Metagenomic Sequences of Three Drinking Water and Two Shower Hose Biofilm Samples Treated with or without Copper-Silver Ionization

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ABSTRACT

We announce five shotgun metagenomics data sets from two Norwegian premise plumbing systems. The samples were shotgun sequenced on two lanes of an Illumina HiSeq 3000 instrument (THRUplex chemistry, 151 bp, paired-end reads), providing an extensive resource for sequence analyses of tap water and biofilm microbial communities.

Water disinfection efficiently reduces the total number of bacteria in drinking water but may also select for disinfection-resistant communities (1). Several common water disinfection methods (2–6) and water flow through metal pipes (7) have been reported to increase the relative abundance of antibiotic-resistant bacteria (ARBs) and genes (ARGs) in drinking water systems.

The rationale for this pilot study was to determine the amount of sequencing required to detect and characterize ARGs in Norwegian premise plumbing systems and to investigate the impact of silver-copper ionization (CSI) on the number and type of antibiotic, biocide, and metal resistance genes detected. CSI is an in-house water disinfection method that works by releasing positively charged silver and copper ions directly into the water stream (8).

We announce three drinking water and two shower hose biofilm metagenomes. Samples were taken from two neighboring buildings in Oslo, Norway, both receiving water from the same drinking water treatment plant and through the same distribution pipes. One building used a CSI system as an additional water disinfection step; the other building did not.

Sampling and DNA isolation protocols are described in reference 9. Previous 16S rRNA gene analyses of the study system revealed five distinct bacterial community clusters (9). DNA from samples within each cluster were pooled in equal amounts prior to library synthesis to produce the metagenome samples described here. Libraries were created using Illumina THRUplex chemistry and were sequenced on two lanes of an Illumina HiSeq 3000 instrument (151 bp, paired-end reads [April 2017]).

To evaluate the results in relation to other human-influenced aquatic habitats known to contain resistance genes, we included the following four published metagenomic data sets in the analyses (Fig. 1): Ref01, inlet of a wastewater treatment plant (WWTP) (ENA accession number ERR1414237) (10); Ref02, river water upstream of a WWTP (SRR5306407); Ref03, river water downstream of a WWTP (SRR5298537) (11); and Ref04, hospital shower hose biofilm from a plumbing system with free chlorine (SRR2751194) (12). Reference data sets were quality treated and analyzed the same way in which the data sets presented here were.

Low-quality bases, reads, and sequencing adapters were trimmed with Trimmomatic

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v.0.36 (default settings, paired-end mode) (13). Exact duplicate reads (--derep: 14, --derep_min: 2) and low-complexity sequences (--lc_method: entropy, --lc_threshold cutoff: 70) were removed with Prinseq v.0.20.4 (14). The data sets were screened for coliphage phiX (GenBank accession number NC_001422.1) and human sequences (hg19) with BBMap v.37.53 (default settings) (15). Unpaired reads were discarded.

DIAMOND v.0.9.24.125 (blastx --max-target-seqs: 1, --id: 90, --query-cover: 90) (16) was employed to search the cleaned read data sets against NCBI's Antimicrobial Resistance Reference Gene Database (downloaded 7 November 2018) and the Antibacterial Biocide and Metal Resistance Genes Database v.2.0 (experimentally confirmed resistance genes) (17). The resulting count data were normalized to “relative gene abundance” following the method of reference 18, which accounts for the total number of reads and average read length in each data set and the subject gene length. Furthermore, cleaned data sets were assembled using MEGAHIT v.1.1.3 (--min-count: 2, --min-contig-len: 200, --k-min: 21, --k-max: 127, --k-step: 6).

The relative abundances of ARGs detected in the five samples were 0.4% (S01) to 4.2% (S04) of that detected in the inlet of a wastewater treatment plant (Fig. 1). Antibacterial biocide and metal resistance (BacMet) genes were considerably more abundant, especially in the biofilm exposed to CSI (S05). Surprisingly, this abundance was due to elevated mercury and not copper or silver resistance genes. A range of full-length mercury resistance genes were detected in the assembled data.

**TABLE 1** ENA accession numbers and sample indices for the five shotgun metagenomes

| Sample no. | Sample description | Sample accession no. | Sample barcode | Run accession no. | No. of reads | Assembly accession no. | No. of contigs >1,000 bp | No. of contigs with \( N_p >1,000 \) bp |
|------------|--------------------|----------------------|----------------|------------------|--------------|-----------------------|---------------------------|--------------------------|
| S01        | Cold inlet water   | ERS1887712            | ATCACGTT       | ERR2105748       | 46,342,789   | ERZ1079234            | 295,161                   | 3,764                     |
| S02        | Warm shower water, building without CSI | ERS1887713 | CGATGGTT | ERR2163668       | 48,595,584   | ERZ1079235            | 300,790                   | 3,756                     |
| S03        | Warm shower water, building with CSI | ERS1887714 | TTAGCCAT | ERR2105754       | 46,497,077   | ERZ1079236            | 303,509                   | 3,712                     |
| S04        | Shower hose biofilm, building without CSI | ERS1887715 | TGACCACCT | ERR2163669      | 57,390,138   | ERZ1079237            | 93,609                    | 14,763                   |
| S05        | Shower hose biofilm, building with CSI | ERS1887716 | ACAGTGGTT | ERR2105756       | 53,219,451   | ERZ1079238            | 54,579                    | 16,742                   |

*GenBank BioProject number PRJEB22193, and EBI metagenomics (MGnify) study accession number MGYS00001968.*

**FIG 1** Relative abundance of antibacterial biocide and metal resistance (BacMet) genes and antibacterial resistance genes (ARGs) in the five metagenomes announced here (S01 to S05) and in four reference data sets. CSI, copper-silver ionization; WWTP, wastewater treatment plant.
sets. The reason for the high abundance of mercury resistance genes remains unclear.

Data availability. All data sets are deposited in ENA (Table 1).

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