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ADRIANA OSIŃSKA[1], PIOTR JACHIMOWICZ[1], SEBASTIAN NIESTĘPSKI[1], MONIKA HARNISZ[1], EWA KORZENIEWSKA[1]

THE EFFECTS OF SEASON AND PROCESSING TECHNOLOGY ON THE ABUNDANCE OF ANTIBIOTIC RESISTANCE GENES IN AIR SAMPLES FROM MUNICIPAL WASTEWATER TREATMENT AND WASTE MANAGEMENT PLANTS

This study aimed to perform a qualitative and a quantitative assessment of the prevalence of genes encoding resistance to beta-lactam, tetracycline, and chloramphenicol antibiotics in samples of DNA isolated from air in a municipal wastewater treatment plant (WWTP) and a municipal waste management plant (WMP). Air samples were collected in the mechanical (MP) and biological (BP) processing units of WWTP and WMP in winter and spring. The samples of air were collected by impingement into PBS solution and subsequently, DNA was isolated. The prevalence of the 16S *r*RNA gene and ARGs was determined by PCR, and the most abundant ARGs were quantified by qPCR. The highest diversity of the analyzed ARGs was noted in air samples collected in the mechanical processing units of the WWTP (winter) and the WMP (spring). The copy of ARGs varied between treatment units and seasons. ARGs were most abundant in air samples collected in spring in the MP units of both the WWTP and the WMP. The study demonstrated that ARGs are ubiquitous in the air in both WWTPs and WMPs. The presence of ARGs in the air can exert a negative impact on the health of plant employees.

1. INTRODUCTION

Bioaerosols contain small biological particles (0.001–100 μm) that are suspended in the gas phase, dispersed in the air. They are composed of pollen, fungi, viruses, and bacteria that are transported by air or are carried by larger non-biological particles. Bioaerosols easily spread between environments due to their small size and weight [1].

[1]Department of Water Protection Engineering and Environmental Microbiology, Faculty of Geoengineering, University of Warmia and Mazury in Olsztyn, ul. Prawocheńskiego 1, 10-720 Olsztyn, Poland, corresponding author A. Osińska, email address: adriana.osinska@uwm.edu.pl

The qualitative and quantitative composition of microorganisms suspended in bioaerosol varies considerably in time and space, and it is influenced by meteorological factors, such as temperature, humidity, solar radiation, wind speed, and direction, as well as landform and land use [2].

Municipal facilities such as wastewater treatment plants (WWTPs), composting plants, biogas plants, and waste management plants (WMPs) are important sources of bioaerosols [3, 4]. In WWTPs, bioaerosols are produced during the aeration, mixing, and flow of sewage through sand filters and bioreactors. Sewage is aerated by diffusers in bioreactors, and the produced air bubbles rise from the bottom to surface (boundary) layers [5]. Upon reaching the surface, air bubbles burst and form small droplets that transport sewage particles and microorganisms from sewage and biofilm into the air [6]. In plants processing municipal solid waste, bioaerosols were produced mainly in waste transport lines and during waste unloading, sorting, and composting [7]. In waste management plants, aerosols are generated when solid waste comes into sudden contact with the mechanical components of processing lines and when pollutant particles are separated from waste by convection [6].

Atmospheric air and indoor air are the key components of the living environment, and they play a very important role in human health. Humans consume around 12–15 m^3 of air daily, which indicates that the quality of ambient and indoor air should be closely monitored. Humans spend around 90% of their time indoors, including 25% at work, and poor air quality can pose a significant health risk [8]. The air in municipal WWTPs and WMPs often contains microorganisms that are dangerous for humans. Han et al. [9] analyzed microbial communities in bioaerosols in a WWTP and identified high counts of potentially pathogenic bacteria belonging to the families *Brucellaceae*, *Alcaligenaceae*, *Neisseriaceae*, *Moraxellaceae*, *Aeromonadaceae*, *Pseudomonadaceae*, *Xanthomonadaceae*, *Enterobacteriaceae*, *Bacillaceae*, *Staphylococcaceae*, and *Micrococcaceae*. The above microorganisms can be resistant to antibiotics, which poses a particular risk for humans because diseases caused by these pathogens can persist for longer periods and could be difficult to treat with the available antimicrobials [10].

Municipal WWTPs and WMPs are regarded as important sources of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) which reach the environment with processed sewage and leachate as also with bioaerosols [5]. The conditions inside WWTPs and WMPs, including high nutrient availability, favorable temperature, and high microbial counts, promote gene transfer [11]. In facilities with high levels of bioaerosols, the employees are particularly exposed to potentially pathogenic bacteria, including ARB. The health risks associated with the transmission of aerosol-borne bacteria are determined by the pathogenicity of bacterial strains, environmental factors, and the gene pool, including ARGs [12]. Municipal WWTPs and WMPs are characterized by a large pool of ARGs that can be transmitted between bacterial species. Antibiotic resistance genes can be also transmitted to respiratory microbiota, thus posing a potential health risk for plant employees.

This article presents the results of a preliminary study aiming to determine the prevalence and concentrations of genes encoding resistance to beta-lactam, tetracycline, and chloramphenicol antibiotics in DNA isolated from air samples in a municipal WWTP and a municipal WMP. Attempts were made to determine the effects of the processing technology (mechanical, biological) and season (winter, spring) on the copy numbers of ARGs in air samples.

2. MATERIALS AND METHODS

Sampling protocol. Air samples were collected in the Łyna Municipal Wastewater Treatment Plant (WWTP) and the Municipal Waste Management Plant (WMP) in Olsztyn, northern Poland. Air was sampled in mechanical (MP) and biological (BP) processing units in both plants. The MP unit consists of a grid compartment (WWTP) and a screened compartment (WMP) for separating waste fractions. The BP unit is composed of aeration tanks (WWTP) and a bio drying compartment (WMP). Air was sampled twice, in February 2019 (winter) and May 2019 (spring). The samples were collected by impingement with the use of the MAS-100 Eco Air Sampler (Merck) placed on a table at a height of 1.30 m. The sampling volume was determined experimentally depending on the expected level of air contamination. To obtain the material for DNA isolation, 6 dm^3 of air were collected with the use of the air sampler onto a Petri plate containing 30 cm^3 of phosphate-buffered saline (PBS) solution. PBS was transferred to sterile screw thread vials, and it was transported to the laboratory. Meteorological conditions, including air temperature and humidity, were recorded during sampling with the Ebro EBI 2-TH-611/6120 Data Logger.

DNA isolation. PBS samples were filtered through white polycarbonate filters (pore size 0.2 μm, diameter 47 mm; type GTTP, Merck, Millipore) in a vacuum. The filters were cut into smaller pieces under sterile conditions and transferred to the Bead Tube (GeneMATRIX Soil DNA Purification Kit, EURx). DNA was isolated according to the manufacturer's instructions. The concentration and quality of the extracted DNA were determined with a Nanodrop spectrophotometer (NanoDrop® ND-1000, NanoDrop Technologies, DE). Genomic DNA was stored at –20 °C for further analysis.

Qualitative analysis of antibiotic resistance genes – PCR. In a preliminary analysis, the presence of four tetracycline resistance genes (*tet*(A), *tet*(B), *tet*(M), *tet*(X)), 10 beta-lactam resistance genes (bla_{TEM}, bla_{SHV}, bla_{OXA}, $bla_{CTX\text{-}M}$, $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}2}$, $bla_{CTX\text{-}M\text{-}9}$, bla_{VEB}, bla_{CMY}, $bla_{AMP\text{-}C}$), four chloramphenicol resistance genes (*flo*R, *cml*A, *fex*A, *fex*B) and the 16S *r*RNA gene was determined by PCR. All primers had been previously validated for primer sequences, amplicon sizes, and annealing temperatures (the references for each sequence and detailed information on PCR conditions are presented in previous studies by Osińska et al. [13] and Potorski et al. [14]).

Quantitative analysis of antibiotic resistance genes – qPCR. The eight most prevalent ARGs identified in the preliminary analysis (*tet*(A), *tet*(B), *tet*(M), bla_{TEM}, $bla_{AMP\text{-}C}$, *cml*A, *fex*A, *flo*R) and the 16S *r*RNA gene were quantified by qPCR. Standard curves were generated by cloning an amplicon from positive control to the pCR2.1-TOPO vector (Invitrogen, USA). Standard curves for qPCR were generated within a range of 10^2–10^8 gene copies/µl. The negative control was distilled-deionized water (ddH_2O) added to the qPCR mixture as a substitute for template DNA. All qPCR reactions were carried out in the Roche LightCycler 480 (Roche Applied Science, USA). Every reaction was run in triplicate with negative and positive controls to ensure repeatability. The specificity of amplification was determined by melting curve analysis within a temperature range of 60–95 °C. The cycle threshold (Ct) was defined as the point at which the fluorescence signal crossed the threshold. Gene concentrations were calculated in 1 m^3 of air and were also expressed in terms of the 16S *r*RNA gene copy number.

Statistical analysis. Genes were quantified in a two-way generalized Poisson log-linear model to account for the effects of season and the applied waste and wastewater processing method. The differences in gene concentrations between air samples collected in different processing units of the WWTP and the WMP (MP and BP) and different seasons (winter, spring) were determined in the Kruskal–Wallis (KW) test. Statistical analyses were carried out in the Statistica 13.1 software package (StatSoft Inc.) at a significance level of $p < 0.05$. The correlations between ARG and 16S *r*RNA concentrations vs. air temperature and humidity were determined by Spearman's rank-order correlation. The correlation matrix was generated in the corrplot package in R Studio v. 1.2.1335. The correlations between the concentrations of the analyzed ARGs and the 16S *r*RNA gene in air samples collected in the WWTP and the WMP in both seasons were determined by Ward's hierarchical clustering method with the use of the gplot package in R Studio. The heatmap was generated with the use of the heatmap.2 package in R Studio.

3. RESULTS

3.1. PHYSICOCHEMICAL PARAMETERS OF AIR IN SAMPLING SITES

In winter, the average temperature of the air in the analyzed WWTP and WMP ranged from 7.5 to 13.5 °C. The average temperature in the mechanical and biological processing units of the WMP was determined at 7.5 and 10.5°C, respectively. The temperature was higher in the MP and BP units of the WWTP (12–13.5 °C) than in the corresponding units of the WMP. In spring, the average temperature in the MP and BP units of the WMP was similar in the range of 15.5–16.5 °C. In the WWTP, considerable temperature differences were observed between the MP and BP units. The average temperature was determined at 15.5 °C in the MP unit and 25 °C in the BP unit. The above

difference could be explained by the fact that bioreactors in the WWTP are exposed to sunlight. In both plants, the temperature was higher in biological units than in mechanical processing ones.

In winter, air humidity was lower in the sampling sites in the WWTP (43–66%) than in the WMP (68–71%). Humidity was lowest in the BP unit of the WWTP. In spring, humidity ranged from 28 to 56%, and similarly to winter, it was lower in the WWTP than in the WMP, in particular in the BP unit. High humidity in the BP unit of the WMP was probably associated with the bio drying technology which removes moisture from waste through a series of biological reactions. Lower humidity in the MP unit of the WWTP can probably be attributed to the applied processing technology where large floating impurities are separated from sewage by grids and screens.

3.2. PREVALENCE OF ANTIBIOTIC RESISTANCE GENES

The analyzed air samples harbored *tet*(A), *tet*(B), and *tet*(M) genes encoding resistance to tetracyclines, as well as bla_{TEM} and $bla_{\text{AMP-C}}$ genes encoding resistance

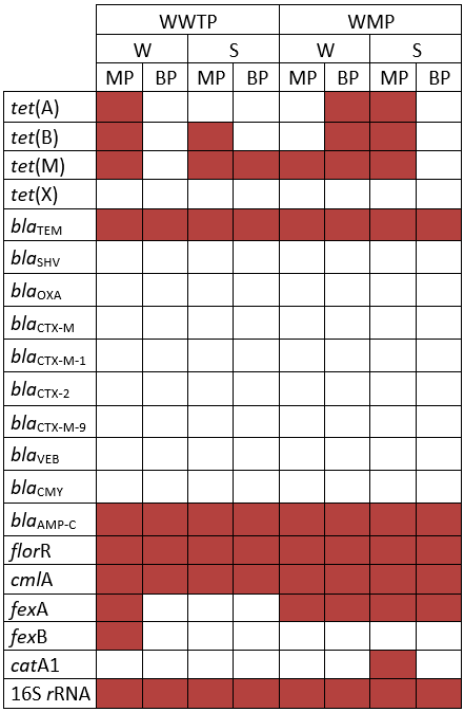

Fig. 1. Quantification of antibiotic resistance genes in air sampling sites by PCR. Red fields denote positive reads

to beta-lactams. The presence of *bla*$_{SHV}$, *bla*$_{OXA}$, *bla*$_{CTX-M}$, *bla*$_{CTX-M-1}$, *bla*$_{CTX-2}$, *bla*$_{CTX-M-9}$, *bla*$_{VEB}$, and *bla*$_{CMY}$ genes was not determined. Five genes encoding resistance to chloramphenicol – *flo*R, *cml*A, *fex*A, *fex*B and *cat*A1 – were identified in the sampled air. The 16 *r*RNA gene was present in all samples (Fig. 1).

The highest number of ARGs (presence of 10 genes per sample) was determined in the samples collected in winter in the MP unit of the WWTP, and in the samples collected in spring in the MP unit of the WMP. In the samples collected in the BP unit of the WWTP in winter, the lowest number of genes was noted (5 genes). The most prevalent ARGs, i.e., *tet*(A), *tet*(B) *tet*(M), *bla*$_{TEM}$, *bla*$_{AMP-C}$, *flo*R, *cml*A and *fex*A, and the 16S *r*RNA gene were quantified by real-time PCR.

3.3. ABUNDANCE OF THE 16S *r*RNA GENE AND ANTIBIOTIC RESISTANCE GENES IN AIR IN THE WMP

The abundance of the 16S *r*RNA gene in air sampled in the WMP ranged from 10^7 to 10^9 copies/m^3 of air in the analyzed processing units and seasons (Fig. 2). The highest and the lowest values were noted in spring in the BP unit and the MP unit, respectively. In the Kruskal–Wallis test, the abundance of the analyzed genes was significantly higher (KW, $p < 0.05$) in the BP unit in both seasons.

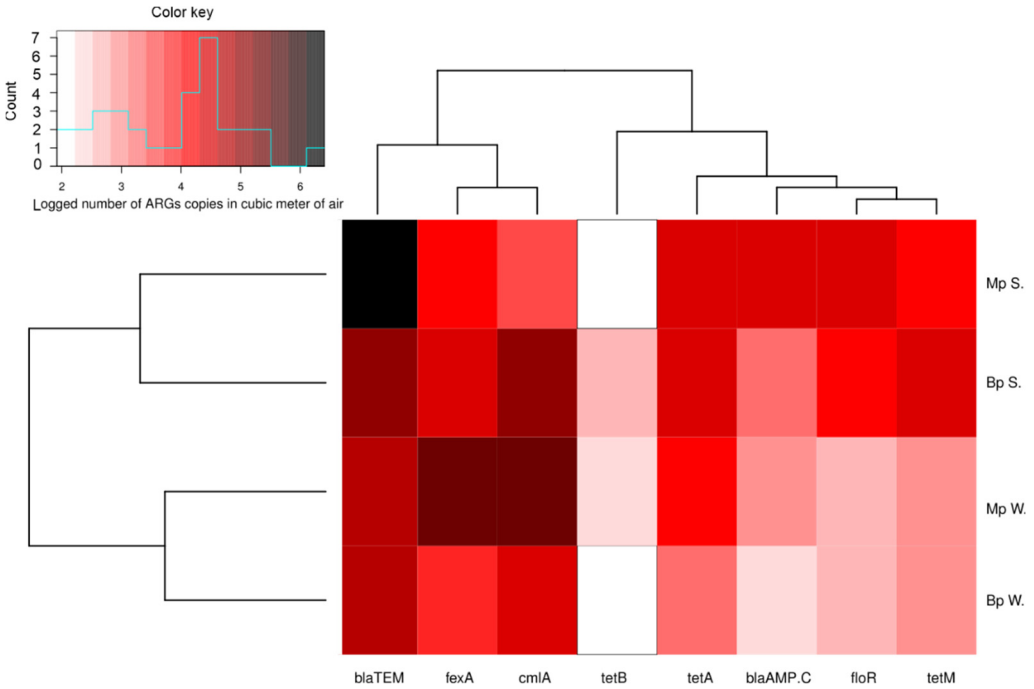

Fig. 2. Heatmap with the logarithmic number of gene copies in the air (m^3) in the WMP; Mp – mechanical processing unit, Bp – biological processing unit, W – winter, S – spring

The copy numbers of tetracycline resistance genes in the WMP ranged from 10 to 10^4 copies/m^3, and *tet*(A) and *tet*(M) genes were more abundant in the air than the *tet*(B) gene. The copy numbers of all *tet* genes in BP and MP units were significantly higher in spring than in winter (KW, $p < 0.05$). In the group of genes encoding resistance to beta-lactams, the most abundant gene was bla_{TEM} (10^4–10^6 copies/m^3 of air) in both processing units and both seasons (Fig. 2). The concentrations of both genes were significantly higher in air samples collected in spring than in winter (KW, $p < 0.05$). The copy numbers of the studied genes were also significantly higher in air sampled from the MP unit than the BP unit (KW, $p < 0.05$).

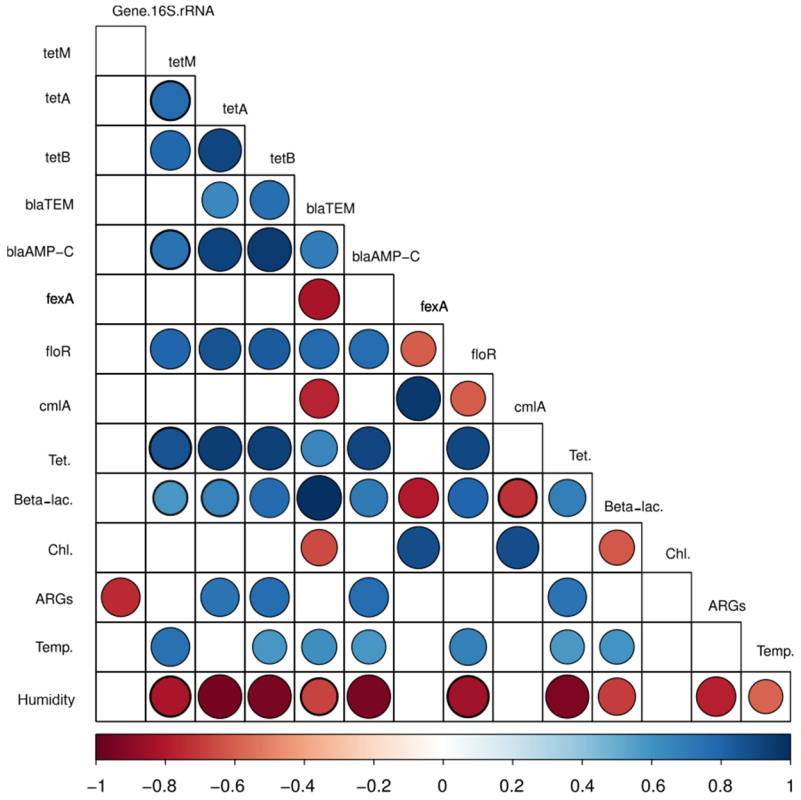

Fig. 3. Spearman's rank correlation coefficient denoting the strength of the relationship between ARG concentrations (copy number/m^3) and physicochemical parameters of air in the WMP ($p < 0.05$). Positive correlations are marked in blue, and negative correlations are marked in red. Color intensity and circle size are proportional to the correlation coefficients (the total copy numbers of tetracycline (Tet), beta-lactam (Beta-lac) and chloramphenicol (Chl) resistance genes are presented; Temp. – temperature)

The prevalence of chloramphenicol resistance genes in air samples differed significantly between processing units and seasons. Their average abundance ranged from 10^2 to 10^5 copies/m^3 of air (Fig. 2). Seasonal variations were noted in the abundance of *fex*A

and *cml*A genes. Their copy numbers were significantly higher in the MP unit in winter and in the BP unit in spring (KW, $p < 0.05$). The concentration of the *flo*R gene in both processing units was higher in spring.

The Wald test involving a generalized linear model (ANOVA) with log-Poisson distribution demonstrated significant ($p < 0.001$) relationships between season and waste processing method vs. the abundance of the 16S *r*RNA and all analyzed ARGs in air samples collected in the WMP.

The cluster analysis involving Ward's method revealed seasonal correlations between ARG abundance in the air in the WMP (Fig. 2). The copy numbers of the examined ARGs were higher in spring than in winter in both MP and BP units. Similarly to the WWTP, *fex*A, *cml*A and bla_{TEM} were the most abundant genes in the WMP, and they were grouped in the same cluster. *tet*(B) was characterized by the lowest concentration in air.

In Spearman's rank correlation test, the abundance of individual genes and the sum prevalence of tetracycline and beta-lactams resistance genes were positively correlated with the *flo*R gene ($r = 0.79–0.90$, $p < 0.05$) and negatively correlated with *fex*A and *cml*A genes ($r = –(0.60–0.83)$, $p < 0.05$) (Fig. 3). The abundance of tetracycline and beta-lactam resistance genes and the *flo*R gene in air was positively correlated with air temperature ($r = 0.58–0.75$, $p < 0.05$) and negatively correlated with air humidity ($r = –(0.68–0.95)$, $p < 0.05$). Air temperature and humidity were bound by negative correlations with the concentrations of all ARGs ($r = –(0.58–078)$, $p < 0.05$).

3.4. ABUNDANCE OF THE 16S *r*RNA GENE AND ANTIBIOTIC RESISTANCE GENES IN AIR IN THE WWTP

The copy numbers of the 16S *r*RNA gene in air samples collected in the Łyna WWTP differed between seasons and processing units. The abundance of the 16S *r*RNA gene was higher in both MP and BP units in winter ($10^7–10^8$ copies/m^3) than in spring. In spring, the prevalence of the analyzed gene did not exceed 10^7 copies/m^3 (Fig. 4). The 16S *r*RNA gene was most abundant in the MP unit in winter and in the BP unit in spring. The tet(A) gene was the most abundant tetracycline resistance gene ($10^2–10^3$ copies/m^3) in air samples collected in the WWTP. The prevalence of *tet*(B) and *tet*(M) genes ranged from 10^0 to 10^2 copies/m^3 of air (Fig. 4). In both seasons, the copy numbers of *tet*(A) and *tet*(M) genes were significantly higher in the MP unit of the WWTP (KW, $p < 0.05$).

The bla_{TEM} gene was the most prevalent beta-lactam resistance gene (10^4 copies/m^3) in both processing units, but its abundance was significantly higher in air samples collected in winter (KW, $p < 0.05$). The copy number of the bla_{AMP-C} gene in was higher in winter, and it ranged from 10^2 to 10^3 copies/m^3 in the analyzed processing units (Fig. 4).

The abundance of chloramphenicol resistance genes in air samples collected in the WWTP ranged from 10^2 to 10^4 copies/m^3 of air (Fig. 4). In winter, the copy numbers of *fex*A and *flo*R genes were significantly higher in the BP unit, whereas the *cml*A gene

was more prevalent in the MP unit. In spring, significant differences were noted only in the abundance of the *fex*A gene whose copy number was higher in the MP unit than in the BP unit (KW, $p < 0.05$). The cluster analysis involving Ward's method revealed significant ($p < 0.001$) correlations between processing technology and season vs. the copy numbers of the 16S *r*RNA gene and all ARGs in air sampled in the Łyna WWTP.

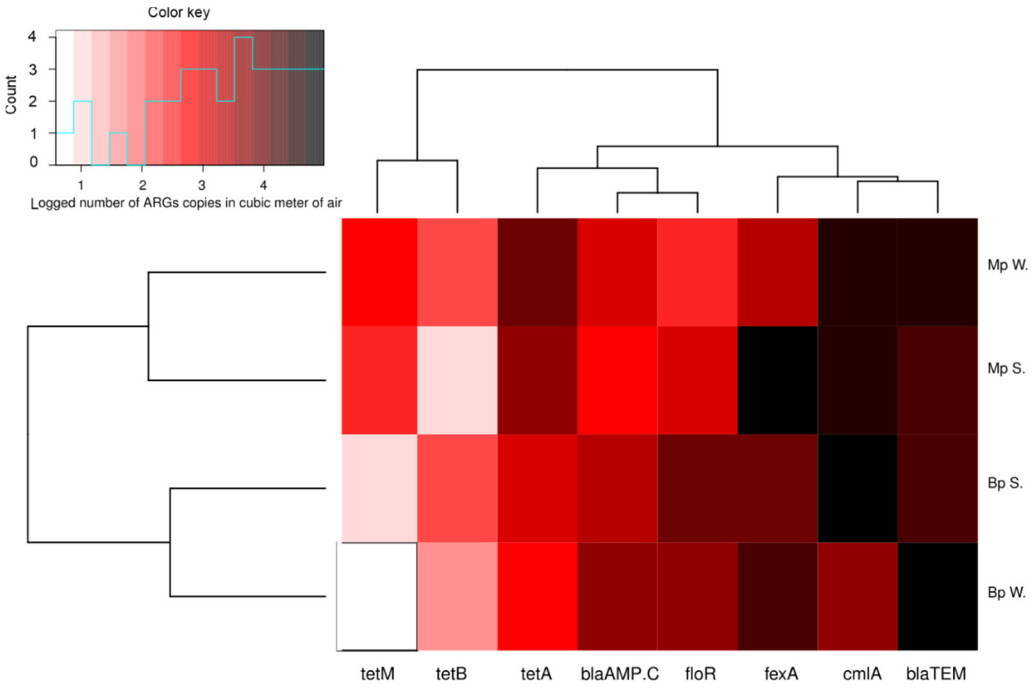

Fig. 4. Heatmap with the logarithmic number of genes copies in air (m^3) in the WWTP

The correlations between ARG concentrations in the analyzed processing units and seasons were determined in Ward's hierarchical cluster analysis, and the results were presented in a heatmap (Fig. 4). Air samples collected in BP and MP units were grouped in separate clusters. The most prevalent ARGs were *fex*A, *cml*A, and bla_{TEM} which were grouped in the same cluster. The copy numbers of *tet*(M) and *tet*(B) genes in 1 m^3 of air were lowest, relative to the remaining analyzed genes.

The Spearman's rank correlation test revealed significant negative correlations between the abundance of chloramphenicol resistance genes and the prevalence of *tet* ($r = –(0.65–0.68)$, $p < 0.05$) and *bla* ($r = –(0.59–0.85)$, $p < 0.05$) genes. No correlations were observed between the copy numbers of genes encoding resistance to tetracyclines and beta-lactams. The concentrations of the 16S *r*RNA gene and beta-lactam resistance

genes were negatively correlated (r = –(0.61–077), $p < 0.05$), and chloramphenicol resistance gene were positively ($r = 0.61$, $p < 0.05$) with air temperature. Reverse correlations were noted concerning air humidity (Fig. 5). Air humidity was also found to decrease with a rise in air temperature.

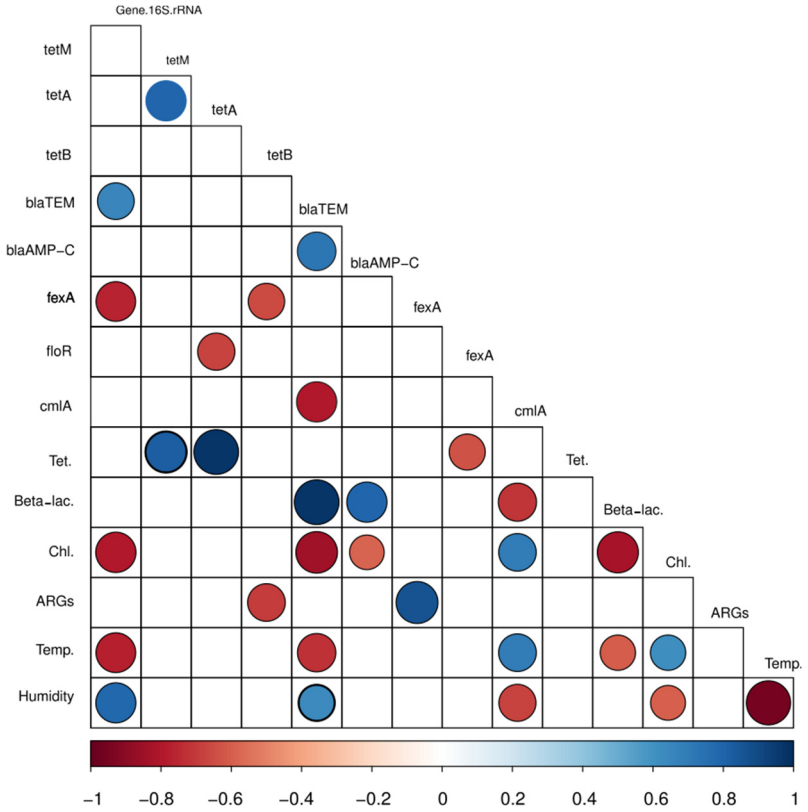

Fig. 5. Spearman's rank correlation coefficient denoting the strength of the relationship between ARG concentrations (copy number/m^3) and the physicochemical parameters of air in the WWTP ($p < 0.05$). Positive correlations are marked in blue, and negative correlations are marked in red. Color intensity and circle size are proportional to the correlation coefficients (the total copy numbers of tetracycline (Tet), beta-lactam (Beta-lac) and chloramphenicol (Chl) resistance genes are presented; Temp. – temperature)

4. DISCUSSION

The use of antibiotics and antibacterial and disinfecting agents in hospitals and outpatient clinics is rigorously controlled. However, the fate of antibiotics cannot be fully monitored in outpatient facilities. The consumed drugs and their metabolic products are

excreted by patients and evacuated to sewage which is processed in wastewater treatment plants. Unused and expired drugs are evacuated with other types of solid waste to waste management plants and landfills. According to research, only 30–60% of pharmaceuticals are disposed of in dedicated collection units [15]. In a study by Rogowska et al. [16], nearly 68% of the respondents admitted to disposing of expired pharmaceuticals with household waste or by flushing them down the toilet. Municipal waste contains up to 7.4–8.1 mg of pharmaceuticals per kilogram [15]. High concentrations of antibiotics were also reported in wastewater treatment plants influents [13, 17].

Municipal waste management plants are regarded as an important reservoir of pollutants, including antibiotics [18] which can exert selective pressure on microorganisms. Selective pressure promotes the transfer of ARGs between bacteria, and it contributes to the emergence of new determinants of antibiotic resistance [11]. Even though antimicrobials reach treatment plants in various forms, including discarded drugs in solid waste (WMP) and metabolic products in sewage (WWTP), all forms contribute equally to the development and transfer of antibiotic resistance.

In this study, the ARGs identified in air samples from different processing units of the WWTP were grouped into clusters with the use of Ward's hierarchical cluster analysis. The observed differences in ARG abundance between processing units could be attributed to the type of processing technology as well as the units' location. The MP unit is situated in a separate building equipped with screens and bars, whereas the BP unit is an outdoor facility (between activated sludge aeration tanks). The BP unit is exposed to a higher number of indoor and outdoor environmental factors, including the type of aeration, wind speed, temperature, and humidity, than the MP unit [19]. The composition of air (including the abundance of ARB and ARGs) in the WWTP is probably affected by the composition of treated wastewater. High concentrations of ARB and ARGs were noted in wastewater samples by other authors [5, 13]. It should also be noted that pollutants are naturally removed from the air by precipitation (rain, drizzle, snow), which can significantly decrease the quantity of bioaerosols. In the presented study, the concentrations of the examined ARGs differed by at least one order of magnitude between processing units in winter, and the relevant differences were only insignificantly smaller in spring. The correlation analysis revealed a significant negative relationship between the abundance of the 16S *r*RNA gene and temperature. Temperature directly affects the metabolic rate and the growth of microorganisms, and it is also correlated with other meteorological and climatic factors (such as air turbulence, time of day, and season) which can influence microbial concentrations in the air [20]. Spearman's rank correlation test revealed a positive correlation between the abundance of the 16S *r*RNA gene and humidity. Humidity is regarded as an important factor in the literature, but studies analyzing the impact of humidity on the microbial abundance in the air often produce contradictory results. However, the survival of specific microbial strains can be highly correlated with both temperature and humidity [21].

In the analyzed WMP, sorted waste is thermally sterilized in the biological reactor. According to many researchers, heat supplied to the waste processing system can potentially decrease the abundance of ARGs [22]. In the present study, the copy numbers of *tet*(A), bla_{TEM}, $bla_{\text{AMP-C}}$, *fex*A, and *cml*A genes were lower in the BP unit than in the MP unit of the WMP. In turn, the prevalence of the 16S *r*RNA, a potential indicator of bacterial abundance, was approximately 95% higher in the BP unit than in the MP unit. The copy numbers of ARGs and the 16S *r*RNA gene were considerably higher than those reported by Gao et al. [23] in a composting plant even though both the analyzed WMP and the cited composting plant processed waste in biological reactors where significant amounts of heat are generated. In a previous study conducted in the same WMP, a similar group of predominant ARGs was identified in the air in MP and BP units [14]. The observed increase in the copy numbers of *tet*(A), *tet*(B), *tet*(M), $bla_{\text{AMP-C}}$, bla_{TEM}, *flo*R genes, and the 16S *r*RNA gene in spring could be attributed to a higher volume of managed waste. Research indicates that WMPs process considerably more waste in spring than in winter (12–37%) and that the composition of waste fractions varies during the annual cycle [24].

Bioaerosols from municipal WWTPs and WMPs could be an important source of ARGs which can be transported across considerable distances and can pose a potential risk to humans. The inhalation of bioaerosols can be the primary means of exposure to ARGs and drug-resistant pathogens [12]. The relevant health risks are particularly high among the employees of WWTPs and WMPs who are exposed to organic dust and bioaerosols containing high concentrations of microorganisms [25].

In the European Union, the exposure to harmful biological substances that affect human health and the relevant health hazards are regulated by Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work, as amended by Commission Directive 2019/1833 of 24 October 2019. The above directives have been incorporated into Polish law by the Regulation of the Minister of Health of 22 April 2005 on harmful biological agents in the occupational environment and the protection of workers against these agents. Employers are legally required to provide the exposed employees with personal protective equipment and to monitor the occupational risks among employees. However, employees often ignore or are unaware of the health risks associated with the absence of personal protective equipment such as face masks or respirators.

The microbiological quality of air in WWTPs and WMPs has to be monitored to minimize the potentially harmful effects of bioaerosols on human health. To the best of the authors' knowledge, the concentrations of ARGs in different segments of WWTPs and WMPs have not been thoroughly investigated to date. Many of the existing studies analyzed only the presence of bacteria and yeasts in air samples or the drug resistance of strains isolated from air. The present study fills in this knowledge gap by analyzing

the concentrations of ARGs in air samples collected from the MP and BP units of a municipal WWTP and a municipal WMP in two seasons.

5. CONCLUSIONS

The results of a preliminary study evaluating the abundance of antibiotic resistance genes in the air in a municipal WWTP and a municipal WMP. Ten out of the 19 examined ARGs were identified in the collected air samples have been presented. The determinants of resistance to chloramphenicol and tetracyclines were most frequently identified. Genes encoding resistance to chloramphenicol (*fex*A and *cml*A) and beta-lactams (bla_{TEM}) were most abundant in air samples from both plants. Tetracycline resistance genes, mainly *tet*(B) and *tet*(M) were the least prevalent. The concentrations of ARGs in the air were influenced by the applied wastewater and waste processing technology and season. The study demonstrated that antibiotic resistance genes are ubiquitous in the air in both plants and that they can exert an adverse effect on employee health. Further and more detailed research is needed to explore the influence of waste and wastewater processing technologies on the abundance of ARGs in the air and their potential effects on the health of municipal plant workers.

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REFERENCES

[1] KIM K.H., KABIR E., JAHAN S.A., *Airborne bioaerosols and their impact on human health*, J. Environ. Sci. (China), 2018, 67, 23–35.

[2] JONES A.M., HARRISON R.M., *The effects of meteorological factors on atmospheric bioaerosol concentrations. A review*, Sci. Total Environ., 2004, 326 (1–3), 151–180.

[3] GOTKOWSKA-PLACHTA A., FILIPKOWSKA Z., KORZENIEWSKA E., JANCZUKOWICZ W., DIXON B., GOLAS I., SZWALGIN D., *Airborne microorganisms emitted from wastewater treatment plant treating domestic wastewater and meat processing industry wastes*, Clean-Soil Air Water, 2013, 41 (5), 429–436.

[4] CHANG M.W., LEE C.R., HUNG H.F., TENG K.S., HUANG H., CHUANG C.Y., *Bioaerosols from a food waste composting plant affect human airway epithelial cell remodeling genes*, Int. J. Environ. Res. Public Health, 2014, 11 (1), 337–354.

[5] KORZENIEWSKA E., *Emission of bacteria and fungi in the air from wastewater treatment plants. A review*, Front. Biosci., 2011, 3, 393–407.

[6] TOMASI C., LUPI A., *Primary and secondary sources of atmospheric aerosol. Atmospheric Aerosols*, Wiley, Weinheim, Germany, 2017.

[7] PILLAI S.D., RICKE S.C., *Bioaerosols from municipal and animal wastes. Background and contemporary issues*, Can. J. Microbiol., 2002, 48 (8), 681–696.
[8] WOLKOFF P., *Indoor air humidity, air quality, and health. An overview*, Int. J. Hyg. Environ. Health, 2018, 221 (3), 376–390.
[9] HAN Y.P., YANG K.X., YANG T., ZHANG M.Z., LI L., *Bioaerosols emission and exposure risk of a wastewater treatment plant with A(2)O treatment process*, Ecotox. Environ. Saf., 2019, 169, 161–168.
[10] ARIAS C.A., MURRAY B.E., *A new antibiotic and the evolution of resistance*, N. Eng. J. Med., 2015, 372 (12), 1168–1170.
[11] SHARMA V.K., JOHNSON N., CIZMAS L., MCDONALD T.J., KIM H., *A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes*, Chemosphere, 2016, 150, 702–714.
[12] MIRHOSEINI S.H., NIKAEEN M., SHAMSIZADEH Z., KHANAHMAD H., *Hospital air. A potential route for transmission of infections caused by beta-lactam-resistant bacteria*, Am. J. Infect. Control, 2016, 44 (8), 898–904.
[13] OSIŃSKA A., KORZENIEWSKA E., HARNISZ M., FELIS E., BAJKACZ S., JACHIMOWICZ P., NIESTEPSKI S., KONOPKA I., *Small-scale wastewater treatment plants as a source of the dissemination of antibiotic resistance genes in the aquatic environment*, J. Hazard. Mater., 2020, 381,121221.
[14] POTORSKI J., KONIUSZEWSKA I., CZATZKOWSKA M., HARNISZ M., *Drug resistance in airborne bacteria isolated from waste management and wastewater treatment plants in Olsztyn*, E3S Web of Conferences, 2019, 00066.
[15] MUSSON S.E., TOWNSEND T.G., *Pharmaceutical compound content of municipal solid waste*, J. Hazard. Mater., 2009, 162 (2–3), 730–735.
[16] ROGOWSKA J., ZIMMERMANN A., MUSZYŃSKA A., RATAJCZYK W., WOLSKA L., *Pharmaceutical Household Waste Practices. Preliminary Findings from a Case Study in Poland*, Environ. Manage., 2019, 64 (1), 97–106.
[17] YOUNES H.A., MAHMOUD H.M., ABDELRAHMAN M.M., NASSAR H.F., *Seasonal occurrence, removal efficiency and associated ecological risk assessment of three antibiotics in a municipal wastewater treatment plant in Egypt*, Environ. Nanotechnol. Monit. Manage., 2019, 100239.
[18] HAN Z.Y., MA H.N., SHI G.Z., HE L., WEI L.Y., SHI Q.Q., *A review of groundwater contamination near municipal solid waste landfill sites in China*, Sci. Total Environ., 2016, 569, 1255–1264.
[19] SCHLOSSER O., *Bioaerosols and health. Current knowledge and gaps in the field of waste management*, Detritus, 2019, 5, 111–125.
[20] ADHIKARI A., REPONEN T., GRINSHPUN S.A., MARTUZEVICIUS D., LEMASTERS, G., *Correlation of ambient inhalable bioaerosols with particulate matter and ozone. A two-year study*, Environ. Poll., 2006, 140 (1), 16–28.
[21] BURROWS S.M., ELBERT W., LAWRENCE M.G., POSCHL U., *Bacteria in the global atmosphere. Part 1. Review and synthesis of literature data for different ecosystems*, Atm. Chem. Phys., 2009, 9 (23), 9263–9280.
[22] XU S.W., SURA S., ZAHEER R., WANG G., SMITH A., COOK S., OLSON A.F., CESSNA A.J., LARNEY F.J., MCALLISTER T.A., *Dissipation of antimicrobial resistance determinants in composted and stockpiled beef cattle manure*, J. Environ. Qual., 2016, 45 (2), 528–536.
[23] GAO M., QIU T.L., SUN Y.M., WANG X.M., *The abundance and diversity of antibiotic resistance genes in the atmospheric environment of composting plants*, Environ. Int., 2018, 116, 229–238.
[24] DEN BOER E., DEN BOER J., JAROSZYNSKA J., SZPADT R., *Monitoring of municipal waste generated in the City of Warsaw*, Waste Manage. Res., 2012, 30 (8), 772–780.
[25] KOZAJDA A., SZADKOWSKA-STANCZYK I., *Selected health complaints, allergic diseases, hygiene behaviors and knowledge of biohazards among workers of waste sorting plants*, Medycyna Pracy, 2009, 60 (6), 491–499.