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Molecular insight into bacterial communities of consumer tap water – a case study

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ABSTRACT

The issue of consumer tap water (i.e., tap water collected without prior flushing – as drawn by consumers from household taps) is often neglected in scientific reports concerning drinking water microbiology. Although water companies usually provide water that is safe to drink, its microbial quality could significantly deteriorate within a building. In this study, four samples collected from taps assembled close to each other within the same building were compared in terms of bacterial community composition, biodiversity, and 16S rRNA gene-denaturing gradient gel electrophoresis and resistome profiling. The results revealed high diversity of samples, indicating the impact of premise plumbing or taps on the microbiome of consumer tap water. All detected bacteria belonged to the phyla Proteobacteria, Firmicutes and Bacteroidetes. However, neither common bacterial core nor resistome was determined among the investigated samples. Each sample presented a unique bacterial community. These results suggest that bacteria dwelling in premise plumbing or taps shape the microbiome of consumer tap water, masking the microorganisms present in tap water provided by a water company. It is known that it is not common practice for consumers to flush tap water before collection. Therefore, consumer health risk could primarily depend on bacteria dwelling in premise plumbing.

Keywords: Premise plumbing; Bacterial community composition; Bacterial biodiversity; Antibiotic resistance genes

1. Introduction

Tap water is one of the main sources of drinking water for consumers. It should be, therefore, safe to drink and free of contaminants threatening human health [1,2]. In many countries, including Poland, water companies are obliged to provide drinking water of appropriate properties only to water meters [3]. It is known, however, that the microbial quality of tap water could deteriorate within premise plumbing due to water stagnation, elevated temperatures, and chlorine decay inside buildings [2,4–12]. Despite that, in many studies, tap water has been flushed prior to sample collection to alleviate the impact of water stagnation, and to depict the drinking water distribution system (DWDS) water quality [13–21]. This approach allows for better understanding of DWDS microbiomes. Nevertheless, it does not contribute to deepening knowledge about bacteria dwelling in premise plumbing. Therefore, little is still known about consumer tap water (i.e., tap water collected without prior flushing – as drawn by consumers from household taps), commonly considered a drinking water source. Such investigations could be limited by the fact that consumer tap water could differ significantly among buildings. Nonetheless, the issue should not be neglected in scientific literature, and this study aimed to fill the gap.

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Few studies have attempted to investigate the influence of water stagnation on tap water microbial quality [5–7,9,10,22], and the development of biofilms inside taps [23–27], shower hoses [18,28], lab-scale [5] and full-scale [12,29] premise plumbing installations, or compared bacterial contamination of tap water collected from nearby taps [9]. Some reports on bacterial diversity within one building are also available [28,30–33]. Over 10 y ago, Rudi et al. [30] compared tap water samples collected from two taps assembled in a Norwegian hospital in terms of bacterial community composition and biodiversity. Similarly, Dias et al. [32] compared bacterial communities among consumer tap water samples collected from 10 taps assembled in a Canadian hospital. Narciso-da-Rocha et al. [31] investigated the biodiversity and antibiotic resistance of sphingomonads isolated from tap water samples collected from 12 taps assembled in a Portuguese hospital. Stüken et al. [28] compared bacterial communities present in hose biofilms and tap water samples collected in a Swiss hospital and research facility, but focusing mostly on hot water. In the last two studies, 1-min flushing was applied. Ling et al. [33] claimed that bacterial community composition of stagnated water clustered with regard to water location within pipes (distinguishing between distal and approximal pipe sections), rather than to different buildings, floors, or time points. Therefore, little is still known regarding the biodiversity of bacterial communities present in cold consumer tap water collected from full-scale non-hospital premise plumbing. Moreover, no comparisons of resistomes in consumer tap water collected within the same building are available, although antibiotic resistant bacteria and antibiotic resistance genes (ARGs) have been formerly found in DWDSs [19,20,31,34–41].

The objectives of this study were to compare consumer tap water collected from four taps assembled close to each other within one building and to reveal the differences in bacterial biodiversity and resistome in each sample. It involved the application of molecular biology techniques, including next generation sequencing (NGS), 16S rRNA gene-denaturing gradient gel electrophoresis (DGGE), and polymerase chain reaction (PCR) of ARGs.

2. Materials and methods

2.1. Sample collection and DNA extraction

Consumer tap water samples were collected from four (I–IV) taps assembled at the Laboratory of Environmental Biotechnology of the Wrocław University of Science and Technology (Poland). The taps were characterised by different frequency of use and age. The details are presented in Table 1.

All taps were located on the same floor, close to each other in adjacent rooms. The new faucets (I–III) were assembled after renovation, one and half a year before sample collection. The old faucet (IV) had been in use for many years. The premise plumbing of the building had been operated for decades.

The external surfaces of taps were disinfected with 96% ethanol (Sigma-Aldrich, St. Louis, MO, USA) to reduce sample contamination. No flushing was applied prior to sample collection, deliberately. Taps were sampled simultaneously at daytime (not after overnight stagnation) to depict the differences in casual consumer tap water. 3 L of water was collected to sterile glass bottles and filtered immediately through mixed cellulose membranes of 0.2 μm pore diameter (Whatman) with the application of a sterile filtration set (Nalgene, Rochester, NY, USA). DNA was extracted from membranes by means of a DNeasy PowerWater kit (QIAGEN, Hilden, Germany), in accordance with manufacturers' instructions. DNA concentration and purity were measured by means of a NanoPhotometer N60 (Implen, München, Germany).

Table 1
Characteristics of the taps

Tap	Characteristics	
	Frequency of use	Age
I	Used intensively every day	New
II	Used several times a week	New
III	Almost unused	New
IV	Used intensively every day	Old

2.2. Bacterial community composition and biodiversity

Environmental DNA samples representing the microbial communities present in consumer tap water collected from four nearby taps were subjected to NGS in order to investigate the bacterial community composition and biodiversity of each sample.

The analyses were conducted as described previously [21], with some modifications. The metagenomic analysis was performed based on hypervariable region V3-V4 of the 16S rRNA coding gene. Gene libraries were prepared with primers 341F and 785R, using Q5 HotStart High-Fidelity DNA Polymerase (NEBNext), in accordance with manufacturer's instructions. The sequencing was conducted on MiSeq (Illumina, San Diego, CA, USA) in paired-end technology (2 × 250 nt), using a MiSeq Reagent kit v2 (Illumina), in accordance with manufacturer's instructions. The bioinformatic analysis was performed by means of Quantitative Insights into Microbial Ecology 2 (QIIME 2) pipeline [42]. Samples were demultiplexed, the adaptors were removed, the quality control was provided, and low-quality sequences (quality < 26, minimum length 30) were discarded with cutadapt [43]. Paired sequences were joined with fastq-join [44]. The sequences were clustered into operational taxonomic units (OTUs) with a 97% similarity threshold and OTUs < 10 were discarded with UCLUST [45]. Chimeras were removed with ChimeraSlayer [46]. The taxonomy was assigned with UCLUST [45], against reference sequences GreenGenes database, version 13_8 [47].

Further bioinformatic analysis was performed by means of R [48]. Alpha diversity was assessed based on rarefaction analyses for each sample in terms of Chao1 estimator and Shannon and Simpson indices [49] with phyloseq and ggplot2 packages. Beta diversity analyses were performed to compare samples using heatmaps generated based on Bray–Curtis distance matrix and clustered

with UPGMA with phyloseq, vegan, and gplots packages. Principal component analysis (PCA) and principal coordinates analysis (PCoA) plots, the latter based on Bray–Curtis distance matrix, were generated to reveal sample clustering with phyloseq, factoextra, ggbiplot, and phyloseq, vegan, ggplot2 packages, respectively.

2.3. DGGE analysis of community structures

The community structures of bacterial consortia were compared by means of DGGE as described previously [20]. Briefly, due to low bacterial biomass in the investigated samples, nested-PCR protocol was applied in Mastercycler Nexus GX2 (Eppendorf). Nearly full 16S rRNA gene was amplified with primer set 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT), and visualised by agarose gel electrophoresis. The post-reaction mixture was used as a DNA template for amplification of an approximately 200-bp 16S rRNA gene fragment, corresponding to region V3, with primer set 338F-GCclamp (5′-GACTCCTACGGGAGGCAGCAG-3′ with a GC clamp attached) and 518R (5′-ATTACCGCGGCTGCTGG-3′). The final nested-PCR products were electrophoresed on denaturing gradient gel electrophoresis systems DGGEK-2001 (CBS Scientific) under conditions identical as described previously [20].

The DGGE profiles were analyzed with CLIQS software (TotalLab, Newcastle Upon Tyne, UK) to get similarity matrices-based on the presence or absence of the bands. The dendrogram was created by the UPGMA. The synthetic line created based on the investigated lines was used as a reference line for dendrogram construction [20].

2.4. Detection of ARGs and other genes in environmental DNA

PCRs were conducted in Mastercycler Nexus GX2 (Eppendorf) to detect ARGs and other genes related to resistance mechanisms or horizontal gene transfer: bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{OXA}, bla_{OXA-48}, *amp*C, *mec*A, *qnr*A, *qnr*B, *qnr*S, *oqx*B, *tet*A, *tet*K, *tet*L, *tet*W, *sul*I, *sul*II, *erm*A, *erm*B, *van*A, *mcr*-1, *mex*A, *flo*R, *qac*EΔ1, *qac*H, *tol*A, *int*I1, *tnp*A in environmental DNA samples, under conditions identical as described previously [41]. The detailed information about primer sequences, amplicon sizes, annealing temperatures, and references is provided in supplementary material (Table S1).

3. Results

3.1. Bacterial community composition and biodiversity

All reads from samples I, II and IV were classified as bacteria. In sample III, bacteria constituted 99.91% of reads, and the remaining reads were unassigned. A total of three phyla (Proteobacteria, Firmicutes and Bacteroidetes) were identified in consumer tap water samples. Bacterial community composition at class level and at family level is presented in Fig. 1.

The bacterial community of sample I was dominated by Comamonadaceae (84.04%) and Bacillaceae (14.19%). In sample II, the vast majority consisted of Moraxellaceae

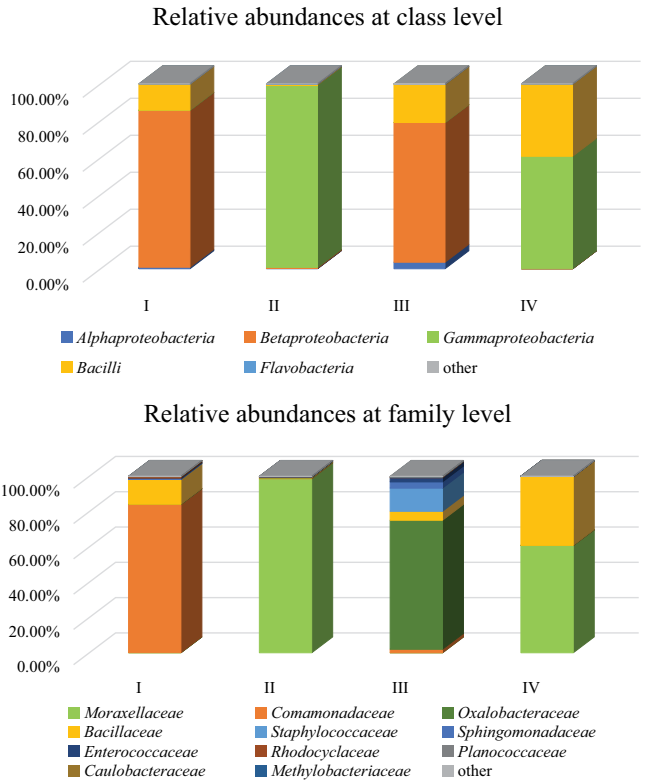

Fig. 1. Relative abundances of bacteria present in consumer tap water collected from the investigated taps (I–IV).

(98.63%); almost all the sequences assigned to this family were further identified as *Acinetobacter* at genus level. The most diverse community was observed in sample III, with Oxalobacteraceae (73.10%) and Staphylococcaceae (13.14%) as the most abundant families; all the sequences were further identified as *Cupriavidus* and *Staphylococcus* at genus level, respectively. The bacterial community of sample IV primarily consisted of Moraxellaceae (60.83%) and Bacillaceae (39.16%); among them, almost all sequences were identified as *Acinetobacter* and *Bacillus* at genus level, respectively. Considering the most abundant bacteria, exceeding 1% of relative abundance, *Acinetobacter* was observed to be common for samples II and IV, Bacillaceae (other than *Bacillus*) and Comamonadaceae were common for samples I and III, and *Bacillus* for samples III and IV, whereas other bacteria were unique for a given sample. No clinically relevant pathogens were found in this study. However, next to the aforementioned bacteria, *Enterococcus* spp. was found in sample III (1.66%) and *Bacillus cereus* in all samples (at relative abundance not exceeding 0.07% in any of them).

Alpha diversity, as well as number of reads and OTUs determined in this study, is presented in Table 2.

Based on rarefaction plots (Fig. S1), the limiting sample was sample I, which consisted of the lowest number of reads and OTUs. The lowest richness (Chaol) was observed in sample I. The highest biodiversity (Shannon and Simpson) was observed in samples III and IV, and lowest in sample II. It is worth emphasising that the discrepancies of the calculated alpha diversity and the results of bacterial

community composition (Fig. 1) originate from the calculation method applied in this study. For the purpose of calculation of alpha diversity, the OTUs were taken into account, whereas only identified taxa are presented in the plots.

The beta diversity heatmap, generated based on Bray–Curtis distance matrix, is presented in Fig. 2. PCA and PCoA plots are presented in Fig. 3.

Considering beta diversity heatmap (Fig. 2), as well as PCA and PCoA plots (Fig. 3), it could be observed that samples II and IV appear to have some similarities at OTUs level, whereas samples I and III seem to be separated. These results stay in accordance with determined bacterial community composition (Fig. 1). Based on *x*-axis, however, covering 57.9% and 57.3% of variance in PCA and PCoA plots (Fig. 3), respectively, samples I and III showed certain mutual similarities.

3.2. DGGE analysis of community structures

The differences in bacterial community structures were also investigated by means of DGGE. The purpose was to compare the community structure, not to perform phylogenetic analyses. The results are presented as an UPGMA dendrogram in Fig. 4.

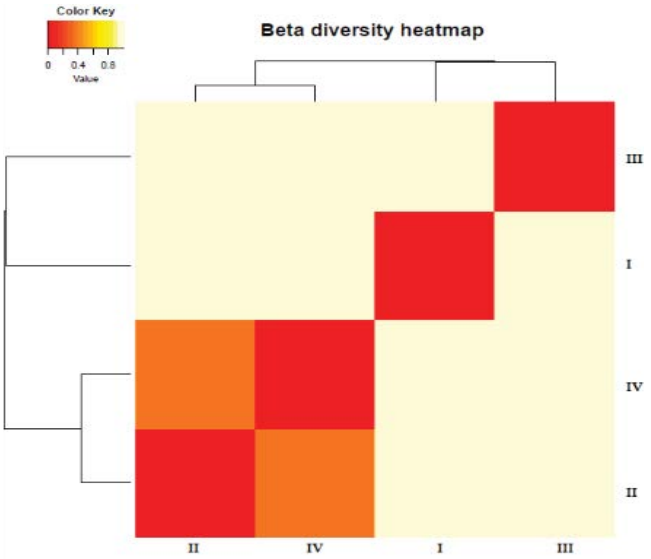

Fig. 2. Beta diversity heatmap based on Bray–Curtis at operational taxonomic units level.

Samples II and IV clustered together with approximately 95% of similarity. Samples I and III appeared different from samples II and IV, which is in accordance with beta diversity results presented above.

3.3. Detection of ARGs and other genes in environmental DNA

The PCR results are presented in Table 3.

PCRs provided positive results only for two samples, namely I and III. Only genes *tet*A, *van*A, *flo*R and *tnp*A were detected in this study, and only gene *van*A was detected in both samples.

4. Discussion

The investigated bacterial communities consisted of classes known to be inhabitants of tap water [14,30,50–52]. The predominance of Alpha-, Beta-, Gammaproteobacteria and Bacilli is in accordance with previous research conducted in Wrocław [21,41] and with other literature reports [2,16,32,53–56]. *Flavobacteria*, detected only in sample II at low abundance, were also found in other studies [21,51,54]. Although at class level sample I appeared similar to sample III, and sample II appeared similar to sample IV, the same similarities were not observed at further taxonomic levels (Fig. 1). Importantly, because the investigated community compositions are highly diverse, no common bacterial core could be determined in this study. The obtained results prove that each collected sample differed from the others. The microbiome of each sample was unique. The probable explanation of those differences could be the fact that tap water stagnating in a building is not microbiologically homogeneous. It could be the effect of entrainment of the fragments of biofilms dwelling on inner surfaces of premise pluming or faucets. As mentioned above, the taxons identified in the study are consistent with commonly found tap water bacteria. Therefore, the microorganisms probably originated from the DWDS, but could have dwelled in some parts of premise plumbing or in taps and further differentiate due to the founder effect. The species which benefited from the actual conditions in premise plumbing could have proliferated and eventually, have been found in consumer tap water samples. The bacteria present in collected samples were able to adapt to the premise plumbing environment. It was shown that microbiome of water stagnating in buildings for 5–6 d is affected mainly by distal small-diameter pipes, which harbored the highest cell counts and deviated most from water supplied to a

Table 2
Number of reads, OTUs, and alpha diversity

Sample	No. of reads classified	No. of OTUs	Alpha diversity		
			Chao1 estimator	Shannon index	Simpson index
I	12,421	33	21.00	0.33	0.89
II	36,950	45	36.75	0.18	0.95
III	31,534	48	35.33	1.14	0.56
IV	37,468	46	35.50	0.80	0.51

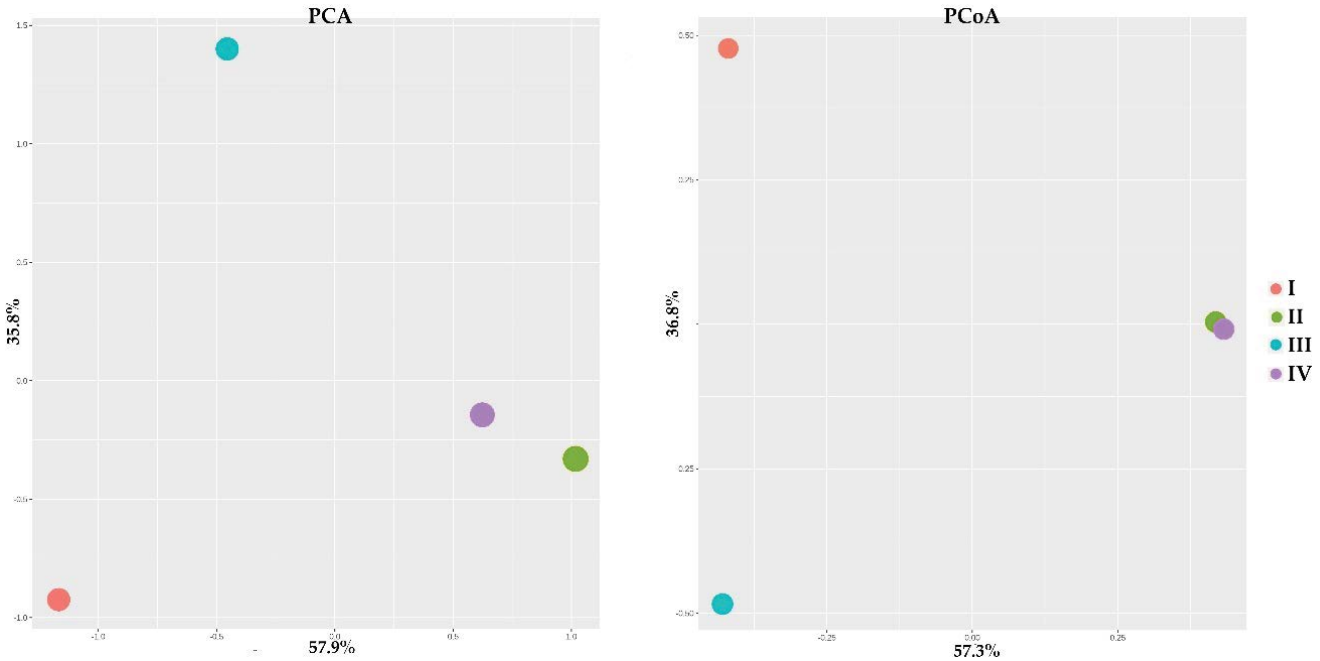

Fig. 3. Principal component analysis and principal coordinates analysis plots at operational taxonomic units level.

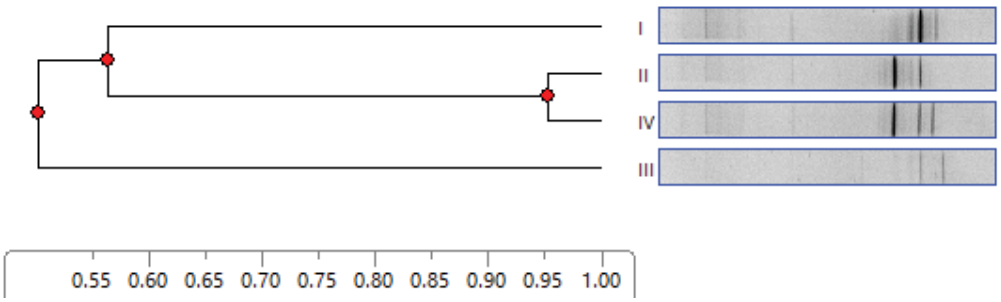

Fig. 4. Dendrogram of denaturing gradient gel electrophoresis profiles.

given building [33]. It could not be, therefore, excluded that the differences observed in the current study origin primarily from the biofilms developed in distal pipe sections, located right behind the taps.

The results of alpha diversity also suggest dissimilarities among the investigated bacterial communities. Higher Shannon and Simpson diversity was found in samples III and IV, completely different in terms of characteristics considered in this study: tap III was almost unused and new, whereas tap IV was used intensively every day and old. It, therefore, appears that frequency of use and age of the faucets were not the factors shaping bacterial alpha diversity. The results of beta diversity, together with the obtained DGGE dendrogram, indicate similarity between samples II and IV. This observation confirms that the biodiversity of bacterial communities investigated in the current study is independent on the faucet's characteristics. Noteworthy, Lipphaus et al. [9] claimed that tap water collected from infrequently used taps presented higher bacterial loads, indicating a relationship between frequency of use of faucets and bacterial contamination of water. Nevertheless, number of bacteria in consumer tap water was not determined in this study. The presence of different bacterial loads in the investigated samples could, therefore, not be excluded. The presented results reveal that consumer tap water differ significantly within the building, even though the samples were collected from taps assembled close to each other, on the same floor. In the current study, the tap use frequency is correlated neither with bacterial community composition nor with alpha and beta biodiversity. However, it deserves attention that the highest Shannon index has been obtained for consumer tap water collected from the almost unused tap III. Nevertheless, on the basis of the presented results, it is impossible to conclude about the relationships between tap characteristics and observed biodiversity. No such correlation could be discerned – perhaps more research is needed. These results highlight how varied consumer tap water is, even though all the taps were supplied by the same source water. The observed differences in bacterial biodiversity indicate the

Table 3
Results of PCRs

Gene	Gene classification, resistance target or mechanisms	I	II	III	IV
bla_{TEM}	β-Lactamase	–	–	–	–
bla_{SHV}		–	–	–	–
bla_{CTX-M}		–	–	–	–
bla_{KPC}		–	–	–	–
bla_{NDM}		–	–	–	–
bla_{OXA}		–	–	–	–
bla_{OXA-48}		–	–	–	–
*amp*C		–	–	–	–
*mec*A	Methicillin	–	–	–	–
*qnr*A	(fluoro)quinolones	–	–	–	–
*qnr*B		–	–	–	–
*qnr*S		–	–	–	–
*oqx*B		–	–	–	–
*tet*A	Tetracyclines	+	–	–	–
*tet*K		–	–	–	–
*tet*L		–	–	–	–
*tet*W		–	–	–	–
*sul*I	Sulfonamide	–	–	–	–
*sul*II		–	–	–	–
*erm*A	Erythromycin	–	–	–	–
*erm*B		–	–	–	–
*van*A	Vancomycin	+	–	+	–
mcr-1	Colistin	–	–	–	–
*mex*A	Efflux	–	–	–	–
*flo*R	Florfenicol	–	–	+	–
*qac*EΔ1	Quaternary ammonium	–	–	–	–
*qac*H	compounds	–	–	–	–
*tol*A	Transmembrane activity	–	–	–	–
*int*I1	Class 1 integron	–	–	–	–
*tnp*A	Transposon	–	–	+	–
Total		2	0	3	0

crucial role of premise plumbing in shaping the microbiomes present in consumer tap water. Determining the cause of the differences is, however, beyond the scope of the current study.

Only several genes were detected in tested consumer tap water. It is at variance with some literature reports [34,35,40]. On the other hand, studies concerning tap water samples collected in the USA reported the presence of only *tet*A and *sul*I genes [57], or no ARGs [58], whereas only bla_{TEM}, *sul*I and *int*I1 were found in tap water in Portugal [38]. Moreover, a previous study conducted on tap water collected in the same building [41] showed the presence of only genes *qnr*B, *tet*W, *erm*B, *qac*EΔ1 and *int*I1 among the tested ones. Therefore, low abundance of ARGs in this study is not surprising. As a result, no common occurrence pattern of ARGs was determined. It was suggested that certain genera of bacteria, for example, *Acinetobacter* and *Methyloversatilis* (from the genera identified in the present study) could be considered resistance vectors in tap water environments [59]. *Acinetobacter* was dominant in samples II and IV; moreover, in sample II *Methyloversatilis* was found (although at very low relative abundance of 0.01%, data not shown). These samples, however, proved free from the tested genes. On the other hand, the detected genes could have also been present as free-DNA molecules [60], regardless of bacterial community composition.

In contrast to the results presented in this paper, a common bacterial core was found for many DWDSs [14,52,55,56,61–63]. Therefore, high diversity of bacterial communities investigated in this study proves the significant impact of bacteria dwelling in premise plumbing on consumer tap water – otherwise, the samples would be more similar to each other, reflecting the microbiome of tap water reaching the building from the distribution system.

Dissimilarities among samples collected within one building were also observed by Rudi et al. [30], who compared bacterial communities present in tap water sampled during winter and summer from kitchen and toilet faucets in a Norwegian hospital, and found a tap-specific, rather than season-specific, colonisation pattern. On the contrary, Dias et al. [32] claimed that among 10 consumer tap water samples collected after overnight stagnation in a Canadian hospital, 8 clustered together in terms of bacterial community composition, and the dissimilarities among the samples were explained by the specific conditions of each location, such as hydraulic regime, frequency of use, and building material of the taps [32].

The findings of Rudi et al. [30], as well as the results of the present study, suggest that the microbiome of consumer tap water is primarily shaped by diversified bacterial communities dwelling in premise plumbing. Such differentiations probably occur over time. For example, biofilms reported to develop inside pipes made of various materials in the building operated for 1 y presented similar abundances of Alpha-, Beta-, and Gammaproteobacteria [29]. On the contrary, Zlatanović et al. [12] claimed that within the same plumbing, biofilms of different compositions were found after 430 d of operation of the installation. In the present study, the age of premise plumbing is not the limiting factor, because the building was constructed decades ago. Moreover, diverse biofilms could have developed inside faucets [23–25], also contributing to differences observed in the investigated bacterial communities. Therefore, the results of this paper could be partially explained by the presence of various biofilms dwelling in faucets or in pipe sections supplying water to each tap of adjacent rooms. However, more research is needed, including the collection and investigation of putative biofilms from inner surfaces of the faucets and pipes, to support that hypothesis.

On the other hand, the high diversity of the investigated samples could be explained by accidental detachment of biofilm fragments, entrained with flowing water. Biofilm is known to be a very complex structure, and its shearings could differ depending on hydraulic regimes [64]. Moreover, bacteria attached to particles [54] randomly reaching the taps could also contribute to the observed diversity of the samples. To confirm this assumption, consumer tap water should be sampled repeatedly on consecutive days from the same taps, and compared in a

further study to exclude or confirm whether bacteria present in the collected sample reach it randomly.

Taking into consideration the results of this study, it seems that threats to consumer health are associated not only with the microbial quality of tap water supplied by a water company, but also, or maybe primarily, with bacteria dwelling in premise plumbing. Noteworthy, no opportunistic pathogens were identified in this study. However, the detection of *Acinetobacter* spp., *Enterococcus* spp. and *Staphylococcus* spp. draws attention, as these genera including many pathogenic species [65]. Nevertheless, the presence of premise plumbing opportunistic pathogens in tap water was widely reported in the literature [8,13,21,30,66–71]. Moreover, because opportunistic pathogens could find suitable conditions to proliferate in premise plumbing, they could pose a real threat to consumers, if develop within a building [8,13,22,72]. Flushing of tap water was suggested to potentially alleviate the adverse impact of water stagnation [6,29], and therefore mitigate the influence of premise plumbing on consumer tap water microbial quality. According to Lautenschlager et al. [6], however, up to 30 L of tap water needs to be flushed to achieve the 'network quality'. This strategy seems to be unattainable for consumers in everyday use of tap water.

To sum up, this study confirms that bacterial communities present in consumer tap water could differ significantly even among samples collected within the same building. Although microbiomes of DWDSs seem to be well known, it is not certain what bacteria would be found in 'a consumer glass of water'. This emphasises the importance of the maintenance of proper conditions in premise plumbing with respect to microbial quality of consumer tap water [22], considering the fact that tap water flushing is not a common practice among consumers. Therefore, the negative impact of water stagnation and bacteria dwelling inside premise plumbing is not alleviated in everyday use of tap water. Although this paper presents a comparison of consumer tap water in a case study, its results may be valid for other premise plumbing systems.

5. Conclusions and final remarks

- Bacterial communities present in consumer tap water collected from nearby taps varied significantly in terms of community composition, biodiversity, and 16S rRNA gene-DGGE and resistome profiles.
- The results of this full-scale case study confirm that the microbiome of consumer tap water is primarily shaped by bacteria dwelling in premise plumbing; although water companies usually supply tap water of sufficient microbial quality, its properties could deteriorate during transportation via premise plumbing, and in some cases even pose a threat to consumer health.
- The presented results did not indicate that tap characteristics (i.e., frequency of use or age) are reflected in the biodiversity of bacterial communities present in consumer tap water; more research is required to elucidate this issue.
- High diversity of the investigated samples suggests that although knowledge on microbiomes of DWDSs is continuously expanding, little is still known about the ecology of bacteria in premise plumbing; nevertheless, this issue is of particular importance to consumer health, as tap water flushing is not a common consumer practice.

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Supporting information

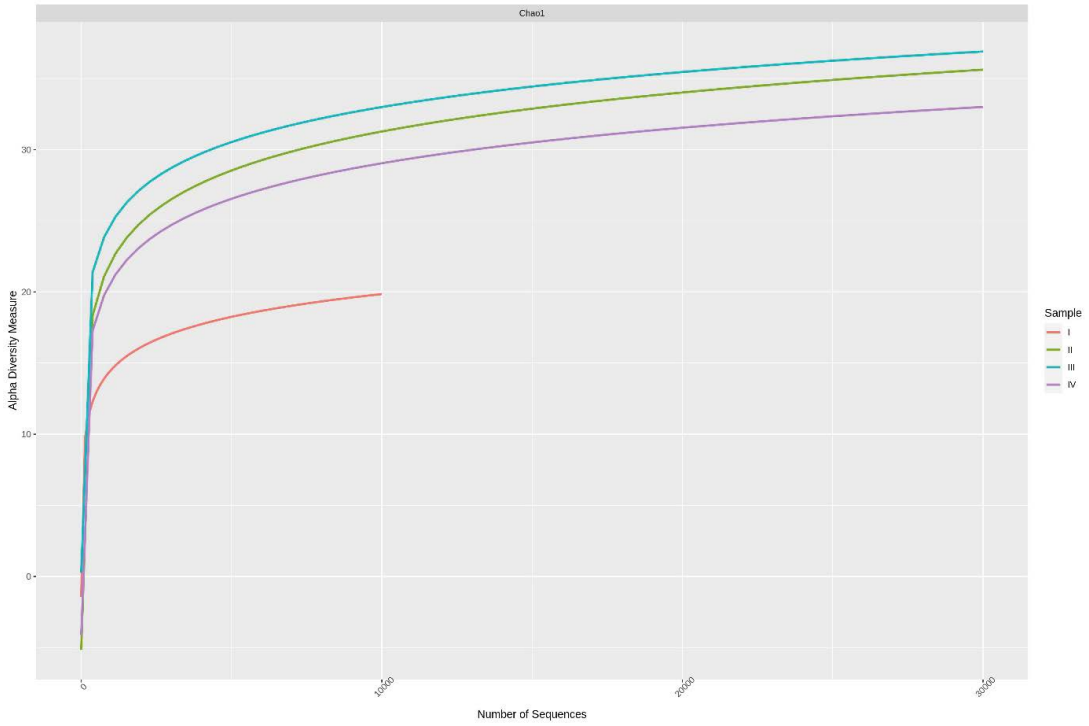

Fig. S1. Continued

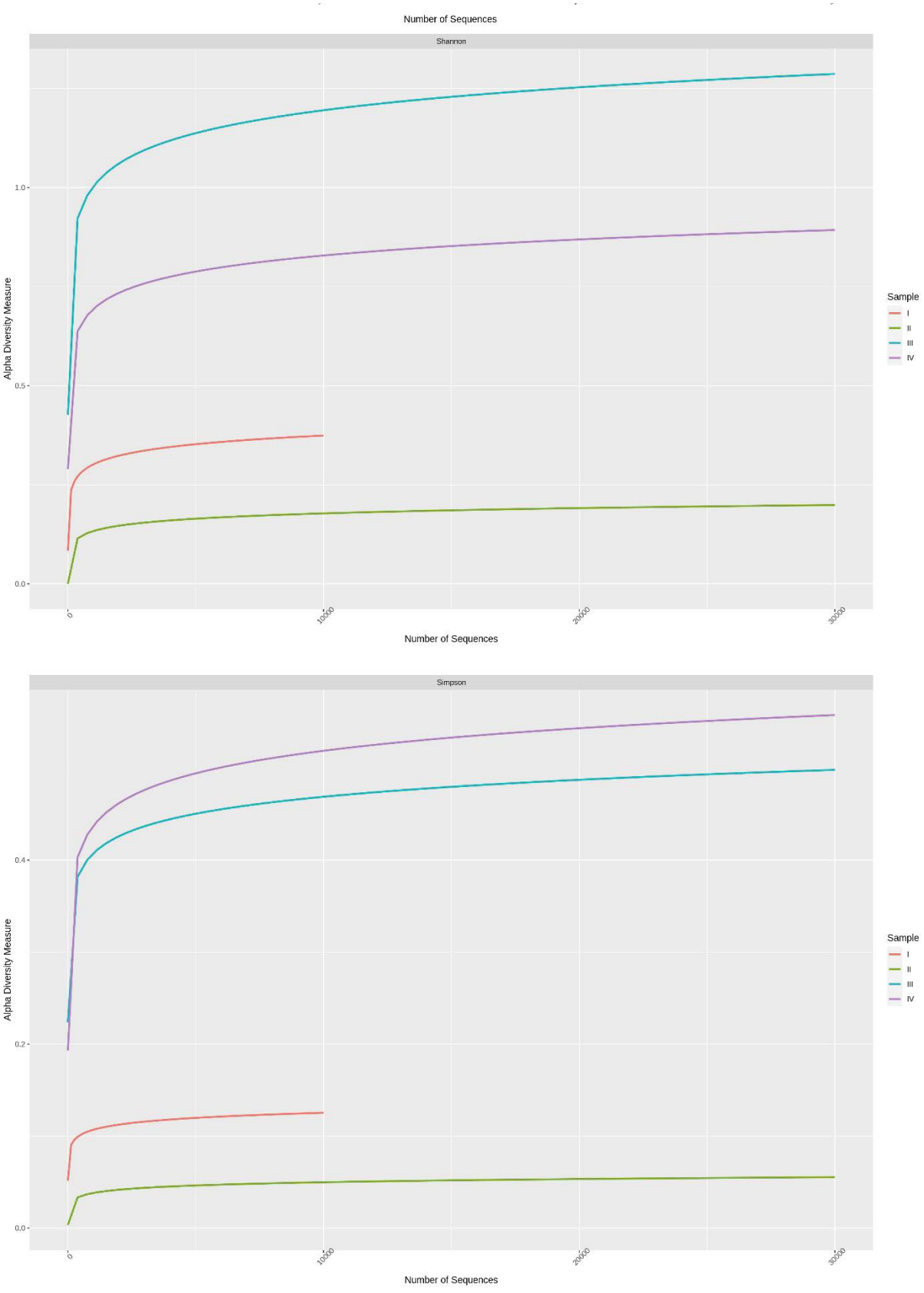

Fig. S1. Rarefaction plots.

Table S1
Annealing temperatures, amplicon sizes, and primer sequences used in PCRs

Gene	T_a (°C)	Amplicon size (bp)	Primer sequences	References
bla_{TEM}	61	247	GCKGCCAACTTACTTCTGACAACG CTTTATCCGCCTCCATCCAGTCTA	[1–4]
bla_{SHV}	61	214	GATGAACGCTTTCCCATGATG CGCTGTTATCGCTCATGGTAA	[5,6]
	61	63	GGAGGCGTGACGGCTTTT TTCAGTGCGATCCAGACGAA	[7–9]
bla_{KPC}	60	196	CAGCTCATTCAAGGGCTTTC GGCGGCGTTATCACTGTATT	[10,11]
bla_{NDM}	60	189	GATTGCGACTTATGCCAATG TCGATCCCAACGGTGATATT	[10,11]
bla_{OXA}	64	296	ATTATCTACAGCAGCGCCAGTG TGCATCCACGTCTTTGGTG	[5,6]
bla_{OXA-48}	64	189	AGGCACGTATGAGCAAGATG TGGCTTGTTTGACAATACGC	[10,11]
*amp*C	58	189	CCTCTTGCTCCACATTTGCT ACAACGTTTGCTGTGTGACG	[12,13]
*mec*A	61	92	CGCAACGTTCAATTTAATTTTGTTAA TGGTCTTTCTGCATTCCTGGA	[14,15]
*qnr*A	63	124	AGGATTTCTCACGCCAGGATT CCGCTTTCAATGAAACTGCAA	[7–9,16,17]
*qnr*B	62	134	CAGATTTYCGCGGCGCAAG TTCCCACAGCTCRCAYTTTTC	[16,18]
*qnr*S	62	118	GACGTGCTAACTTGCGTGAT TGGCATTGTTGGAAACTTG	[4,19–21]
*oqx*B	64	131	TCCTGATCTCCATTAACGCCCA ACCGGAACCCATCTCGATGC	[5,16,22,23]
*tet*A	58	210	GCTACATCCTGCTTGCCTTC CATAGATCGCCGTGAAGAGG	[5,12,24–26]
*tet*K	58	169	TCGATAGGAACAGCAGTA CAGCAGATCCTACTCCTT	[5,24,27]
*tet*L	58	267	TCGTTAGCGTGCTGTCATTC GTATCCCACCAATGTAGCCG	[5,24,27]
*tet*W	60	168	GAGAGCCTGCTATATGCCAGC GGGCGTATCCACAATGTTAAC	[1,16,25–28]
*sul*I	63	162	CGCACCGGAAACATCGCTGCAC TGAAGTTCCGCCGCAAGGCTCG	[1,4,7,8,16,27,29–31]
*sul*II	63	190	TCCGGTGGAGGCCGGTATCTGG CGGGAATGCCATCTGCCTTGAG	[1,16,26,27,29,31]
*erm*A	60	185	ATGTCTGCATACGGACACGG ACTTCAACTGCCGTTATCGC	[12,13,32]
*erm*B	63	139	AAAACTTACCCGCCATACCA TTTGGCGTGTTTCATTGCTT	[16,33]
*van*A	60	65	CTGTGAGGTCGGTTGTGCG TTTGGTCCACCTCGCCA	[14,20]
mcr-1	60	120	ACACTTATGGCACGGTCTATG GCACACCCAAACCAATGATAC	[34]
*mex*A	58	79	AGGACAACGCTATGCAACGAA CCGGAAAGGGCCGAAAT	[9,17,35,36]
*flo*R	62	61	ATTGTCTTCACGGTGTCCGTTA CCGCGATGTCGTCGAACT	[7–9,17,36]
*qac*EΔ1	63	226	ATCGCAATAGTTGGCGAAGT CAAGCTTTTGCCCATGAAGC	[7,8.37,38]
*qac*H	60	59	GTGGCAGCTATCGCTTGGAT CCAACGAACGCCCACAA	[7–9,17,35]
*tol*A	60	134	GATCTGGAGTTCGTTCGATGAG CGCTTGATTCCTGGCTTTG	[39]
*int*I1	63	146	GGCTTCGTGATGCCTGCTT CATTCCTGGCCGTGGTTCT	[31,33,40,41]
*tnp*A	63	102	AATTGATGCGGACGGCTTAA TCACCAAACTGTTTATGGAGTCGTT	[7–9,17,35]

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