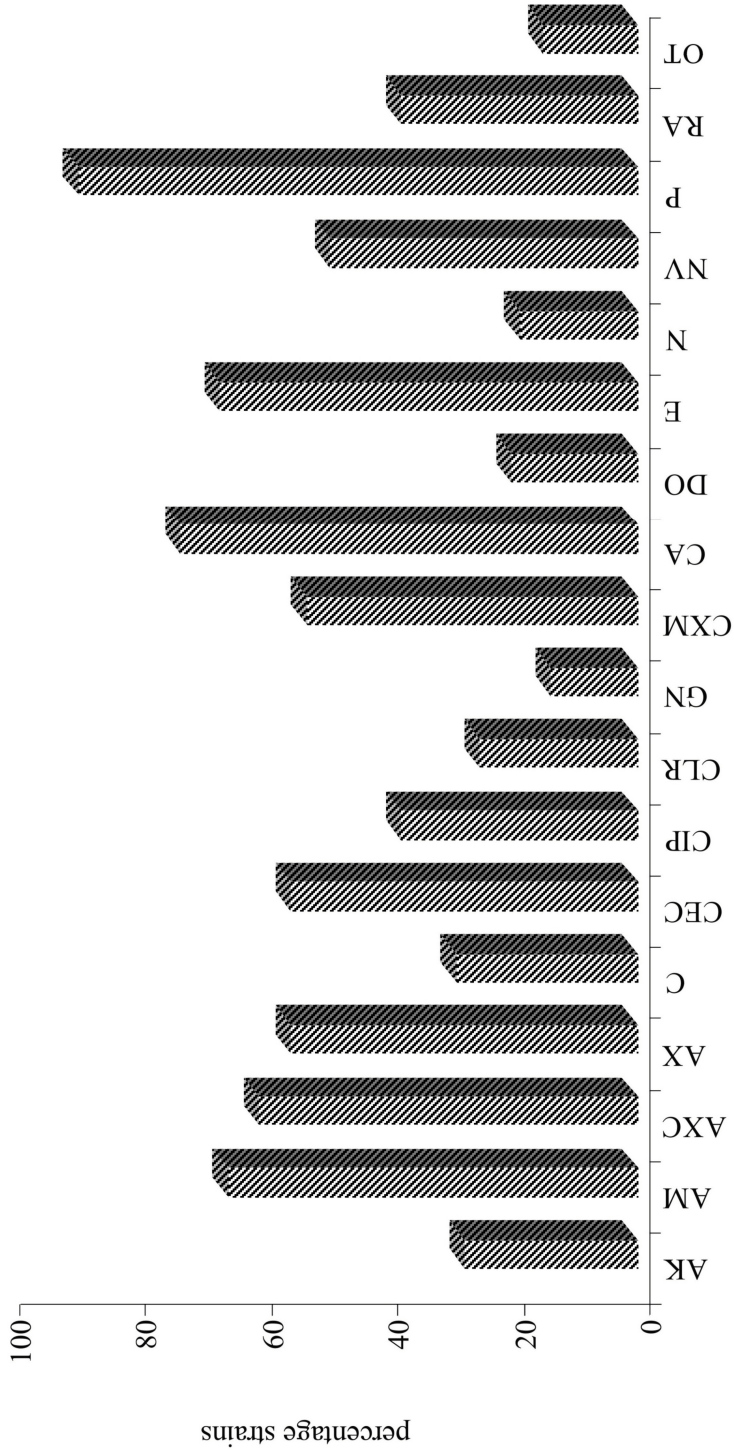

Fig. 1. Resistance to different antibiotic among bacteria isolated from water of the Łabędzi Pond (percentages derived from the pooled of both water layers)

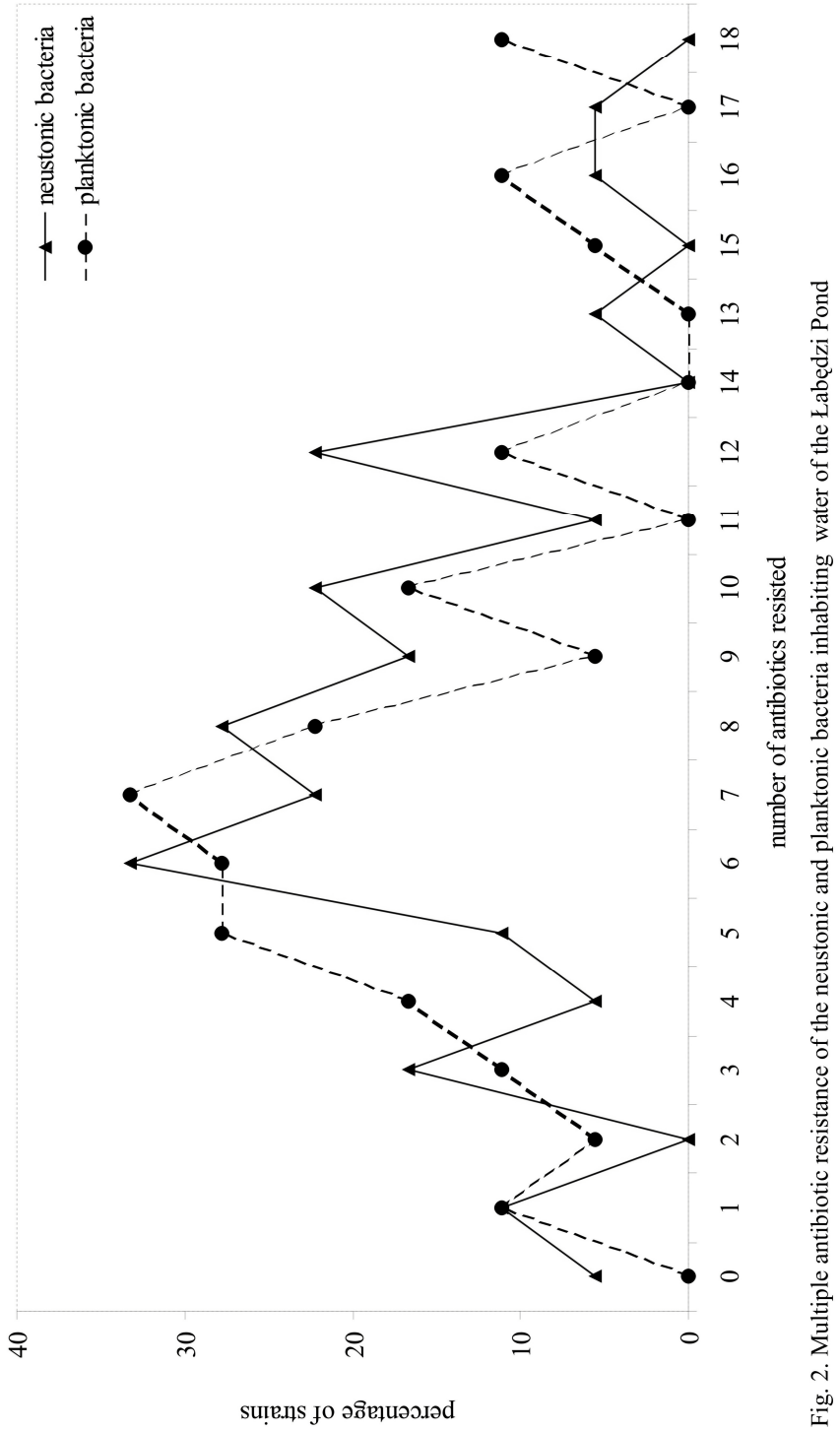

Fig. 2. Multiple antibiotic resistance of the neustonic and planktonic bacteria inhabiting water of the Łabędzi Pond

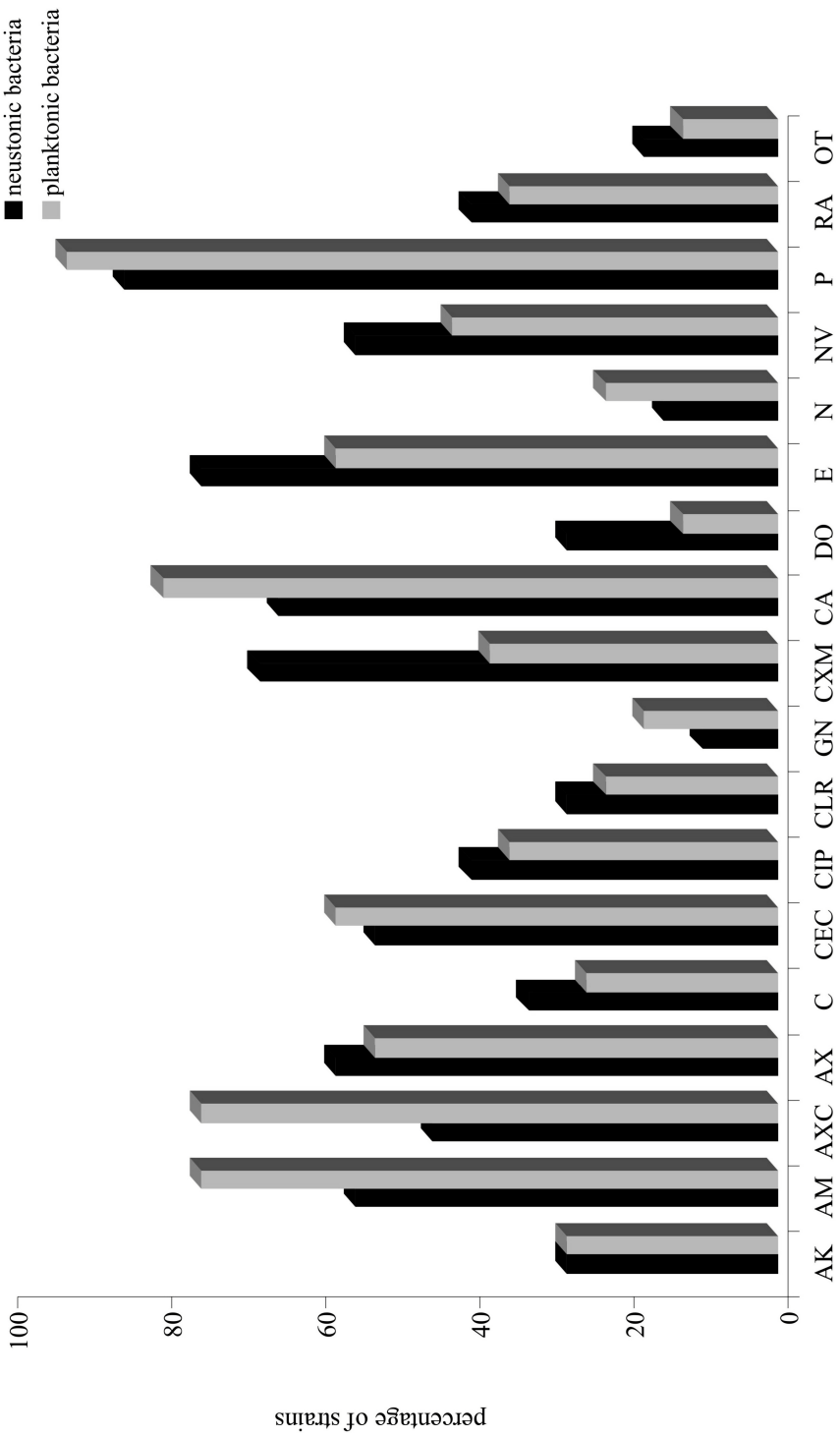

Fig. 3. Resistance to the antibiotics of the neustonic and planktonic bacteria

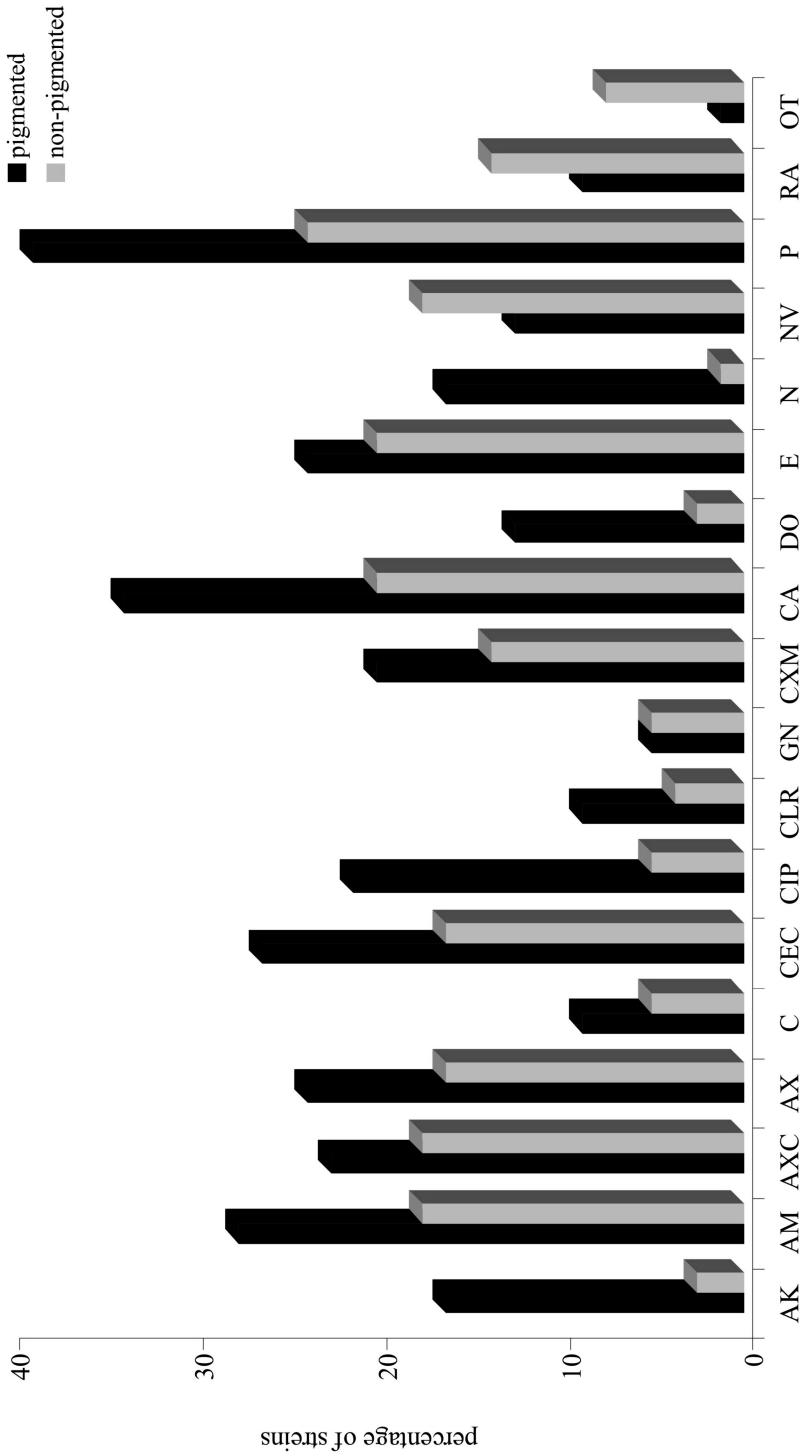

Fig. 4. Differences in the resistance of pigmented and non-pigmented bacteria to tested antibiotics

Gardno, Walczak and Donderski (2004) in lake Jeziorak Mały show presence of high percentage (70-90%) of the penicillin resistant bacteria. According to Hermansson et al. (1987) and Herwig et al. (1997) the resistance of bacteria to penicillin lies in their ability to synthesize extracellular enzyne penicillinase which can transform the hydrolysis of the β-lactam bond penicillin into antibiotically inactive penicilloic acid.

Bacteria inhabiting many water bodies are resistant to only a small number of antibiotics (Nair et al. 1992, Herwig et al. 1997, Miranda and Zemelman 2002). In the present study however, majority of bacteria inhabiting the waters of the Łabędzi Pond were found to be characterised by multiantibiotic resistance. The majority of microorganisms inhabiting this water basin were resistant to 6-8 antibiotics used in this study. That means they are perfectly capable of detoxicating those pharmaceutical organic compounds. Adaptive responses of bacterial communities to several antibiotics observed in the present investigation may have possible implications for the public health (Qureshi and Quershi 1992) and may reflect the history of antibiotic application (Hsu et al. 1992).

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 lake Jeziorak Mały showed that bacteria inhabiting the surface microlayers were much more resistant to antibiotics than those isolated from subsurface water. By contrast, as a rule the results obtained in the present study do not show any differences in antibiotic resistance between neustonic and planktonic bacteria. Mudryk and Skórczewski (1998) and Mudryk (2002) obtained identical results, during their investigation of bacterial resistance to antibiotics in marine bay and estuarine lake. According to Nemi et al. (1983) the level of antibiotic resistance of bacteria depends on their taxonomic position rather than their origin.

The studies carried out by Hermansson et al. (1987) along the Swedish west coast, Nair et al. (1992) in different regions of the Arabian Sea and De Souza et al. (2006) in Antarctic marine waters showed that pigmented bacteria were more resistant to antibiotics than non-pigmented ones. Similar results have been obtained during this study.

The results presented in this paper have proven that antibiotics are significant selection factor of neustonic and planktonic bacterial strains inhabiting water of pond. For this reason, further research is necessary concerning the interaction of bacteria and antibiotics in surface microlayer and subsurface water basins.

CONCLUSIONS

The above considerations give the ground to formulate the following conclusion:

- Bacteria isolated from water of the Łabędzi Pond are characterized by large differences in the level of resistance to antibiotics. The highest number of bacterial strains was resistant to penicillin.
- Bacteria inhabiting the waters of the Łabędzi Pond were found to be characterized by multiantibiotic resistance.

- As a rule, there were no significant differences in antibiotic resistance between neustonic and planktonic bacteria.
- Pigmented bacteria were more antibiotic resistant than non-pigmented ones.

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WYSTĘPOWANIE ANTYBIOTYKOOPORNYCH BAKTERII W WODZIE ŚRÓDMIEJSKIEGO STAWU

Streszczenie

W pracy przedstawiono wyniki badań dotyczących występowania antybiotykoopornych bakterii w powierzchniowych i podpowierzchniowych warstwach wody śródmiejskiego stawu zlokalizowanego w Słupsku. Uzyskane wyniki badań wykazały duże zróżnicowanie poziomu oporności bakterii na testowane antybiotyki. Największy procent bakterii charakteryzował się opornością wobec penicyliny, a najmniejszy wobec gentamycyny, neomycyny oraz oxytetracykliny. Większość szczepów bakterii wykazywała oporność na 6-8 antybiotyków. Nie wykazano znaczących różnic poziomu antybiotykooporności między bakteriami nesutonowymi i planktonowymi. Bakterie syntetyzujące barwniki karotenoidowe wykazywały większą antybiotykooporność niż bakterie bezbarwne.