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Analysis of the presence and drug resistance of bacteria from the *Enterobacteriaceae* family and the genus of *Enterococcus* in treated wastewater from a selected wastewater treatment plant

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ABSTRACT

Sanitary cleanness of water is one of the most important components of contemporary water management. However, many potentially hazardous microorganisms are often found in water. These include bacteria such as *Escherichia coli* and enterococci (former streptococci). Both enterococci and many intestinal bacteria can lead to several diseases which are hazardous to humans and animals. These microorganisms are a leading cause of infections in the gastrointestinal tract, with its symptoms including diarrhoea, fevers, intestinal and digestive problems. However, in certain cases of the above infections, tissues which are important to the function of human body can be affected (e.g., joints, lungs, soft tissues, kidneys, endocardium, etc.). These bacteria, especially if drug-resistant, can lead to sepsis (especially in people with weak immunological system), which is potentially hazardous to human life. For this reason, both intestinal bacteria and enterococci should be eliminated through a water treatment step in municipal wastewater treatment plants. The material was sampled from a medium-sized wastewater treatment plant in the southern part of Poland. The plant uses a biological wastewater treatment process based on activated sludge. After pure water is separated from sewage sludge in this process, it is transferred to the water receiver (river). The examinations revealed the presence of indicator bacteria, that is, intestinal bacteria and enterococci in the analysed water samples following the treatment process.

Keywords: Water; Pathogenic bacteria; *Enterobacteriaceae*; *Enterococcus*; Drug-resistant bacteria

1. Introduction

The wastewater treatment process is one of the key elements of contemporary water management. Unfortunately, many potentially hazardous microorganisms are often found in water. The most characteristic microorganisms represent water contamination indicators. These include such bacteria as *Escherichia coli* and enterococci (former streptococci). These and other indicator bacteria are used to determine microbiological contamination of surface waters during routine laboratory tests [1].

Both enterococci and many intestinal bacteria can lead to several diseases which are hazardous to humans and animals. They represent a leading cause of infections in the gastrointestinal tract and manifest themselves by diarrhoeas, fevers and abdominal pain. In more serious cases, other important tissues which are critical to the functioning of human body can be affected, for example, joints, lungs, soft tissues, kidneys, endocardium, etc. Unfortunately, these bacteria can lead to sepsis, especially in people with weak immunological system, which is potentially hazardous to human life [2]. A particularly dangerous problem is the

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phenomenon of resistance of many types of bacteria present in the environment to various types of drugs, including antibiotics. Wastewater, sewage sludge, fertilizers derived from animal matter and water purified in wastewater treatment plants can represent reservoirs for such forms of microorganisms [3–5].

The aim of the research was to determine the presence of sanitary indicators (intestinal bacteria and enterococci) in the analysed sewage samples after the purification process. Their drug resistance was also determined. This is an important aspect of the research, because the sewage after the treatment processes is discharged directly into the river.

2. Materials and methods

The research was carried out for effluent (after the process of wastewater treatment) discharged to the river. The samples were taken from a municipal wastewater treatment plant in a town with around 33,000 people. Wastewater in this location is treated by means of biological processes using activated sludge. Next, sewage sludge is separated with belt presses and effluent is discharged to the river.

The wastewater treatment plant is located in the northern part of the city with ca. 33,000 inhabitants at the left bank of the river. The plant has a permit required by Water Law Act for discharging the treated wastewater to the river fork with the amount of Q_{mean} of 9,000 m^3/d (Q_{maxd} – 15,000 m^3/d) and with the content not exceeding:

General suspension	35 mg/L
BOD_5	15 mg O_2/L
CODCr	125 mg O_2/L
Total nitrogen	15 mg N/L
Total phosphorus	2 mg P/L

The basal (standard) values of chemical pollutants in the treated wastewater do not exceed the permissible values. Technological processes in this plant are performed based on biological aerobic treatment (activated sludge) in the aeration chambers. The sewage sludge is dewatered on belt presses and solar dried after liming. After treatment, the wastewater is discharged to the river. Equivalent number of inhabitants is 44,000.

The samples of wastewater discharged to the river that were used for the examinations were sampled in three repetitions with the amount of 1 dm^3. Next, the averaged sample was supplied directly to the laboratory and subjected to examinations in the period of 2 h from sampling.

The effluent samples were analysed microbiologically. A series of dilutions in saline solution (with decimal progression) were prepared at the first stage. Next, 0.1 mL of each of the above dilutions was spread on Endo, BEA and nutrient agars with three repetitions. Incubation was performed for 24 h at 37°C. Samples were taken and analysed three times at monthly intervals (from August to the end of October). In this time, wastewater temperature in the (open) bioreactor was reduced from 21°C to ca. 14°C, which was connected with natural cooling in the period of autumn.

Identification of individual groups of microorganisms used the respective selective agar mediums. Endo agar is used for quantitative determination of bacteria from coli group and other intestinal bacteria from water, wastewater, etc. Lactose-fermenting bacteria grow in the form of claret or dark red colonies. The bacteria without such properties produce colourless or light pink colonies. BEA agar was used for selective isolation of enterococci. These bacteria grow on this agar in the form of small colonies with grey or black colour with characteristic blackened zone around. This is caused by the esculin capabilities to hydrolyse to esculetin. Unlike staphylococci, enterococci hydrolyse this compound in several hours after inoculation and do not produce catalyse [6,7]. Agar medium (M-PA) was used to determine the total count of mesophilic microorganisms in the samples.

After bacterial colonies were grown, they were sieved for three times using the reduction method in order to obtain pure strains. With various and microbiologically reach environments of wastewater and water after treatment, individual colonies on the microbiological agars are often formed by more than one species, which can make process of identification very difficult. This can be observed for Gram-stained preparations. For this reason, it is necessary to perform at least three reduction inoculations.

Identification of intestinal bacteria was based on biochemical Microgen GN-ID A + B multitests which take into consideration analysis of the capability of decomposition of 24 substrates. Enterococci were identified by means of Microgen Strep ID biochemical multitests. As recommended by the manufacturer of the multitests, enterococci were incubated for 24 h at temperature of 37°C. The results obtained were analysed by means of Microgen MID 60 software.

Drug resistance test of intestinal bacteria and enterococci isolated from effluent was performed using the disc diffusion method. The basic criterion for selection of antibiotics for examinations of drug sensitivity of the strains was their activity in clinical conditions with respect to the *Enterobacteriaceae* family and *Enterococcus* genus. Current recommendations concerning their choice, published by EUCAST (European Committee on Antimicrobial Susceptibility Testing), were also used. The guidelines of these institutions were based on current scientific research and numerous experiences of clinical microbiologists that have performed such examinations routinely.

Antibiograms were prepared based on cell concentration (in the bacteria suspension) of ca. 0.5 on the McFarland scale. The scale is used for determination of the density of cell suspension and recommended in the methodology of analysis of sensitivity of microorganisms to antibiotics using the disc diffusion test. The previously purchased standardised reference models were used to determine the degree of opaqueness and density of bacteria suspension that provides on average information on cell count which is similar to the aforementioned methodology.

The Mueller–Hinton agar recommended in clinical diagnostics was used for the purpose. In the case of intestinal bacteria, analysis of drug susceptibility to amikacin, co-amoxiclav, cefazolin, ceftazidime, cefuroxime, ciprofloxacin, ampicillin and gentamicin was performed. Furthermore, in the case of enterococci, tests were performed to examine susceptibility to ampicillin, ciprofloxacin, penicillin, erythromycin, streptomycin, vancomycin, chloramphenicol, tetracycline, linezolid and imipenem [8,9]. Concentrations of the

Table 1
Results of antibiograms for individual bacteria from the *Enterobacteriaceae* family isolated from the effluent

Species of bacteria isolated from the effluent	Amikacin (AK30)	Co-amoxiclav (AK30)	Gentamicin (CN10)	Cefazolin (CZ30)	Ciprofloxacin (CIP5)	Ceftazidime (CAZ30)	Cefuroxime (CXM30)	Ampicillin (AM10)
Escherichia coli	S	S	S	S	S	S	R	S
Morganella morganii	R	S	S	R	S	S	MS	R
Enterobacter cloacae	S	S	S	S	S	S	R	R
Klebsiella oxytoca	S	S	S	S	S	S	S	R
Klebsiella ozaenae	R	S	S	S	S	S	R	R
Serratia odorifera	S	S	S	S	S	S	R	R
Enterobacter aerogenes	S	S	S	S	S	S	S	R

Note: S, susceptible, MS, medium susceptible, R, resistant.

antibiotics tested resulted from guidelines used. For the bacteria from the *Enterobacteriaceae* family, the content of antibiotic in a single disc is presented in Table 1, with abbreviation of the antibiotic's name (in μg). Furthermore, for the bacteria from the *Enterococcus* genus, the content of antibiotic in a single disc is presented in Table 2, with abbreviation of the antibiotic's name (in μg).

After even spreading of the suspensions of the bacteria isolated on the Mueller–Hinton agar surface (on Petri dishes), the dishes soaked with the respective antibiotics were applied and the incubation was performed at the temperature of 37°C for 1 d. The results concerning susceptibility to individual antibacterial substances were read from the interpretation tables for minimal inhibiting concentrations and the size of growth inhibition zones developed by the European Committee for Determination on Antimicrobial Susceptibility Testing [10].

In the methodology of determination of drug sensitivity to antibiotics using the disc diffusion test, the measurement was performed for the growth inhibition zone on the agar where a bacterial suspension was spread and the discs with antibiotics were inserted, with the value presented in millimetres. With standardised conditions of incubation, the antibiotic diffuses to the agar at a specific rate. A concentration gradient zone is formed around the disc. The sensitive microorganism grows only to the place where the minimum inhibitor concentration value is reached. The more sensitive the organism, the greater the distance from the disc where it can grow on agar. This translates into the magnitude of the growth inhibition zone: the greater the zone, the more active the antibiotic. No inhibition zone or the zone is smaller than indicated in the standards for reading the antibiogram means that this microorganism is resistant to drug concentrations used in clinical conditions. These standards are regularly published by EUCAST and National Reference Center for Microbial Susceptibility. In some regions of the world, individual values can slightly differ depending on the adopted guidelines.

3. Results and discussion

The quantitative analysis revealed (Table 3) that total mesophilic bacterial count in the effluent discharged to the river from the wastewater treatment plant was $1.3–3.6 \cdot 10^5$ in 1 cm^3. The intestinal bacterial count ranged from $1 \cdot 10^4$ in the third sample taken in October to $8.1 \cdot 10^4$ in the first sample from the end of August (Table 4). Presence of the following intestinal bacteria was found in the samples: *Escherichia coli*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Klebsiella oxytoca*, *Klebsiella ozaenae*, *Serratia odorifera*, *Morganella morganii* (Table 1). Apart from these bacteria, typical saprophytic genera present in the environment were isolated on the Endo agar, including *Pseudomonas stutzeri*, *Pseudomonas fluorescens* and *Alcaligenes faecalis*. Qualitatively, they represented only an insignificant percentage of all the bacteria obtained on this microbiological agar.

Enterococci (streptococci) were present only in the form of individual cells per 1 cm^3 of the effluent samples (Table 4). Presence of *Enterococcus faecalis* was found in the first sample, the second sample of effluent did not contain these bacteria, whereas the third contained only *Enterococcus gallinarum*. Other species of this type were not found in the samples studied (Table 2).

Sanitary safety of water is an important problem. Sewage treatment should reduce or eliminate pathogenic microorganisms which are particularly hazardous to animal and human health. Apart from bacteria from the *Enterobacteriaceae* family which are always present in the human gastrointestinal tract, various amounts of enterococci are also transferred to municipal wastewater. After treatment, water from the wastewater treatment plants is usually discharged to natural water reservoirs, for example, rivers. Therefore, the risk of the spread of pathogenic forms in the environment seems to be real [11].

The condition of the effluent discharged to water receivers depends on the type of technological processes used to treat wastewater. Further processing of sewage sludge generated as a final waste after processes of wastewater treatment may impact on migration of contaminants to waters. The most frequent processes used to limit the amount of pathogens include methane fermentation of both wastewater and sewage sludge [12,13]. Another biological method used to treat wastewater is activated sludge.

In the case of indicator bacteria such as those from *Enterobacteriaceae* family and *Enterococcus* genus, their presence was demonstrated in the analysed effluent samples after the treatment process. Intestinal bacterial count

Table 2
Results of antibiograms for bacteria from the *Enterococcus* genus isolated from the effluent

Species of bacteria isolated from the effluent	Chloramphenicol (C30)	Ciprofloxacin (CIP5)	Ampicillin (AM10)	Erythromycin (E15)	Penicillin (P10)	Streptomycin (S300)	Linezolid (LNZ30)	Tetracycline (TE30)	Vancomycin (VA30)	Imipenem (IMP10)
Enterococcus Faecalis	S	S	S	R	MS	R	R	S	S	S
Enterococcus gallinarum	S	S	S	S	MS	S	S	S	S	S

Note: S, susceptible; MS, medium susceptible; R, resistant.

Table 3
Total mesophilic bacterial count in effluent samples for the set temperature at the sewage outfall (CFU – colony-forming unit/mL)

Type of isolated microorganisms (CFU/mL)	Series 1	Series 2	Series 3
Total mesophilic bacterial count	$2.6 \cdot 10^5$	$1.4 \cdot 10^5$	$1.3 \cdot 10^5$
Effluent temperature during sampling	21°C	17.5°C	14°C

Table 4
Enterobacteriaceae and *Enterococcus* bacterial count in the effluent samples for the set temperature at the sewage outfall (CFU – colony-forming unit/mL)

Type of isolated microorganisms [CFU/mL]	Series 1	Series 2	Series 3
Enterobacteriaceae	$8.1 \cdot 10^4$	$5.5 \cdot 10^4$	$1 \cdot 10^4$
Enterococcus	7	0	2
Effluent temperature during sampling	21°C	17.5°C	14°C

(*Enterobacteriaceae*) was at a worrying high level. Total count of *Enterobacteriaceae* bacteria in treated sewage ranged from $1 \cdot 10^4$ to $8.1 \cdot 10^4$ CFU/mL. Total heterotrophic bacterial count ranged from $1.3 \cdot 10^5$ to $2.6 \cdot 10^5$ CFU/mL. However, in their study on the degree of water pollution and quality of fish, Al-Bahry et al. [14] demonstrated greater total bacterial count. It was on average $3.5 \cdot 10^6$ CFU/mL.

Presence of enterococci was also found. However, only very small amounts (only in the first and third samples) were observed for these bacteria. Therefore, they do not represent a serious epidemiological problem in the water discharged to the river analysed in the study. However, the intestinal bacteria can pose a potential hazard in the case of their contact with the water during, for example, a bathe in the river in summer or watering farming animals. From 0 to 7 CFU/mL bacteria were isolated. Although greater *Enterococcus* bacterial count was found in a study concerning the analysis of presence of these bacteria in treated sewage from 14 sewage treatment plants in Portugal and their resistance to antibiotics published by Costa et al. [15]. The researchers isolated from $0.1 \cdot 10^1$ to $3.2 \cdot 10^4$ CFU/mL of these microorganisms. In this study of the *Enterococcus* type, the researchers isolated *E. faecalis* and *E. gallinarum*. In the treated sewage, Costa et al. [15] found *Enterococcus faecium*.

It was found during the examinations that the decline in the ambient temperature and water in the wastewater treatment plant led to a gradual reduction in the mesophilic microorganism count, including those from *Enterobacteriaceae* family. Therefore, it can be assumed that water cleaned in the wastewater treatment plant (using the activated sludge method) will pose the highest hazard in summer months. At low temperatures, these bacteria are relatively quickly ousted by the group of psychrophiles and do not remain in waterways for a long time.

The study also found a faster decline of intestinal bacterial count compared with other microorganisms that can grow at the temperature of 37°C. Bacteria which belong to the *Pseudomonas*, *Acinetobacter* or *Aeromonas*, are usually characterised by a wider thermal optimum for the development compared with the microorganisms adapted to temperatures similar to the physiological temperature of humans and warm-blooded animals, such as *Escherichia*, *Klebsiella*, *Salmonella*, *Enterobacter*, *Proteus*, *Morganella*, *Citrobacter* and *Hafnia*. This results in improved adaptation to variable physical parameters of the environment [2].

Examinations of drug resistance of the bacteria of the *Enterobacteriaceae* family demonstrated that such properties can be found in five of seven tested species. In a study of treated sewage, the bacteria isolated from the *Enterobacteriaceae* family included: *Escherichia coli*, *Morganella morganii*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Klebsiella ozaenae*, *Serratia odorifera*, *Enterobacter aerogenes.* The particularly dangerous is the presence of the bacteria of the *Klebsiella* genus. This is so-called "signal" bacteria, which signals the infection risk. Toxins produced by the bacteria of this type can lead to serious diseases. It produces beta-lactamase and therefore is resistant to penicillin and ampicillin. It is often present in the intestines, but it can be also found in other parts of human body. The most frequent infections with these bacteria occur in hospital. However, as demonstrated in this study, the source of these bacteria can be sewage, even those subjected to treatment. Hospital wastewater can be particular source of bacteria resistant to ciprofloxacin and other antibiotics, especially those of *Klebsiella* spp. and *Aeromonas* spp. [16,17].

Similar results of the examinations in this study were obtained by Al-Bahry et al. [14]. The researchers isolated similar bacterial species from treated wastewater and water. Most of them were of *Klebsiella* genus whereas less frequent were *Serratia*, *Escherichia*, *Enterobacter* and *Vibrio*. The authors of the cited examinations also obtained similar results of resistance of isolated bacteria to the antibiotics as in this study. Percentage of ampicillin-resistant bacteria was in both cases almost 100%.

Apart from *Escherichia coli*, all of them are naturally little susceptible to ampicillin. *Escherichia coli* was resistant to cefuroxime, *Morganella morganii* to amikacin, cefazolin and medium susceptible to cefuroxime. *Enterobacter cloacae* showed resistance to cefuroxime, *Klebsiella ozaenae* to amikacin and cefuroxime, and *Serratia odorifera* to cefuroxime. Proper drug resistance profile was found for *Enterobacter aerogenes* and *Klebsiella oxytoca*. Similar findings concerning the spread of drug-resistant forms of gram-negative bacteria containing plasmid-encoded extended-spectrum beta-lactamases (ESBL) was obtained by Korzeniewska and Harnisz [18]. ESBL decompose antibiotics that contain the beta-lactam ring. Beta-lactam antibiotics include all the antibiotics excluding cephamycin and carbapenems, that is, penicillin, cephalosporins and monobactams. Bacterial strains that can synthesise ESBL include the group of so-called alert pathogens. These include the bacteria from the *Enterobacteriaceae* family (e.g., *Escherichia coli*, *Salmonella enterica*, *Klebsiella pneumoniae*). They are often characterised by drug resistance, which leads to limitation of the opportunities for choosing the efficient antibiotic therapy. The cited authors found genes transferred by plasmids that encode susceptibility to beta-lactams in nearly 10% of bacteria isolated from effluent after wastewater treatment. The authors demonstrated that these genes are released to the environment, which can help them spread among the environmental bacteria. Examinations demonstrated that despite treatment, municipal wastewater and purified water can represent the microorganisms resistant to antibiotics and genes resistant to drugs. This might represent a threat to human health. This problem requires extending the evaluation and control of processes of wastewater treatment.

In other research, Harnisz et al. [19] analysed quality of water in the Drwęca river and found that the variety of tetracycline-resistant genes increased in the bacteria they determined. The researchers found that the bacteria from *Aeromonas* sp. and *Acinetobacter* sp. were able to transfer 6 of 13 resistance genes of *E. coli,* which can contribute to the spread of resistance to antibiotics in the water environment. Bacteria belonging to the genus *Serratia* isolated from effluent and water can also transmit plasmid-encoded beta-lactamases in the environment as was demonstrated in the studies [20].

In the case of bacteria from the *Enterococcus* family, *E. gallinarum* was characterised by the profile of susceptibility characteristic for wild strain whereas *E. faecalis* was resistant to erythromycin, streptomycin and linezolid and medium susceptible to penicillin. Xua et al. [21] demonstrated that plasmids can mediate transfer of genes that cause resistance to certain drugs between gram-negative bacteria (with phylogenetically distant genomes) and *E. faecalis* and *E. faecium.*

Both isolated species of bacteria of the *Enterococcus* genus were susceptible to ampicillin. Different results of examinations were obtained by Costa et al. [15], who isolated this type of bacteria from the treated waste. The results of the examinations reflect greater resistance of isolated microorganisms to ampicillin. There were from 0% to 17.2% bacteria resistant to this antibiotic. The authors cited also demonstrated a worrying high percentage degree of resistance of bacteria to vancomycin (from 0% to 3.1%), tetracycline (from 11.9% to 53.1%), chloramphenicol (from 0% to 13.4%) and ciprofloxacin (from 6.0% to 28.1%) compared with authors of the present study. Both examinations demonstrated that processes of sewage treatment are unable to ensure complete elimination of drug-resistant enterococci in water environment. A study by Monticelli et al. [22] also emphasised resistance of *E. gallinarum* to vancomycin, which is used to treat serious bacterial infections (mainly in hospital settings).

The wastewater was biologically treated using the activated sludge method. The benefits of treatment by means of activated sludge include high effectiveness at insignificant land demand. Removal of BZT5 (BOD-Biological Oxygen Demand) and suspension amounts to 95%, whereas reduction in pathogenic bacterial count at properly performed process reaches 98%. Based on the literature data, one can conclude that in the processes of mechanical treatment, the removal concerns around 20% of total bacterial count, 90% of *Salmonella* and *Mycobacterium tuberculosis* bacteria. Furthermore, in the processes of biological wastewater treatment using sewage sludge, these percentages are 90%–98% of reduction of total bacterial count, 55%–98% reduction in *Salmonella* and 45% reduction in the count of *Mycobacterium tuberculosis* [23].

In the municipal wastewater treatment plant analysed in this study, no additional effluent is made after completion of the entire process, for example, using UVC-emitting lamps, which is likely to be the major cause of such a high bacterial count, including the *Enterobacteriaceae* family that represent a sanitary hazard.

The basis for classification of the condition of surface and underground waters is currently defined by the Water Framework Directive (WFD). After adoption of the WFD by Poland, the evaluation of purity and utility of waters was replaced by the assessment of the ecological state. Therefore, the evaluation concerns the condition of water considered not only as economic resources but also as an element of the ecosystem. According to the Ordinance of the Ministry of the Environment as of 21 July 2016 on classification of the condition of uniform parts of surface waters and environmental quality standards for priority substances, the classification adopts five quality classes [24].

The examinations of river water to which treated wastewater is discharged are performed by the analysed treatment plant and point to the fourth class of quality of surface water. This suggests poor ecological quality of water. Discharge of the treated wastewater does not lead to increased degree of pollution. However, presence of drug-resistant bacteria in this wastewater can contribute to their spread in the water and soil environment.

4. Conclusion

The results containing the quantitative and qualitative presence of bacteria from the *Enterobacteriaceae* family and *Enterococcus* genus in the effluent samples after purification in the municipal wastewater treatment plant lead to the following conclusions:

- The samples contained various mesophilic bacteria, including those from the *Enterobacteriaceae* family.
- Amount of intestinal bacteria discharged to the river with effluent from the wastewater treatment plant was at a worrying high level.
- Presence of drug-resistant forms of both intestinal bacteria and enterococci was observed in the samples.
- Percentage share of bacteria from the *Enterobacteriaceae* and other mesophilic bacteria decreased noticeably with reduction in the temperature of wastewater in the treatment process.
- *Enterobacteriaceae* were the most sensitive to the reduction in temperature compared with other mesophilic bacteria present in the effluent samples.
- Lower effluent temperatures during the treatment process are conducive to limitation of the mesophilic bacterial count, including *Enterobacteriaceae* family.
- The amount of enterococci in the effluent samples was at a very low level.

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References

[1] Z. Libudzisz, K. Kowal, Z. Żakowska, Technical microbiology, Polish Scientific Publishers PWN, Warszawa, 2015 (in Polish).

[2] M. Zaremba, J. Borowski, Medical Microbiology, Medical Publishers PZWL, Warszawa, 2013 (in Polish).

[3] Y. Ji-Ran, Y. Soon-Uk, K. Chang-Gyun, Quantification of residual antibiotics in cow manure being spread over agricultural land and assessment of their behavioral effects on antibiotic resistant bacteria, Chemosphere, 182 (2017) 771–780.

[4] E. Stańczyk-Mazanek, T. Nalewajek, M. Zabochnicka, Drug-resistant microorganisms in soils fertilized with sewage sludge, Arch. Environ. Prot., 38 (2012) 97–102.

[5] T.J. Everage, R. Boopathy, R. Nathaniel, G. LaFleur, J. Doucet, A survey of antibiotic-resistant bacteria in a sewage treatment plant in Thibodaux, Louisiana, USA, Int. Biodeterior. Biodegrad., 95 (2014) 2–10.

[6] J. Borowski, Medical Microbiology, Medical Publishers, PZWL, Warszawa, 2004 (in Polish).

[7] E.M. Szewczyk, Bacteriological Diagnosis, Polish Scientific Publishers PWN, Warszawa, 2011 (in Polish).

[8] W. Hryniewicz, A. Sulikowska, K. Szczypa, J. Krzystoń-Russjan, M. Gniadkowski, Recommendations for the Selection of Tests for the Determination of Bacterial Susceptibility to Antibiotics and Chemotherapeutics, National Reference Center for Microbial Susceptibility, 2001 (in Polish).

[9] W. Hryniewicz, D. Żabicka, Position of the Working Group on the Determination of Susceptibility in Accordance with the EUCAST Recommendations on the Most Frequently Asked Questions Regarding the Use of Recommendations hEUCAST 2016, 2016. Available at: www.korld.edu.pl/pdf/StanowiskaZespołuRoboczego_2016.pdf (in Polish).

[10] Interpretation Tables for Minimum Inhibitory Concentrations (MICs) and Size of Growth Inhibition Zones, The European Committee on Antimicrobial Susceptibility Testing - (EUCAST), Version 5.0., 2015.

[11] Z. Markiewicz, Z.A. Kwiatkowski, Bacteria, Antibiotics, Drug Resistance, Polish Scientific Publishers PWN, 2017 (in Polish).

[12] P. Wolski, I. Zawieja, Hybrid conditioning before anaerobic digestion for the improvement of sewage sludge dewatering, Desal. Wat. Treat., 52 (2014) 3725–3731.

[13] P. Wolski, M. Małkowski, Dewatering of initially conditioned excess sludge after fermentation, Desal. Wat. Treat., 52 (2014) 3973–3978.

[14] S.N. Al-Bahry, I.Y. Mahmoud, K.I.A. Al-Belushi, A.E. Elshafie, A. Al-Harthy, C.K. Bakheit, Coastal sewage discharge and its impact on fish with reference to antibiotic resistant enteric bacteria and enteric pathogens as bio-indicators of pollution, Chemosphere, 77 (2009) 1534–1539.

[15] P.M. Costa, P. Vaz-Pires, F. Bernardoc, Antimicrobial resistance in *Enterococcus* spp. isolated in inflow, effluent and sludge from municipal sewage water treatment plants, Water Res., 40 (2006) 1735–1740.

[16] J. Ory, G. Bricheux, A. Togola, J.L. Bonnet, F. Donnadieu-Bernard, L. Nakusi, Ch. Forestier, O. Traore, Ciprofloxacin residue and antibiotic-resistant biofilm bacteria in hospital effluent, Environ. Pollut., 214 (2016) 635–645.

[17] M. Harnisz, E. Korzeniewska, The prevalence of multidrug-resistant *Aeromonas* spp. in the municipal wastewater system and their dissemination in the environment, Sci. Total Environ., 626 (2018) 377–383.

[18] E. Korzeniewska, M. Harnisz, Extended-spectrum beta-lactamase (ESBL)-positive Enterobacteriaceae in municipal sewage and their emission to the environment, J. Environ. Manage., 128 (2013) 904–911.

[19] M. Harnisz, E. Korzeniewska, I. Gołas, The impact of a freshwater fish farm on the community of tetracycline resistant bacteria and the structure of tetracycline resistance genes in river water, Chemosphere, 128 (2015) 134–141.

[20] M. Xue-Jiao, Y. Hai-Fei, L. Yan-Yan, M. Qing, Y. Ying, L. Hong-ru, Ch. Jun, L. Jia-Bin, ACT-10, a novel plasmid-encoded class C β-lactamase in a *Serratia marcescens* isolate from China, Diagn. Microbiol. Infect. Dis., 80 (2014) 343–344.

[21] Z. Xua, L. Lia, M.E. Shirtliffb, B.M. Petersb, Y. Penge, M.J. Alamf, S. Yamasakia, L. Shi, First report of class 2 integron in clinical *Enterococcus faecalis* and class 1 integron in *Enterococcus faecium* in South China, Diagn. Microbiol. Infect. Dis., 68 (2010) 315–317.

[22] J. Monticelli, A. Knezevich, R. Luzzati, S. Di Bella, Clinical management of non-faecium non-faecalis vancomycin resistant enterococci infection. Focus on *Enterococcus gallinarum* and *Enterococcus casseliflavus/flavescens*, J. Infect. Chemother., 24 (2018) 237–246.

[23] G. Tchobanoglous, F.L. Burton, H.D. Stensel, Wastewater Engineering: Treatment and Reuse, 4th ed., Metcalf & Eddy, 2004.

[24] The Ordinance of the Ministry of the Environment as of 21 June 2016 on classification of the condition of uniform parts of surface waters and environmental quality standards for priority substances, 2016 (in Polish).