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Treated sewage as an additional source of dangerous bacteria in rivers such as *Listeria* spp.

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ABSTRACT

Purified water daily discharged to surface water from local sewage treatment plants can cause illnesses people and animals in nearby areas. Sometimes they also contribute to the development of diseases in much bigger region range. Often the cause of such infections are pathogenic *Listeria* rods especially *Listeria monocytogenes*, which are not eliminated in the technological process of wastewater treatment carried out by local sewage treatment plants. Therefore, the purpose of the study was to investigate the influence of the sewage treatment plant on the presence of *Listeria* bacteria and to estimate the potential hazard resulting from its occurrence in the water of the Warta River. Research points were located on the Warta River before and after the discharge of purified sewage through the treatment plant, also a direct exit of purified wastewater was examined. Samples were filtered and then estimated the growth characteristics of typical colonies on the selected diagnostic media were estimated and isolated microorganisms were characterized. Samples were filtered and then it had been defining the growth characteristics of typical colonies on the selected diagnostic media, finally isolated microorganisms were described. Studies have shown that the local wastewater treatment plant has a strong influence on the microbial quality of the Warta River and has shown strong impurity with *Listeria* rods, including pathogenic strains. Bearing in mind the results obtained, it can be stated that direct or indirect contact with receivers of the water of purified sewage poses a real threat to the health and even life of humans and animals.

***Keywords*: *Listeria monocytogenes*, *Listeria*, listeriosis, wastewater, treated sewage, sewage sludge**

1. INTRODUCTION

In most developed countries, liquid waste is converted by sewage treatment plants and then discharged into rivers and collected in sewage sludge, which are burned and increasingly used for fertilization of agricultural soils [1]. Wastewater contains significant amounts of microorganisms including pathogens, the majority of which are removed by purification [2]. Unfortunately in Poland The specificity of wastewater treatment technology does not eliminate all the risks flowing with municipal sewage and industrial sewage from the operated agglomerations. Despite significant reductions in microbiological danger, even biologically purified wastewater is still hazardous due to the presence of certain bacteria, viruses and parasites for which the purification process is insufficient [3]. An example is *Listeria monocytogenes*, a gram-positive bacterium that also adapts in the environment of wastewater [4], inter alia by transmission from diseased and vector organisms. It has been shown that wastewater entering the treatment plant containing *Listeria* rods is the main cause of the spread of these bacteria and their presence in river receivers and sewage sludge [1]. Increasing volume and prohibition of storing sediment generated in treatment plants introduced since 1 January 2013 have led to the necessity for further processing, which among other things has led to their agricultural use [5].

The use of this type of waste to fertilization of agriculture soils contributes to permanent contamination of plants consumed by humans and animals. The faeces of infected animals containing significant amounts of bacteria is also used as a fertilizer for growing crops especially in rural households, which is also a major microbiological risk. In addition, flow from such polluted fields to nearby reservoirs and watercourses results in the presence of these bacteria in the surrounding water [6]. The genus of *Listeria* currently contains of 18 species, but the greatest risk is pathogenic *Listeria monocytogenes* [7]. Aerobic and relatively anaerobic rod-shaped bacterium with an optimal growth temperature of 30-37 °C [8], insensitive to many physicochemical factors. It is isolated from various environments and food products. Bacteria have high adaptability, their growth does not deter cold storage, it grow well in a wide pH range especially with high water content and also tolerate high levels of sodium chloride, sodium nitrate, and organic acids. It survive short-term pasteurisation, freezing and prolonged drying [9]. Causes symptomatic infections called listeriosis in people and animals and each year contribute to epidemic diseases of digestive system, often with tragic consequences. Bacteria is usually dangerous only for a certain group of people (YOPI - Young, Old, Pregnant, Immunocompromised) [10] and the annual incidence is not so high, However it is characterized by sharp run and significant mortality (20, 30%) [11,12]. The vast majority of confirmed cases are those over 65 years of age. The disease is particularly dangerous for pregnant women resulting in miscarriage, dead or premature childbirth and dangerous fetal infections [10]. The health effects range from influenza-like conditions to life-threatening conditions that reveal as meningitis or septicemia [13,14].

As a result of environmental protection investments, new wastewater treatment plants are being put into use every year, effectively reducing the uncontrolled release of raw sewage into rivers. In 2015, the percentage of the population using wastewater treatment plants in Poland amounted to almost 73% and so far the main source of water of the national economy is surface water which covering 85% of the population's needs. In Poland, there is a great deal of legislation governing the sanitary status of both sewage and sludge, however no disinfection is required as a means of effectively destroying microbes that may pose an

epidemiological threat. Disinfection of wastewater is a standard process used in developed countries and has been proven to significantly reduce the spread of diseases by water [3]. In our country only infectious hospitals are obliged to disinfect sewage prior to their releasing into the sewage system. However, according to the reports of the Sanitary Inspectorate, many of them have been failing to comply with the imposed duty and they have been discharging sewage directly into the municipal sewage system, increasing the amount of hazardous microorganisms reaching the sewage plant [15]. Therefore it is important to take care of the microbiological quality of wastewater leaving the sewage treatment plant and the receivers of water by adjusting the sanitary and epidemiological standards to the real threats to the aquatic environment [4].

2. MATERIALS AND METHODS

In order to determine the impact of WARTA SA wastewater sewage treatment plant located in Częstochowa on the presence of *Listeria* bacteria, the water samples from Warta River were collected in three points: before the point of discharge of sewage purified by the treatment plant (50°49'35.9" N, 19°09'36.5" E), directly from the outlet (50°49'37.0" N, 19°09'39.4" E) and behind the place of the dump (50°49'38.8" N, 19°09'41.9" E). Samples of water (1 ml, 10 ml, 50 ml, 100 ml) were filtered through a microbial filter of 0.45μm pore diameter then filter was placed in a semi-Fraser medium for multiplication. Following the incubation period (24 h, 37 °C) were sown by spread plate method on CHROMagar Listeria. The resulting single colonies were sifted on CHROMagar identification Listeria. In addition, a test for hemolysis and Gram staining were performed.

3. RESULTS

The study found variation in the number of *Listeria* bacteria depending on the volume of the filtered sample and the point of collected water. The highest amount of *Listeria* bacteria (presence in 1ml) was found at the point of collection samples out of the Warta River behind the discharge of purified sewage to the river by the WARTA S.A sewage treatment plant in Czestochowa. The study was conducted with simultaneous determination of genus *Enterococcus* in Slanetz – Bartley agar and sodium azide and crystal violet medium. None of the samples tested had shown the presence of genus *Enterococcus*.

Table 1. Growth of the bacteria after filtering a specified volume (1, 10, 50, 100 ml) from each sample.

Sampling point	Volume of filtered sample			
	1 ml	10 ml	50 ml	100 ml
Before point of sewage discharge on Warta River	-	+	+	+

Behind the point of sewage discharge on Warta River	+	+	+	+
Direct outlet ejecting sewage	-	+	+	+

(- no growth, + growth).

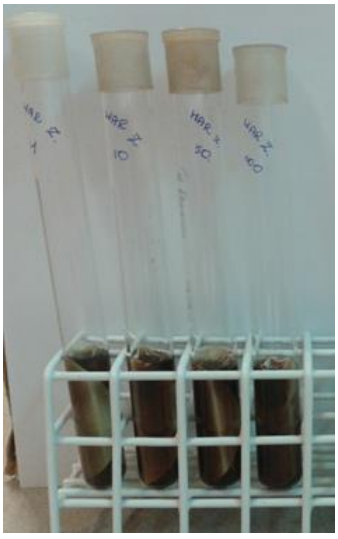

Figure 1. Semi-Fraser medium after incubation with visible filter discs. *Listeria spp.* hydrolyse the esculin from the semi-Fraser medium to esculetin and glucose. Esculetin reacts with the iron ions contained in the substratum, which results in darkening of medium.

A. B. C.

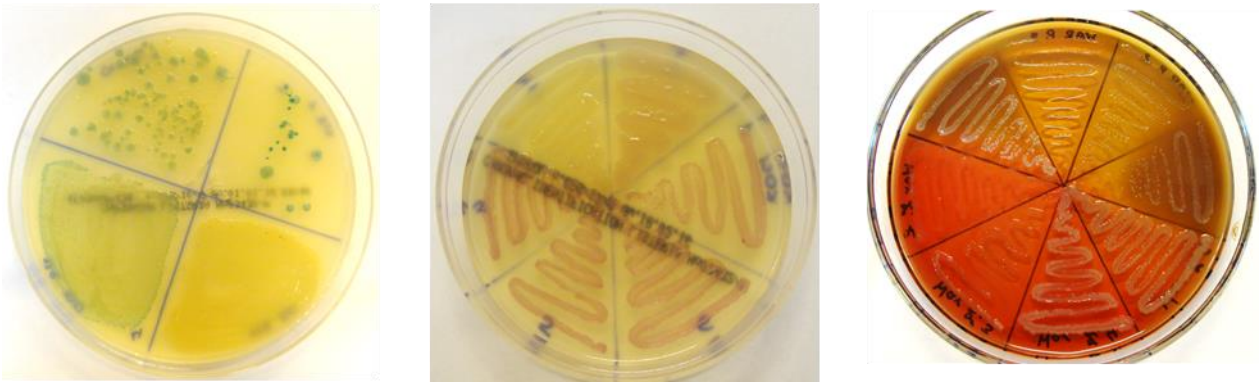

Figure 2. Growth of *Listeria spp.* on selected mediums. **A.** CHROMagar Listeria medium. *Listeria spp.* grows in form of blue-green colony. **B.** CHROMagar Identification Listeria medium. *Listeria monocytogenes* grow in the form of pink colonies surrounded by brighter zones. **C.** Blood Agar medium. *Listeria monocytogenes* expresses a beta hemolysin.

Inoculation on selected diagnostic medium allows detection of hemolytic strains of *Listeria monocytogenes*.

Figure 3. Gram stain of *Listeria monocytogenes* obtained from direct outlet of wastewater purified from sewage treatment plant.

4. CONCLUSIONS

Studies have shown that sewage treatment plants WARTA S.A. has a strong influence on the presence of Listeria bacteria in the Warta River and consequently affects the local population. Warta River has been serving residents of the city for many years as a place of recreation and entertainment, organized are here kayaking and fishing tournaments. In addition, local people use river water to watering crops, watering animals and also as a source of water to nearby fish breeding ponds. For *Listeria* spp. including pathogen species, this creates excellent living conditions and may contribute to development in humans and animals disease. Although there is no evidence of direct infection by human through contaminated water, there are data on the development of the disease after drinking such water by animals. Contaminated water can be an indirect cause of diseases in humans through polluted plants or aquatic animals whose further processing and consumption can cause local epidemics especially among people at risk group. Such situation needs immediate steps to check the microbiological state of the water used by the population and local authorities should inform local residents of the real risks of exposure to contaminated water. Optimization of wastewater treatment processes and proper management of sewage sludge can minimize the occurrence in the environment of pathogenic species of *Listeria* genus.

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