

OxiTop microcosm model as a possible tool to study the effects of antibiotic exposure on the microbial community

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Abstract

The bacterial community of surface waters is a key element of self-cleansing, but under antibiotic pressure, it should be closely monitored to evaluate the emergence of antimicrobial resistance. According to the World Health Organization (WHO), antibiotic resistance is one of the three most serious threats to human health. Antibiotic resistance can develop due to the selective pressure of antibiotics on the natural microbial ecosystem discharged through the effluents of wastewater treatment plants (WWTPs). This study aimed to find a link between sewage load and the biological activity of the bacterial community with and without antibiotic pressure. To reach this goal, an OxiTop microcosm experiment was set using surface water samples originating from a Hungarian site with and without sewage load. Samples were supplemented with different combinations of antibiotics relevant in human and veterinary therapy (colistin, florfenicol, imipenem, ceftazidime). During the incubation period, biological oxygen demand (BOD) was regularly measured and was paired with traditional cultivation and next-generation sequencing. Based on our results, the bacterial diversity decreased under antibiotic pressure, but the viable cell counts were not necessarily affected. The microbial community of surface water with sewage load had a higher level of adaptability to the antibiotics. The key taxa under antibiotic pressure that are possible hosts of multiple antibiotic resistance genes were *Cellvibrio*, *Aquabacterium*, and *Flavobacterium*.

Keywords

Antibiotics, antibiotic resistance, microbial community, OxiTop microcosm model, sewage, next generation sequencing, 16S rRNA.

OxiTop mikrokozmosz modell, mint lehetséges eszköz az antibiotikumok mikrobiális közösgre gyakorolt hatásainak vizsgálatára

Kivonat

A felszíni vizek bakteriális közössége kulcsfontosságú eleme a szennyvízbevezetések helyén zajló öntisztulásnak, azonban az antibiotikum rezisztencia kialakulása körükben is mind nagyobb figyelmet érdemel. Az Egészségügyi Világszervezet (WHO) szerint az antibiotikum-rezisztencia az emberi egészséget fenyegető három leg súlyosabb veszély egyike. Antibiotikum-rezisztencia kialakulhat a szennyvíztisztító telepek kibocsátásával a természetes mikrobiális ökoszisztémára gyakorolt szelektív nyomás következtében. A tanulmány célja, hogy kapcsolatot találjon a szennyvízterhelés és a bakteriális közösség biológiai aktivitása között antibiotikum terhelés mellett és anélkül, melynek érdekében OxiTopTM mikrokozmosz kísérletet állítottunk be egy magyarországi, szennyvízterhelés alatt álló víztestből származó minták felhasználásával. A mintákhoz humán és állatgyógyászati szempontból jelentős antibiotikumok különböző kombinációit adtuk (Kolisztin, Florfenikol, Imipenem, Ceftazidim). Az inkubációs időszak során folyamatosan mértük a minták mikrobaközösségének biológiai aktivitását, melyet hagyományos tenyésztési módszerekkel és újgenerációs szekvenálással egészítettünk ki. Eredményeink alapján az antibiotikum terhelés hatására csökkent a bakteriális diverzitás, de a bakteriális sejtszám nem. A szennyvízterheléssel érintett felszíni víz mikrobiális közössége nagyobb alkalmazkodóképességet mutatott az antibiotikumokkal szemben, mint a háttér minta. Eredményeink alapján az antibiotikumok hatására dominánssá váló taxonok – amelyek potenciális hordozói lehetnek többféle antibiotikum-rezisztencia génnek – a *Cellvibrio*, az *Aquabacterium* és a *Flavobacterium* voltak.

Kulcsszavak

Antibiotikumok, antibiotikum rezisztencia, mikrobiális közösség, OxiTop mikrokozmosz modell, szennyvíz, újgenerációs szekvenálás, 16S rRNS gén.

INTRODUCTION

The World Health Organization has listed antimicrobial resistance (AMR) as one of the top 10 threats to public health (WHO 2023). Due to the gradually growing pharmaceutical (González Peña et al. 2021) and antibiotic use all over the world (Klein et al. 2018), antibiotic-resistant bacteria (ARB) are distributed widely not only in healthcare institutes (Serra-Burriel et al. 2020) but in various environments, such as soil (Bombaywala et al. 2021) and

freshwater systems (Nnadozie and Odume 2019). Wastewater treatment plants (WWTPs) are verified sources of antibiotic load (Rodríguez-Mozaz et al. 2020), resistant microorganisms (Rodríguez-Molina et al. 2019), and resistance genes (Li et al. 2022). Therefore, WWTPs are potential hotspots for ARB spread into the environment (Rizzo et al. 2013). At the same time, the available information on the effect of last-resort antibiotics, such as colistin (polymyxin E) (El-Sayed et al. 2020) and broad-

spectrum antibiotics, like carbapenems (Armstrong *et al.* 2021) and florfenicol (Zhang *et al.* 2020) on the environmental microbiome is limited, despite the scientific evidence suggesting that wastewater effluent is frequently polluted with colistin (Smyth *et al.* 2020) and carbapenem-resistant bacteria (Araújo *et al.* 2021). In Hungary, the bacterial community structure of wastewater effluents (Benedek *et al.* 2014) and the effect of antibiotic pressure on the forming microbial community is poorly understood, and the impact of the simultaneous load of wastewater and antibiotics on the recipient surface water's microbiome and its activity has not been examined in detail.

Based on this background, this work aimed to compare two sections of a chosen surface water body located in Hungary, with and without sewage load, and to evaluate the effect of antibiotic pressure on the biological activity and composition of the cultivable and non-cultivable microbial communities. To reach this goal, a microcosm model was set using different treatments with four “Watch” and “Reserve” types of antibiotics (colistin, florfenicol, imipenem, ceftazidime) according to the WHO classification for evaluation and monitoring (WHO 2019). For

antibiotic treatment, sewage-affected and background surface water samples were used. The initial hypothesis of this study was that the microbial communities of environmental samples affected by sewage inlets show a higher level of adaptability to antibiotics and a higher number of cultivable ARB.

MATERIALS AND METHODS

Sampling

The sampling strategy was to choose a surface water stream with and without the effect of urban sewage effluent. Our choice was the section of the Rákos stream localized between Gödöllő and Isaszeg, Hungary, receiving the effluent of Gödöllő WWTP (wastewater treatment plant), with a capacity of 50000 population equivalent (10000 m³/day). As background, an unaffected area of the same stream was sampled 200 m upstream (see Figure 1). Samples were taken on the 30th of March 2021 aseptically into sterile 1 L volume bottles and immediately transported to the MATE - Institute of Aquaculture and Environmental Safety, Department of Environmental Safety laboratory for analysis. Antibiotic concentrations were not measured in the native samples.



Figure 1. Location of the examined WWTP and the sampling site, Gödöllő, Hungary (©2023 Google Map, ©2000-2021 Michelin Travel Partner)

Notes: Blue mark – background (unaffected) area (GPS coordinates: 47°33'54.8"N 19°22'03.0"E); orange mark – WWTP effluent affected area (GPS coordinates: 47°33'53.6"N 19°22'02.9"E)

1. ábra. A vizsgált szennyvíztisztító telep és a mintavételi hely elhelyezkedése, Gödöllő, Magyarország (©2023 Google Térkép, ©2000–2021 Michelin Travel Partner)

Megjegyzés: Kék jelölés – háttér (nem érintett) terület (GPS koordináták: 47°33'54.8"N 19°22'03.0"E); narancssárga jelölés – a szennyvíztisztító telep kifolyása által érintett terület ((GPS koordináták: 47°33'53.6"N 19°22'02.9"E); narancssárga nyíl – a víz áramlásának iránya

Experimental settings

To reveal the effect of the chosen antibiotics and their combinations on the microbial community and biological activity of the examined (sewage-affected and background) samples, a laboratory experiment was set in three replicates using 0.5 L sterile bottles inoculated with 200 mL volume of native samples. At the beginning of the experiment, samples were treated with three different

combinations of antibiotics using MAST disks (Mast Diagnostica, Germany) to reach a final 300 µg/L antibiotic concentration (Table 1). The chosen sub-lethal concentrations were higher than the previously detected concentrations of antibiotics in the environment (Wang *et al.* 2025), because we aimed to reach the inhibitory concentration of the sensitive organisms and therefore, to perform a persistent selective pressure on the microbial community.

Table 1. Sample types and antibiotic treatments in the experimental settings
1. táblázat. A kísérleti beállítások során használt mintatípusok és antibiotikum kezelések

Sample identifiers and sample types	Treatment
B (Background) SA (Sewage affected) surface waters	Native (non-treated)
B-COL (Background + Colistin) SA-COL (Sewage affected + Colistin)	Colistin (300 µg/L)
B-FLOR (Background + Florfenicol) SA-FLOR (Sewage affected + Florfenicol)	Florfenicol (300 µg/L)
B-MIX (Background + antibiotic mixture) SA-MIX (Sewage affected + antibiotic mixture)	Colistin (100 µg/L) Imipenem (100 µg/L) Ceftazidime (100 µg/L)

OxiTop™ System to measure biological oxygen demand

To determine the biological oxygen demand (BOD) of the microbial community under the effect of antibiotics, a manometric method measuring pressure differences via piezoresistive electronic pressure sensors (OxiTop™, WTW, Weilheim, Germany) was used (González Peña *et al.* 2021). Samples were kept in a sealed container fitted with a pressure sensor. During the incubation, oxygen was consumed, and the biologically active microorganisms released carbon dioxide. The pressure decreased because sodium hydroxide pellets were added to a separate container above the sample level to absorb carbon dioxide.

The incubation temperature was 15°C, and the incubation time was 7 days (168 h) with continuous stirring using the platforms of the OxiTop™ system. Negative pressure (that correlates with the microbial activity, i.e., BOD value of the sample) was measured automatically in every 30 minutes during the incubation period. At the end of our experiment, outlier bottles (with more than ±10 Pa deviation compared to the average of the replicates) were excluded from further data analysis in each setting (altogether 2 bottles in the whole experiment). Pressure curves of the remaining technical replicates with acceptable deviation in pressure values were averaged and further analyzed.

Cultivation-based methods

Traditional cultivation aimed to determine the colony-forming units (CFUs) of bacteria from the treated and non-treated environmental samples. A tenfold series of dilutions was prepared, and the diluted samples were used for the pour plate technique. Preliminary experiments were performed to evaluate the sufficient media for the cultivation and isolation of antibiotic-resistant microorganisms originating from environmental samples. Based on these preliminary tests, R2A (Agar, 15.0 g; Casein acid hydrolysate, 0.5 g; Dextrose, 0.5 g; Dipotassium phosphate, 0.3 g; Magnesium sulphate, 0.024 g; Proteose peptone, 0.5; Sodium pyruvate, 0.3 g; Starch (soluble), 0.5 g; Yeast extract, 0.5 g, Distilled water, 1,000 ml) was the most chosen for cultivation (data not shown). After incubation at 28°C for 96h, colony-forming units (CFUs) of the tenfold dilutions were counted and averaged to calculate the CFU/ml values of the original (100%) samples.

Amplicon sequencing

For amplicon sequencing, at the end of the 7-day OxiTop™ experiment, we averaged the technical replicate samples, and aseptically filtered 200-300 ml volume of each experimental setting using a 0.22 µm pore size, hydrophilic MCE membrane filter, 47 mm diameter, white, Triton-free (Millipore). We extracted community DNA from the filtrate using the DNeasy PowerSoil DNA extraction kit (Qiagen, Germany) according to the manufacturer's instructions.

During our experiment, we amplified bacteria domain-specific genetic elements from the community DNA. For paired-end 16S rRNA amplicon sequencing, the variable V3 and V4 regions of the 16S rRNA gene were amplified using primers containing overhang Illumina adapters. Paired-end fragment reads (2x250 bp read length) were generated on the Illumina MiSeq platform using MiSeq Reagent Kit v3 by Biomi Ltd., Hungary. Reads were trimmed according to their quality and length using the CLC Genomics Workbench Tool; the minimum required length of nucleotides was set to 50. Trimmed sequences were processed using mothur v 1.41.1 (Schloss *et al.* 2009), an open-source, platform-independent, community-supported software for describing and comparing microbial communities. Sequences were assorted based on their alignment using the SILVA 138.2 database (Quast *et al.* 2013) followed by the chimera detection and the removal of singleton reads. The standard 97% similarity threshold was used to determine operational taxonomic units (OTUs) as suggested by Tindall *et al.* (2010) for prokaryotic species delineation. After data processing, the mothur software summarized taxonomy and diversity results of the examined samples in data files. Data was exported to Excel for further analysis.

RESULTS AND DISCUSSION

Based on the examination of environmental samples with and without sewage load of the same geographical location (CW and SL samples) and their treatment with different types of antibiotics, we gained information about the composition and activity of the microbial community. Hereby we present results by cell counts (colony-forming units - CFU), BOD values, microbial community (amplicon sequencing).

Cell counts (colony-forming units) under antibiotic treatment

With traditional cultivation, colony-forming units were

determined on R2A medium at the beginning and at the end of the 7-day (168h) long experiment. CFU values are summarized in *Figure 2*.

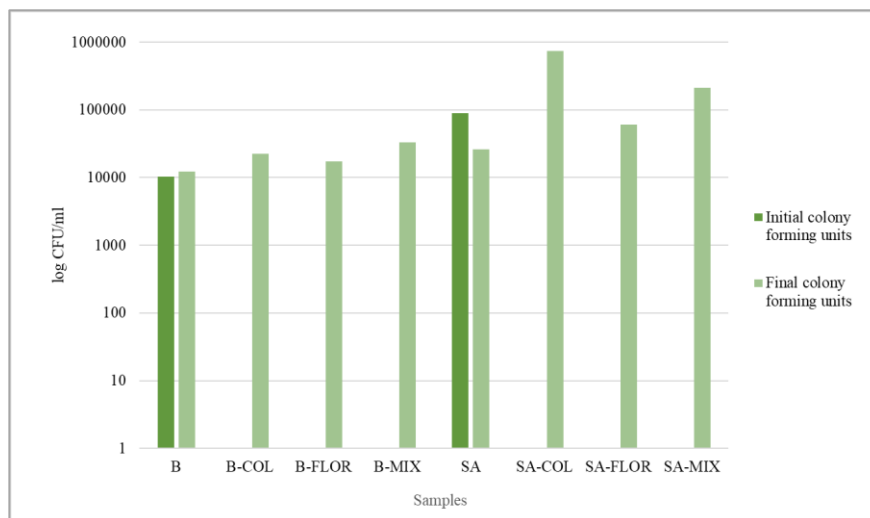


Figure 2. Log CFU values of the averaged samples of the experimental settings using background (B) and sewage affected (SA) surface waters with and without antibiotic supplementation on R2A agar.

Note: Initial colony forming units - 0h; Final colony forming units - 168h; COL - Colistin (300 µg/L); FLOR - Florfenicol (300 µg/L); MIX - Colistin, Imipenem, Ceftazidime mixture (100-100 µg/L)

2. ábra. A háttér (B) és a szennyvízzel terhelt (SA) felszíni vizek kísérleti beállításainak átlagolt mintáin mért log CFU értékek antibiotikum-kiegészítéssel és anélkül R2A agaron

Megjegyzés: Kiindulási CFU értékek - 0 óra; Záró CFU értékek - 168 óra; COL - Kolisztin (300 µg/L); FLOR - Florfenikol (300 µg/L); MIX - Kolisztin, Imipenem, Cefotazidim keverék (100-100 µg/L)

In *Figure 2*, the cell count of the background surface water sample (without sewage load) was stable; there were no significant increases or decreases in CFU values after 7 days of incubation in the OxiTop™ system. At the same time, SA samples, especially with colistin treatment, increased their cultivable microbial numbers, which was an unexpected outcome of antibiotic treatment. The difference was higher in the case of sewage-affected surface water samples, which is in accordance with previous findings that in the sewage microbial community, the adaptability and resistance to antibiotics are higher (*Rizzo et al. 2013*). However, the stable or increasing cell counts were unexpected: we hypothesized that antibiotic treatment would reduce the number of cultivable microorganisms. A possible explanation is that with the effect of antibiotics, sensitive microorganisms were eradicated, and without the competition, resistant, fast-growing bacteria could easily proliferate till the 7-day-long experiment; consequently, the CFU values increased.

Microbiological activity under antibiotic treatments

During the 7-day-long experiment, we continuously measured the changes of negative pressure in each bottle to evaluate the microbial activity. The result of biological oxygen demands (BODs) of the eight different settings (B and SA samples, the other 6 samples treated by antibiotics and their combinations (B-/SA-COL, B-/SA-FLOR, B-/SA-MIX) during the incubation period are summarized in *Figure 3*, supplemented with the average value obtained by the replicate flasks (2-3 replicates/settings) after the exclusion of the outliers.

In *Figure 3*, the pressure goes down when the microbial community is consuming a significant amount of oxygen, which means that it is biologically active. If the curve is flat, it means there was only minimal biological activity because the microbial community was not consuming much oxygen. This graph showed that the lowest level of biological activity was in the untreated Background (B) surface water sample. The biological activity was also lower than the average value in the background (B) samples supplemented with colistin (B-COL), florfenicol (B-COL), and with the mixture of colistin, imipenem, and ceftazidime (B-MIX). According to the BOD values of the Background samples, we could conclude that the antibiotic treatment did not cause a significant change in the biological activity of the microbial community, which correlates with the stable CFU values of these samples.

Among the sewage affected (SA) sample types, the untreated sample (SA) had the lowest degree of biological activity and was the only one that stayed below the average value during the 168h incubation. The biological activity of the organisms in the sewage-affected samples supplemented with antibiotics was much higher than the average: florfenicol (SA-FLOR) and mixed (SA-MIX with colistin, imipenem, and ceftazidime) antibiotic treatment induced a similar, significant increase in BOD values, while colistin (SA-COL) sample had the highest biological activity among all sample types, with a significant BOD increase especially in the second half of our experiment. The extremely high biological oxygen demand of the SA-COL sample can be a result of the higher microbial cell counts verified by the plate counting technique.

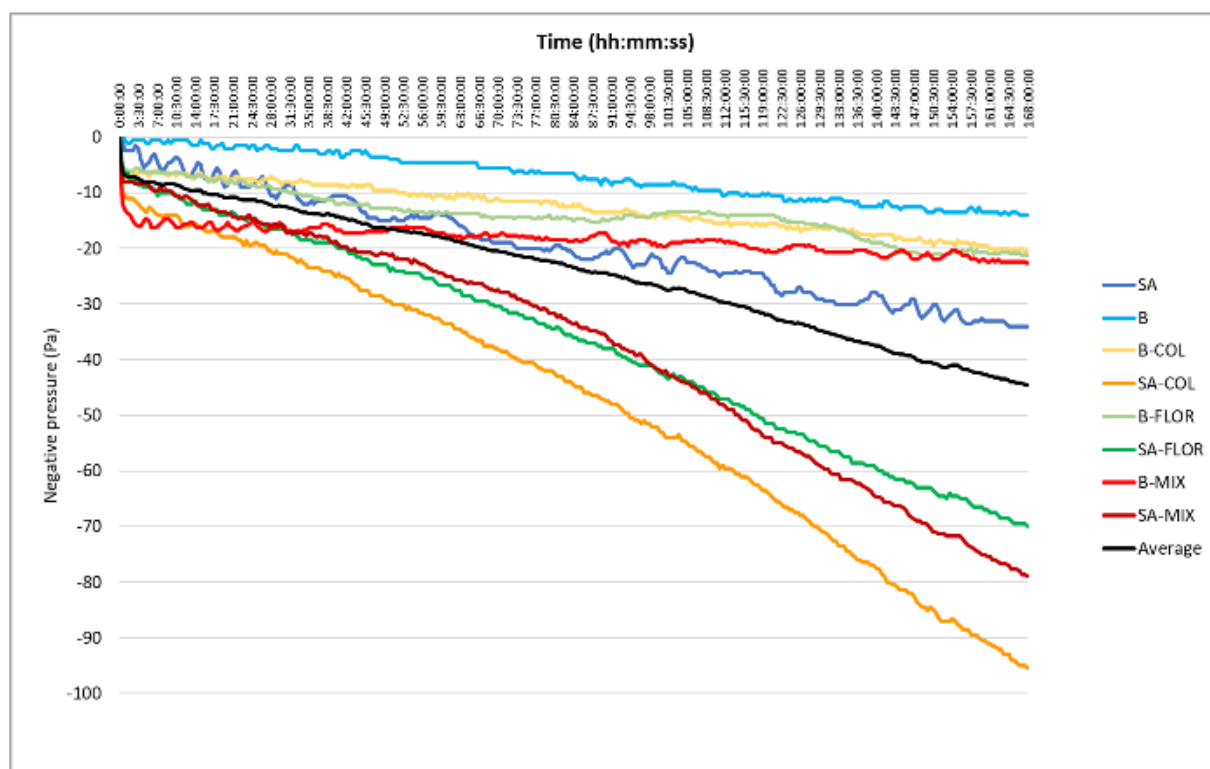


Figure 3. Microbiological activity of the examined samples expressed in negative pressure measured by OxiTop system

Note: SA-sewage affected, B-background, COL-Colistin; FLOR-Florfenicol; MIX-antibiotic mixture

3. ábra. Az OxiTop rendszerben vizsgált minták biológiai aktivitása negatív nyomás értékben kifejezve

Megjegyzés: SA-szennyvízterhelés alatt álló víz; B-háttér minta; COL - Kolisztin; FLOR - Florfenikol; MIX-antibiotikum keverék

In the background (B) water group, samples without antibiotic treatment had the lowest microbiological activities. When a solo or combined antibiotic treatment was used, the biological activity of the microorganisms is increased. The three combined antibiotics of colistin, imipenem, and ceftazidime had the greatest impact on microbial activity in the background sample during the incubation, but after 168h only minor differences could be detected between the different treatments.

Antibiotics have a significant impact on microbial activity, particularly in the case of colistin, but after the first 24h, stagnating biological activity, the determined effect was not inhibition. BOD is the measure of the amount of oxygen taken up by aerobic microorganisms that decompose organic waste matter in water. A high BOD indicates the presence of a large number of microorganisms, which suggests a high level of biodegradable organic matter, such as drug residues or other organic pollutants (Ijoma 2010).

Therefore, we can presume that the applied concentrations of antibiotics during a long-term incubation are not only environmental pollutants, but potentially carbon sources for fast-growing microorganisms and their presence does not necessarily decrease the biological activity or inhibit the bacterial growth.

Compared to scientific literature, there are only a few similar studies regarding the comparison of BOD values of

microbial communities under different antibiotic treatments. Panta and Doerrler described colistin resistance as a complex and multifactorial phenomenon noting that colistin resistance can be linked to the pH and oxygen level of the medium (Panta and Doerrler 2021). Li and her co-authors mentioned that the relative abundances of antibiotic resistance genes (ARGs) were negatively correlated with temperature and BOD (Li *et al.* 2020). In our case, the temperature was constant during the incubation and pH was not measured; therefore, we could not draw similar conclusions. Based on the biodegradation rates of the examined antibiotics, carbapenems and colistin reach a significantly higher degradation rate in wastewater than in natural water; physicochemical factors such as temperature and pH greatly affect the degradation rates (Sharma *et al.* 2023). Consistent with this, we observed a higher biological activity in wastewater-affected samples, suggesting that antibiotic degradation rates in these environments are likely to be higher.

Diversity and taxonomy of water samples with and without antibiotic treatment

The bacterial diversity of the samples was determined by the Mothur algorithm using the Illumina next-generation sequencing data and was expressed with the commonly used Shannon diversity index that considers both the number and distribution of the detected species. Shannon diversity indices are summarized in Figure 4.

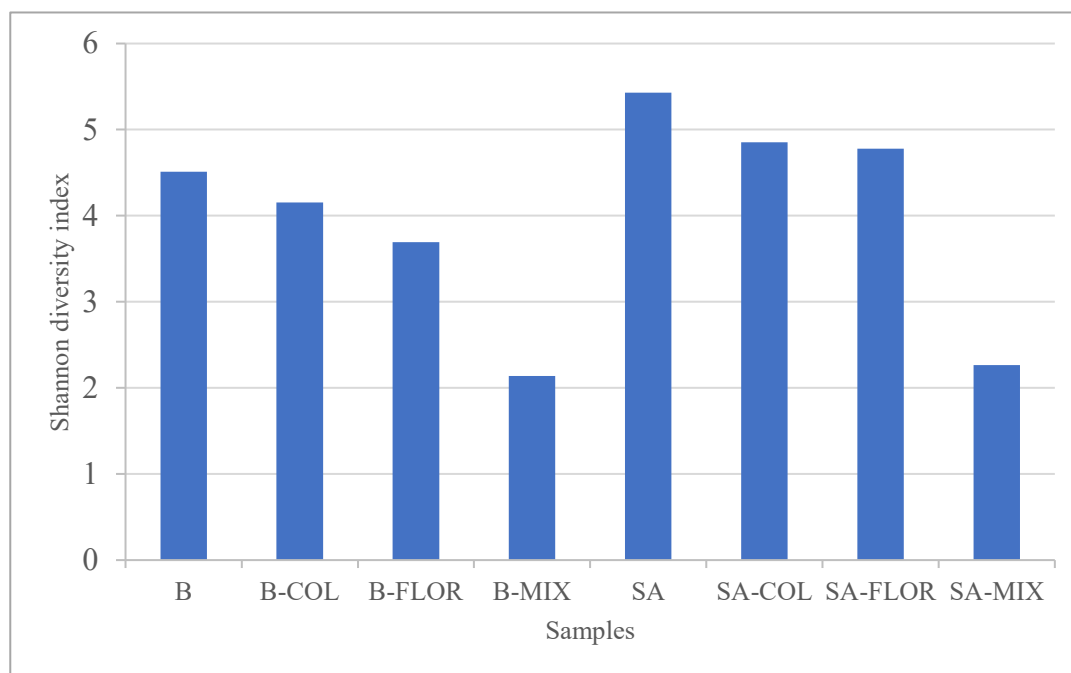


Figure 4. Shannon diversity index of the examined samples after 168h incubation with and without antibiotics

Note: SA-sewage affected, B-background, COL-Colistin; FLOR-Florfenicol; MIX-antibiotic mixture

4. ábra. A vizsgált minták Shannon diverzitás index értékei 168 órás inkubációt követően antibiotikum kiegészítéssel és anélkül
Megjegyzés: SA-szennyvízterhelés alatt álló víz; B- háttér minta; COL - Kolisztin; FLOR - Florfenikol; MIX-antibiotikum keverék

As Figure 4 shows, after 7 days of incubation, the Background (B) samples had a lower level of microbial diversity, while the surface water sample with sewage load (SA) had the highest Shannon index. Compared to native (B and SA) samples, all antibiotic treatments led to a lower level of bacterial diversity: according to our results, the SA-COL setting had the highest, and B-MIX/SA-MIX samples had the lowest diversity of all antibiotic treatments. Compared to the determined CFU values of each sample (Figure 2), there is an inverse correlation between diversity and cell counts. According to this information, we could state that antibiotic treatment caused a decrease in the microbial diversity of the examined samples, while most of the time the microbial counts remained the same or increased. A possible explanation is that antibiotic treatment eradicated the sensitive microorganisms and opened a niche to the fast-growing, antibiotic-resistant species.

To determine the key members of the microbial community, at the end of the experiment, the genus-level composition of the bacterial community under different treatments was determined by next-generation sequencing. The composition of the top 20 identified bacterial genera of each sample is summarized in Figure 5, which shows that the Background (B) and Sewage affected (SA) samples had different microbial communities. B sample contained typical surface water-related taxa such as *Perlucidibaca* and *Aquabacterium*. *Aquabacterium* species such as *A. citratiphilum*, *A. commune* and *A. parvum* were previously isolated from biofilm from the Berlin drinking water system (Pham et al. 2015), while *A. fontiphilum* and *A. limnoticum* were found in freshwater springs in China (Chen et al. 2012, Lin et al. 2009). Some potentially pathogenic species of the

genus had the highest abundances in biofilm samples and were described as hosts harbouring many resistance genes (Zhao et al. 2021). The Gram-negative freshwater bacterial genus *Perlucidibaca* was described in 2008 (Song et al. 2008). Together with *Aquabacterium*, *Perlucidibaca* belong to the family *Moraxellaceae* and species; they presumably play a role in the early colonization of microplastics travelling in wastewater (Martínez-Campos et al. 2021). In contrast to the background (B) sample, the sewage-affected (SA) surface water contained many sewage-originating organisms, such as *Ferruginibacter* (Kong et al. 2024).

Antibiotic treatment induced significant changes in both sample types. With antibiotic treatment, the dominance of the *Nevskia* genus was suppressed in both B and SA samples. *Nevskia* species have a wide nutritional spectrum and can utilize sugars as growth substrates (Gutierrez et al. 2012), but based on the scientific literature, *Nevskia* species are not discussed in context with antibiotic resistance, which is supported by our results.

At the same time, the proportions of the *Perlucidibaca* and in the background samples, *Aquabacterium* and *Hydrogenophaga* species remained similar under COL and FLOR treatment; only the MIX antibiotic setting eradicated the members of these genera. The preponderance of some of these genera in COL samples is understandable, given that *Perlucidibaca*, *Hydrogenophaga* and *Aquabacterium* are members of the *Comamonadaceae* and *Moraxellaceae* families and are innately resistant to colistin. At the same time, we couldn't find any information about their florfenicol resistance, which should be investigated further in the future.

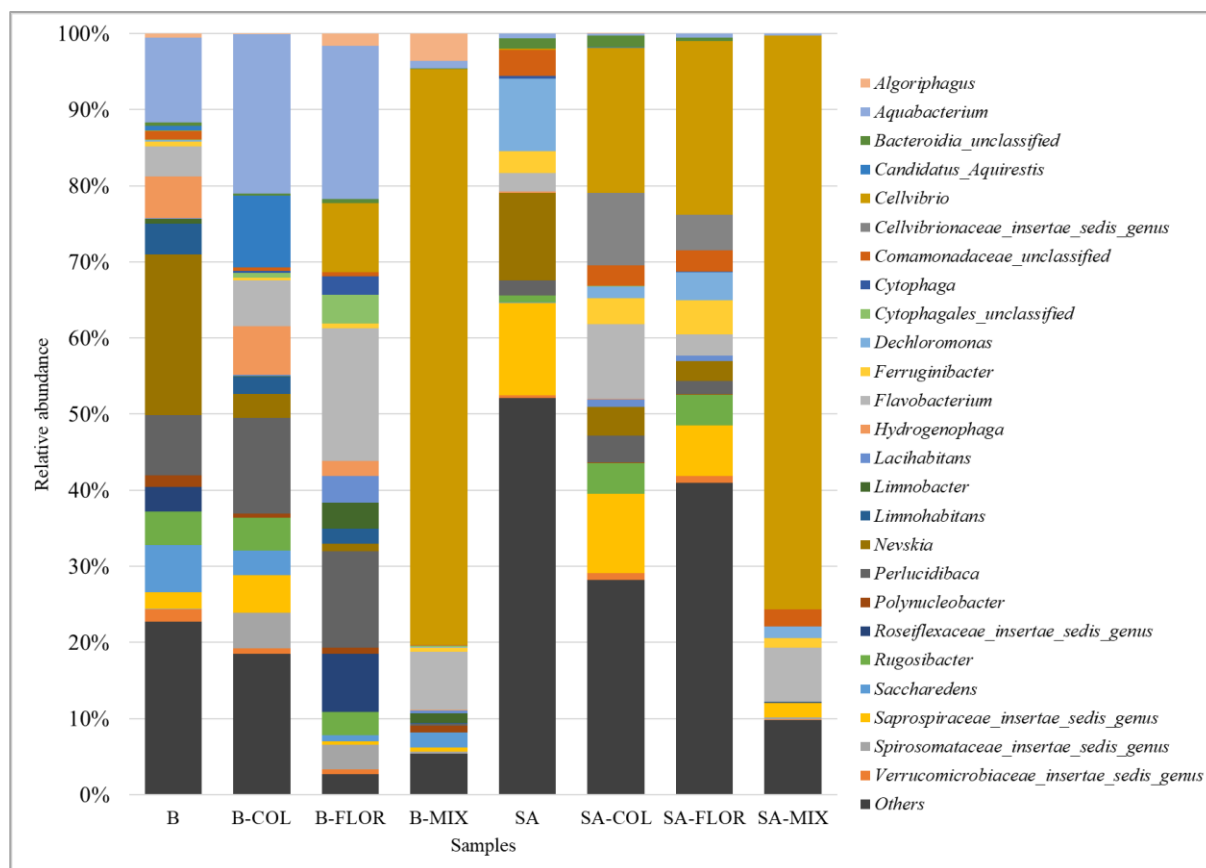


Figure 5 The composition of the top25 bacterial taxa with >1% abundance of the examined samples after 168h incubation with and without antibiotics

Note: SA-sewage affected, B-background, COL-Colistin; FLOR-Florfenicol; MIX-antibiotic mixture

5. ábra. A vizsgált mintabeállításokban 1%-nál nagyobb abundanciát elérő, baktériumnemzetségek top25 képviselője 168 óra inkubációt követően, antibiotikum kiegészítéssel és anélkül

Megjegyzés: SA-szennyvízterhelés alatt álló víz; B- háttér minta; COL - Kolisztin; FLOR - Florfenikol; MIX-antibiotikum keverék

Interestingly, *Cellvibrio* species, which had a negligible proportion in the native samples, reached an outstanding abundance in the B-MIX and SA-MIX samples, followed by B-FLOR, SA-FLOR and SA-COL treatment. They were only absent in the B-COL setting. This genus is involved in the breakdown of cellulose and its presence correlates with the frequency of antibiotic resistance genes - according to the results of Han and his co-workers, *Cellvibrio* species are possible hosts of ARGs in agricultural soils treated with different sources of animal manure, conferring resistance to aminoglycoside, tetracycline, and MLSB (Macrolide-lincosamide-streptogramin B) (Han et al. 2018). Recently, it was proven that the abundance of *Cellvibrio* species can grow under chronic antibiotic exposure in the gut microbiome of zebrafish (*Danio rerio*) as well, which correlates with our results (Yang et al. 2024).

Flavobacterium, which was the second most abundant genus in SA samples and also frequently detectable in B samples, reached at least 5% abundance in all examined settings, and it was the only genus that remained abundant in both sample types under all antibiotic treatment. The presence of *Flavobacterium* species was previously described to correlate with the presence of various ARGs, in-

cluding aminoglycoside, tetracycline, MLSB, FCA (fluroquinolone, quinolone, chloramphenicol, florfenicol and amphenicol) resistance genes, sulfonamide, β -lactam and multidrug resistance genes (Zhang et al. 2022). Moreover, *Flavobacterium* species were previously described as antibiotic degraders (Ma et al. 2022) that can explain their presence under antibiotic pressure.

Among the less abundant genera, *Ferruginibacter* and *Dechloromonas* were detectable in SA samples regardless of the antibiotic treatment; many of them were previously cultured from wastewater, activated sludge and granular activated carbon filters in water treatment (Kong et al. 2024, Mulder and Deinema 2006, Magic-Knevez et al. 2009). In the Background samples, *Hydrogenophaga* species were the predominant bacteria in the B, B-COL, and B-FLOR samples and were the most abundant in the B-COL setting. The members of this genus have a significant nutritional versatility and capacity utilize various substrates (Garrity et al. 2005, Magic-Knevez and van der Kooij 2006). *Hydrogenophaga* was described as a new host of ARGs in samples after O_3/Cl_2 disinfection (Zhang et al. 2019) and harboured Class 1 integrons that are playing a significant role in the worldwide problem of antibiotic resistance (Gillings et al. 2008).

CONCLUSIONS

Based on the OxiTop™ microcosm experiment using surface water samples taken from the Rákos stream with and without sewage load, the diversity of the microbial community was correlated with the origin of the sample (background and sewage-affected section). Antibiotic treatments with clinically and veterinary relevant antibiotics (colistin, florfenicol, imipenem, ceftazidime) significantly influenced the biological activity, bacterial diversity, and composition. It was verified that antibiotic-resistant bacteria that are non-susceptible to the four examined antibiotics are present in the untreated surface water samples and easily proliferate when the selective pressure of antibiotics opens a niche for them. Sewage affected (SA) microbial community had a higher diversity, showed an increased level of adaptability to the antibiotic treatments, therefore reached a higher cell count and biological activity until the end of the 168h-long experiment due to the dominance of fast-growing microorganisms such as *Cellvibrio* species. Based on the overview of the scientific literature, many of the identified bacterial genera (such as *Flavobacterium*, *Cellvibrio* and *Aquabacterium*) have been proven to be antibiotic-resistant or to harbour antibiotic resistance genes. Since the sensitive bacteria have been eradicated by antibiotics, the growth rate of the resistant bacteria has accelerated due to reduced competition, increasing the number and biological activity of potentially antibiotic-resistant microorganisms.

The OxiTop™ system has been adapted and optimized for the examination of antibiotic treatment using natural freshwater samples with and without sewage load. Under the controlled environment (temperature) provided by this microcosm model, the OxiTop™ system can be optimal to evaluate the long-term effects of antibiotic exposure not only on the composition, diversity and cell count of a bacterial community, but to measure the biological activity of microorganisms.

In the future, the developed methodology can be further improved by measuring the effect of pH and other temperature settings, as these factors also affect the relative abundance of ARGs (Panta and Doerrler 2021, Li et al. 2020). Quantifying various antibiotic resistance genes using molecular methods, complemented by traditional cultivation and characterization of antibiotic-resistant bacteria, can provide valuable insights into the dissemination of antimicrobial resistance in sewage-affected surface waters and contribute to improved environmental safety.

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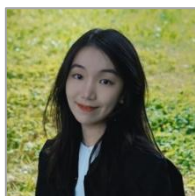
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