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Community Composition and Antibiotic Resistance of Tap Water Bacteria Retained on Filtration Membranes

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Abstract: Community composition and antibiotic resistance of tap water bacteria are still not known well enough. This study fills the gaps in knowledge regarding this matter. To provide representativeness of collected samples, tap water bacteria were concentrated from huge amounts of water, using filtration membranes monthly during the continuous, semi-annual study, covering winter and spring seasons. Biomass was investigated both using a culture-based method (for total and antibiotic-resistant culturable bacteria counts) and metagenomic DNA sequencing (for taxonomic identification of bacteria). The results showed that bacteria resistant to ceftazidime were the most prevalent among the studied resistance phenotypes, whereas bacteria resistant to amoxicillin, ciprofloxacin, and tetracycline were scarce. On average, 20,059 and 26,200 CFU/mL per month was counted in the winter and spring season, respectively, whereas in terms of antibiotic-resistant bacteria, average counts were 14,270 and 9435 CFU/mL per month in the winter and spring season, respectively. In terms of bacterial community composition, *Cyanobacteria*, *Proteobacteria* and *Actinobacteria* were the most abundant phyla, reaching up to 77.71%, 74.40% and 21.85%, respectively, which is supported by previous studies conducted on the same water supply network and other drinking water distribution systems across the world. No season-dependent variations were observed for culturable antibiotic-resistant bacteria or bacterial community composition. The prevalence of culturable antibiotic-resistant bacteria was not correlated with any of the identified taxa.

Keywords: drinking water; bacterial community composition; antibiotic-resistant bacteria; membrane filtration

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1. Introduction

In the majority of European countries, tap water is considered to be safe for drinking. Nevertheless, it is not—and probably never will be—free of microbial contamination. Despite more and more advanced treatment processes (including disinfection) being applied in water treatment plants (WTPs), treated water entering a distribution system still contains microorganisms [1,2]. Some bacteria present in raw (untreated) water show resistance to chlorine or other disinfectants commonly used at WTPs [3,4]. One of the alternative solutions for the removal of microorganisms from treated water is the membrane process. However, due to high costs and energy consumption, they are not commonly used, though some authors suggest that operating costs could be compensated by significant decrease of disinfectants and coagulant consumptions, as well as by a decrease in operational costs of sand filters [5].

Furthermore, microbial quality of water flowing through pipelines could significantly deteriorate, due to the presence of some nutrients and biofilms [6–9] throughout a pipe network. It was suggested that bacteria could proliferate and exchange the genetic material in water distribution systems [10]. Moreover, it was proven that the chlorination process promoted the horizontal transfer of plasmids by natural transformation [11]. It is associated

with a risk of spread of unfavourable features (such as resistance or virulence) within the microbial consortia, than the antimicrobial resistance among tap water bacteria. Though bacteria commonly present in drinking water systems are considered to be saprophytic, it cannot not be excluded that some pathogens or opportunistic pathogens enter into a network. Even if present at relatively low concentrations in tap water, they can pose a risk to immunocompromised consumers [12]. Recently, antibiotic resistance of bacteria was considered as an emerging contamination of drinking water [13,14].

The biodiversity of tap water bacteria was investigated in many studies across the world [6,15–17]. A few of them focus on biofilms gathering on domestic sanitary installations (taps, shower hoses, seals) [18–22] or tubercles in municipal pipeline networks [15,23,24]. To date, many authors performed long-term research, taking into account seasonal variation of bacterial biodiversity [6,16,25–27]. There are also some papers presenting the biodiversity of drinking water bacteria in artificial laboratory installations or bioreactors [28–31]. Nevertheless, field studies are always of importance, giving insight into community composition of microorganisms in full-scale systems. It is worth noting that thanks to the results of field studies, it is possible to evaluate whether the results obtained in artificial systems truly reflect the actual microbial communities in drinking water systems.

The aim of this study was to perform semi-annual, continuous research on the community composition of bacteria present in tap water with regard to seasonal variability and the presence of cultivable antibiotic-resistant bacteria (ARB). The groups of ARB selected for investigation in this study consisted of bacteria resistant to the antibiotics more commonly consumed in Poland [4]: amoxicillin (β-lactams, β), ciprofloxacin (fluoroquinolones, FQ), ceftazidime (third generation cephalosporins, 3GC) and tetracycline (T). To ensure the representativeness of tap water samples, biomass was concentrated from many litres of water by means of filtration membranes. The obtained results fulfil the knowledge on the community composition of bacteria present in tap water in the European country (Poland) with a temperate climate.

2. Materials and Method

2.1. Tap Water Filtration Set

Tap water microorganisms were collected from membranes mounted in the commonly used and available 4-stage under-counter filtration systems (Aquafilter FP3-HJ-K1N, Łódź, Poland). The system (or similar sets) could be used to treat water by end-point users in whole Europe. Water flowing through these systems is subjected to 3 stages of pre-treatment and then flows through the membrane (as presented on Figure 1). Filter 1 is a sedimentation (mechanical) cartridge made of polypropylene fabric, formed in such a way that the density of the filter increases towards the core. It stops all kinds of mechanical impurities, such as sand, silt, rust, etc., with a grain thickness of up to 5 microns. Filter 2 is responsible for softening and removing iron from tap water through an active ion-exchange bed. Filter 3 is a coconut shell carbon cartridge, which removes chlorine, pesticides, and heavy metals from tap water. Moreover, this activated carbon filter shows bacteriostatic properties, what ensures that captured bacteria should not proliferate during the sample collection. The set was connected directly to the water pipe in the laboratory at Wroclaw University of Science and Technology, and all cartridges and a membranes were changed every month (both the sampling point and the water treatment plant were marked in Figure 2). Water flowed continuously for 48 h. Prior to the installation of new cartridges and a membrane, water was drained at maximum flow for 15 min, to avoid contamination resulting from water stagnation. Detailed information on the filtration set was presented elsewhere [32].

Samples were collected during two seasons (winter and spring), from December 2020 to June 2021. Samples marked with numbers from 1 to 4 were winter samples (membranes installed in December, January, February and March). Samples marked with numbers from 5 to 7 were spring samples (membranes installed in April, May and June). In total, 7 samples were collected. In the study, only cold tap water was sampled every month

from the same tap. Before reaching the tap, water was treated by the municipal WTP, with the processes as follow: ground infiltration, aeration, filtration, ozonation, adsorption on activated carbon, pH correction and disinfection by chlorine and chlorine dioxide.

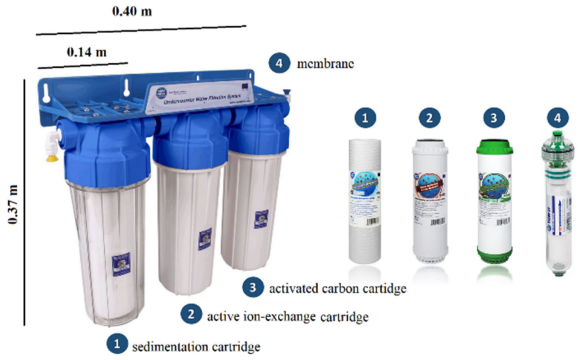

Figure 1. Tap water filtration set [33].

Figure 2. The location of the sampling point and the water treatment plant providing tap water (the distance of about 2 km), source: https://www.google.pl/maps, accessed on 20 January 2023.

After 48 h of water filtration, the membrane was disassembled, cut from the top and placed in a sterile physiological solution (1,100 mL, 0.85% NaCl, BTL, Łódź, Poland) in a large flask so that all fibres were covered with the solution. The flask was secured with parafilm. To rinse (recover) microorganisms from the membrane to the solution, the flask was shaken at 150 rpm (ELPIN, Lubawa, Poland) for 24 h at room temperature (22 °C). All the steps were undertaken in a sterile manner, excluding the possibility of external microbial contamination of the prepared suspension.

2.2. Heterotrophic Plate Counts and Antibiotic-Resistant Bacteria

To determine heterotrophic plate counts (HPC) and antibiotic-resistant bacteria (ARB), 10-fold dilution series of the obtained suspension were prepared in a physiological solution (0.85% NaCl, BTL, Łódź, Poland), ranging from 10^0–10^{-4}. One hundred microlitres of each dilution were plated on R2A (BTL, Łódź, Poland) for HPC, and on R2A supplemented with an antibiotic (amoxycillin (β) 8 mg/L, ciprofloxacin (FQ) 2 mg/L, ceftazidime (3GC) 8 mg/L or tetracycline (T) 16 mg/L, Sigma-Aldrich, St. Louis, MO, USA) for ARB enumeration. The concentrations were adopted from the literature [32,34]. On each type of medium, the suspension was plated in triplicate. Plates were incubated at 22 °C for 7 days, as was performed in the previous reports concerning the same sampling area [2]. The colonies were counted at the appropriate dilution, usually ranging from 30 to 300 CFU on a plate.

2.3. DNA Extraction

The obtained suspension (400 mL) was concentrated by filtration through mixed cellulose membranes of 0.2 µm pore diameter (Whatman, Maidstone, UK), by means of a sterile filtration set (Nalgene). The environmental DNA was extracted from the membrane using the E.Z.N.A. Water DNA Kit (Omega Bio-tek, Norcross, GA, USA), in accordance with the manufacturers' instructions with minor changes: vortexing time was increased to 15 min and penetration time of the elution buffer was also increased by an additional 10 min, at room temperature. The concentration and purity of the obtained DNA were checked on the NanoPhotometer N60 (IMPLEN, München, German).

2.4. Bacterial Community Analysis

The metagenomic analysis of the bacterial population was carried out on the basis of the V3–V4 region of the 16S rRNA gene. Specific primer sequences 341F and 785R [34] were used to amplify the selected region and prepare the library. PCR was performed using Q5 Hot Start High-Fidelity 2X Master Mix, at reaction conditions according to the manufacturer's recommendations. Sequencing was performed on a MiSeq apparatus, in paired-end (PE) technology, 2 × 300 nt, using the Illumina v3 kit. Bioinformatics analysis, ensuring the classification of reads to the species level, was carried out with the QIIME 2 software package based on the database of reference sequences Silva 138. Identification of sequences of biological origin from those newly generated in the sequencing process and isolation of amplicon sequence variants was performed using the DADA2 package.

The quality control of the readings together with the analysis of the error profile of individual samples was performed with the FIGARO tool. However, the initial data processing was performed using the Cutadapt tool (removal of adapter sequences and shortening of readings, minimum value 30 nt). The unique ASV sequences were selected by: filtering out sequences containing errors during the sequencing process (denoising), combining paired reads (to increase sequencing accuracy they were carried out in paired-end mode), dereplication (merging identical, unique sequences while maintaining the number of their occurrences and quality profile) and chimera filtering (getting rid of constructs resulting from incorrect sequence assembly during PCR). Assignment of taxonomy to ASV sequences based on the Silva reference database, vsearch, and sklearn search engines, was performed (machine learning).

2.5. Statistical Analysis

The analysis of variance using the Statistica (v.13) (StatSoft Polska, Cracow, Poland) program was used to determine the relationship between the number of ARB and the community composition of bacteria in the seasonal aspect. The normality of the data distribution was determined based on the Shapiro–Wilk test, while the Levene's test was used to assess the homogeneity of the variance.

3. Results

3.1. Heterotrophic Plate Counts and Antibiotic-Resistant Bacteria

Both the greatest HPC and most abundant ARB were determined in February, March and June. On average, a greater HPC was determined in the spring (26,200 CFU/mL per month) than in the winter (20,059 CFU/mL per month), contrary to ARB (14,270 CFU/mL per month in winter and, 9435 CFU/mL per month in spring), as shown in Figure 3. Nevertheless, it is worth to note that both HPC and ARB counts decreased in April and May, which are warmer months than February and March, in a moderate climate.

In general, among all types of resistant bacteria tested in the current study, the most abundant were bacteria resistant to ceftazidime (3GC), reaching more than 90% of HPC in sample 4. In some samples, bacteria resistant to amoxicillin (β) were observed, while bacteria resistant to ciprofloxacin and tetracycline were absent or present only as single colonies (even in the non-diluted suspensions).

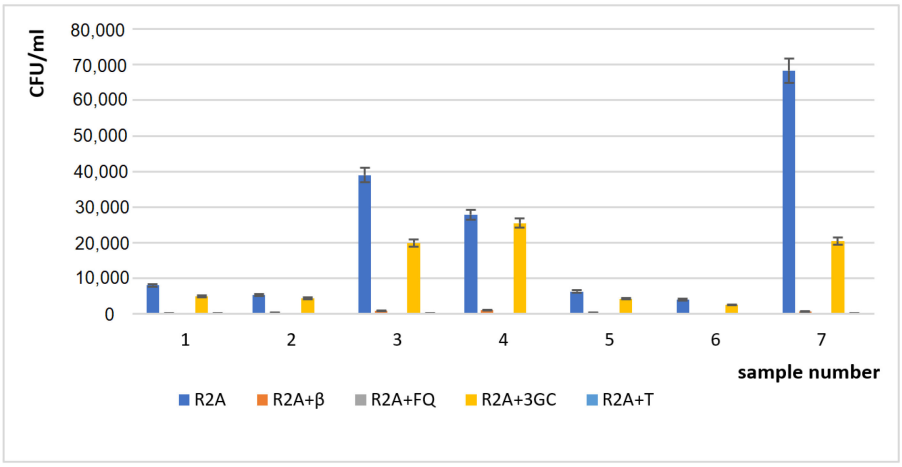

Figure 3. Quantified HPC and ARB, shown as CFU/mL.

3.2. Bacterial Community Analysis

The most common phylum in the samples was *Cyanobacteria* (17.86–77.71%), followed by *Proteobacteria* (up to 74.40% in sample 6, collected in May) and *Actinobacteriota* (up to 21.85% in sample 7, collected in June). Detailed information of each sample composition at the phylum level is presented in Figure 4. At the class level, three leading taxa could be observed: *Vampirivibrionia*, *Alphaproteobacteria*, and *Actinobacteria*, as presented in Figure 5. In addition, the *Mycobacterium* genus was detected in each sample ranging from 4.37% (sample 5) to 21.81% (sample 7). The identified *Mycobacterium* genera are considered to be non-tuberculous mycobacteria, commonly found in water, air and soil.

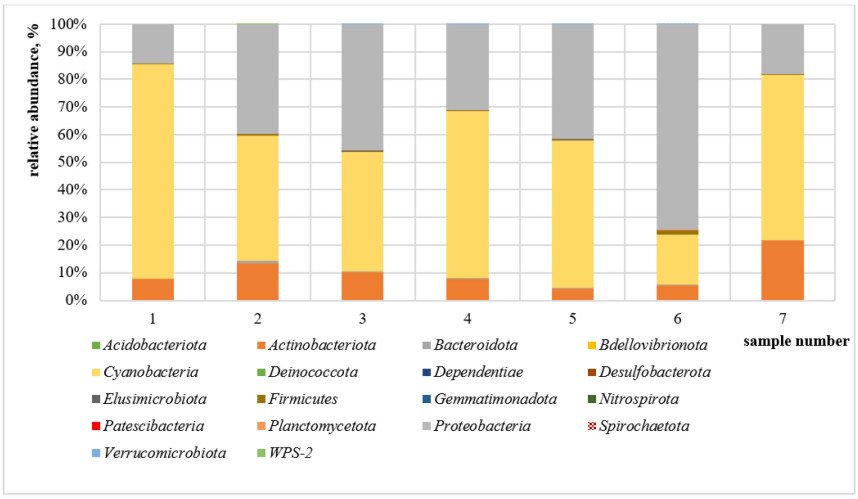

Figure 4. Bacterial communities in all samples at the phylum level.

Typical water pathogens, which are known to be indicators of tap water pollution (such as *Escherichia coli*, enteroccoci, *Clostridium perfringers* and *Pseudomonas aeruginosa*, included in the Polish Ministry of Health Regulations [35]), were not detected by means of culture-based methods (data from routine laboratory analyses performed by the municipal water company). Nevertheless, sequence analysis allowed for their identification. *Clostridium* bacteria were detected in each sample. Though their relative content was below 1%, given the ability of this group to produce spores, this pathogen poses a high risk to consumers. *Pseudomonas* bacteria were also identified in the winter samples (1 and 2). It is also worth noting that in samples 2, 3, 4, 5, 6 and 7, the genus *Legionella* was identified.

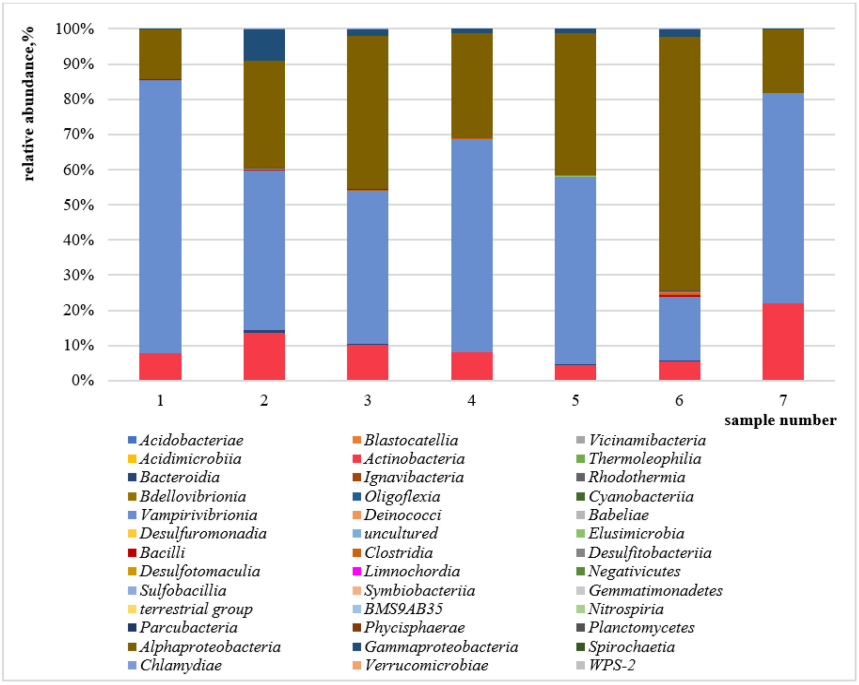

Figure 5. Bacterial communities in all samples at the class level.

3.3. Statistical Analysis

The analysis of variance (ANOVA) showed no significant correlations between ARB and the bacterial community composition of the samples in terms of the season ($p < 0.05$). The homogeneous groups were not distinguished (too little variability).

4. Discussion

In this study, the community composition and antibiotic resistance of tap water bacteria collected from huge volumes of water in a semi-annual study were presented. Samples were collected monthly, which allowed for the continuous monitoring of temporal variation. Nevertheless, no clear season-dependent difference between samples collected during winter and spring could be observed.

In terms of HPC and ARB, the highest numbers were noticed in February, March and June, two colder and one warmer month; while the lowest in December, January, April and May. Similar observations were made in the previous study performed at the same sampling point, in which no season-dependent trend was found [32]. Interestingly, 3GC-resistant bacteria were the most prevalent, reaching from 40.46% in spring to 99.86% in summer, while β- and T-resistant bacteria were found only occasionally (up to 3.80% and 1.16%, respectively), and FQ-resistant bacteria were almost absent [32], consistent with the results obtained in the current study. Similar results were reported in the study in which bulk water samples were collected across the same DWDS, whereby the highest relative abundances were observed in order: 3CG, β, FQ, and T resistance [2]. It is worth noting that in the mentioned previous study, relative abundances of 3GC- and T-resistant bacteria were statistically significantly season-dependent, with higher prevalence in winter and summer, respectively [2]. In the other study performed in the same DWDS and focusing on biofilm scrapped from multiple points across the water supply network, it was suggested that in general, ARB-relative abundances were higher in summer than in winter; however, only FQ- and 3GC-resistant bacteria relative abundances were statistically significantly season-dependent [34]. All the mentioned observations highlight that 3GC-resistant bacteria, especially bacteria resistant to ceftazidime, are the most prevalent ARB in the studied DWDS, and, at the same time, no reliable season-dependent variability of culturable ARB could be observed. The results of the current study confirm that other factors than season

are probably responsible for the shaping of the culturable antibiotic-resistant microbiome in collected tap water. However, more studies are needed to find and determine these factors.

Additionally, the results of sequencing of the hypervariable 16S rRNA gene region are consistent with those obtained in the previous studies conducted at the same sampling point. Though only two samples (spring and summer) were sequenced in the previous study [32], the most prevalent phyla were *Proteobacteria*, *Actinobacteria* and *Cyanobacteria*, respectively. This suggests that at the same sampling point, the core bacterial microbiome was stable during the past 2 years, though some changes within these three phyla could be noted. Precisely, *Cyanobacteria* were more prevalent in the current, than in the previous study, at the expense of *Proteobacteria*. As noticed by Montoya-Pachongo et al. [15], "since drinking water pipes are dark environments, how Cyanobacteria survive in these is not clear yet". In the large study comprising 15 water samples collected from the same DWDS, *Proteobacteria, Firmicutes*, and *Actinobacteria* were the most prevalent [36], whereas in biofilm samples scrapped from the above-mentioned water supply network, *Proteobacteria* were clearly dominating [34]. In another study performed on the consumer tap water collected in the same building (as in the current paper), *beta-* and *gamma-proteobacteria* dominated tap water samples [37]. Therefore, it could be concluded, that in the studied DWDS, the most prevalent bacteria belong to the *Proteobacteria* phylum, but samples collected from various sampling points across the water supply network could differ. It seems that bacterial community composition could be shaped locally in the DWDS, probably due to the presence of nutrients or residual disinfectants [7].

Perrin et al. [16] performed a one-year large study, comprising 31 sampling points in the DWDSs in Paris, France, and concluded that bacterial biodiversity was not significantly affected neither by spatial nor by physico–chemical parameters, though temporal parameters probably played a role during the year, due to flooding events. The most dominant class in all samples was *Alphaproteobacteria*, with a high proportion of two orders, namely *Rhizobiales* and *Sphingomonadales*, which is reaffirmed by a high proportion of *Alphaproteobacteria* identified in the current study. A two-year study conducted in a large, full-scale South African drinking water distribution system that uses three successive disinfection strategies (i.e., chlorination, chloramination, and hypochlorination) showed that *alpha-* and *beta-proteobacteria* dominated the bulk water bacterial communities [6]. Moreover, the authors observed a higher diversity in the samples collected during South African winter, than in summer. In the current study, no differences between the two seasons were observed. In the study of Jiang et al. [17], about 1000 L of tap water samples collected from the DWDS in Nanjing, China, were filtered through 0.22 µm micropore membranes. At the same time, water from the corresponding WTP was collected. The results showed that *Actinobacteria* was the predominant phylum in source water, while *Proteobacteria* dominated after chlorine disinfection. Moreover, the genera *Phreatobacter*, *Undibacterium*, *Pseudomonas* and *Sphingomonas* within the *Proteobacteria* phylum were greatly enriched after chlorination. Similar findings were presented by Bertelli et al. [38], who claimed that chlorine disinfection created a homogeneous bacterial population, dominated by *Pseudomonas*. Nevertheless, *Proteobacteria* were dominant also in an unchlorinated DWDS located in the northern part of the Netherlands [39]. Another one-year study performed in Xi'an, China, showed that *Proteobacteria* dominated tap water samples, followed by *Actinobacteria* and *Firmicutes* [27]. *Proteobacteria* were also dominating the DWDS in Yancheng, China [40]. In a tropical DWDS, Montoya-Pachongo et al. [15] found that *Proteobacteria, Firmicutes,* and *Actinobacteria* were dominant in bulk water and biofilm samples (wherein in bulk water samples *Cyanobacteria* were also common). The above-mentioned results imply that similar bacterial taxa can be found in treated water across the world.

Similar to the results presented in this paper, Ley et al. found *Legionella* spp. and *Mycobacterium* spp. in premise plumbing water samples; however, the authors noticed their highest detection in the summer [26]. *Legionella* spp., including *L. pneumophila*, was previously detected in hot tap water in the same sampling area as in the current study [41]. It is worth noting that the *Mycobacterium* genus is often detected in chloraminated drinking

water [24,42]. Although no pathogenic species were found in the current study, the presence of genera comprising many opportunistic pathogens should arise attention. According to Zhang et al. [43], opportunistic pathogens increase the risk of disease, especially in stagnant water.

The obtained results highlight the need to implement more advanced methods, such as metagenomic DNA sequencing, into routine laboratory analyses, as it was demonstrated that some opportunistic pathogens (such as *Clostridium* spp.) identified by the sequencing approach were not detected by standard methods. The strong discrepancy between cultivation-based techniques and metagenomic sequencing was underlined also by Perrin et al., who claimed that only 1.8% of bacterial biodiversity was recovered through cultivation [16]. Nevertheless, it could not be excluded that the sequences ascribed to bacteria identified in the current study originated from dead cells. Indeed, the perfect method for bacterial biodiversity determination should distinguish between live and dead bacterial cells. For this purpose, DNA extraction could be preceded by using propidium monoazide, for example [44]. The lack of differentiation between live and dead bacteria could partially explain the presence of *Cyanobacteria* in the collected samples and it cannot not be excluded that members of this phylum were dead.

No correlation between culturable ARB and bacterial community composition was observed. The highest number of 3GC-resistant bacteria was counted in the samples collected in February, March, and June. At the same time, *Cyanobacteria* were the most abundant in samples collected in December, March, and June, while *Proteobacteria* were most abundant in samples collected in May. It is worth to note that almost all *Cyanobacteria* were further identified as the *Vampirivibrionia* class, to date, found in the microbiota of rats [45], dogs [46] and, recently, on polyethylene coupons in a drinking water biofilm-formation study [47]. Nevertheless, to elucidate whether *Cyanobacteria* were continuously transferred (live or dead) from the WTP to the sampling point for the period of 7 months, or whether they were proliferating within the distribution system close to the sampling point, more research is needed, including continuous sampling the other points along water transfer from the WTP. Further research could contribute to a better understanding of the presence of these bacteria in the dark environment of drinking water distribution systems. For now, the issue seems to be uncertain.

It seems that the abundance of culturable ARB is not associated with these phyla. More deep analyses, including whole-genome sequencing, are needed to elucidate which taxa of bacteria are the drivers of antibiotic resistance in collected water samples. Interestingly, Lugli et al. [48] performed shotgun metagenomics analysis and demonstrated that the majority of DNA sequences obtained from tap water could not be ascribed to a known bacterial species. Nevertheless, the authors suggest that tap water bacteria shape human gut microbiota due to colonization and horizontal transmission. Therefore, the knowledge of drinking water bacteria biodiversity and antibiotic resistance is of great importance.

The application of membrane filtration in order to collect tap water samples could be the easiest and affordable way to provide reliable, averaged samples for microbiome research. By means of a water filtration set, huge volumes of tap water could be collected and, therefore, more microbials captured within each sample. Moreover, samples collected in this way represent the microbiome present in a given sampling point for a period of a few hours to even a few days (depending on the research needs), which further reduces the randomness of sampling. The situation, in which the microorganisms captured accidentally in a small-volume sample does not represent the actual microbiome of a given sampling point, could be easily avoided. The microbials concentrated from huge volumes of tap water depict the real community composition more reliably. The method—applied as presented in the current study or further ameliorated—could contribute to improving future tap water microbial research.

It is known that ARB could be especially dangerous for immunocompromised patients [12]. Therefore, their presence in tap water provided to hospitals is particularly undesired. Nevertheless, antibiotic-resistant strains were found in samples collected in

hospitals in Europe [49,50]. Moreover, it was proven that mcr-1 gene (conferring resistance to colistin) could be transferred to healthy mouse gut from drinking water [51]. Therefore, further studies are needed to evaluate the risk associated with the presence of ARB and resistance genes in drinking water. Precisely, it should be determined what doses of these contaminants in tap water pose a serious threat. Furthermore, thorough monitoring of their concentrations in hospital drinking water should be implemented to ensure safety for patients. To achieve these goals, methods for the determination of ARB in tap water should be standardized. At the same time, the standardization will allow to perform the research, with results that would be easily comparable.

5. Conclusions

In this study, tap water bacteria were concentrated from huge amounts of water, using filtration membranes. The samples were collected monthly in a semi-annual study. The sequencing analysis of the hypervariable 16S rRNA region showed that *Cyanobacteria*, *Proteobacteria*, and *Actinobacteria* were the most abundant phyla, which is consistent with other reports concerning the drinking water bacterial community composition. Bacteria resistant to ceftazidime were the most prevalent, whereas bacteria resistant to amoxicillin, ciprofloxacin, and tetracycline were scarce. No season-dependent variations were observed, neither for culturable antibiotic resistant bacteria, nor for bacterial community composition.

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References

1. El-Chakhtoura, J.; Saikaly, P.E.; Van Loosdrecht, M.C.M.; Vrouwenvelder, J.S. Impact of distribution and network flushing on the drinking water microbiome. *Front. Microbiol.* **2018**, *9*, 2205. [CrossRef] [PubMed]
2. Siedlecka, A.; Wolf-Baca, M.; Piekarska, K. Spatiotemporal Changes of Antibiotic Resistance and Bacterial Communities in Drinking Water Distribution System in Wrocław, Poland. *Water* **2020**, *12*, 2601. [CrossRef]
3. Luo, L.W.; Wu, Y.H.; Yu, T.; Wang, Y.H.; Chen, G.Q.; Tong, X.; Bai, Y.; Xu, C.; Wang, H.B.; Ikuno, N.; et al. Evaluating method and potential risks of chlorine-resistant bacteria (CRB): A review. *Water Res.* **2021**, *188*, 116474. [CrossRef] [PubMed]
4. Siedlecka, A.; Wolf-Baca, M.J.; Piekarska, K. Antibiotic and disinfectant resistance in tap water strains—Insight into the resistance of environmental bacteria. *Pol. J. Microbiol.* **2021**, *70*, 57–67. [CrossRef]
5. Bodzek, M.; Konieczny, K.; Rajca, M. Membranes in water and wastewater disinfection—Review. *Arch. Environ. Prot.* **2019**, *45*, 3–18. [CrossRef]
6. Potgieter, S.; Pinto, A.; Sigudu, M.; du Preez, H.; Ncube, E.; Venter, S. Long-term spatial and temporal microbial community dynamics in a large-scale drinking water distribution system with multiple disinfectant regimes. *Water Res.* **2018**, *139*, 406–419. [CrossRef]
7. Nescerecka, A.; Juhna, T.; Hammes, F. Identifying the underlying causes of biological instability in a full-scale drinking water supply system. *Water Res.* **2018**, *135*, 11–21. [CrossRef]
8. Liu, G.; Verberk, J.Q.J.C.; Van Dijk, J.C. Bacteriology of drinking water distribution systems: An integral and multidimensional review. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 9265–9276. [CrossRef]
9. Wingender, J.; Flemming, H.C. Biofilms in drinking water and their role as reservoir for pathogens. *Int. J. Hyg. Environ. Health* **2011**, *214*, 417–423. [CrossRef]
10. Kimbell, L.K.; Wang, Y.; McNamara, P.J. The impact of metal pipe materials, corrosion products, and corrosion inhibitors on antibiotic resistance in drinking water distribution systems. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 7673–7688. [CrossRef]
11. Jin, M.; Liu, L.; Wang, D.N.; Yang, D.; Liu, W.L.; Yin, J.; Yang, Z.W.; Wang, H.R.; Qiu, Z.G.; Shen, Z.Q.; et al. Chlorine disinfection promotes the exchange of antibiotic resistance genes across bacterial genera by natural transformation. *ISME J.* **2020**, *14*, 1847–1856. [CrossRef]

12. Wright, G.D. The antibiotic resistome: The nexus of chemical and genetic diversity. *Nat. Rev. Microbiol.* **2007**, *5*, 175–186. [CrossRef]
13. Pruden, A.; Pei, R.; Storteboom, H.; Carlson, K.H. Antibiotic resistance genes as emerging contaminants: Studies in northern Colorado. *Environ. Sci. Technol.* **2006**, *40*, 7445–7450. [CrossRef]
14. Sanderson, H.; Fricker, C.; Brown, R.S.; Majury, A. Antibiotic Resistance Genes as an Emerging Environmental Contaminant. *Environ. Rev.* **2016**, *24*, 2. [CrossRef]
15. Montoya-Pachongo, C.; Douterelo, I.; Noakes, C.; Camargo-Valero, M.A.; Sleigh, A.; Escobar-Rivera, J.C.; Torres-Lozada, P. Field assessment of bacterial communities and total trihalomethanes: Implications for drinking water networks. *Sci. Total Environ.* **2018**, *616–617*, 345–354. [CrossRef]
16. Perrin, Y.; Bouchon, D.; Delafont, V.; Moulin, L.; Héchard, Y. Microbiome of drinking water: A full-scale spatio-temporal study to monitor water quality in the Paris distribution system. *Water Res.* **2019**, *149*, 375–385. [CrossRef]
17. Jiang, R.; Li, Z.; Li, Q.; Liu, Y.; Zhu, Y.; Chen, Z.; Liu, P.; Jia, S.; Ren, H.; Zhang, X.-X. Metagenomic insights into the variation of bacterial communities and potential pathogenic bacteria in drinking water treatment and distribution systems. *Natl. Sci. Open* **2022**, *1*, 20220015. [CrossRef]
18. Stüken, A.; Haverkamp, T.H.A.; Dirven, H.A.A.M.; Gilfillan, G.D.; Leithaug, M.; Lund, V. Microbial Community Composition of Tap Water and Biofilms Treated with or without Copper-Silver Ionization. *Environ. Sci. Technol.* **2018**, *52*, 3354–3364. [CrossRef]
19. Proctor, C.R.; Reimann, M.; Vriens, B.; Hammes, F. Biofilms in shower hoses. *Water Res.* **2018**, *131*, 274–286. [CrossRef]
20. Liu, R.; Yu, Z.; Guo, H.; Liu, M.; Zhang, H.; Yang, M. Pyrosequencing analysis of eukaryotic and bacterial communities in faucet biofilms. *Sci. Total Environ.* **2012**, *435–436*, 124–131. [CrossRef]
21. Lin, W.; Yu, Z.; Chen, X.; Liu, R.; Zhang, H. Molecular characterization of natural biofilms from household taps with different materials: PVC, stainless steel, and cast iron in drinking water distribution system. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 8393–8401. [CrossRef] [PubMed]
22. Zupančič, J.; Turk, M.; Črnigoj, M.; Ambrožič Avguštin, J.; Gunde-Cimerman, N. The dishwasher rubber seal acts as a reservoir of bacteria in the home environment. *BMC Microbiol.* **2019**, *19*, 300. [CrossRef] [PubMed]
23. Chen, L.; Jia, R.B.; Li, L. Bacterial community of iron tubercles from a drinking water distribution system and its occurrence in stagnant tap water. *Environ. Sci. Process. Impacts* **2013**, *15*, 1332–1340. [CrossRef] [PubMed]
24. Waak, M.B.; Hozalski, R.M.; Hallé, C.; Lapara, T.M. Comparison of the microbiomes of two drinking water distribution systems—With and without residual chloramine disinfection. *Microbiome* **2019**, *7*, 87. [CrossRef] [PubMed]
25. Pinto, A.; Schroeder, J.; Lunn, M.; Sloan, W.; Raskin, L. Spatial-temporal survey and occupancy-abundance modeling to predict bacterial community dynamics in the drinking water microbiome. *mBio* **2014**, *5*, e01135-14. [CrossRef] [PubMed]
26. Ley, C.J.; Proctor, C.R.; Singh, G.; Ra, K.; Noh, Y.; Odimayomi, T.; Salehi, M.; Julien, R.; Mitchell, J.; Nejadhashemi, A.P.; et al. Drinking water microbiology in a water-efficient building: Stagnation, seasonality, and physicochemical effects on opportunistic pathogen and total bacteria proliferation. *Environ. Sci. Water Res. Technol.* **2020**, *6*, 2902–2913. [CrossRef]
27. Zhang, H.; Xu, L.; Huang, T.; Yan, M.; Liu, K.; Miao, Y.; He, H.; Li, S.; Sekar, R. Combined effects of seasonality and stagnation on tap water quality: Changes in chemical parameters, metabolic activity and co-existence in bacterial community. *J. Hazard. Mater.* **2021**, *403*, 124018. [CrossRef] [PubMed]
28. Gomes, I.B.; Simões, M.; Simões, L.C. An overview on the reactors to study drinking water biofilms. *Water Res.* **2014**, *62*, 63–87. [CrossRef]
29. Douterelo, I.; Boxall, J.B.; Deines, P.; Sekar, R.; Fish, K.E.; Biggs, C.A. Methodological approaches for studying the microbial ecology of drinking water distribution systems. *Water Res.* **2014**, *65*, 134–156. [CrossRef]
30. Zlatanović, L.; van der Hoek, J.P.; Vreeburg, J.H.G. An experimental study on the influence of water stagnation and temperature change on water quality in a full-scale domestic drinking water system. *Water Res.* **2017**, *123*, 761–772. [CrossRef]
31. Hu, Y.; Dong, D.; Wan, K.; Chen, C.; Yu, X.; Lin, H. Potential shift of bacterial community structure and corrosion-related bacteria in drinking water distribution pipeline driven by water source switching. *Front. Environ. Sci. Eng.* **2021**, *15*, 28. [CrossRef]
32. Siedlecka, A.; Wolf-Baca, M.; Pierkarska, K. Seasonal variabilitiy of antibiotic resistance and biodiversity of tap water bacteria in Wrocław, Poland. *Environ. Prot. Eng.* **2020**, *46*, 93–109. [CrossRef]
33. Available online: https://aquafilter.com/pl/product/25/88/fp3-hj-k1n (accessed on 30 December 2022).
34. Siedlecka, A.; Wolf-Baca, M.; Piekarska, K. Microbial communities of biofilms developed in a chlorinated drinking water distribution system: A field study of antibiotic resistance and biodiversity. *Sci. Total Environ.* **2021**, *774*, 145113. [CrossRef] [PubMed]
35. Polish Ministry of Health. *Regulation of the Minister of Health from December 7, 2017 on the Quality of Water Intended for Human Consumption*; Polish Ministry of Health: Warsaw, Poland, 2017; p. 2294. (In Polish)
36. Wolf-Baca, M.; Piekarska, K. Biodiversity of organisms inhabiting the water supply network of Wroclaw. Detection of pathogenic organisms constituting a threat for drinking water recipients. *Sci. Total Environ.* **2020**, *715*, 136732. [CrossRef]
37. Siedlecka, A.; Wolf-Baca, M.; Piekarska, K. Molecular insight into bacterial communities of consumer tap water—A case study. *Desalin. Water Treat.* **2021**, *222*, 114–126. [CrossRef]
38. Bertelli, C.; Courtois, S.; Rosikiewicz, M.; Piriou, P.; Aeby, S.; Robert, S.; Loret, J.F.; Greub, G. Reduced chlorine in drinking water distribution systems impacts bacterial biodiversity in biofilms. *Front. Microbiol.* **2018**, *9*, 2520. [CrossRef]

39. Liu, G.; Bakker, G.L.; Li, S.; Vreeburg, J.H.G.; Verberk, J.Q.J.C.; Medema, G.J.; Liu, W.T.; Van Dijk, J.C. Pyrosequencing reveals bacterial communities in unchlorinated drinking water distribution system: An integral study of bulk water, suspended solids, loose deposits, and pipe wall biofilm. *Environ. Sci. Technol.* **2014**, *48*, 5467–5476. [CrossRef]
40. Bian, K.; Wang, C.; Jia, S.; Shi, P.; Zhang, H.; Ye, L.; Zhou, Q.; Li, A. Spatial dynamics of bacterial community in chlorinated drinking water distribution systems supplied with two treatment plants: An integral study of free-living and particle-associated bacteria. *Environ. Int.* **2021**, *154*, 106552. [CrossRef]
41. Wolf-Baca, M.; Siedlecka, A. Detection of pathogenic bacteria in hot tap water using the qPCR method: Preliminary research. *SN Appl. Sci.* **2019**, *1*, 840. [CrossRef]
42. Gomez-Smith, C.K.; Lapara, T.M.; Hozalski, R.M. Sulfate reducing bacteria and mycobacteria dominate the biofilm communities in a chloraminated drinking water distribution system. *Environ. Sci. Technol.* **2015**, *49*, 8432–8440. [CrossRef]
43. Zhang, H.; Liu, X.; Huang, T.; Ma, B.; Sun, W.; Zhao, K.; Sekar, R.; Xing, Y. Stagnation trigger changes to tap water quality in winter season: Novel insights into bacterial community activity and composition. *Sci. Total Environ.* **2022**, *844*, 157240. [CrossRef]
44. Jäger, T.; Alexander, J.; Kirchen, S.; Dötsch, A.; Wieland, A.; Hiller, C.; Schwartz, T. Live-dead discrimination analysis, qPCR assessment for opportunistic pathogens, and population analysis at ozone wastewater treatment plants. *Environ. Pollut.* **2018**, *232*, 571–579. [CrossRef]
45. Diviccaro, S.; Giatti, S.; Cioffi, L.; Falvo, E.; Piazza, R.; Caruso, D.; Melcangi, R.C. Paroxetine effects in adult male rat colon: Focus on gut steroidogenesis and microbiota. *Psychoneuroendocrinology* **2022**, *143*, 105828. [CrossRef]
46. Díaz-Regañón, D.; García-Sancho, M.; Villaescusa, A.; Sainz, Á.; Agulla, B.; Reyes-Prieto, M.; Rodríguez-Bertos, A.; Rodríguez-Franco, F. Characterization of the Fecal and Mucosa-Associated Microbiota in Dogs with Chronic Inflammatory Enteropathy. *Animals* **2023**, *13*, 326. [CrossRef]
47. Zhang, X.Y.; Lin, T.; Jiang, F.C.; Zhang, X.; Wang, S.Y.; Zhang, S. Impact of pipe material and chlorination on the biofilm structure and microbial communities. *Chemosphere* **2022**, *289*, 133218. [CrossRef]
48. Lugli, G.A.; Longhi, G.; Mancabelli, L.; Alessandri, G.; Tarracchini, C.; Fontana, F.; Turroni, F.; Milani, C.; van Sinderen, D.; Ventura, M. Tap water as a natural vehicle for microorganisms shaping the human gut microbiome. *Environ. Microbiol.* **2022**, *24*, 3912–3923. [CrossRef]
49. Narciso-da-Rocha, C.; Vaz-Moreira, I.; Manaia, C.M. Genotypic diversity and antibiotic resistance in Sphingomonadaceae isolated from hospital tap water. *Sci. Total Environ.* **2014**, *466–467*, 127–135. [CrossRef]
50. Vincenti, S.; Quaranta, G.; De Meo, C.; Bruno, S.; Ficarra, M.G.; Carovillano, S.; Ricciardi, W.; Laurenti, P. Non-fermentative gram-negative bacteria in hospital tap water and water used for haemodialysis and bronchoscope flushing: Prevalence and distribution of antibiotic resistant strains. *Sci. Total Environ.* **2014**, *499*, 47–54. [CrossRef]
51. Khan, H.; Miao, X.; Liu, M.; Ahmad, S.; Bai, X. Behavior of last resort antibiotic resistance genes (mcr-1 and blaNDM-1) in a drinking water supply system and their possible acquisition by the mouse gut flora. *Environ. Pollut.* **2020**, *259*, 113818. [CrossRef]