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PRELIMINARY EXAMINATION OF RESISTANCE OF BACTERIA ISOLATED FROM DRINKING WATER TO ANTIBACTERIAL AGENTS

Susceptibility of microorganisms isolated from drinking water to antibacterial agents, including antibiotics, chemotherapeutic agents and sulphonamides, was examined. The samples were taken in the area run by the Municipal Water Supply and Sewage Service (MPWiK) in Wrocław at the central pumping station. The aim of the work was to establish the bacterial species present in treated water and to determine the resistance of microorganisms to some chosen antibacterial agents. A total of 41 strains of saprophytic bacteria, 7 strains of conditional-pathogenic bacteria and 1 strain of pathogenic actinomycetes were isolated and subjected to examination. It was found that majority of isolated microorganisms were resistant to antibacterial agents used at present in health service. Besides, the microorganisms showed resistance to several preparations used at the same time.

1. INTRODUCTION

The increase of morbidity rate observed in people and animals, caused by infections with pathogenic microorganisms resistant to antibiotics and other antibacterial agents, creates serious and complex microbiological and medical problem.

Discovering the mechanism of resistance conditioned by the presence of the factor R and the possibility of its transfer from plasmidic to non-plasmidic strains we can perceive the essence of resistance in bacteria. The possibility of transferring the factor R within a given species or even between particular species or types provides in turn evidence of the ease with which resistance develops (DOMARADSKII et al. [6], HEDGES and JACOB [8], KOWALCZYK and LORKIEWICZ [9]).

It is very likely that a significant role in spreading the drug resistance is also played by saprophytic bacteria, including the ones existing in extracorporal medium. For instance, BURZYŃSKA and STEC [4] point to participation of saprophytes occurring in foodstuffs

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in transferring this feature within human organism. At the same time, there is a complete lack of information about the possible role played in this process by microflora of water and sewage. It is plain that surface waters, being a receiving body of farm and house impurities and sewage from animal farms, contain both microorganisms resistant to antibacterial agents, as well as residue of different antibiotics, chemotherapeutic agents and disinfecting agents which can stimulate resistance. These are favourable conditions for the spread of resistance in saprophytic microorganisms inhabiting aqueous medium. When such water is used in water supply systems as it happens in the case of the city of Wrocław for which water is taken by the Municipal Water Supply and Sewage Service from the river Oława – the presence of resistant bacteria makes the disinfection process difficult and less efficient. As a result, the water used for household purposes is not free from bacteria.

The river Oława is seriously contaminated by rural and urban wastewaters. Apart from that, at a distance of several kilometers from the water-bearing areas, the Zootechnical Experimental Farm "Czechnica" is situated from which waste products of animal breeding go into the river Oława.

The aim of this work was to establish the species of bacteria in water for domestic supply and to determine their resistance to some antibacterial agents used at present in medical treatment of people and animals.

2. EXPERIMENTAL

Bacteria were isolated from water supplied for domestic consumption from the reservoir situated in the area run by MPWiK Wrocław. Species of strains were identified using the standard tests (BERGEY [2], COWAN and STEEL [5]).

Susceptibility to antibacterial agents was determined by the method of absorbent paper disks on the Müller–Hinton medium, making use of disks prepared by Serum and Vassine Production Plant in Warsaw. The resistance to the following antibiotics was examined: penicillin, ampicillin, cloxacillin, naphcyllin, oxytetracycline, doxycyclin, streptomycin, gentamycin, chloramphenicol, collistine and riphampicyn as well as chemotherapeutic agents: biseptol, nalidix acid and nitrofurantoin and one sulphonamide, i.e. sulphathiazol.

The sets of 3 disks saturated with appropriate compound were placed with aseptic forceps on the surface of agar medium (Müller–Hinton) inoculated earlier with 18-hour broth culture of pure strain. The plates prepared in this way were left for 30 min at room temperature and then incubated for 16–18 hours at the temperature of 310 K (37°C). *Staphylococcus aureus* PCM-2054, *Staphylococcus aureus* PCM-520, *Escherichia coli* PCM-2057 and *Pseudomonas aeruginosa* PCM-2058, obtained from the collection of strains at the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences, were used as control strains.

After incubation, the area of bacteriostasis around the absorbent paper disks was measured. A degree of susceptibility was read from the tables enclosed with each series of disks.

3. RESULTS

Altogether 48 bacteria strains belonging to 5 types and 17 species and one actinomycete strain were isolated (table). *Bacillus* species was the most numerous and, as was shown before, it was significantly resistant to the process of water disinfection supervised by MPWiK (TRACZEWSKA et al. [13]).

The majority of bacteria present in drinking water were resistant to penicillin (77.5%) and its semisynthetic derivatives – ampicillin (91.8%), cloxacillin (89.7%) and naphcylin (69.3%), and antibiotics from the group of polymyxins – colistyne (85.7%). Resistance of the strains to penicillin and ampicillin can be connected with the natural or acquired ability of their enzymatic decomposition by penicillin, whereas resistance to cloxacillin and naphcylin must be of different character.

The examination showed that doxycycline is remarkably efficient in inhibiting the growth of bacteria isolated from treated water, which was an 87.7% of the total number of bacteria. The above agrees with the data found in the literature, as regards susceptibility of coccidians and oxygen bacilli (PODLEWSKI, CHWALIBOGOWSKA-PODLEWSKA [2]).

Besides, a number of strains were observed to be sensitive to aminoglycoside antibiotics – gentamicin (67.3%), synthetic derivative of rimphamycin – rimphampicin (65.3%), and chloramphenicol (53%), antibiotic which is rarely used in health service due to its high toxicity.

Of the group of chemotherapeutic agents, bisepitol was the most efficient (61.2%), a drug which inhibits activity of enzymes taking part in biosynthesis of folic acid in bacteria. Nitrofurantoin another efficient agent (48.9%) – the drug with bacteriocidal effect on many microorganisms, both gram-positive and gram-negative.

Summing up, among microorganisms isolated from water, two types of conditional-pathogenic bacteria were present, namely, *Pseudomonas fluorescens* and *Bacillus cereus* and one strain of pathogenic actinomycetes, *Arachnia propionica*. *Pseudomonas fluorescens* was sensitive only to aminoglycoside antibiotics – gentamycin and streptomycin and to doxycycline belonging to tetracyclines and sulphonamid – sulphathiazol which is rarely used at present due to mass crystallurgy. All the strains belonging to *Bacillus cereus* were resistant to collistine and majority of them were also resistant to such antibiotics as penicillin and its semisynthetic derivatives – ampicillin, cloxacillin and naphcylin.

All of the strains under examination were sensitive to doxycyclin and ryphampicin. Resistance to chemotherapeutic agents and sulphonamids was not a characteristic of species, it different for each individual strain.

Actinomycete, *Arachnia propionica*, was resistant to penicillin and its semisynthetic derivatives (ampicillin and cloxacyclin), oxytetracycline, streptomycin, collistine, ryphampicine, being sensitive only to very powerful antibiotics such as doxycyclin, gentamycin and chloramphenicol and bisepitol from the group of chemotherapeutic agents.

4. DISCUSSION

It has been shown that the majority of microorganism strains isolated from drinking water were resistant to penicillin and its semisynthetic derivatives, especially ampicillin. These drugs are commonly used in medical treatment of people and animals, which in turn

Resistance of microorganism strains isolated from drinking water to antibacterial agents

Types and species	Total number of strains in a given species	Sensibility to antibacterial agents	Number of strains resistant and moderately sensitive to antibacterial agents applied														Chemotherapeutic agents	Sulphonamide
			Antibiotics															
			Pe	Am	Cl	Nc	T	Dc	S	Ge	C	Cs	Rf	Bs	Na	Nf		
<i>Bacillus subtilis</i>	2	R	0	2	2	2	0	0	0	0	1	2	1	0	2	2	1	
		MS	1	0	0	0	0	0	2	1	0	0	0	0	0	0	0	
		S	1	0	0	0	2	2	0	1	1	0	1	2	0	0	0	
<i>Bacillus cirroflagelosus</i>	2	R	2	2	2	2	1	0	1	1	1	2	1	0	0	2	1	
		MS	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	
		S	0	0	0	0	1	2	0	1	1	0	1	2	2	0	1	
<i>Bacillus acidocaldarius</i>	1	R	-	1	0	1	0	0	0	0	0	1	0	0	1	1	0	
		MS	-	0	1	0	0	0	0	0	0	0	0	0	0	0	0	
		S	-	0	0	0	1	1	1	1	1	0	1	1	0	0	1	
<i>Bacillus laterosporus</i>	1	R	1	1	1	-	1	0	0	0	1	0	1	0	1	0	1	
		MS	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	
		S	0	0	0	-	0	1	1	1	0	1	0	1	0	1	0	
<i>Bacillus globisporus</i>	1	R	1	1	1	-	1	0	1	0	1	1	0	0	1	1	0	
		MS	0	0	0	-	0	0	0	1	0	0	0	0	0	0	0	
		S	0	0	0	-	0	1	0	0	0	0	1	1	0	0	1	
<i>Bacillus megaterium</i>	9	R	9	9	9	9	6	2	6	0	3	7	5	7	6	5	5	
		MS	0	0	0	0	1	0	0	3	0	0	0	0	0	4	0	
		S	0	0	0	0	2	7	3	6	6	2	4	2	3	0	4	
<i>Bacillus brevis</i>	5	R	4	5	5	5	2	0	2	1	0	4	1	3	3	2	5	
		MS	0	0	0	0	1	0	1	1	1	1	0	0	0	3	0	
		S	1	0	0	0	2	5	2	3	4	0	4	2	2	0	0	
<i>Bacillus coagulans</i>	7	R	6	6	5	5	4	2	2	0	2	5	2	0	2	1	3	
		MS	0	0	1	1	2	0	1	2	2	2	1	0	0	4	0	
		S	1	1	1	1	1	5	4	5	3	0	4	7	5	2	4	
<i>Bacillus firmus</i>	5	R	4	4	5	4	3	1	2	0	1	5	1	2	3	0	2	
		MS	0	0	0	0	1	0	0	1	1	0	0	0	1	5	0	
		S	1	1	0	1	1	4	3	4	3	0	4	3	1	0	3	
<i>Bacillus cereus</i>	3	R	2	3	2	3	1	0	1	0	0	3	0	1	2	2	0	
		MS	0	0	1	0	0	0	1	0	2	0	1	0	0	0	0	
		S	1	0	0	0	2	3	1	3	1	0	2	2	1	1	3	

<i>Bacillus cereus</i> var. <i>mycoides</i>	3	R	3	3	3	3	0	0	0	0	1	3	0	2	1	1	2	
		MS	0	0	0	0	1	0	1	1	0	0	0	0	0	0	2	0
		S	0	0	0	0	2	3	2	2	2	0	3	1	2	0	1	0
<i>Bacillus</i> sp. (Wolf and Barker) gr. 1	1	R	-	0	0	-	3	0	0	0	0	1	0	1	1	0	1	
		MS	-	0	1	-	0	1	0	0	1	0	0	0	0	1	0	
		S	-	1	0	-	0	0	1	1	0	0	1	0	0	0	0	
<i>Bacillus sphaericus</i>	1	R	-	1	1	-	1	0	1	1	0	1	0	1	1	0	1	
		MS	-	0	0	-	0	0	0	0	1	0	0	0	0	1	0	
		S	-	0	0	-	0	1	0	0	0	0	0	0	0	0	0	
<i>Sphaerotilus natans</i>	4	R	4	3	4	-	0	0	1	2	1	3	1	2	1	2	1	
		MS	0	1	0	-	0	0	2	0	2	0	0	0	0	0	2	0
		S	0	0	0	-	4	4	1	2	1	1	3	3	3	0	3	
<i>Oscillospira</i> <i>guilliermondi</i>	1	R	-	1	1	-	0	0	0	1	0	1	0	0	1	0	1	
		MS	-	0	0	-	0	0	0	0	0	0	0	0	0	1	0	
		S	-	0	1	-	1	1	0	1	0	0	1	0	0	1	0	
<i>Pseudomonas</i> <i>fluorescens</i>	1	R	1	1	1	-	1	0	0	0	1	1	0	1	1	1	0	
		MS	0	0	0	-	0	0	0	0	0	0	1	0	0	0	0	
		S	0	0	0	-	0	1	1	1	0	0	0	0	0	0	1	
<i>Sporolactobacillus</i> sp.	1	R	0	1	1	-	0	0	1	1	0	1	0	0	1	0	1	
		MS	0	0	0	-	1	0	0	0	0	0	0	0	0	1	0	
		S	1	0	0	-	0	1	0	0	1	0	1	1	0	0	0	
<i>Arachnia</i> <i>propionica</i> <i>Actinomycetes</i>	1	R	1	1	1	-	1	0	1	0	0	1	1	0	1	1	1	
		MS	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	
		S	0	0	0	-	0	1	0	1	1	0	0	1	0	0	0	
Percentage of bacteria resistant to particular antibacterial agents		R	77.5	91.8	29.7	69.3	36.9	10.2	38.7	12.2	26.5	66.7	28.57	38.77	58.8	42.85	53.06	
		MS	2.04	2.04	8.16	2.04	14.2	2.04	18.36	20.4	20.4	6.1	6.1	0	2.04	48.07	0	
		S	12.2	6.1	2.04	2.04	38.7	87.7	42.85	67.3	53.06	5.1	65.3	65.3	38.7	8.16	46.93	
	Total 49																	

Pe - penicillin, Am - ampicillin, Cl - cloxacillin, Nc - naphcilin, T - oxytetracycline, Dc - doxycyclin, S - streptomycin, Ge - gentamycin, C - chloramphenicol, Cs - collistine, Rf - rifampicin, Bs - bisseptol, Na - nalidix acid, Nf - nitrofurantoin, St - sulphathiazol.

provides favourable conditions for the development of resistance. This problem refers to most antibiotics and chemotherapeutic agents and produces a series of epidemiologic and ecological consequences. As regards ecological issues, one point is worth stressing, namely the possibility that microflora resistant to drugs and disinfection agents may penetrate with wastewaters into surface waters and then into waters supplied for drinking, since it is more likely to survive during disinfecting process. The presence of many species in pure water confirms the above statement.

Another question to be raised is the spreading of resistance both in aqueous medium, as well as in human organisms after water consumption.

These are very important questions and they mainly refer to resistance conditioned by the presence of plasmides R, in the transfer of which the intestinal microflora plays an important role. For instance, LINTON [10] states that the quantity of bacteria from coli group excreted with feces and possessing plasmides R is large and it is, for instance, 67% in children treated with antibiotics and 46% in adults in relation to the total number of bacteria. These plasmides are transferred to sensitive cells of the same species or different species and even different types.

Antibiotic-resistant strains of *Escherichia coli* (WILLIAMS, SMITH [14]) were isolated from surface waters; they were able to transfer the resistance determinants discussed to the bacteria *Salmonella typhi* and *Salmonella typhimurium*. Other saprophytes can also play a significant role in the spreading of drug resistance (ANDERSON [1], BOBROWSKI, LACHMAJSTER-LUTOSŁAWSKA [7], DZIERŻANOWSKA [7]).

Finally, antibiotics in active form as residues after treatment of people and animals happen to occur in surface water flowing with wastewaters from households and farms. In natural medium, they are the factors stimulating development of drug resistance and bringing about a selection of microflora such that resistant species are predominant.

Summing up, the bacterial resistance to some pharmacologic and disinfectant agents, important from the medical point of view, affects ecological and technological conditions, especially during disinfection of water supplied for drinking.

5. CONCLUSIONS

1. A total of 48 strains of bacteria belonging to 13 species of *Bacillus* type, 4 strains from *Sphaerotilus natans*, 1 strain from *Oscillospira quilliermondi* and 1 strain from *Pseudomonas fluorescens*, 1 strain from *Sporolactobacillus* species and 1 strain of actinomycete *Arachnia propionica* were isolated from 1 cm³ of treated water for domestic supply.

2. The microorganisms were resistant to most of the antibiotics used in the examination. Their resistance varied, and as shown in the table, the percentage of bacteria resistant to individual preparations calculated with regard to the total number of the strains isolated was as follows:

- penicillin 77.5%,
- ampicillin 91.8%,
- cloxacillin 89.7%,
- naphcylin 69.3%,
- oxytetracycline 36.9%,

doxycyclin 10.2%,
 streptomycin 38.7%,
 gentamycin 12.2%,
 chloramphenicol 26.5%,
 collistine 85.7%,
 riphamycin 28.5%,
 biseptol 38.7%,
 nalidix acid 58.8%,
 nitrofurantoina 42.8%,
 sulphathiazol 53.0%.

3. It has been observed that resistances to antibiotics varied both in different species and in different strains within the same species.

4. It appeared that all bacteria strains were, to a varying degree, resistant to antibiotics used in the examination.

5. The fact that there were so many strains resistant to antibiotics in water disinfected both with chlorine and ozone points to their resistance also to disinfection agents, as it can be assumed that resistance to antibiotics is connected with resistance to disinfection agents.

6. Apparently two mechanisms influenced the results obtained, as far as resistance of bacteria to disinfection agents is concerned. One is of plasmide character or consists in mutation occurring in bacteria genome. The other results from the bacterial ability to form endospores.

7. The presence of such a great number of bacteria resistant to antibiotics and disinfectant agents in drinking water proves that the disinfection process is not efficient enough, and what is even more important, it shows that bacteriological quality of water taken for domestic supply is very bad.

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WSTĘPNE BADANIA NAD OPORNOŚCIĄ NA ŚRODKI PRZECIWBAKTERYJNE BAKTERII WYIZOLOWANYCH Z WODY PITNEJ

Zbadano wrażliwość drobnoustrojów wyizolowanych z wody pitnej na środki przeciwbakteryjne, takie jak antybiotyki, chemioterapeutyki i sulfonamidy. Próbkę wody pobierano na terenie MPWiK we Wrocławiu na stanowisku pompownia centralna. Celem pracy było ustalenie składu jakościowego mikroflory w wodzie uzdatnionej oraz określenie oporności drobnoustrojów na wybrane środki przeciwbakteryjne. Ogółem wyizolowano i zbadano 41 szczepów bakterii saprofitycznych, 7 szczepów bakterii warunkowo patogennych oraz 1 szczep patogennego promieniowca. Stwierdzono, że większość wyizolowanych drobnoustrojów była oporna nie tylko na pojedyncze stosowane w lecznictwie środki przeciwbakteryjne, ale także na kilka preparatów zastosowanych równocześnie.

ПРЕДВАРИТЕЛЬНЫЕ ИССЛЕДОВАНИЯ УСТОЙЧИВОСТИ К АНТИБАКТЕРИАЛЬНЫМ СРЕДСТВАМ БАКТЕРИЙ, ВЫДЕЛЕННЫХ ИЗ ПИТЬЕВОЙ ВОДЫ

Исследована устойчивость микроорганизмов, выделенных из питьевой воды, к антибактериальным средствам, таким как: антибиотики, химиотерапевтические средства и сульфонамиды. Пробы воды брали на территории МПВиК во Wrocławie, на стенде — центральная насосная станция. Целью работы было установление качественного состава микрофлоры в подготовленной воде, а также определение устойчивости микроорганизмов к избранным антибактериальным средствам. Выделили и исследовали 41 сапрофитных штаммов бактерий, 7 штаммов условно-патогенных бактерий, а также 1 штамм патогенного актиномицета. Установили, что большинство выделенных микроорганизмов было устойчиво не только к отдельным, применяемым в медицине антибактериальным средствам, но также к нескольким препаратам, применяемым одновременно.