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Relationship between modification of activated sludge wastewater treatment and changes in antibiotic resistance of bacteria

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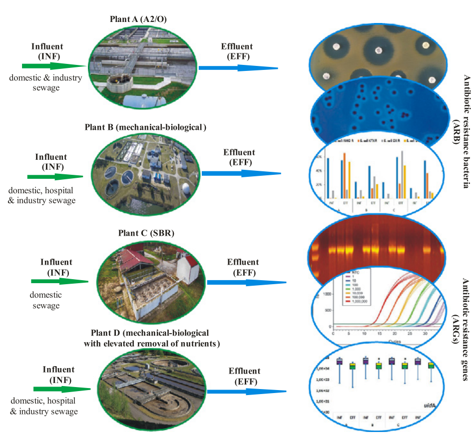
HIGHLIGHTS

- The number of ARB and ARGs in 13 WWTPs' influents & effluents were investigated
- The percentage of ARB in total bacteria counts increased in effluents compared to WWTP's influents
- The highest counts of ARGs copies in wastewater samples were observed for *sul*1, *tet*(A) and *qep*A
- The abundances of ARB and ARGs were related to the applied modification of sewage treatment
- The significant increase in ARB and ARGs number was observed in WWTP's effluents with A2/O and SBR system

GRAPHICAL ABSTRACT



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ABSTRACT

Biological treatment processes at wastewater treatment plants (WWTPs), which are the most common methods of sewage treatment, could cause selective elimination and/or changes in the proportions of phenotypes/genotypes within bacterial populations in effluent. Therefore, WWTPs based on activated sludge used in sewage treatment constitute an important reservoir of enteric bacteria which harbour potentially transferable resistance genes. Together with treated wastewater, these microorganisms can penetrate the soil, surface water, rural groundwater supplies and drinking water. Because of this, the aim of this study was to determine the impact of various modification of sewage treatment (the conventional anaerobic/anoxic/oxic (A2/O) process, mechanical-biological (MB) system, sequencing batch reactors (SBR), mechanical-biological system with elevated removal of nutrients (MB-ERN)) on the amount of antibiotic resistant bacteria (ARB) (including *E. coli*) and antibiotic resistance genes (ARGs) in sewage flowing out of the 13 treatment plants using activated sludge technology. There were no significant differences in ARB and ARGs regardless of time of sampling and type of treated wastewater ($p > 0.05$). The highest percentage of reduction (up to 99.9%) in the amount of ARB and ARGs was observed in WWTPs with MB and MB-ERN systems. The lowest reduction was detected in WWTPs with SBR. A significant increase ($p < 0.05$) in the percentage of bacteria resistant to the new generation antibiotics (CTX and DOX) in total counts of microorganisms was observed in effluents (EFF) from WWTPs with A2/O system and with SBR. Among all ARGs analyzed, the highest prevalence of ARGs copies in EFF samples was observed for *sul*1, *tet*(A) and *qep*A, the lowest for bla_{TEM} and bla_{SHV}. Although, the results of presented study demonstrate

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high efficiency of ARB and ARGs removal during the wastewater treatment processes, especially by WWTPs with MB and MB-ERN systems, EFF is still an important reservoir of ARGs which can be transferred to other microorganisms.

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1. Introduction

Antimicrobial resistance due to the continuous selective pressure from widespread use of antimicrobials in humans, animals and agriculture has been a growing problem for decades (Bengtsson-Palme et al., 2018; Berendonk et al., 2015). Antibiotic resistance is not restricted to pathogenic bacteria. Several studies show that antibiotic resistant clinical strains and/or their modes of resistance often originate from bacteria living in the natural environment, within soils and water (Finley et al., 2013; Singer et al., 2016). These environmental antibiotic resistant bacteria (ARB) can transfer the resistance genes (ARGs) to human pathogens causing resistant infections to become more difficult or even impossible to treat with current antibiotics, leading to infections causing higher morbidity and mortality. Intrinsic resistance in bacteria of the hospital environment is problematic because it limits the therapeutic options (Sengupta et al., 2013). Antibiotics including the groups of beta-lactams, tetracyclines, fluoroquinolones and sulfonamides comprise the largest share of antibiotics for human and animal use in the world. The metabolism of those active compounds in humans and animals varies widely. Some of compounds are metabolized in 90% or more, while others are metabolized in only 10% or even less (Kümmerer, 2009). They are then excreted as parent compounds or metabolites in urine and faeces into wastewater, in the case of human medicines, (X Guo et al., 2017; Le Corre et al., 2012; Leung et al., 2012) or, in the case of veterinary, as a fertilizer in animal farms (Wei et al., 2011; R Marti et al., 2014; Tien et al., 2017). Antibiotics and their transformation products (TPs) entering the environment can affect the evolution of the bacterial community structure (Aminov and Mackie, 2007; Baran et al., 2011) which plays a significant role in the ecosystem (Grenni et al., 2018; Thiele-Bruhn and Beck, 2005). However, apart from antibiotics and their TPs, antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) have been identified as emerging pollutants of concern. They also enter ecosystems with treated wastewater, specifically if influents include hospital wastewater (Korzeniewska and Harnisz, 2013a; Lien et al., 2017; Wang et al., 2018). The low efficacy of hospital sewage treatment or lack of any sewage treatment may contribute to the dissemination of multi-drug resistant bacteria (MDR) from hospital effluents to the municipal sewage and then to the environment either with treated sewage or directly into the water bodies (lakes/rivers) (Ahn and Choi, 2016; Korzeniewska et al., 2013; Lekunberri et al., 2017).

The activated sludge process was developed over 100 years ago and is primarily used for removal of biodegradable organic compounds, which could otherwise cause oxygen depletion of receiving waters if discharged in the treatment plant effluent. In activated sludge process wastewater containing organic matter is aerated in an aeration basin in which micro-organisms metabolize the suspended and soluble organic matter. The amounts of air and sludge used can be modify to control the level of treatment obtained. Part of organic matter is synthesized into new cells and part is oxidized to carbon dioxide and water to derive energy. In activated sludge systems the new cells formed in the reaction are removed from the liquid stream in the form of a flocculent sludge in settling tanks. A part of this settled biomass, described as activated sludge is returned to the aeration tank and the remaining forms waste or excess sludge (Modin et al., 2016; Xu et al., 2018).

In sewage, especially in untreated sewage and activated sludge, where the bacterial density is very high, microorganisms have access to a large pool of itinerant genes which move from one bacteria cell to another (horizontal and vertical transfer) (Jiao et al., 2017; Summers, 2006). The genes may spread through bacterial populations via plasmids and a variety of mobile genetic elements, such as transposons or integrons (Chamosa et al., 2017; Fletcher, 2015; Patel, 2016), carrying genes which encode resistance to other antimicrobial agents (von Wintersdorff et al., 2016). Therefore, biological treatment processes at sewage treatment plants could cause selective elimination, and/or changes in the proportions of phenotypes/genotypes within bacterial populations in effluent. Korzeniewska and Harnisz (2013b) and Osińska et al. (2017) found higher frequency of multiple resistant *Escherichia coli* bacteria in treated than in untreated wastewater. These findings agree with studies of Szczepanowski et al. (2009), who detected a reduction of susceptibility to selected antimicrobial drugs in bacteria isolated from activated sludge compared to those isolated from the effluent of WWTP. Ferreira da Silva et al. (2007) and Osińska et al. (2017) demonstrated higher percentage of amoxicillin and tetracycline resistance in *E. coli* isolated from treated effluent than in *E. coli* isolated in the inflow of the same WWTP. The wastewater treatment plant constitutes, therefore, an important reservoir of enteric bacteria which carry potentially transferable resistance genes. Together with treated sewage these microorganisms and their genes can penetrate the soil, surface water, rural groundwater supplies and drinking water (Khan et al., 2016; Su et al., 2018). It creates a potential risk to human and animal health because ARGs and ARB transported to the environment can be transferred back to people and animals.

Biological treatment processes at wastewater treatment plants (WWTPs) are the most common methods of sewage treatment. At the end of 2016 in Poland there were 3319 working WWTPs, including 22 mechanical, 2461 biological and 836 with increased nutrients removal system (GUS, 2017). Biological WWTPs are mainly located in villages and small cities (2089) and in Poland most of them use activated sludge technology with sequencing batch reactors (SBR). SBRs are a type of activated sludge process designed for the treatment of domestic wastewater in WWTPs with low capacity. Tanks which make the installation consist of a series of walls or baffles which direct the flow either from side to side of the tank or under and over consecutive baffles. This helps to mix the incoming influent and the returned activated sludge, beginning the biological digestion process before the liquid enters the main part of the tank. As the bacteria multiply and die, the sludge within the tank increases. The quantity and age of sludge within the tank can have a marked effect on the treatment process and the presence of ARB and ARGs in WWTPs' effluents (Di Cesare et al., 2016; J Guo et al., 2017). The anaerobic/anoxic/oxic (A2/O) process, the oldest biological method with elevated removal of nutrients have gained popularity in many countries in the 90s. This process, the most often in a three-stage system, is employed mainly for organic matter and phosphorus removal as well as for denitrification. The three-stage system consists of chambers connected in series: anaerobic (phosphorus removal process), anoxic (denitrification process) and oxic (nitrification process). Using a several-phase cycle and desired biochemical transformations are accomplished at flow system to force fluctuation of organics and nutrient concentrations in process reactors (Lai et al., 2011; Wang et al., 2011). To control and decrease eutrophication of receiving water bodies, various modifications of wastewater treatment with elevated biological nutrient removal of nitrogen and phosphorus are also widely used in wastewater treatment practices, both for the upgrade of existing wastewater treatment facilities and the design of new facilities. An example of this is the additional use of a pre-denitrification chamber or alternate aeration system in nitrification/denitrification chambers. However, implementation of biological nutrient removal activated

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