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Short Communication

Pilot study of seasonal occurrence and distribution of antibiotics and drug resistant bacteria in wastewater treatment plants in Slovakia

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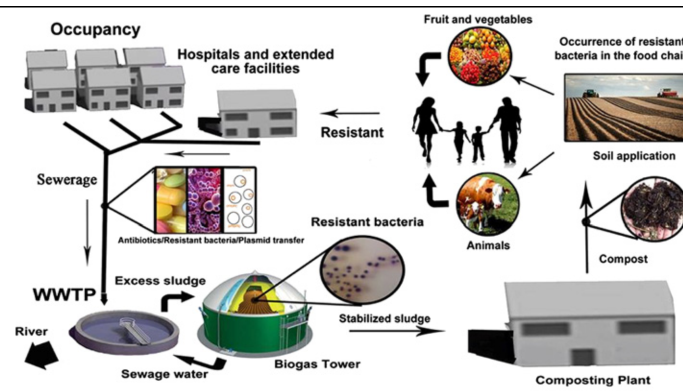
HIGHLIGHTS

- Analysis of selected antibiotics in influent and effluent wastewater in Slovakia.
- Determination of antibiotic resistant coliforms prevalence in sewage sludge.
- Comparison of seasonal occurrence of antibiotics and resistants in two WWTPs.

GRAPHICAL ABSTRACT



Dissemination of antibiotic resistance in the environment

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ABSTRACT

This work presents environmental and quality-control data from the analyses of 33 antibiotics in influent and effluent water from two waste water treatment plants (WWTPs) in the capital and the biggest city of Slovakia. Seeing that consumption of antibiotics depends on epidemiological season, samples were collected during February and August. Among assessed antibiotics ciprofloxacin and clarithromycin were detected in highest concentrations in influent water. Seasonal changes were observed only in plant A when antibiotic concentrations decreased. On the other hand an increase in some cases was observed in plant B. Insufficient degradation of some macrolides, sulfonamides and trimethoprim was detected according to their higher concentrations in effluent water. Contact of antibiotics in subinhibitory concentrations and sludge bacteria in WWTPs represent the base for the development of significant levels of microbial resistance. Simultaneously, antibiotic resistance of fecal coliforms and fecal streptococci from sewage sludge was evaluated. Majority of coliform bacteria were found to be resistant to ampicillin and gentamicin. A significant seasonal difference was determined only in case of high-level resistance. In summer samples, an increase in the strains resistant to concentrations higher than the resistance breakpoints established by EUCAST and NCCLS was observed. No antibiotic resistance in streptococci was observed. However, as a part of sewage sludge is mixed with compost and utilized in agriculture, better processing of sludge should be considered.

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1. Introduction

The predominant role of human activities in the generation of environmental reservoirs of antibiotic resistance cannot be disputed. Since the 1940s, the ever-increasing amounts of antibiotics designated for human application have been manufactured, used clinically, released into the environment, and widely disseminated thus providing constant selection and maintenance pressure for populations of resistant strains in all environments (Davies and Davies, 2010). Among the aquatic environments, wastewater habitats represent the most important reservoirs of antimicrobial resistant bacteria and genes. This type of water contains human and animal excretion with abundant doses of commensal and pathogenic antimicrobial-resistant bacteria (Ye and Zhang, 2011; Novo et al., 2013). Since antimicrobials are not fully degraded in the human and animal body, antimicrobial compounds, their metabolites and transformation products are abundant in urban sewage treatment plants (Segura et al., 2009; Michael et al., 2013). Although a proportion of the antimicrobials is transformed and degraded in the environment, the occurrence of these micropollutants is reported worldwide, with the antimicrobials of all classes in wastewater habitats being detected in concentrations ranging from ng L^{-1} to mg L^{-1} (Michael et al., 2013). Simultaneously, urban wastewater contains resistant bacteria together with other pollutants, such as pharmaceutical and personal hygiene products and heavy metals, whose effects on the antibiotic resistance selection are still not very clear (Graham et al., 2011; Oberlé et al., 2012; Novo et al., 2013). Often, wastewater treatment does not sufficiently eliminate the antimicrobial residues entering the system (Michael et al., 2013). The consequence is that such micropollutants, exerting selective pressure, may facilitate the selection of antimicrobial resistant bacteria or the acquisition of resistance genes by horizontal transfer (Martinez, 2009).

Microflora of sludge in a WWTP is constantly in contact with different types of drugs in sub-inhibitory concentrations. These substances can generate selective pressure on sludge microbiota which can result in the selection of antibiotic resistant strains (Cantas et al., 2013). Not only antibiotics but also biocides like triclosan may affect this unwanted process (Birošová and Mikulášová, 2009). These substances are frequently used in households and so higher levels of the biocide concentrations are present in wastewater during the whole year. In Slovakia, the annual production of sludge is of about 58 000 tons and the majority is usually transferred to composting plants where it is mixed with compost and applied to soil. A part of this compost is utilized in agriculture. In 2012, 36 832 tons of sludge was processed to compost and 1139 tons of sludge from WWTPs was applied directly to agriculture soil in Slovakia (Kozáková and Šumná, 2013). This sludge treatment process can also contribute to the resistance dissemination in the environment.

The aim of this study was to analyze the occurrence and levels of selected antibiotics and antibiotic resistant strains in two WWTPs in Slovakia. According to the seasonal differences in antibiotic consumption, antibiotic concentrations in influent and effluent water as well as antibiotic resistance of coliforms and streptococci in sewage sludge were compared.

2. Materials and methods

2.1. Wastewater treatment plants and sampling

Samples were collected from two WWTPs which receive wastewater from urban households, industries, hospitals and other facilities. Plant A currently serves for 125 000 inhabitants and treats up to 33 000 m^3/day of municipal wastewater. Plant B currently serves a population of 350 000 inhabitants and treats up to 150 000 m^3/day of municipal wastewater. The treatment process in both plants includes mechanical pre-treatment and an activated sludge system which is followed by a secondary clarifier. Sludge is anaerobically stabilized and the generated biogas is processed to energy. Sampling points were situated on the influent and effluent stream. Samples were taken every 15 min by an automatic sampling device and mixed as 24-hour composite samples. The samples of wastewater were frozen and transported to the laboratory. Samples of anaerobically stabilized and dewatered sludge were taken directly from a chamber filter press to a sterile falcon tube and transported to the laboratory for microbiological analysis.

2.2. Antibiotic resistance detection

Bacteria were detected in samples of stabilized sludge collected in sterile tubes and transferred to the laboratory within 1 h. Samples from the two WWTPs were collected during February and August 2013. Samples of sewage sludge were aspirated and collected in the last stage of the process before leaving the plant. Consequently, 1 g of the sample was mixed with 10 mL of physiological saline. Serial 10-fold dilutions of samples in physiological saline were prepared and 0.1 mL aliquots were spread on selective diagnostic agar plates. Tryptone soy agar was applied for the selective cultivation of total aerobes; for the coliforms, they were Chromocult Coliform Agar and Levine EMB Blue Agar, and SlanetzBartley Agar was applied for streptococci. Antibiotic resistant strains were detected after the cultivation on plates containing diagnostic media with different antibiotics. Before bacteriological counting, the plates were incubated for 24 h at 37 °C for coliforms and for 48 h at 42 °C for fecal streptococci. Each experiment was run in triplicate and was repeated five times.

2.3. Wastewater analysis

Wastewater samples were transported to the laboratory and defrosted at 18 °C. Consequently, the samples were homogenized and filtered (GFC, 0.45 μm). Isotope marked internal standards were added to 10 mL of the sample and the analysis was performed in one step using in line SPE; liquid chromatography coupled with a hybrid mass analyzer Q-Exactive (quadrupole coupled to Orbitrap high resolution MS, Thermo Scientific) was employed. Relative standard deviations for the used procedures were under 30%. In case of real wastewater, matrix LOD and LOQ values strongly differ from sample to sample. The limits of quantification (LOQs) ranged from 0.05 to 50 ng L^{-1} (median 5 ng L^{-1}). The use of matrix-matched standards led to the elimination of ionization enhancement or suppression (Grabic et al., 2012; Khan et al., 2012).

3. Results and discussion

Antibiotics are predominantly water soluble and enter the aquatic environment through sewerage systems following consumption and excretion by humans and in effluent from farms, abattoirs and landfills. Between 30% and 90% of an administered dose of most antibiotics to humans and animals are excreted in the urine as an active substance (Costanzo et al., 2005). According to this fact, concentrations of some antibiotics in influent and effluent waste water from two WWTPs in the capital of Slovakia—Bratislava, were analyzed. Bratislava WWTPs were chosen because they process wastewater from approximately 10% of the Slovak population. This represents the highest wastewater loading in Slovakia. Also, winter and summer wastewater compositions were compared. For winter sampling, February was chosen because of regular flu epidemic in Slovakia (Public Health Authority of Slovak Republic, 2013). Results are in Tables 1 and 2.

In February, high levels of ciprofloxacin were detected in the influent water (1.35 μg L^{-1} in plant A; 2.12 μg L^{-1} in plant B). Concentrations of levofloxacin and norfloxacin were also higher during winter. This can be a consequence of frequent prescription of fluoroquinolones. Higher levels of clarithromycin were also observed in both WWTPs. In summer samples from plant A, the concentration of most antibiotics in wastewater decreased. It can be assumed that seasonal change in plant A is caused by the increased appearance of epidemics during the winter