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Exploring the presence and characteristics of antibiotic resistance genes and bacteria present in water environments of Uppsala, Sweden

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List of content:

Abstract:

Antibiotics are one of the greatest discoveries in medicine, and emerged resistances have become a global threat. It is theorized that a big part of the antibiotic resistance genes come from the environment, and wastewater treatment plants and hospitals are considered a great breeding ground for the spread of these. The aim of this project is to analyse the microbiome and resistome of the wastewater of Uppsala and to evaluate the efficiency in the elimination of antibiotic resistance genes and bacteria. Samples from the University Hospital and the influents, sand filter and effluent of the Wastewater Treatment Plant were collected, DNA was extracted and sequenced to be analysed through metagenomics to explore them taxonomically and looking for resistance genes. Bacteria were also isolated, and their resistances were analysed. Taxonomical differences became noticeable in Order, Family, Genus and Species, with an increase of diversity in the Effluent samples. A total of 233 resistance genes were found in all the samples. There was a clear reduction in the number of resistance genes in the Effluent samples. However, there was an important number of genes carried in these and some prevail through all the path. Within all the isolates collected, from a total of 11, three *E. coli* isolates, one *C. freundii* and one *E. cloacae* presented resistances. Our study shows that the effluent of the wastewater treatment plant of Uppsala is potentially causing a negative impact on the environment, flushing out water not completely free of antibiotic resistance genes and resistant bacteria.

Keywords:

Resistome, microbiome, metagenomics, taxonomy, wastewater, wastewater treatment plant, hospital.

Abbreviations:

Wastewater Treatment Plant (WWTP), Clinical Wastewater (CWW), Antibiotic Resistance Gene (ARG), Antibiotic Resistant Bacteria (ARB), Horizontal Gene Transfer (HGT), Mobile Genetic Element (MGE).

Popular Science Summary:

Clean but not enough: how the effluents of the wastewater treatment plant of Uppsala contribute to the dissemination of antibiotic resistance

Antibiotics are one of the most important discoveries in medicine of the last century, but with the rise and spread of resistance, their efficacy is threatened. Dissemination of antibiotic resistance in the environment is important as new resistance mechanisms can be recruited from commensal bacteria. Hospital wastewater is especially interesting as crucial, last-line antibiotics are only used there.

In this study, the wastewater of the Uppsala University Hospital and the wastewater treatment plant of the city, Kungsängsverket; are analysed in order to know the characteristics of the bacteria population and how it looks in terms of antibiotic resistance. To achieve this, water samples were collected at different time points in both the hospital and the wastewater treatment plant, in the last more precisely in the influent, in the sand filter (early filtration step), and in the effluent. These samples were prepared to be processed with a technique known as metagenomics or population genetics, that allow us to look into them as a whole and not by the individuals that constitute them; and later analysed by comparation with databases that gave us information about their composition and the presence of antibiotic resistance genes.

Our results showed us that the wastewater have similar characteristics until it reaches the effluent, after all the wastewater treatment processes. In terms of which type of bacteria are found and how they are classified, in the initial taxonomic ranks there is not much variability, but the more specialized we go into these classifications, the more differences we found, and, as commented before, the biggest differences are in the effluent samples, with a more broader pool of different bacteria. In terms of antibiotic resistance, our results show that there is a clear reduction in the number of resistances present in the effluent samples. However, there is still a significant number of resistance genes, and we could also see that some of these resistances are carried over all the way from the hospital.

In summary, our study illustrates that our wastewater, even after is processed; is potentially causing a negative impact on the environment. We pour into our surroundings water not completely free of antibiotic resistance, and the way we are dealing with it is not satisfactory. It is necessary to carry out more complete and long-term studies to evaluate the situation and suggest solutions that could lower our impact, because it is our responsibility as habitants of this planet to take care of the place we live in and reduce the burden that we generate with our way of living as much as we can.

Introduction:

Antibiotics are one of the greatest discoveries in medicine in the twentieth century, not only affecting the severity of infectious diseases, but also improving the outcome of surgery and other medical interventions, for example, reducing the infection rate after surgery from 40% to 2% (1). The discovery of Penicillin in 1928, and the later synthesis of Sulphonamides in 1935 by Bayern scientists, started the Golden Age of antibiotics, that lasted until the beginning of the 2000s, when antibiotic resistance started to become a more common problem (2). The introduction of antibiotics, for example Streptomycin in 1943; significantly reduced the mortality rate of infectious diseases up to 8.2% per year in the USA from 1938 to 1952. This was mainly due to the decrease in pneumonia and tuberculosis (3). Antibiotics use different cellular mechanisms to act against bacteria and inhibit its growth. Depending on the characteristics of the compound and how the bacteria respond to it, antibiotics can present a bactericidal or a bacteriostatic behaviour, killing the bacteria (more effective in patients with a diminished immune system) or stopping or slowing bacterial grow (adequate for people with a normal immune system), respectively (4). The main mechanisms of antibiotic action can be divided into four big groups: disruption of the cell wall, inhibition of the synthesis of proteins through disruption of one of the ribosome components, inhibition of the synthesis of nucleic acids, and antimetabolites **(Figure 1)** (5).

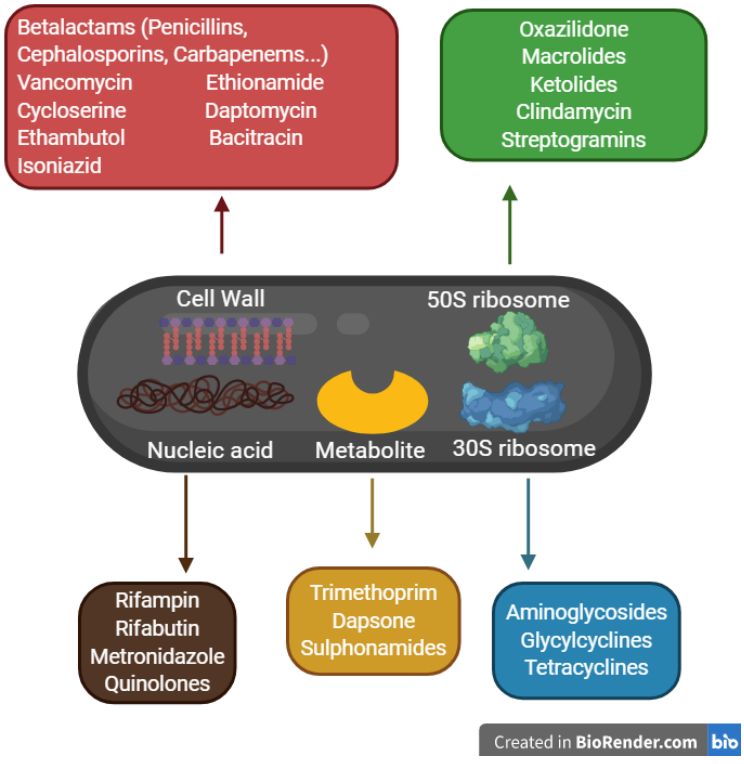

Figure 1 .- Antibiotic groups by their mechanism of action. The main groups of antibiotics can be grouped depending on which cell component they action against: the cell wall (red), the nucleic acids (brown), one of the ribosome components (green, blue) or metabolites (yellow). Created in BioRender.com

The ability of bacteria to evolve resistance mechanisms was reported soon after Penicillin was discovered, and the first cases of resistance in patients were already reported in 1942. More than 80% of the community- and hospital acquired strains of *Staphylococcus* aureus gained this characteristic by the 1960s, showing how quickly it spreads through the bacterial community (6). The importance of antibiotic treatment in terms of reducing mortality and morbidity from infectious diseases is unquestionable, but their extensive use, and misuse in numerous cases; in

humans and animals have contributed to the emanation of resistance in all classes of pathogenic bacteria (7,8). The development and spread of resistant bacteria have become an urgent global threat, being responsible of hundreds of thousands of deaths yearly (9). Over 25000 deaths in Europe are linked to antibiotic resistant bacteria infections (10). According to a report from the UK Review on Antimicrobial Resistance, 700000 people die yearly all over the world due to resistant bacteria, with an expected rise to over 10 million people by 2050 (11). Despite the decline in use of antibiotics in both animal and human medicine in, for example, Sweden; the reports of infections with antibiotic resistant bacteria are still increasing (10). The antibiotic resistance mechanisms that bacteria can develop or acquire can be divided in four big groups according to the mechanism involved: inactivation of the antibiotic molecule, reducing antibiotic levels at the target by decreasing internalization or actively expelling the compound; transforming and/or replacing the target site, and adaptation of global cell processes **(Figure 2)** (12).

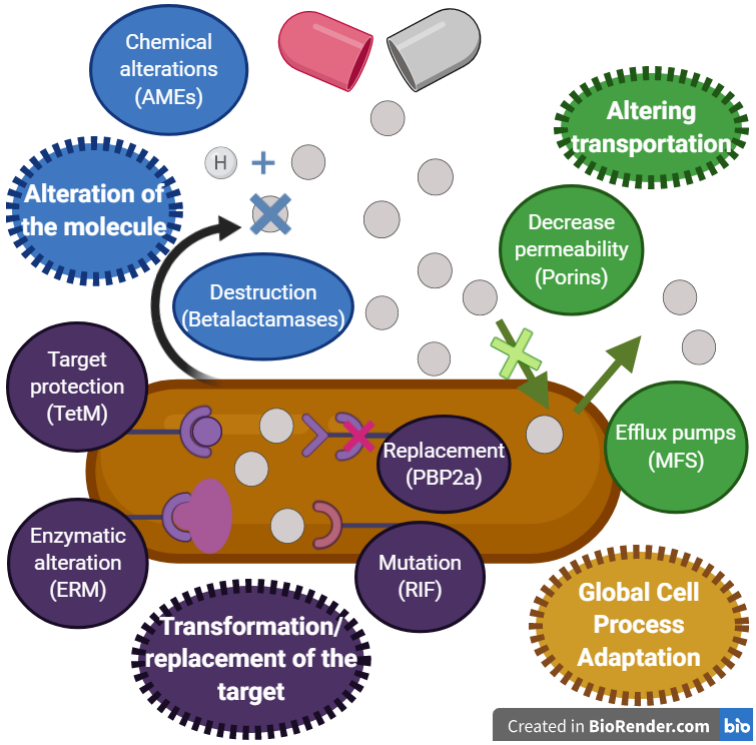

Figure 2 .- Antibiotic Resistance Mechanisms. Antibiotic resistance can be mediated through four main mechanisms: inactivation of the antibiotic molecule (blue), alteration of the transport (green), transformation or replacement of the target of the antibiotic (purple), or modifying global cell process (yellow). Some examples of antibiotic resistance genes that use each mechanisms are given.Created in BioRender.com

The presence of antimicrobial compounds as a threat for bacteria in the environments is one of the reasons of the selection of antibiotic resistance in both commensal and pathogenic bacteria, among others like antibiotic treatment in humans and animals. This scenario favours the survival of bacteria that have suffered random mutations in their genes that decrease the susceptibility against the compound. However, these mutations generally present a fitness cost, and are only maintained in the population if needed (12). Nonetheless, the resistance genes are often acquired and disseminated through Horizontal Gene Transfer (HGT), a faster and more efficient method to respond to an unfavourable situation. Conjugation is the main mechanism that occurs in clinical settings, but transformation and transduction also contribute (13) **(Figure 3)**. Resistance genes can be spread when contained in Mobile Genetic Elements (MGE), such as insertion sequences, transposons, integrative conjugative elements, plasmids, etc. Despite differences within Gram positive and Gram negative bacteria or different species, there are many similarities and a general commonality in the elements involved. Further studies of HGT

and MGE will be important in the discovery, control, and fight against antibiotic resistance in the future (14). Some studies have reported that there is a higher abundance of integrons of MGE in human influenced environments (8).

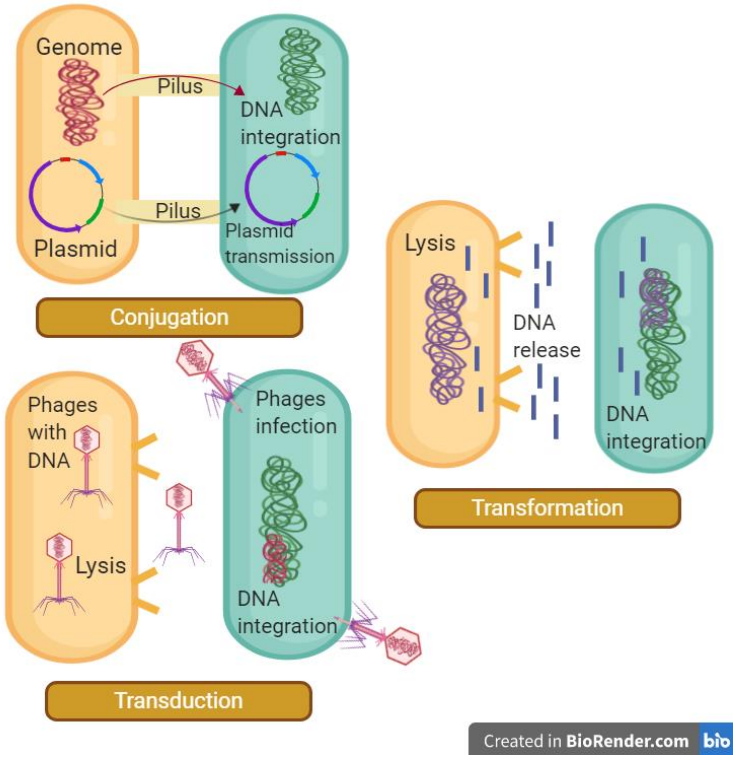

Figure 3 .- Horizontal Gene Transfer (HGT) methods. There are three main HGT mechanisms for bacteria to disseminate genetic information: Conjugation (most common in clinical settings), Transduction and Tranformation. Created in BioRender.com

It is hypothesized that the majority of the antibiotic resistance genes (ARGs) carried by pathogenic bacteria came from environmental bacteria, highlighting the role of those in the spreading of resistance (8). The presence of Antibiotic Resistance Bacteria (ARB) in surface water has been associated with anthropological activity (15), and the widespread antibiotic use has resulted in the contamination by antibiotics and ARGs of most of the surface and ground water sources of the world (16). It has been demonstrated that multi-resistant bacteria present in water environments are able to propagate ARGs to other bacteria, even from different genera (17). ARG presence in wastewater from different origins has been reported as ubiquitous (8). In terms of antibiotic resistance, wastewater treatment plants (WWTPs) are considered a great breeding ground for the spread of ARGs, HGT being highly favoured in the processes the water is treated under, despite the differences between practices (7,10,18). Conditions like high bacterial density, contaminant substances causing stress like heavy metals, and the formation of biofilms; are suggested as positive factors for the dissemination of these genes (18,19). Their effluent, highly influenced by human activity regardless of the treatment they have been under, is mixed after being flushed out with environmental bacteria, favouring spread and development of resistances. Additionally, WWTPs are not very competent in destroying antibiotics carried in the water, being those also flushed out into the environment, creating selective pressures even at low concentrations (8,20). The presence of antibiotics has been shown to favour mutation, transposon activity, recombination, and mobilization of DNA (9). Hospital wastewater, carrying one of the highest burdens of ARGs and ARB, in addition to contaminants like heavy metals or antibiotic residues; can be suggested as one of the most important sources causing the situation in urban wastewater. However, this statement is debatable, because their discharge represents less than 5% of the total of the wastewater produced by a city in most of

the cases, and most antibiotic consumption occurs at home and in other circumstances (pets and livestock). On the other hand, ARGs can easily proliferate from these low percentage to the total of the wastewater, being a favoured pathway for their distribution. Additionally, important last-line antibiotics are principally used in clinal environments, and the concentration of antibiotics and ARGs in hospital wastewater is higher due to the high number of patients gathered in the same place. The clinical situation needs to be more deeply studied and clarified (21)

Culture-based methods to evaluate the presence of ARGs in wastewater are limited due to the small fraction of bacteria capable of growing in laboratory conditions (19). Metagenomics, through the sequencing of the DNA of the whole community, have been suggested as a great tool to study ARGs in the environment. This technique can elucidate the whole resistome of a bacterial community and is not restricted to choosing a limited number of genes (18). However, because it is relatively new, it presents some challenges to overcome, like the reliability on known genes present in databases, the assembly of the genes obtained through sequencing, and the low prevalence of those genes (19).

Taxonomy in biology is defined as the science that classifies biological systems in a way that reflects the relationships between individuals (22). A taxonomic study of a wastewater sample is a good way to analyse its composition and observe how different processes at the distribution, mixing or WWTP treatment, for example, could affect it. Secondary treatments of sewage water involve the growth of different type of microorganisms, like ammonia-oxidizing or nitrifying bacteria (23), that consume suspended solids and organic compounds (24). Additionally, the aerobic treatment also done in WWTPs reduce the burden of faecal and pathogenic anaerobic bacteria (9). These can affect the final bacterial composition of the wastewater effluent. Additionally, the different origins of the sewage water bring different bacterial composition per se. Studies show that a high heterogeneity in the effluent samples of WWTPs is commonly reported (10,25,26).

ARGs in hospitals and WWTPs have been previously reviewed in numerous studies. Hospital wastewater is reported as abundant in ARGs and in some cases as the place with the highest prevalence. Its resistome is normally rich, acting as a reservoir of numerous and unique ARGs (10). The presence of betalactam resistance genes, like *blaCTX-M* or *blaOXA* genes, and genes of other classes like *mcr* or *sul*; is common (26). WWTP samples are characterized by a higher prevalence and diversity of ARGs in all the samples collected before treatment as compared to samples collected after treatment (8,9,19,27). In WWTP samples, a high prevalence of betalactam resistance genes is also common (25,28).

A total of six samples sites were used in this project **(Figures 4 and 5)**:

- **Uppsala University Hospital**: the Uppsala University Hospital (or "Akademiska Sjukhuset" in Swedish), was the first university hospital built in Sweden in 1708 and is one of the biggest hospitals in the country (S-751 85, Uppsala, Sweden). It brings together a large number of services as a county, specialist, training and research hospital, with over 700000 patients every year, 8000 employees and 1000 beds. During the dates of the sample collection, an Ozone Treatment Station was provided by Ozonetech (Hägersten, Sweden) to test this disinfection method in order to get rid of ARBs and ARGs with part of the wastewater of the hospital. The samples were obtained from this facility prior to the ozone treatment. (29).
- **Kungsängsverket**: Kungsängsverket is the WWTP in charge of the water originating from businesses, households, and other sites, like the Uppsala University Hospital; from Uppsala, Bälinge and Lövstalöt. It is located near the river (Fyrisån), in the area of

Kungsängen, southeast of the centre of the city (Stallängsgatan 3, 753 23 Uppsala). It has the capacity to treat water equivalent to the consumption of 20000 people, taking care of 19 million m^3 of water every year. It has two inputs (AB and C) that collect water from two different points, receive the same initial treatment and come together after the pre-treatment, being flushed out at a unique output in the river Fyrisån. (30) The treatment process takes about 20 hours to be complete, and consists of:

- Pre-treatment: big- and small-particle filtration, including a sand filter; phosphorus precipitation with ferric chloride; pre-sedimentation.
- Biological treatment and nitrogen removal: decomposition of organic material and creation of activated sludge with high concentration of microorganisms in aeration tanks, separation of ammonium nitrogen, separation of organic nitrogen by sedimentation.
- Chemical treatment: ferric chloride to decrease the concentration of phosphorus.
- Sludge treatment: thickening because of removing of water, digestion at 37ºC, formation of biogas, storage. The sludge is later reused in agriculture as fertilizer.
- Energy recovery: cool the water before it is flushed into the river, energy used in the plant for production of district heating and cooling. (30)

- **Fjällnora**: recreational area located 15km east from Uppsala (59°50'01.8"N 17°54'30.3"E), close to the lakes Trehörningen and Ramsen, that is very popular with locals during the warm months of the year thanks to a variety of activities offered, especially linked to the water. It is a point of interest for us because of its isolation from other urban water environments, but not being free of human influence. (31).
- **Svandammen**: urban pond located in the centre of Uppsala, close to the hospital facilities and the river (59°51'15.6"N 17°38'24.1"E). The water of the pond is provided from a close point in the river and returned to the river further downstream. It is a point of interest in the city due to its proximity and the variety of birds you can find on it, having a heavy anthropogenic influence with activities like feeding the animals or ice skating in winter. Other studies have examined the presence of ARBs in the water and the animals present there (32).
- **Fyrisån**: river that runs through Uppsala and ends in the Mälaren lake. It has a length of 80km, a basin size of 1982 km^2 and it discharges an average of 14 m^3/s. Kungsängsverket flushes out the treated wastewater into the river, so after passing Uppsala, the river is heavily influenced by the city, in addition to other activities that are carried out on it. Samples were obtained at two different points in the river: Upstream of city centre and the WWTP (59°51'49.3"N 17°37'49.4"E); and Downstream of the point where Kungsängsverket flushes out the treated wastewater (59°50'31.0"N 17°39'23.7"E) (33).

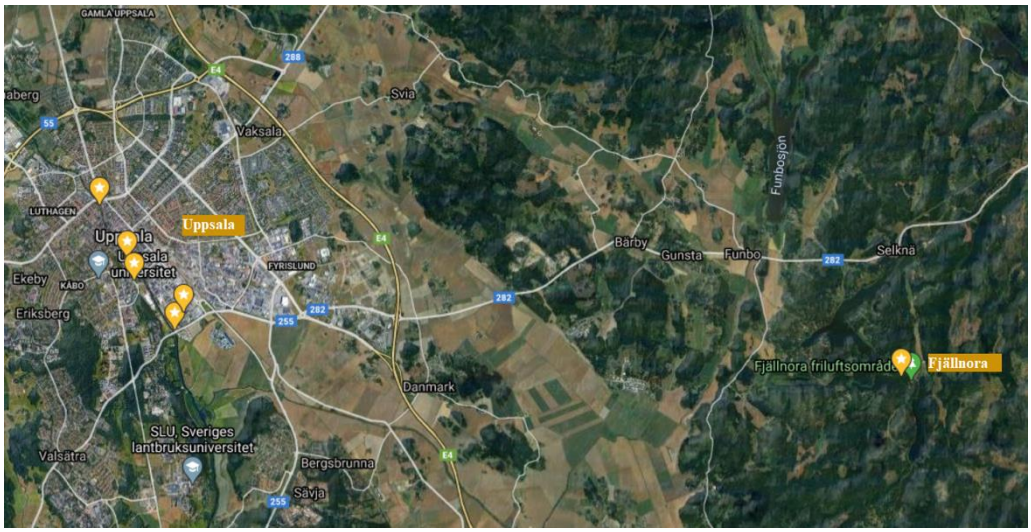

Figure 4 .- Map of samples sites including Fjälnora. Map of an extended view to show all of the samples sites use in the study in yellow, including Fjälnora. Created in google maps.

Figure 5 .- Map of samples sites in Uppsala. Closer look to the samples sites in the city of Uppsala in yellow: River Upstream and Downstream, Svandammen, Uppsala University Hospital and Kungsängsverket Created in google

Aim:

The first aim of this project is to analyse the microbiome and resistome of the wastewater of the University Hospital and the whole city of Uppsala using Metagenomics and culture-related tools. The second aim is to assess the efficiency of the WWTP in eliminating ARGs present in the water and to measure the human impact in the urban water environments in terms of ARGs.

Material and Methods:

Sample collection, conservation, and processing:

Three sample collections were gathered in the project

- **Clinical Wastewater samples (CWW)**: during six non-consecutive weeks of November-January 2019 once a week, 10 sterile tubes of 50ml were collected from the Ozone Treatment Station, located in the exterior of the Uppsala University Hospital, before the water was treated. These tubes were processed immediately after collecting them.
- **Wastewater Treatment Plant samples (WWTP)**: during six weeks (with the last one separated by 3 weeks from the others) of November-January 2019 once a week, three 500ml bottles (normally filled with 200ml) were collected by a WWTP employee from the four collection points: Input AB (SNTAB), Input C (SNTC), Sand Filter C (SFC) and Output (UTS). From each bottle, 3 to 4 tubes of 50ml were obtained, with a total of 10 tubes per samples site per day. The samples from the first three weeks were processed immediately after collecting them, and the samples from the last three weeks were frozen at -20ºC.

Every collection of 10 tubes in both the CWW and WWTP correspond to 3 samples and an additional tube that is conserved as a backup. Every three tubes were mixed together after the first incubation on the DNA extraction, forming a sample. For example, the tubes 1, 2 and 3 picked up on day 1 on the CWW collection form the sample CWW_1_1. This can be seen for all the samples in **Tables 3 and 4**.

- **Environmental Water samples (EW)**: during four consecutive weeks of December-January 2019 (with a break of two weeks between week 2 and 3) a 250ml sterile bottle was collected from the four collection locations: River Upstream, River Downstream, Svandammen and Fjällnora. From each bottle, 4 tubes of 50ml were obtained, with a total of 16 tubes per day. These samples were frozen at -20ºC.

All the CWW samples and the samples of the first 3 weeks of the WWTP were concentrated via centrifugation (Thermo Scientific Heraeis Megafige 40R Centrifuge) at 4700rpm for 30min. The supernatant was discarded, and the sediment conserved in 2ml sterile tubes frozen at -80ºC prior to DNA extraction.

DNA extraction, quantification and purity measure:

The DNA extraction was performed with QIAamp FAST DNA Stool Mini Kit (Qiagen, Hilden, Germany) following a modified protocol (**Appendix 1**). The DNA concentration was measured using the Qubit dsDNA BR assay kit (Invitrogen, California, USA) following the standard protocol (34) and reading it in the Qubit Fluorometer (Invitrogen, California, USA), with a concentration goal of ≥4ng/µl. DNA purity was also measured using a NanoDrop One/OneC Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific,

Massachusetts, USA), with an ideal range of 1.8-2.1 (A260/A280). Before sequencing the samples (half of the volume extracted or all of it, depending of the concentration obtained), they were dried into a dry pellet. The samples were sent to Novogene (Cambridge, UK) for short gun 150 pair-level DNA sequence using the Illumina platform.

Metagenomics analysis:

The following bioinformatic tools and databases were used in this project:

- **FastQC**: a tool consisting of a group of quality control tests that makes certain that the raw data is safe to use, without biases or any other problems. It is prepared to detect issues in the source data or from the process of sequencing. Some examples of the data that can be obtained by this tool are duplicate or overrepresented sequences, N or GC content per base, etc. (35).
- **MultiQC**: a tool that assembles multiple FastQC analysis data files from a group of samples into a singular report, easy to read and organized. This was used to have access to the data and visualize it in a simpler way (36).
- **TRIM GALORE**: a script tool prepared to automatize the process of quality control. It trims unreliable reads in one or both ends in order to leave only high-quality regions of the sequence; and also performs adaptive trimming (37).
- **SPAdes**: an assembly toolkit that consists of different assembly pipelines that correct read errors for Illumina and IonTorret, assemble short-read genomes, and enhance mismatches and short indel rates (38).
- **MG-RAST**: high-performance pipeline that compares metagenomic data with both nucleotide and protein databases, generating summaries and comparative graphs (25).
- **KmerResistance:** database created by the Center for Genomic Epidemiology (Denmark Technical University (DTU)) that identifies acquired ARGs in bacterial data using Kmer alignment (40,41).
- **CARD 2020**: CARD or the Comprehensive Antibiotic Resistance Database is a tool that provides reference data about bacterial antibiotic resistance (42).

The first part of the bioinformatic work with the sequence raw data was done by my co-supervisor, Rachel Hickman, bioinformatician for the Zoonosis Science Center. The raw reads obtained from Illumina were first quality-checked by FastQC, and then trimmed and quality checked again by TRIM GALORE. MultiQC was then used to generate a unique complete report of all the samples. Lastly, the trimmed sequences were assembled with SPAdes.

The second part of the bioinformatics work consisted on the analysis of the assembled contigs, comparing them with existing databases looking for ARGs and taxonomic characteristics of the samples. The databases used were the ones included in the MG-RAST pipeline; and KmerResistance. The CARD 2020 database was also used to find descriptions and other information like species prevalence about the different genes found in the samples.

Bacteria Isolation:

Bacteria was isolated from the 3 last rounds of Output samples, looking for antibiotic-resistant *E. coli* and *Klebsiella pneumoniae*. For one round of samples, the excess water from the sampling bottles was filtered (Sarsted; Filtropur BT25 250ml 0.45µm). The filters then were cut and put into 5ml of peptone water, that was vortexed. After that, a 1:10 dilution was made and incubated for 16-18h at 37ºC on a CHROMagar C3G^R plate (CHROMagar, Paris, France) looking for the growth of pink (*E. coli*) or blue (*K. pneumoniae, Enterobacter spp.*) colonies. For the other two rounds of samples, 100µl of the water from each bottle was directly put on a CHROMagar C3G^R plate and incubated for 16-18h at 37ºC. If colonies grew, they were picked

up (up to 3 colonies per colour per plate), pure-streaked in CHROMagar C3G^R plates, and frozen in a mix of 80% Lysogeny broth (Sigma-Aldrich, Missouri, USA) and 20% glycerol (Sigma-Aldrich, Missouri, USA).

To identify the isolates, they were cultured first on Mueller Hinton 2 agar (Sigma-Aldrich, Missouri, USA) and then on CHROMagar C3G^R; before they were analysed with mass spectrometry (Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight; MALDI-TOF). The identified species were classified in order of interest in four priority groups according to their relevance as human pathogens **(Table 1)**.

Priority	Bacteria
1	*Escherichia coli*
2	*Klebsiella pneumoniae*
3	*Hafnia alvei; Acinetobacter baumanii; Citrobacter freundii; Enterobacter cloacae*
4	*Enterobater asburiae; Lelliottia amnigena; Rahnella aquatitis; Enterobacter kobei; Aeromonas veronii; Pantoea agglomerans; Citrobacter murlinae*

Table 1.- Priority groups of bacteria isolated from the water samples. The possible bacteria that could have been found in the isolates from the samples were classified in four priority groups according to their relevance as human pathogens, giving the most importance to *E. coli* and *K. pneumoniae*.

Antimicrobial Susceptibility Testing was performed on the isolates in groups 1, 2 and 3, obtaining MIC values using Sensititre GNX2F plates (Thermo Scientific, Massachusetts, USA). Cultured bacteria were dissolved into 5ml of a 0.9% NaCL (Sigma-Aldrich, Missouri, USA) solution achieving a turbidity of 0.5 in the McFarland Scale tested by measuring them in a Sensititre Nephelometer (Thermo Scientific, Massachusetts, USA). Then, 10µl of the solution was added to 10ml of Mueller Hinton 2 broth (Sigma-Aldrich, Missouri, USA), and it was mixed thoroughly to create a starting inoculum of 0.5-1 x 10^6 CFU/ml. After that, using a multichannel pipette, each well of the plate was filled with 50µl of the broth, and it was incubated for 16-18h at 37ºC. The results were later evaluated by eye using a mirror and were compared with the clinical breakpoints reported by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (**Table 2**) (43).

Antibiotic	AMI	DOX	GEN	MIN	TOB	TGC	CIP	SXT	LEVO	AZT	IMI
S ≤ (µg/ml)	8	-	2	-	2	0.5	0.25	2	0.5	1	2
R > (µg/ml)	8	-	2	-	2	0.5	0.5	4	1	4	2
Antibiotic	FEP	MERO	COL	POL	TAZ	FOT	A/S2	DOR	P/T4	TIM2	
S ≤ (µg/ml)	1	2	2	-	1	1	8	0.015	8	8	
R > (µg/ml)	4	8	2	-	4	2	8	0.12	16	16	

Table 2.- Antibiotic Clinical Breakpoints for Enterobacteria (35). Clinical breakpoints reported by EUCAST for Amikacin, Doxycycline, Gentamycin, Minocyclin, Tobramycin, Tigecycline, Ciprofloxacin, Trimethoprim /Sulfamethoxazole, Levofloxacin, Aztreonam, Imipenem, Cefepime, Meropenem, Colistin, Polymixin B, Ceftazidime, Cefotaxime, Ampicilin/ Sulbactam 2:1, Doripenem, Piperacillin/ tazobactam cte.4, and Ticarcillin/ Clavulanic Acid cte. 2; antibiotics used in the AST testing. The ones in blank did not have an specified clinical breakpoint.

Results:

Samples

Water samples were collected from the Uppsala University Hospital wastewater, the Uppsala WWTP, and different water environments of the city in order to analyse them as metagenomic samples exploring the characteristics of the bacterial population and the presence of ARGs. The objectives were to both explore the resistome and microbiome of water environments of Uppsala and to study how the treatments at the WWTP affect them.

A total of 332 tubes (60 from the Hospital, 240 from the WWTP, and 32 from the Environmental sources) constituted a collection of 106 samples (18 from the Hospital, 72 from the WWTP, and 16 from the Environmental sources). Because of the time available for the project, we did not work with all the samples collected. The main analyses were made with the Hospital samples and the WWTP samples from the first three weeks. The information of the samples used is contained in **Tables 3 and 4**. The sample "UTS_2_1" was discarded for metagenomic analysis because its low quality, so a total of 17 samples where used (8 from the Hospital and 9 from the WWTP). The samples used ranged after assembly from 764681495 to 1445858879 base pairs, with 1770514 to 3724091 sequences 360 to 432 base pairs long. A summary of this information is located in **Tables 5 and 6**.

Sample	Samples from	DNA extraction	DNA quantification (ng/µl)	Total DNA (ng)	Date	DNA purity (A260/A280)	Date
CWW_1_1	1.1+1.2+1.3 (09/10)	.-	9.56	1912	12-Nov	2.01	12-Nov
CWW_1_2	1.4+1.5+1.6 (09/10)	11-Nov	10.3	6180	12-Nov	2.01	12-Nov
CWW_1_3	1.8+1.9+1.10 (09/10)	11-Nov	1.54	924	12-Nov	1.85	12-Nov
CWW_2_1	2.1+2.2+2.3 (14/10)	11-Nov	5.25	1050	12-Nov	2.05	12-Nov
CWW_3_1	1.1+1.2+1.3 (21/10)	12-Nov	15.7	3140	13-Nov	2.07	13-Nov
CWW_4_3	CWW_B_4_7-9 (15/11)	18-Nov	12.7	2540	18-Nov	2.06	18-Nov
CWW_5_1	CWW_B_5_1-3 (19/11)	21-Jan	4.47	894	21-Jan	2	21-Jan
CWW_6_3	CWW_B_6_7-9 (22/11)	21-Jan	3.47	694	21-Jan	1.99	21-Jan

Table 3.- Hospital Wastewater samples. Data about the CWW samples used in this study: tubes they came from, date of the DNA extraction, DNA concentration, total DNA, DNA purity and dates of those analyses.

Sample	Samples from	DNA extraction	DNA quantification (ng/µl)	Total DNA (ng)	Date	DNA purity (A260/A280)	Date
SNTAB_1_1	SNTAB_1_1-3 (22/11)	18-Dec	38.2	7640	19-Dec	1.89	19-Dec
SNTC_1_1	SNTC_1_1-3 (22/11)	18-Dec	18.8	3760	19-Dec	2.05	19-Dec
SFC_1_1	SFC_1_1-3 (23/11)	18-Dec	37.7	7540	19-Dec	2.11	19-Dec
UTS_1_1	UTS_1_1-3 (24/11)	18-Nov	2.19	438	19-Dec	1.98	19-Dec
SNTAB_2_1	SNTAB_2_1-3 (27/11)	22-Jan	15.7	3140	22-Jan	2.08	22-Jan
SNTC_2_1	SNTC_2_1-3 (27/11)	22-Jan	19.6	3920	22-Jan	2.11	22-Jan
UTS_2_1	UTS_2_1-3 (28/11)	19-Dec	3.14	628	19-Dec	2.05	19-Dec
SNTAB_3_1	SNTAB_3_1-3 (04/12)	22-Jan	8.91	1782	22-Jan	2.01	22-Jan
SNTC_3_1	SNTC_3_1-3 (04/12)	22-Jan	7.88	1576	22-Jan	2.08	22-Jan
UTS_3_1	UTS_3_1-3 (05/12)	19-Dec	3.18	636	19-Dec	1.98	19-Dec

Table 4.- Wastewater Treatment Plant samples. Data about the WWTP samples (Inputs (SNTAB, STNC), Sand Filter (SFC) and Output (UTS)) used in this study: tubes they came from, date of the DNA extraction, DNA concentration, total DNA, DNA purity and dates of those analysis.

	CWW_1_1	CWW_1_2	CWW_1_3	CWW_2_1	CWW_3_1	CWW_4_3	CWW_5_1	CWW_6_3
Sequences	1479362	1603195	1647642	1910765	2089290	2506453	2402848	1770514
Mean length	437 bp	426 bp	415 bp	416bp	406bp	381bp	393bp	432bp
Base pairs	647021644	683106230	683367678	794867667	847922484	954993665	943474476	764681495
Size of the file	0.697 GB	0.737 GB	0.739 GB	0.860 GB	0.919 GB	1.041 GB	1.026 GB	0.825 GB

Table 5- Hospital Wastewater sequenced samples information. Number of sequences and their mean length, number of base pairs and size of the file of the CWW samples after being sequenced.

	SNTAB_1_1	SNTC_1_1	SFC_1_1	UTS_1_1	SNTAB_2_1	SNTC_2_1	SNTAB_3_1	SNTC_3_1	UTS_3_1
Sequences	2079072	2544039	3310513	3724091	2149477	2754510	2391301	2476613	32798017
Mean length	390bp	379bp	351bp	388bp	387bp	372bp	408bp	360bp	414bp
Base pairs	810798775	962977009	1163416621	1445858879	831018610	1025739031	975869639	892596765	1358535109
Size of the file	0.884 GB	1.051 GB	1.279 GB	1.575 GB	0.905 GB	1.121 GB	1.058 GB	0.978 GB	1.471 GB

Table 6.- Wastewater Treatment Plant sequenced samples information. Number of sequences and their mean length, number of base pairs and size of the file of the WWTP samples (Inputs (SNTAB, STNC), Sand Filter (SFC) and Output (UTS)) after being sequenced.

Taxonomic data:

With the metagenomic data obtained from the samples of both the Hospital and the WWTP, a taxonomic analysis was done using the MG-RAST pipeline, in order to explore the characteristics of the bacteria population present in the sewage water at the sampled locations and how it changes through the wastewater flow until it is flush out into the river. Information about the domain, phylum, class, order, family, and genus of bacteria contained in the samples was obtained. Additionally, the alpha diversity, or mean species diversity, was analysed within four different groups: CWW samples (CWW), Inputs of the WWTP (INPUT), Output of the WWTP (OUTPUT), and samples theoretically belonging to the same day (22-Nov).

Bacteria was always the most prominent **Domain**, with a mean of 98.22% of the reads. Among the **Phylum**, *Proteobacteria*, *Bacteroidetes* and *Firmicutes* were the most predominant, with a mean of 45.82%, 31.39% and 15.92% of the reads, respectively. *Proteobacteria* dominated the reads of all the WWTP samples, while in the CWW samples either *Proteobacteria* or *Bacteroidetes* dominated. Other Phylum never constituted more than 4% of the reads. In terms of **Class**, *Bacteroidia*, *Betaproteobacteria*, *Gammaproteobacteria* and *Clostridia* were the most predominant, with a mean of 24.26%, 17.63%, 15.24% and 12.62% of the reads, respectively. All the rest of the Classes generally had a percentage of reads not reaching 10%. The Output samples presented variations, with an increase in *Betaproteobacteria* or *Alphaproteobacteria*, and a decrease in *Bacteroidia* or *Clostridia*. In terms of **Order,** there were bigger differences between CWW and WWTP samples. *Bacteroidales* was generally the most predominant order, with a mean of 24.94% of the reads. In the Output samples, *Burkholderiales* was the most prevalent Order, with a mean of 17.25% of the reads versus a 10.15% within all the samples. *Clostridiales* and *Pseudomonales* were other prevalent orders. *Clostridiales* was the second most prevalent in all the CWW samples and within the top 4 in the WTTP samples with a 13.23% of the reads. *Pseudomonales* stood out within the Inputs and the Sand Filter samples of the WWTP, with a 11.48% of the reads versus an 8% within all the samples. For **Family**, *Bacteroidaceae* dominated in prevalence in 15 of the 17 samples, with a mean of 15.64% of the reads. The difference in prevalence within families was reduced the further the water was processed. In the Output samples, the highest prevalence was 9%, while the mean highest prevalence was 16%. *Prevotellacea* and *Ruminococcaeae* were other predominant Families within the CWW samples. *Pseudomonaceae* and *Campylobacteraceae*, with percentages of 7.58% and 6.51% of the reads versus a mean of 4.6% and 2.94%, respectively, were prevalent in the Input and Sand Filter samples. In the Output samples, *Comamonadecae* stood out (9.49% of the reads versus a mean of 6.21%). Finally, in terms of **Genus**, *Bacteroides* was the most prevalent in 16 of 17 of the samples, with a mean of 17.82% of the reads. Like the previous group, the difference in prevalence within genus was reduced the further the water was processed. *Prevotella*, *Acinetobacter*, *Pseudomonas* and *Arcobacter* were other predominant Genera, the last two with particularly higher percentages of reads in the Input and Sand Filter samples (7.27% and 5.95% versus a mean of 4.89 and 3.27%,

respectively). In the **Figure 6,** the representation of this data can be seen, using the 4 Domain groups, and the 14 more prevalent Phylum/Class/Order/Family/Genera.

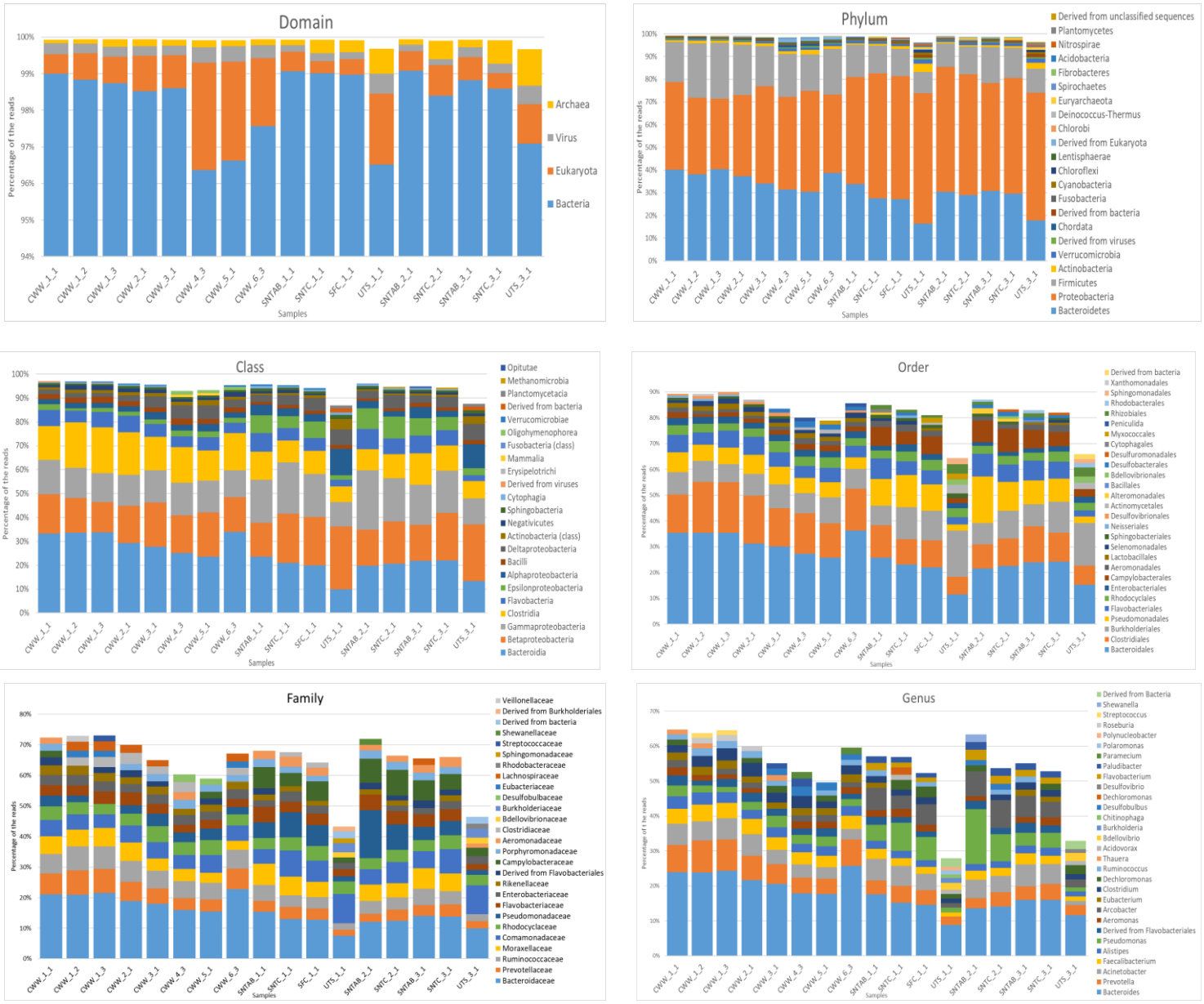

Figure 6.- Taxonomic distribution. Information about the Domain, Phylum, Class, Order, Family and Genus distribution of the reads of all the samples in the study (Clinical Wastewater samples (CWW); and Wastewater Treatment Plant samples ((Inputs (SNTAB, STNC), Sand Filter (SFC) and Output (UTS)), containing the 4 possible groups in the Domain; and the 14 most predominant groups in the rest.

The **alpha diversity** showed that the highest diversity of species was in the Output of the WWTP samples with a mean of 822 (±12) species, while the lowest was in the CWW samples with a mean of 437 (±59) species. In the Input of the WWTP samples there was a mean of 473 (±24) species, and in the "22-Nov" group there was a mean of 541 (±154) species, showing a clear increase in the number of species from the CWW sample to the Output of the WWTP sample. The highest diversity in species in the Output samples support the indications that there was a general higher heterogeneity in these samples in terms of taxonomy. **(Figure 7)**

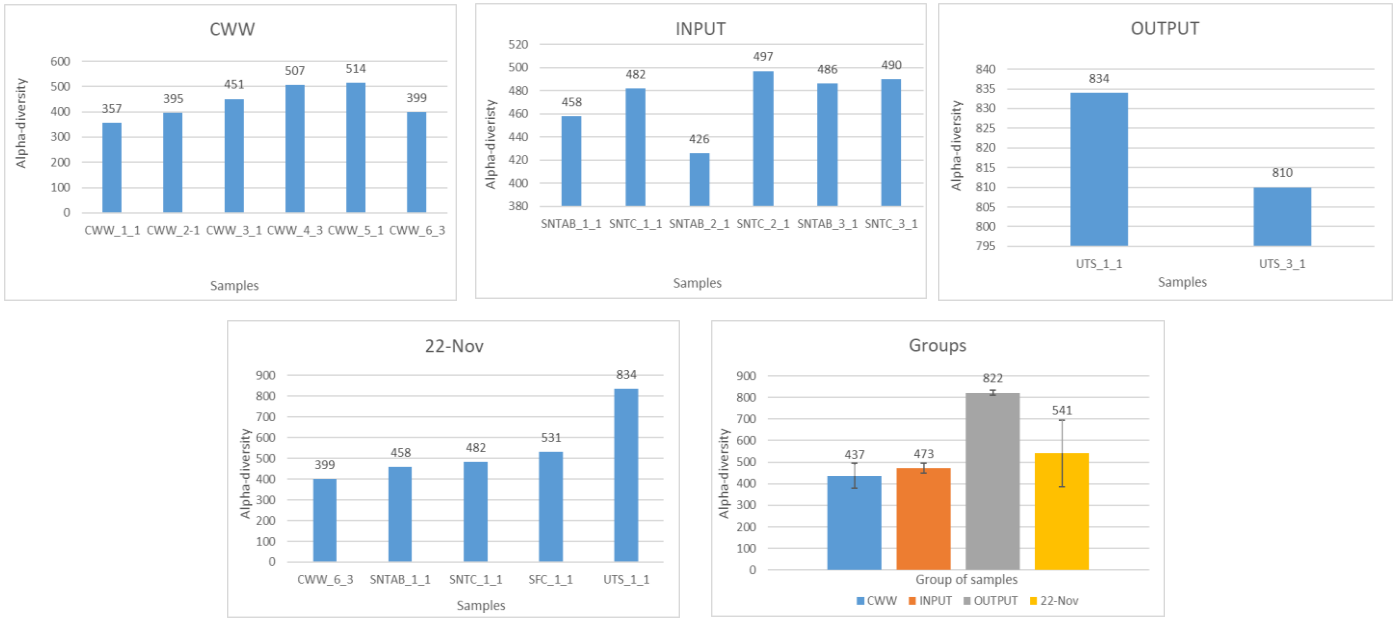

Figure 7.- Alpha-diversity. Species richness, or alpha-diversity, data within the samples in the CWW, Input (SNTAB, SNTC), Output (UTS) and 22-Nov groups (Sand FIlter (SFC)); and mean of all the groups and their standard deviation.

In summary, there was a low variability in terms of taxonomy in the first ranks analysed. The variability was higher in Family, Genus and Species, with a clear increase in bacterial diversity in the Output of the WWTP samples.

Antibiotic Resistance Genes:

The metagenomic data obtained from the Hospital and WWTP samples was also used to, through the KmerResistance database, analyse the presence of ARGs in the samples, exploring the particular characteristics of each location sampled, and studying the effect the wastewater treatment cause to them. This way we could explore how much ARGs are coming from the Hospital and from the whole city, and if an impact is produced in the environment in terms of antibiotic resistance thanks to the water flushed out after it is processed in the WWTP.

A total of 233 genes were found in all the samples, of which 131 were shared between CWW and WWTP samples, 59 were unique of the CWW samples and 43 of the WWTP samples. Genes from the classes Aminoglycoside (47), Betalactam (94), Rifamycin (2), Chloramphenicol (11), Diaminopyrimidine (12), Macrolide (11), MLSb phenotype (5), Lincosamide (4), Polypeptide (7), Nitroimidazole (4), Fluoroquinolone (6), Glycopeptide (2), Sulphonamide (3), Tetracycline (17), Mixed (6) and Other (2) resistance were detected. Some of the most remarkable resistance genes found, because of their importance and/or their presence in almost all the samples; are *aph(3'')-Ib* (Aminoglycoside resistance), *blaOXA-10*, *CfxA* (betalactam resistance), *ermB (*MSLb genotype*)*, *mef(C)* (Macrolide resistance), *sul1* (Sulphonamide resistance) and *msr(E)* (Mixed resistance). The complete list of genes found is located in **Appendix 4 and 5**.

The **CWW** samples presented a total of 190 different genes with a mean per sample of 103 (±10) genes. All the classes of resistance genes were present: Aminoglycoside (43), Betalactam (68), Rifamycin (2), Chloramphenicol (10), Diaminopyrimidine (8), Macrolide (9), MLSb phenotype (5), Lincosamide (3), Polypeptide (4), Nitroimidazole (4), Fluoroquinolone (4),

Glycopeptide (2), Sulphonamide (3), Tetracycline (17), Mixed (6) and Other (2). From the total, 50 (26.31%) genes were present in all the samples. The main classes of resistance genes found in these samples are displayed in **Table 7**. The **WWTP** samples presented a total of 174 different genes with a mean per sample of 81 (±18). The Input samples had a mean of 88 (±9) genes per sample, and the Output samples, 53 (±1) genes per sample. Neither Rifamycin or Glycopeptide resistance genes were present in these samples: Aminoglycoside (29), Betalactam (69), Chloramphenicol (9), Diaminopyrimidine (9), Macrolide (11), MLSb phenotype (5), Lincosamide (4), Polypeptide (5), Nitroimidazole (1), Fluoroquinolone (6), Sulphonamide (3), Tetracycline (17), Mixed (5) and Other (1). From the total, 21 (12.07%) genes were present in all the samples. The main families of resistance genes found in these samples are displayed in **Table 8**. The difference in the number of genes between the groups was mainly caused by the decrease in the WWTP samples of Aminoglycoside resistance genes. Some classes even suffer an enrichment in diversity, like Betalactam, Diaminopyrimidine, or Macrolide genes. There was also a clear reduction of total number of resistance genes in the different sampling locations if we compare the CWW samples with the Output of the WWTP. **(Figure 8)**

ampH	*ampS*	*blaACI*	*blaAER*	*blaBEL*	*blaCARB*	*blaDES*	*blaEBR*	*blaFOX*	*blaGES*	*blaIMP*	*blaLCR*	*blaMOX*	*blaNPS*
blaOCH	*blaOKP*	*blaOXA*	*blaPER*	*blaSHV*	*blaTEM*	*blaTLA*	*blaVEB*	*blaVIM*	*blaZ*	*cepA*	*CfxA*	*cphA*	*imiH*
aac	*aadA*	*ant*	*aph*	*str*	*arr*	*cat*	*cml*	*floR*	*dfrA*	*dfrB1*	ere	mef	mph
erm	lnu	mcr	*nim*	oqx	qnr	sul	*tet*	Van	*lsa*	*mdf*	*msr*	fos	

Table 7.- Main families of antibiotic resistance genes present in the CWW samples. These give resistance to, in order, Betalactam (ochre), Aminoglycoside (red), Rifamycin (orange), Chloramphenicol (navy), Diaminopyrimidine (grey), Macrolide (light blue), MLSb phenotype (turquoise), Lincosamide (brown), Polypeptide (yellow), Nitroimidazole (green), Fluoroquinolone (light green), Sulphonamide (pink), Tetracycline (pale pink), Glycopeptide (dark green), Mixed (purple) and Other (white) antibiotics.

blaACI	*blaAER*	*blaBEL*	*blaBRO*	*blaCARB*	*blaCMY*	*blaDES*	*blaEBR*	*blaFOX*	*blaGES*	*blaIMP*	*blaKHM*	*blaLCR*	*blaMOX*
blaNPS	*blaOXA*	*blaPER*	*blaSHV*	*blaTEM*	*blaTLA*	*blaVEB*	*ampH*	*ampS*	*cepA*	*cfiA*	*CfxA*	*cphA*	*aac*
aadA	*ant*	*aph*	*cat*	*cml*	*dfrA*	*dfrB*	ere	mef	mph	*erm*	*lnu*	*mcr*	*nim*
oqxA	*qnr*	*sul*	*tet*	*cfr*	*lsa*	*mdf*	*msr*	*fos*					

Table 8.- Main families of antibiotic resistance genes present in the WWTP samples. These give resistance to, in order, Betalactam (ochre), Aminoglycoside (red), Chloramphenicol (navy), Diaminopyrimidine (grey), Macrolide (light blue), MLSb phenotype (turquoise), Lincosamide (brown), Polypeptide (yellow), Nitroimidazole (green), Fluoroquinolone (light green), Sulphonamide (pink), Tetracycline (pale pink), Mixed (purple) and Other (white) antibiotics.

In the samples gathered in the group "22-Nov" we found a total of 155 different genes, where 31 were present in all the samples, 49 were common in both CWW and WWTP samples within the group, 20 were only present in the CWW sample, and 55 were present only in the WWTP samples. There was a mean of 86 (±21) genes per sample, with 99 genes in the CWW sample, 80 and 98 genes in the Input AB and C samples, respectively, and 52 in the Output sample. All the classes were present except Glycopeptide resistance genes: Aminoglycoside (30), Betalactam (58), Rifamycin (1), Chloramphenicol (6), Diaminopyrimidine (6), Macrolide (10), MLSb phenotype (4), Lincosamide (3), Polypeptide (3), Nitroimidazole (2), Fluoroquinolone (6), Sulphonamide (3), Tetracycline (17), Mixed (5) and Other (1). It is interesting to mention that the genes that were persistent within all the samples belonged to the classes Aminoglycoside (*aph(3)*, *aph(6)*), Betalactam (*blaAER*, *blaOXA*, *CfxA*), MLSb phenotype (*erm*), Lincosamide (*Inu*), Macrolide (*mef*, *mph*), Fluoroquinolone (*qnrS*), Sulphonamide (*sul*), Tetracycline (*tet*) and Mixed resistance(*Isa*, *msr*). As said before, there was a reduction of the

Figure 8.- Total and per class antibiotic resistance genes per sample in CWW and WWTP samples. On graphs A and C information about the total number of antibiotic resistance genes of each samples is showed. On graphs B and D the data is complemented with information about each of the Antibiotic Resistance Gene classes present in the samples. (Clinical Wastewater samples (CWW); Wastewater Treatment Plant samples (Inputs (SNTAB, STNC), Sand Filter (SFC) and Output (UTS))

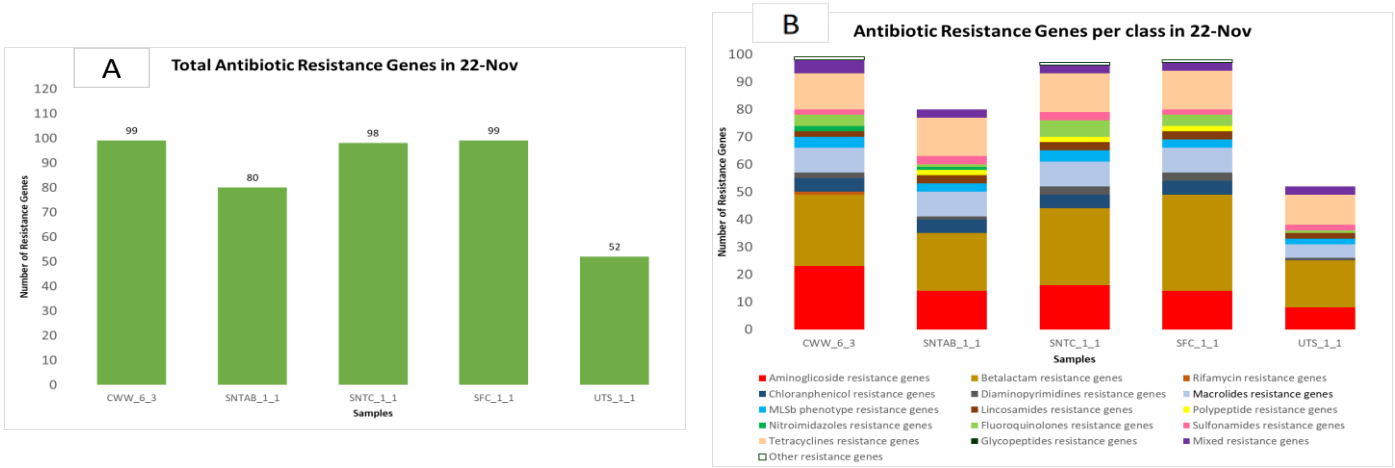

Figure 9.- Total and per class antibiotic resistance genes per sample in the group "22-Nov". On graph A information about the total number of antibiotic resistance genes of each samples is showed. On graph B the data is complemented with information about each of the Antibiotic Resistance Gene classes present in the samples. (Clinical Wastewater samples (CWW); Wastewater Treatment Plant samples (Inputs (SNTAB, STNC), Sand Filter (SFC) and Output (UTS))

total number of genes in the samples during the WWTP processes, but there was still a considerable number of genes carried, and 31 of those were conserved during all the path. Other remarkable differences were the reduction on the Aminoglycoside resistance genes along the path, and the increase of Betalactam resistance genes in the Input C and Sand Filter of the WWTP samples compared to the CWW sample, that can be seen in **Figure 9**

Isolates:

Resistance bacteria were isolated from the last three time points of the Output samples of the WWTP looking for species of interest. Then, Antimicrobial Susceptibility Tests were made to explore which resistances they expressed. The objective of this part was to explore in an alternative way the presence of ARBs in the water that is flush out into the river.

A total of 14 isolates were obtained from the last three collected Output of the WWTP samples: four isolates from the samples collected on the 10th of December (10E1, 10E2, 10K1, 10K2), four from the samples collected on the 17th of December (17K1, 17K2, 17K3, 17E1), and six from the samples collected on the 13th of January (13K1, 13K2, 13K3, 13E1, 13E2, 13E3). Three of them were discarded because they did not grow back after being frozen (10K1, 10K2, 17K1). The remaining isolates were identified as the following species: *Escherichia coli*, *Citrobacter freundii*, *Enterobacter cloacae*, *Citrobacter braaki* and *Raoultella ornithinolytica*.

		10E2: *E. coli*	17E1: *E. coli*	13E3: *E. coli*	10E1: *C. freundii*	13E1: C. freundii	13E2: C. freundii	17K3: *E. cloacae*	13K1: E. cloacae
Antimicrobial Susceptibility Tests (µg/ml)	Amikacin	≤4	≤4	≤4	≤4	≤4	≤4	≤4	≤4
	Doxycycline	>16	≤2	8	≤2	≤2	≤2	≤2	≤2
	Gentamycin	>8	≤1	≤1	≤1	≤1	8	≤1	≤1
	Minocyclin	>16	16	≤2	≤2	≤2	2	≤2	≤2
	Tobramycin	>8	≤1	≤1	≤1	≤1	≤1	≤1	≤1
	Tigecycline	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
	Ciprofloxacin	>2	≤0.06	0.25	≤0.06	0.25	0.12	≤0.06	≤0.06
	Trimethoprim /Sulfamethoxazole	>4/76	≤ 0.5/9.5	>4/76	≤ 0.5/9.5	1/19	≤ 0.5/9.5	≤ 0.5/9.5	≤ 0.5/9.5
	Levofloxacin	8	≤1	≤1	≤1	≤1	4	≤1	≤1
	Aztreonam	8	16	≤2	≤2	≤2	≤2	≤2	≤2
	Imipenem	≤1	≤1	≤1	≤1	≤1	4	≤1	≤1
	Cefepime	4	≤2	≤2	≤2	≤2	≤2	≤2	≤2
	Meropenem	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1
	Colistin	1	≤0.25	≤0.25	≤0.25	0.25	≤0.25	>4	≤0.25
	Polymixin B	1	≤0.25	≤0.25	≤0.25	0.5	≤0.25	>4	≤0.25
	Ceftazidime	4	>16	≤1	≤1	≤1	≤1	≤1	≤1
	Cefotaxime	>32	4	4	≤2	≤2	≤2	≤2	≤2
	Ampicilin/ Sulbactam 2:1	16/8	64/32	8/4	≤4/2	≤4/2	≤4/2	32/16	4/2
	Doripenem	2	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
	Piperacillin/ tazobactam cte.4	≤8/4	≤8/4	≤8/7	≤8/7	≤8/7	≤8/7	≤8/7	≤8/7
	Ticarcillin/ Clavulanic Acid cte. 2	≤16/2	32/2	≤16/2	≤16/2	≤16/2	≤16/2	≤16/2	≤16/2
Number of resistances		9	5	2	0	0	3	2	0

Table 9.- Antimicrobial Susceptibility Testing results. Results of the AST from the obtained isolates are showed in this table in µg/ml. Green = sensible. Orange = intermediate. Red = resistance. Ochre = no clinical breakpoint specified.

Antimicrobial Susceptibility Testing was done to the isolates belonging to the species of groups 1, 2 and 3. The results can be seen in **Table 9**. The three *E. coli* isolates presented 9, 5 and 2 resistances respectively, while only one of the *C. freundii* (3 resistances) and *E. cloacae* (2 resistances) presented resistance to the antibiotics analysed. Not all the isolates were resistant to the third-generation cephalosporin antibiotics (Ceftazidime, Cefotaxime), present in the media used to isolate the bacteria. This may be due to downregulation of an inducible *AmpC* resistance gene.

Discussion:

In this project, the microbiome and resistome of the wastewater of the University Hospital and the whole city of Uppsala were studied using mainly metagenomics. The anthropogenic influence through the effluent of WWTPs in the emergence of ARGs in the environment has been suggested by multiple articles (7,8,10,18,25,27). Thus, it is important to study and analyse the characteristics of the wastewater of a city and a hospital that is flushed out into our surrounding environment.

Taxonomic data:

Our results reflect the bacteria composition and diversity of the wastewater originated from the hospital and in the before- and after-processed water in the WWTP. Although the differences between samples were not very pronounced in Domain, Phylum and Class; they became more noticeable in Order, Family, Genus and Species; with an increase of diversity in the Output of the WWTP samples. In **Phylum**, our results are comparable with other studies, were in Hospital and WWTP samples *Proteobacteria* is the dominant group with percentages like 90% (27) or 75% (28) of the total population studied. *Proteobacteria* is one of the most frequent groups of bacteria found in water and are involved in infections in humans (44). In terms of **Class**, the ones belonging to the Phylum P*roteobacteria*, like *Gammaproteobacteria* and *Betaproteobacteria,* were abundant in the WWTP samples, like in *Marathe et al*. (28); compared to the CWW samples, where *Bacteroidia* and *Clostridia* were also prominent. In **Order**, our results, with *Bacteroidales*, *Clostridiales* and *Pseudomonas* as most predominant orders; are comparable with *Hultman J et al.* (19) The exception is the predominance of *Burkholderiales* in the Output of the WWTP samples. *Burkholderiales* are Gram negative bacteria belonging to the Phylum *Proteobacteria* that are normally found in soil and water. They are capable of cause human infections and present natural resistances to some common antibiotics. (45,46). In terms of **Family**, *Bacteroidaceae* was the most predominant in almost all the samples. *Bacteroidaceae* is a family of anaerobic Gram-negative bacteria where *Bacteroides* is their most notable genus, important member of the human microbiota. (47,48). This is consistent with the Genus results, where *Bacteroides* was the most predominant Genus. *Moraxellaceae* was quite predominant within CWW, Input and Sand filter of the WTTP samples. In the Input and Sand filter samples, *Pseudomonaceae* and *Campylobacteraceae* were also noteworthy, and in the Output of the WWTP samples *Comamonadecae* stood out. These results are comparable with other articles, but with differences like a lower predominance of *Streptococcaceae* or *Pseudomonadaceae* in the Input and the Output samples, respectively. (10,36). Finally, in **Genus**, as described before, *Bacteroides* was the most predominant genera. *Acinetobacter* was also abundant in almost all the samples, and *Pseudomonas* in the Input and Sand Filter samples. This is also reflected in the literature (26–28). However, there are two notable differences: the lack of other highly predominant genera within the Output of the WWTP samples, and the occurrence of *Arcobacter* within the Input and Sand Filter samples. *Arcobacter* are wide-spread Gram-negative bacteria with a strongly pathogen character in both humans and animals and a known profile of antibiotic resistance. (49)

Regarding **alpha-diversity**, our results show that the highest diversity in species was present in the Output samples, reinforcing the idea that there is a general higher heterogeneity in these samples in terms of taxonomy. Other articles confirm the same statement (10,25,26). Additionally, there was a cohesion among the samples taken from the same site, and similar values on the unprocessed samples, with some small differences probably due to their different origins. These taxonomic results are very useful to know the characteristics of the bacterial

community present in the Uppsala wastewater and how the hospital with its discharge and the WWTP with its processing is impacting it. However, without data on abundance of these bacteria, the real impact of these operations in the bacterial community cannot be measured. The possibility of having more extended data per rank, or of linking the taxonomic data with the presence of ARGs on each of the detected, at least, genus or species, could be nice additions for a future and more complete study.

Antibiotic Resistance Genes:

A total of 233 resistance genes were found in all the samples, where 131 were shared between CWW and WWTP samples, 59 were unique of the CWW samples and 43 of the WWTP samples. While the number of genes present in the samples within the CWW and Input of the WWTP were relatively stable, there was a clear reduction in the number of genes present in the Output of the WWTP samples. However, there was still a high number of genes carried in these samples, with representation of resistance to most antibiotic classes, and some of them persistent through all the sampling sites. The reduction caused by the WWTP treatment is shared within numerous articles that worked with similar samples. In some cases, this reduction is analysed in terms of concentration (8) or relative abundance (19,26,27). In *Khan et al.*, from 84 genes that were detected, 55 were shared between all the sampling sites, 47 were found in the hospital samples, and a considerable reduction was detected in the effluent water of the WWTP, with 33 genes (10). In *Gupta et al.*, from a total of 397 genes detected, 308 were detected in the influent and 293 in the effluent of the WWTP. In *Ekwanzala et al.* 156 genes were detected in the hospital wastewater, 202 in the influent and 116 in the effluent of the WWTP (26).

The resistance gene classes with high prevalence in our study were Betalactam, Aminoglycoside and Tetracycline resistance genes, with 94, 47 and 17 different genes each, respectively. A similar distribution can be seen in other studies (26–28). It is known that hospitals are hotspots for antibiotic-resistant bacteria (21), and it is also displayed in our results, with a higher mean per sample and a higher diversity of resistance genes present compared with the samples collected from the WWTP. However, their impact in the global picture of the wastewater is debated (18), and our study cannot conclusively determine the origin of the genes found in the Inputs of the WWTP. However, a majority of the resistome of both places is shared (133 of 233 genes (57%) in common), suggesting that the hospital wastewater constitutes an important contribution to ARGs in the WWTP in our study.

Some of the genes found in our samples are of interest because of their importance and/or their presence in almost all the samples. *aph(3'')-Ib* is an Aminoglycoside phosphotransferase resistance gene that has been found also in a high frequency in other studies (9), is encoded in both mobile genetic elements and chromosomes, and is most commonly found in *E. coli* or *P. aeruginosa*, among other bacteria (42). *blaOXA* genes is one of the most numerous families of resistance genes found in our samples, with 39 different genes within all the samples. They confer resistance to cephalothin and ampicillin, have a characteristic hydrolytic action against cloxacillin and oxacillin, and are not well inhibited by clavulanic acid (42). In particular, *blaOXA-10* is a commonly found gene within our samples and other studies (28); and common in *Acinetobacter baumannii* or *P. aeruginosa*, among others (42). The *sul* family of genes, conferring a sulphonamide resistant dihydropteroate synthase (enzyme implicated in the synthesis of folate) (42); is reported within all our samples, in particular the genes *sul1* and *sul2*. More specifically *sul1*, that is mostly found in Gram negative bacteria (42), is widely reported in literature as part of the majority of their collection of samples (9,26,50), or even as the most abundant gene found (28). Other genes that are broadly reported in our samples, like *ermB, mef(C),* or the *Tet* family; are also reported in other articles (7–9,19,21,26,50)

Some antibiotic resistance genes reported commonly in other similar studies are not present in this project. An example within the Betalactam resistance family is *blaOXA-48*, a clinically important gene due to its capacity to induce pandrug resistance (38). It is found in WWTP samples, most prevalently in the aeration tank (38,39); and reported in Sweden as one of the causes of the presence of carbapenemases in Gram negative bacteria in the environment (10). Another important betalactam resistance gene family that is not found in any of our samples is *blaCTX-M*. This family of genes, which act against oxymino-beta-lactam substrates including cefotaxime, are widely spread in clinical environments and easily circulate between bacteria thanks to plasmids (29). Because of their clinical importance they are genes generally targeted in other studies (7) and their prevalence is high (21,38,45).

The results in our study in terms of ARGs help us to illustrate the situation in the wastewater of Uppsala in terms of how many genes are present, how the genes persisted in a period of time and how many genes survived to the wastewater process. A reduction is clearly reported in the number of genes within the process of clearing the water in the WWTP. However, a study in terms of abundance of the total and each gene in particular would help to elucidate the real power of the WWTP. Additionally, our results shown how the effluent is not totally clear of ARGs and a source of anthropogenic contamination that could worsen the environmental resistome. It could be interesting to explore different methods of processing the water in WWTP, their efficacy in eliminating these genes; and evaluate the addition of an further step in the process particularly dedicated to the eradication of ARGs and the bacteria that carry them. Finally, the database used has been very useful, with the discovery of a great number of different genes within our samples. Nonetheless, the lack of some widely reported and clinically important genes like the family *blaCTX-M* or *blaOXA-48*, suggests that for future studies the use of more than one database could be a valuable addition.

Isolates:

Within all the isolates collected from our Output of the WWTP samples, the three *E. coli* isolates presented 9, 5 and 2 resistances respectively, while only one of the *C. freundii* (3 resistances) and *E. cloacae* (2 resistances) presented resistance to the antibiotics analysed. Through the sampling and isolating of bacteria from wastewater samples, is common to find isolates with expanded resistances, like methicillin-resistance *S. aureus* or ESBL enterobacteria (15,18). The species we could isolate are commonly reported by other studies (10,15). Although our results are quite limited, they reflect the survival of resistant bacteria through the processes in a WWTP and is another fact that support the role of WWTP effluents as anthropogenic contamination to the environment. The aerobic treatment included within the course of the wastewater is supposed to reduce considerably the burden of faecal bacteria, but it is reported as not totally efficient, not affecting facultative anaerobe pathogens (9). Our results could be consistent with contribution of the wastewater from the hospital to the wastewater microbiome as it likely contains a higher percentage of resistant bacteria, On the other hand, earlier studies have demonstrated that hospital sewage is diluted within the rest of the wastewater, and is reported to only contribute to a maximum of a 9% (15). Finally, studying isolates from the different steps on the WWTP and even from the origin of the wastewater would be an important addition for a future and more complete study, and a way to evaluate the evolution of resistant bacteria along the path. Furthermore, a genetic study of the isolates through, for example, whole genetic sequencing could be a good approach to explore the ARGs present in them and to compare these results with the ones obtained through metagenomics.

In conclusion, our study shows that the effluent of the WWTP of Uppsala contains ARGs and ARBs, and this has the potential to have a negative impact on the environment. There is a clear reduction in the number of different genes carried, and a modification in the microbiome

of the water through the WWTP process, but it is not enough to remove ARGs and ARBs. More complete and long-term studies need to be carried out to completely evaluate the situation and suggest solutions that could lower our impact in our surroundings. It is our responsibility as habitants of this planet to take care of the place we live in and reduce the environmental burden that we generate with our way of living as much as we can.

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Appendix 1.- DNA extraction Protocol (*QIAamp Fast DNA Stool Mini Kit*):

The extraction will be done using together three samples at the same time. **Avoid contamination** as much as possible (change tip of the pipette, change gloves when necessary…).

1. Take three samples tubes and centrifuge all 1 min 14000 rpm.
2. Discard the supernatant and add to each tube **330µl of InhibitEX Buffer**. Mix all the samples in one tube.
3. Incubate the sample for 5 min at 70ºC in a heat block. Prepare a new 1.5ml tube adding **15µl of Proteinase K**.
4. Centrifuge the sample 1 min 14000 rpm. Add **600µl of the supernatant** into the 1.5ml tube containing the enzyme.
5. Add **600µl of Buffer AL** into the tube and mix. Incubate the sample at 70ºC for 10 min.
6. Prepare a new tube with **600µl of ethanol (96-100%)**. Pipette the lysate into that tube.
7. Move **600µl of the lysate** to a **QIAamp spin column** and centrifuge it 1min 14000rpm. Discard the filtrate. Add and centrifugate the rest of the lysate. Discard the tube.
8. Put the column into a new tube. Add **500µl of Buffer AW1** and centrifuge it 1min 14000rpm. Discard the filtrate.
9. Add **500µl of Buffer AW2**. Centrifugate it 1 min 14000 rpm. Discard the tube and place the column into a new one. Centrifugate it 2 min 14000 rpm.
10. Discard the tube and place the column into a 1.5ml labelled tube. Add **200µl of Buffer ATE** and incubate 1 min at room temperature. Centrifuge it 1 min 14000 rpm.
11. Place the filtrate again into the column and centrifuge again 1 min 14000rpm. **Use new gloves with each sample in this step**. Discard the column and conserve (froze) the tube with the filtrate.

Appendix 2.- CWW Taxonomic data:

	CWW_1_1	CWW_1_2	CWW_1_3	CWW_2_1	CWW_3_1	CWW_4_3	CWW_5_1	CWW_6_3
Domain	Bacteria - 791,816 (99.00%) Eukaryota - 4,238 (0.53%) Viruses - 2,498 (0.31%) Archaea - 716 (0.09%)	Bacteria - 837,722 (98.84%) Eukaryota - 6,067 (0.72%) Viruses - 2,168 (0.26%) Archaea - 1,098 (0.13%)	Bacteria - 807,541 (98.74%) Eukaryota - 5,932 (0.73%) Viruses - 2,186 (0.27%) Archaea - 1,720 (0.21%)	Bacteria - 966,903 (98.53%) Eukaryota - 9,474 (0.97%) Viruses - 2,459 (0.25%) Archaea - 1,825 (0.19%)	Bacteria - 1,053,475 (98.61%) Eukaryota - 9,579 (0.90%) Viruses - 2,684 (0.25%) Archaea - 1,830 (0.17%)	Bacteria - 1,059,487 (96.37%) Eukaryota - 32,206 (2.93%) Viruses - 4,735 (0.43%) Archaea - 2,072 (0.19%)	Bacteria - 1,126,116 (96.63%) Eukaryota - 31,500 (2.70%) Viruses - 4,935 (0.42%) Archaea - 1,976 (0.17%)	Bacteria - 887,208 (97.57%) Eukaryota - 16,894 (1.86%) Viruses - 3,168 (0.35%) Archaea - 1,454 (0.16%)

Table A.1.- Domain data of the CWW samples.

	CWW_1_1	CWW_1_2	CWW_1_3	CWW_2_1	CWW_3_1	CWW_4_3	CWW_5_1	CWW_6_3
Phylum	Bacteroidetes - 293,613 (40.23%) Proteobacteria - 280,607 (38.44%) Firmicutes - 128,973 (17.67%) Actinobacteria - 6,962 (0.95%) Verrucomicrobia - 2,690 (0.37%) unclassified (derived from Viruses) - 2,498 (0.34%) Chordata - 1,820 (0.25%) unclassified (derived from Bacteria) - 1,571 (0.22%) Fusobacteria - 1,125 (0.15%) Cyanobacteria - 1,059 (0.15%) Chloroflexi - 847 (0.12%) Lentisphaerae - 821 (0.11%) unclassified (derived from Eukaryota) - 725 (0.10%) Chlorobi - 692 (0.09%)	Bacteroidetes - 292,281 (38.08%) Proteobacteria - 258,405 (33.66%) Firmicutes - 184,322 (24.01%) Actinobacteria - 9,305 (1.21%) Verrucomicrobia - 4,512 (0.59%) Chordata - 2,782 (0.36%) unclassified (derived from Viruses) - 2,168 (0.28%) unclassified (derived from Bacteria) - 1,473 (0.19%) Euryarchaeota - 1,021 (0.13%) Fusobacteria - 1,000 (0.13%) Chloroflexi - 966 (0.13%) unclassified (derived from Eukaryota) - 949 (0.12%) Deinococcus-Thermus - 944 (0.12%) Cyanobacteria - 914 (0.12%)	Bacteroidetes - 299,661 (40.34%) Proteobacteria - 231,146 (31.12%) Firmicutes - 182,215 (24.53%) Actinobacteria - 7,499 (1.01%) Verrucomicrobia - 3,501 (0.47%) Chordata - 2,403 (0.32%) unclassified (derived from Viruses) - 2,186 (0.29%) Euryarchaeota - 1,631 (0.22%) Fusobacteria - 1,491 (0.20%) unclassified (derived from Bacteria) - 1,334 (0.18%) unclassified (derived from Eukaryota) - 1,126 (0.15%) Cyanobacteria - 960 (0.13%) Chloroflexi - 877 (0.12%) Deinococcus-Thermus - 678 (0.09%)	Bacteroidetes - 330,699 (37.33%) Proteobacteria - 316,476 (35.72%) Firmicutes - 195,500 (22.07%) Actinobacteria - 10,850 (1.22%) Verrucomicrobia - 4,675 (0.53%) Chordata - 3,228 (0.36%) Chloroflexi - 2,707 (0.31%) unclassified (derived from Bacteria) - 2,483 (0.28%) unclassified (derived from Viruses) - 2,459 (0.28%) Fusobacteria - 2,181 (0.25%) unclassified (derived from Eukaryota) - 1,930 (0.22%) Euryarchaeota - 1,693 (0.19%) Cyanobacteria - 1,349 (0.15%) Spirochaetes - 1,017 (0.11%)	Proteobacteria - 412,546 (43.05%) Bacteroidetes - 325,237 (33.94%) Firmicutes - 167,509 (17.48%) Actinobacteria - 12,312 (1.28%) Verrucomicrobia - 7,513 (0.78%) unclassified (derived from Bacteria) - 2,820 (0.29%) unclassified (derived from Viruses) - 2,684 (0.28%) Chordata - 2,662 (0.28%) Lentisphaerae - 2,614 (0.27%) unclassified (derived from Eukaryota) - 2,247 (0.23%) Chloroflexi - 2,194 (0.23%) Fusobacteria - 2,088 (0.22%) Cyanobacteria - 1,772 (0.18%) Euryarchaeota - 1,652 (0.17%)	Proteobacteria - 398,264 (40.90%) Bacteroidetes - 304,876 (31.31%) Firmicutes - 183,083 (18.80%) Unclassified (derived from Eukaryota) - 15,168 (1.56%) Actinobacteria - 12,778 (1.31%) Verrucomicrobia - 10,996 (1.13%) Chordata - 10,185 (1.05%) unclassified (derived from Viruses) - 4,734 (0.49%) Spirochaetes - 3,600 (0.37%) Lentisphaerae - 3,468 (0.36%) Fusobacteria - 3,322 (0.34%) Fibrobacteres - 3,236 (0.33%) Cyanobacteria - 2,532 (0.26%) unclassified (derived from Bacteria) - 2,325 (0.24%)	Proteobacteria - 461,523 (44.47%) Bacteroidetes - 315,021 (30.36%) Firmicutes - 167,249 (16.12%) Actinobacteria - 20,219 (1.95%) Unclassified (derived from Eukaryota) - 12,609 (1.22%) Verrucomicrobia - 11,722 (1.13%) Chordata - 10,898 (1.05%) unclassified (derived from Viruses) - 4,935 (0.48%) Lentisphaerae - 4,679 (0.45%) Spirochaetes - 3,272 (0.32%) Fusobacteria - 3,244 (0.31%) unclassified (derived from Bacteria) - 3,109 (0.30%) Cyanobacteria - 2,220 (0.21%) Fibrobacteres - 1,937 (0.19%)	Bacteroidetes - 317,183 (38.75%) Proteobacteria - 281,929 (34.44%) Firmicutes - 165,784 (20.25%) unclassified (derived from Eukaryota) - 10,945 (1.34%) Actinobacteria - 9,667 (1.18%) Verrucomicrobia - 7,628 (0.93%) unclassified (derived from Viruses) - 3,168 (0.39%) Chordata - 3,090 (0.38%) Lentisphaerae - 2,585 (0.32%) unclassified (derived from Bacteria) - 2,449 (0.30%) Fusobacteria - 1,995 (0.24%) Cyanobacteria - 1,351 (0.17%) Euryarchaeota - 1,323 (0.16%) Spirochaetes - 1,235 (0.15%)

Table A.2.- Phylum data of the CWW samples.

	CWW_1_1	CWW_1_2	CWW_1_3	CWW_2_1	CWW_3_1	CWW_4_3	CWW_5_1	CWW_6_3
Class	Bacteroidia - 218,454 (33.24%)	Bacteroidia - 233,418 (33.55%)	Bacteroidia - 227,929 (33.75%)	Bacteroidia - 233,052 (29.38%)	Bacteroidia - 234,673 (27.67%)	Bacteroidia - 216,581 (25.16%)	Bacteroidia - 215,007 (23.52%)	Bacteroidia - 251,032 (34.04%)
	Betaproteobacteria - 108,673 (16.54%)	Clostridia - 133,020 (19.12%)	Clostridia - 128,904 (19.09%)	Clostridia - 141,598 (17.85%)	Betaproteobacteria - 158,260 (18.66%)	Betaproteobacteria - 135,738 (15.77%)	Betaproteobacteria - 170,138 (18.61%)	Clostridia - 114,900 (15.58%)
	Gammaproteobacteria - 94,240 (14.34%)	Betaproteobacteria - 101,949 (14.65%)	Betaproteobacteria - 85,722 (12.69%)	Betaproteobacteria - 122,626 (15.46%)	Clostridia - 118,856 (14.02%)	Clostridia - 127,789 (14.85%)	Gammaproteobacteria - 121,860 (13.33%)	Betaproteobacteria - 106,828 (14.49%)
	Clostridia - 92,881 (14.13%)	Gammaproteobacteria - 87,242 (12.54%)	Gammaproteobacteria - 81,756 (12.11%)	Gammaproteobacteria - 102,397 (12.91%)	Gammaproteobacteria - 114,054 (13.45%)	Gammaproteobacteria - 117,167 (13.61%)	Clostridia - 115,603 (12.65%)	Gammaproteobacteria - 82,033 (11.13%)
	Flavobacteria - 44,058 (6.70%)	Flavobacteria - 33,220 (4.77%)	Flavobacteria - 44,157 (6.54%)	Flavobacteria - 55,404 (6.98%)	Flavobacteria - 45,546 (5.37%)	Deltaproteobacteria - 48,304 (5.61%)	Deltaproteobacteria - 52,572 (5.75%)	Flavobacteria - 32,423 (4.40%)
	Epsilonproteobacteria - 15,834 (2.41%)	Alphaproteobacteria - 16,213 (2.33%)	Bacilli - 17,239 (2.55%)	Deltaproteobacteria - 19,394 (2.45%)	Deltaproteobacteria - 39,090 (4.61%)	Flavobacteria - 38,895 (4.52%)	Flavobacteria - 49,507 (5.42%)	Deltaproteobacteria - 24,202 (3.28%)
	Alphaproteobacteria - 13,806 (2.10%)	Bacilli - 15,425 (2.22%)	Alphaproteobacteria - 13,802 (2.04%)	Alphaproteobacteria - 18,149 (2.29%)	Alphaproteobacteria - 26,623 (3.14%)	Bacilli - 22,652 (2.63%)	Alphaproteobacteria - 28,008 (3.06%)	Alphaproteobacteria - 16,514 (2.24%)
	Bacilli - 13,634 (2.07%)	Deltaproteobacteria - 13,852 (1.99%)	Negativicutes - 13,452 (1.99%)	Bacilli - 15,840 (2.00%)	Bacilli - 15,841 (1.87%)	Epsilonproteobacteria - 21,485 (2.50%)	Epsilonproteobacteria - 22,317 (2.44%)	Bacilli - 15,273 (2.07%)
	Deltaproteobacteria - 12,266 (1.87%)	Negativicutes - 13,307 (1.91%)	Epsilonproteobacteria - 11,755 (1.74%)	Epsilonproteobacteria - 13,312 (1.68%)	Epsilonproteobacteria - 15,601 (1.84%)	Alphaproteobacteria - 19,589 (2.28%)	Actinobacteria (class) - 20,219 (2.21%)	Epsilonproteobacteria - 15,029 (2.04%)
	Actinobacteria (class) - 6,962 (1.06%)	Actinobacteria (class) - 9,305 (1.34%)	Deltaproteobacteria - 10,876 (1.61%)	Negativicutes - 12,858 (1.62%)	Actinobacteria (class) - 12,312 (1.45%)	Oligohymenophorea - 12,783 (1.49%)	Bacilli - 19,685 (2.15%)	Negativicutes - 14,103 (1.91%)
	Negativicutes - 6,216 (0.95%)	Epsilonproteobacteria - 6,911 (0.99%)	Actinobacteria (class) - 7,499 (1.11%)	Actinobacteria (class) - 10,850 (1.37%)	Negativicutes - 12,155 (1.43%)	Actinobacteria (class) - 12,778 (1.48%)	Oligohymenophorea - 10,854 (1.19%)	Oligohymenophorea - 10,092 (1.37%)
	Sphingobacteria - 5,642 (0.86%)	Sphingobacteria - 4,393 (0.63%)	Sphingobacteria - 5,067 (0.75%)	Sphingobacteria - 8,274 (1.04%)	Sphingobacteria - 7,989 (0.94%)	Negativicutes - 9,797 (1.14%)	Negativicutes - 9,944 (1.09%)	Actinobacteria (class) - 9,667 (1.31%)
	Cytophagia - 2,563 (0.39%)	Verrucomicrobiae - 3,840 (0.55%)	Verrucomicrobiae - 3,007 (0.45%)	Cytophagia - 3,859 (0.49%)	Cytophagia - 4,613 (0.54%)	Mammalia - 8,966 (1.04%)	Mammalia - 9,501 (1.04%)	Sphingobacteria - 5,891 (0.80%)
	unclassified (derived from Viruses) - 2,498 (0.38%)	Erysipelotrichi - 2,454 (0.35%)	Erysipelotrichi - 2,883 (0.43%)	Verrucomicrobiae - 3,735 (0.47%)	Verrucomicrobiae - 3,944 (0.47%)	Sphingobacteria - 7,613 (0.88%)	Sphingobacteria - 8,165 (0.89%)	Verrucomicrobiae - 5,054 (0.69%)

Table A.3.- Class data of the CWW samples.

	CWW_1_1	CWW_1_2	CWW_1_3	CWW_2_1	CWW_3_1	CWW_4_3	CWW_5_1	CWW_6_3
Order	Bacteroidales - 218,454 (35.46%)	Bacteroidales - 233,418 (35.40%)	Bacteroidales - 227,929 (35.42%)	Bacteroidales - 233,052 (31.24%)	Bacteroidales - 234,673 (30.09%)	Bacteroidales - 216,581 (27.27%)	Bacteroidales - 215,007 (25.68%)	Bacteroidales - 251,032 (36.26%)
	Clostridiales - 90,691 (14.72%)	Clostridiales - 130,046 (19.72%)	Clostridiales - 126,076 (19.59%)	Clostridiales - 138,282 (18.54%)	Clostridiales - 115,759 (14.84%)	Clostridiales - 123,960 (15.61%)	Clostridiales - 112,172 (13.40%)	Clostridiales - 111,903 (16.16%)
	Burkholderiales - 53,536 (8.69%)	Burkholderiales - 53,069 (8.05%)	Burkholderiales - 44,967 (6.99%)	Burkholderiales - 63,070 (8.45%)	Burkholderiales - 72,074 (9.24%)	Burkholderiales - 62,696 (7.89%)	Burkholderiales - 83,607 (9.98%)	Burkholderiales - 53,754 (7.76%)
	Pseudomonadales - 47,241 (7.67%)	Pseudomonadales - 41,861 (6.35%)	Flavobacteriales - 42,059 (6.54%)	Pseudomonadales - 54,543 (7.31%)	Pseudomonadales - 53,453 (6.85%)	Pseudomonadales - 47,268 (5.95%)	Pseudomonadales - 49,449 (5.91%)	Pseudomonadales - 30,723 (4.44%)
	Flavobacteriales - 41,871 (6.80%)	Flavobacteriales - 31,651 (4.80%)	Pseudomonadales - 41,291 (6.42%)	Flavobacteriales - 52,732 (7.07%)	Flavobacteriales - 43,069 (5.52%)	Flavobacteriales - 36,627 (4.61%)	Flavobacteriales - 46,694 (5.58%)	Flavobacteriales - 30,591 (4.42%)
	Rhodocyclales - 22,971 (3.73%)	Rhodocyclales - 21,574 (3.27%)	Rhodocyclales - 18,217 (2.83%)	Rhodocyclales - 23,690 (3.18%)	Rhodocyclales - 34,105 (4.37%)	Rhodocyclales - 28,472 (3.59%)	Rhodocyclales - 34,671 (4.14%)	Rhodocyclales - 21,188 (3.06%)
	Enterobacteriales - 16,904 (2.74%)	Enterobacteriales - 18,192 (2.76%)	Enterobacteriales - 17,380 (2.70%)	Enterobacteriales - 16,557 (2.22%)	Enterobacteriales - 19,677 (2.52%)	Enterobacteriales - 20,837 (2.62%)	Campylobacterales - 19,427 (2.32%)	Enterobacteriales - 18,292 (2.64%)
	Campylobacterales - 13,995 (2.27%)	Selenomonadales - 13,307 (2.02%)	Lactobacillales - 13,821 (2.15%)	Selenomonadales - 12,858 (1.72%)	Campylobacterales - 13,221 (1.70%)	Desulfobacterales - 18,486 (2.33%)	Enterobacteriales - 19,135 (2.29%)	Selenomonadales - 14,103 (2.04%)
	Aeromonadales - 11,608 (1.88%)	Lactobacillales - 12,217 (1.85%)	Selenomonadales - 13,452 (2.09%)	Campylobacterales - 11,956 (1.60%)	Desulfobacterales - 12,248 (1.57%)	Campylobacterales - 18,247 (2.30%)	Desulfobacterales - 15,914 (1.90%)	Campylobacterales - 12,699 (1.83%)
	Lactobacillales - 10,932 (1.77%)	Aeromonadales - 8,933 (1.35%)	Campylobacterales - 10,529 (1.64%)	Lactobacillales - 11,630 (1.56%)	Selenomonadales - 12,155 (1.56%)	Aeromonadales - 16,941 (2.13%)	Lactobacillales - 14,071 (1.68%)	Aeromonadales - 12,121 (1.75%)
	Selenomonadales - 6,216 (1.01%)	Desulfovibrionales - 7,169 (1.09%)	Aeromonadales - 7,234 (1.12%)	Aeromonadales - 8,425 (1.13%)	Lactobacillales - 11,831 (1.52%)	Lactobacillales - 15,963 (2.01%)	Aeromonadales - 14,023 (1.67%)	Lactobacillales - 11,018 (1.59%)
	Sphingobacteriales - 5,642 (0.92%)	Campylobacterales - 6,082 (0.92%)	Desulfovibrionales - 5,984 (0.93%)	Sphingobacteriales - 8,274 (1.11%)	Aeromonadales - 10,982 (1.41%)	Peniculida - 11,390 (1.43%)	Actinomycetales - 13,363 (1.60%)	Peniculida - 9,626 (1.39%)
	Neisseriales - 5,253 (0.85%)	Xanthomonadales - 5,536 (0.84%)	Sphingobacteriales - 5,067 (0.79%)	Desulfovibrionales - 7,810 (1.05%)	Desulfovibrionales - 10,381 (1.33%)	Selenomonadales - 9,797 (1.23%)	Myxococcales - 12,700 (1.52%)	Desulfobacterales - 9,612 (1.39%)
	Desulfovibrionales - 4,725 (0.77%)	Neisseriales - 5,026 (0.76%)	Neisseriales - 4,564 (0.71%)	Actinomycetales - 6,538 (0.88%)	Sphingobacteriales - 7,989 (1.02%)	Actinomycetales - 8,257 (1.04%)	Selenomonadales - 9,944 (1.19%)	Desulfovibrionales - 5,923 (0.86%)

Table A.4.- Order data of the CWW samples.

	CWW_1_1	CWW_1_2	CWW_1_3	CWW_2_1	CWW_3_1	CWW_4_3	CWW_5_1	CWW_6_3
Family	Bacteroidaceae - 107,489 (21.08%)	Bacteroidaceae - 113,484 (20.94%)	Bacteroidaceae - 114,161 (21.51%)	Bacteroidaceae - 116,471 (18.97%)	Bacteroidaceae - 117,527 (18.05%)	Bacteroidaceae - 106,401 (15.92%)	Bacteroidaceae - 108,049 (15.52%)	Bacteroidaceae - 131,891 (22.82%)
	Prevotellaceae - 34,721 (6.81%)	Prevotellaceae - 43,361 (8.00%)	Prevotellaceae - 41,692 (7.86%)	Ruminococcaceae - 42,184 (6.87%)	Ruminococcaceae - 37,752 (5.80%)	Ruminococcaceae - 37,290 (5.58%)	Comamonadaceae - 40,188 (5.77%)	Prevotellaceae - 38,842 (6.72%)
	Ruminococcaceae - 32,732 (6.42%)	Ruminococcaceae - 42,714 (7.88%)	Ruminococcaceae - 39,092 (7.37%)	Prevotellaceae - 37,808 (6.16%)	Comamonadaceae - 34,468 (5.29%)	Comamonadaceae - 30,540 (4.57%)	Ruminococcaceae - 38,103 (5.47%)	Ruminococcaceae - 35,304 (6.11%)
	Moraxellaceae - 29,264 (5.74%)	Moraxellaceae - 29,477 (5.44%)	Moraxellaceae - 32,064 (6.04%)	Moraxellaceae - 37,299 (6.07%)	Rhodocyclaceae - 34,105 (5.24%)	Rhodocyclaceae - 28,467 (4.26%)	Rhodocyclaceae - 34,662 (4.98%)	Comamonadaceae - 28,845 (4.99%)
	Comamonadaceae - 27,249 (5.34%)	Comamonadaceae - 26,781 (4.94%)	Comamonadaceae - 23,435 (4.42%)	Comamonadaceae - 33,872 (5.52%)	Prevotellaceae - 32,439 (4.98%)	Prevotellaceae - 26,481 (3.96%)	Prevotellaceae - 26,703 (3.83%)	Rhodocyclaceae - 21,188 (3.67%)
	Rhodocyclaceae - 22,971 (4.51%)	Rhodocyclaceae - 21,574 (3.98%)	Flavobacteriaceae - 21,998 (4.15%)	Flavobacteriaceae - 25,846 (4.21%)	Pseudomonadaceae - 27,029 (4.15%)	Moraxellaceae - 26,051 (3.90%)	Pseudomonadaceae - 26,062 (3.74%)	Enterobacteriaceae - 18,292 (3.16%)
	Pseudomonadaceae - 17,732 (3.48%)	Enterobacteriaceae - 18,192 (3.36%)	Rhodocyclaceae - 18,217 (3.43%)	Rhodocyclaceae - 23,690 (3.86%)	Moraxellaceae - 26,110 (4.01%)	Clostridiaceae - 21,690 (3.24%)	Moraxellaceae - 23,034 (3.31%)	Moraxellaceae - 17,088 (2.96%)
	Flavobacteriaceae - 17,558 (3.44%)	Flavobacteriaceae - 17,964 (3.31%)	Enterobacteriaceae - 17,380 (3.28%)	Clostridiaceae - 21,851 (3.56%)	Flavobacteriaceae - 20,741 (3.19%)	Pseudomonadaceae - 20,869 (3.12%)	Flavobacteriaceae - 19,585 (2.81%)	Flavobacteriaceae - 15,623 (2.70%)
	Enterobacteriaceae - 16,904 (3.32%)	Rikenellaceae - 16,598 (3.06%)	Clostridiaceae - 17,022 (3.21%)	Pseudomonadaceae - 16,894 (2.75%)	Enterobacteriaceae - 19,677 (3.02%)	Enterobacteriaceae - 20,837 (3.12%)	Enterobacteriaceae - 19,135 (2.75%)	Lachnospiraceae - 15,433 (2.67%)
	Rikenellaceae - 16,221 (3.18%)	Lachnospiraceae - 16,205 (2.99%)	Lachnospiraceae - 16,321 (3.08%)	Enterobacteriaceae - 16,557 (2.70%)	Rikenellaceae - 16,773 (2.58%)	Porphyromonadaceae - 19,824 (2.97%)	Rikenellaceae - 15,930 (2.29%)	Rikenellaceae - 14,390 (2.49%)
	unclassified (derived from Flavobacteriales) - 12,889 (2.53%)	Clostridiaceae - 14,990 (2.77%)	Rikenellaceae - 14,323 (2.70%)	Lachnospiraceae - 16,389 (2.67%)	Clostridiaceae - 16,347 (2.51%)	Flavobacteriaceae - 17,074 (2.55%)	Porphyromonadaceae - 15,437 (2.22%)	Clostridiaceae - 13,681 (2.37%)
	Campylobacteraceae - 11,341 (2.22%)	Pseudomonadaceae - 12,132 (2.24%)	Eubacteriaceae - 11,404 (2.15%)	Rikenellaceae - 14,143 (2.30%)	Porphyromonadaceae - 15,369 (2.36%)	Desulfobulbaceae - 16,851 (2.52%)	Campylobacteraceae - 14,937 (2.14%)	Pseudomonadaceae - 13,478 (2.33%)
	Porphyromonadaceae - 11,303 (2.22%)	Eubacteriaceae - 11,578 (2.14%)	unclassified (derived from Flavobacteriales) - 10,535 (1.99%)	unclassified (derived from Flavobacteriales) - 13,909 (2.27%)	Lachnospiraceae - 13,416 (2.06%)	Aeromonadaceae - 16,344 (2.45%)	Desulfobulbaceae - 14,920 (2.14%)	Porphyromonadaceae - 12,562 (2.17%)
	Aeromonadaceae - 10,900 (2.14%)	Veillonellaceae - 10,453 (1.93%)	Streptococcaceae - 10,048 (1.89%)	Porphyromonadaceae - 12,863 (2.09%)	unclassified (derived from Flavobacteriales) - 11,490 (1.76%)	Rikenellaceae - 14,231 (2.13%)	unclassified (derived from Flavobacteriales) - 14,175 (2.04%)	Eubacteriaceae - 11,555 (2.00%)

Table A.5.- Family data of the CWW samples.

	CWW_1_1	CWW_1_2	CWW_1_3	CWW_2_1	CWW_3_1	CWW_4_3	CWW_5_1	CWW_6_3
Genus	Bacteroides - 107,489 (23.97%)	Bacteroides - 113,484 (23.86%)	Bacteroides - 114,161 (24.36%)	Bacteroides - 116,471 (21.61%)	Bacteroides - 117,527 (20.58%)	Bacteroides - 106,401 (17.88%)	Bacteroides - 108,049 (17.67%)	Bacteroides - 131,891 (25.75%)
	Prevotella - 34,718 (7.74%)	Prevotella - 43,359 (9.12%)	Prevotella - 41,690 (8.90%)	Prevotella - 37,807 (7.02%)	Prevotella - 32,437 (5.68%)	Prevotella - 26,476 (4.45%)	Prevotella - 26,701 (4.37%)	Prevotella - 38,841 (7.58%)
	Acinetobacter - 27,164 (6.06%)	Acinetobacter - 26,020 (5.47%)	Acinetobacter - 28,533 (6.09%)	Acinetobacter - 33,494 (6.21%)	Acinetobacter - 23,123 (4.05%)	Acinetobacter - 22,905 (3.85%)	Faecalibacterium - 20,457 (3.35%)	Faecalibacterium - 19,414 (3.79%)
	Faecalibacterium - 19,275 (4.30%)	Faecalibacterium - 22,897 (4.81%)	Faecalibacterium - 20,668 (4.41%)	Faecalibacterium - 23,313 (4.33%)	Faecalibacterium - 20,437 (3.58%)	Clostridium - 21,083 (3.54%)	Acinetobacter - 19,992 (3.27%)	Acinetobacter - 15,219 (2.97%)
	Alistipes - 16,221 (3.62%)	Alistipes - 16,598 (3.49%)	Clostridium - 16,553 (3.53%)	Clostridium - 21,249 (3.94%)	Pseudomonas - 18,199 (3.19%)	Faecalibacterium - 20,547 (3.45%)	Pseudomonas - 17,492 (2.86%)	Alistipes - 14,389 (2.81%)
	Pseudomonas - 13,713 (3.06%)	Clostridium - 14,587 (3.07%)	Alistipes - 14,320 (3.06%)	Alistipes - 14,142 (2.62%)	Alistipes - 16,772 (2.94%)	Desulfobulbus - 15,863 (2.67%)	Alistipes - 15,930 (2.61%)	Clostridium - 13,267 (2.59%)
	unclassified (derived from Flavobacteriales) - 12,886 (2.87%)	Eubacterium - 11,363 (2.39%)	Eubacterium - 11,195 (2.39%)	unclassified (derived from Flavobacteriales) - 13,904 (2.58%)	Clostridium - 15,858 (2.78%)	Pseudomonas - 15,310 (2.57%)	unclassified (derived from Flavobacteriales) - 14,170 (2.32%)	Eubacterium - 11,333 (2.21%)
	Aeromonas - 10,152 (2.26%)	Ruminococcus - 10,269 (2.16%)	unclassified (derived from Flavobacteriales) - 10,530 (2.25%)	Pseudomonas - 12,111 (2.25%)	unclassified (derived from Flavobacteriales) - 11,487 (2.01%)	Alistipes - 14,231 (2.39%)	Desulfobulbus - 14,083 (2.30%)	Aeromonas - 9,953 (1.94%)
	Arcobacter - 9,650 (2.15%)	Pseudomonas - 9,383 (1.97%)	Ruminococcus - 9,934 (2.12%)	Ruminococcus - 10,698 (1.98%)	Dechloromonas - 11,245 (1.97%)	Paludibacter - 13,753 (2.31%)	Eubacterium - 11,919 (1.95%)	Pseudomonas - 9,759 (1.91%)
	Eubacterium - 9,479 (2.11%)	Roseburia - 7,686 (1.62%)	Roseburia - 7,688 (1.64%)	Eubacterium - 9,568 (1.78%)	Desulfobulbus - 10,669 (1.87%)	Aeromonas - 12,453 (2.09%)	Aeromonas - 11,822 (1.93%)	Paramecium - 9,626 (1.88%)
	Clostridium - 9,449 (2.11%)	Aeromonas - 7,648 (1.61%)	Arcobacter - 7,366 (1.57%)	Arcobacter - 8,373 (1.55%)	Eubacterium - 10,298 (1.80%)	Eubacterium - 12,046 (2.02%)	Arcobacter - 11,660 (1.91%)	Desulfobulbus - 8,383 (1.64%)
	Dechloromonas - 7,077 (1.58%)	Thauera - 6,789 (1.43%)	Pseudomonas - 7,060 (1.51%)	Roseburia - 7,561 (1.40%)	Thauera - 9,049 (1.58%)	Paramecium - 11,389 (1.91%)	Clostridium - 11,628 (1.90%)	Ruminococcus - 8,191 (1.60%)
	Ruminococcus - 6,775 (1.51%)	unclassified (derived from Flavobacteriales) - 6,721 (1.41%)	Streptococcus - 6,736 (1.44%)	Dechloromonas - 7,478 (1.39%)	Paludibacter - 8,847 (1.55%)	unclassified (derived from Flavobacteriales) - 10,740 (1.80%)	Dechloromonas - 10,232 (1.67%)	unclassified (derived from Flavobacteriales) - 7,737 (1.51%)
	Thauera - 6,540 (1.46%)	Streptococcus - 6,237 (1.31%)	Aeromonas - 6,149 (1.31%)	Aeromonas - 7,294 (1.35%)	Ruminococcus - 8,809 (1.54%)	Arcobacter - 10,239 (1.72%)	Ruminococcus - 9,534 (1.56%)	Arcobacter - 7,191 (1.40%)

Table A.6.- Genus data of the CWW samples.

Appendix 3.- WWTP Taxonomic data:

	SNTAB_1_1	SNTC_1_1	SFC_1_1	UTS_1_1	SNTAB_2_1	SNTC_2_1	SNTAB_3_1	SNTC_3_1	UTS_3_1
Domain	Bacteria - 1,139,678 (99.07%)	Bacteria - 1,351,260 (99.01%)	Bacteria - 1,690,703 (98.97%)	Bacteria - 1,139,027 (96.52%)	Bacteria - 1,125,496 (99.09%)	Bacteria - 1,430,267 (98.40%)	Bacteria - 1,255,241 (98.82%)	Bacteria - 1,305,455 (98.59%)	Bacteria - 1,084,021 (97.09%)
	Eukaryota - 6,137 (0.53%)	Archaea - 5,031 (0.37%)	Eukaryota - 7,295 (0.43%)	Eukaryota - 22,727 (1.93%)	Eukaryota - 5,851 (0.52%)	Eukaryota - 12,256 (0.84%)	Eukaryota - 8,017 (0.63%)	Archaea - 8,553 (0.65%)	Eukaryota - 12,099 (1.08%)
	Viruses - 2,085 (0.18%)	Eukaryota - 4,468 (0.33%)	Archaea - 5,653 (0.33%)	Archaea - 8,195 (0.69%)	Viruses - 2,135 (0.19%)	Archaea - 7,365 (0.51%)	Viruses - 3,608 (0.28%)	Eukaryota - 5,567 (0.42%)	Archaea - 11,001 (0.99%)
	Archaea - 1,708 (0.15%)	Viruses - 2,987 (0.22%)	Viruses - 3,178 (0.19%)	Viruses - 6,516 (0.55%)	Archaea - 1,579 (0.14%)	Viruses - 2,372 (0.16%)	Archaea - 2,506 (0.20%)	Viruses - 3,424 (0.26%)	Viruses - 5,747 (0.51%)

Table A.7.- Domain data of the WWTP samples.

	SNTAB_1_1	SNTC_1_1	SFC_1_1	UTS_1_1	SNTAB_2_1	SNTC_2_1	SNTAB_3_1	SNTC_3_1	UTS_3_1
Phylum	Proteobacteria - 488,047 (47.23%)	Proteobacteria - 677,773 (55.19%)	Proteobacteria - 814,786 (54.20%)	Proteobacteria - 535,907 (57.53%)	Proteobacteria - 566,542 (54.94%)	Proteobacteria - 688,856 (53.18%)	Proteobacteria - 538,101 (47.55%)	Proteobacteria - 601,389 (51.02%)	Proteobacteria - 496,220 (56.29%)
	Bacteroidetes - 349,130 (33.79%)	Bacteroidetes - 337,027 (27.44%)	Bacteroidetes - 408,095 (27.15%)	Bacteroidetes - 152,892 (16.41%)	Bacteroidetes - 314,228 (30.47%)	Bacteroidetes - 375,367 (28.98%)	Bacteroidetes - 347,505 (30.71%)	Bacteroidetes - 349,169 (29.62%)	Bacteroidetes - 157,384 (17.85%)
	Firmicutes - 145,725 (14.10%)	Firmicutes - 149,347 (12.16%)	Firmicutes - 180,890 (12.03%)	Firmicutes - 86,931 (9.33%)	Firmicutes - 107,722 (10.45%)	Firmicutes - 158,075 (12.20%)	Firmicutes - 180,135 (15.92%)	Firmicutes - 153,888 (13.05%)	Firmicutes - 91,987 (10.43%)
	Verrucomicrobia - 12,499 (1.21%)	Verrucomicrobia - 12,298 (1.00%)	Actinobacteria - 16,220 (1.08%)	Actinobacteria - 33,896 (3.64%)	Verrucomicrobia - 9,064 (0.88%)	Verrucomicrobia - 11,794 (0.91%)	Verrucomicrobia - 14,106 (1.25%)	Verrucomicrobia - 13,329 (1.13%)	Actinobacteria - 23,691 (2.69%)
	Actinobacteria - 6,576 (0.64%)	Actinobacteria - 10,027 (0.82%)	Verrucomicrobia - 14,066 (0.94%)	Verrucomicrobia - 16,671 (1.79%)	Actinobacteria - 5,114 (0.50%)	Actinobacteria - 8,734 (0.67%)	Actinobacteria - 9,350 (0.83%)	Actinobacteria - 10,205 (0.87%)	Verrucomicrobia - 15,196 (1.72%)
	Fusobacteria - 4,430 (0.43%)	unclassified (derived from Bacteria) - 5,098 (0.42%)	Nitrospirae - 6,467 (0.43%)	unclassified (derived from Bacteria) - 12,983 (1.39%)	Fusobacteria - 3,658 (0.35%)	Euryarchaeota - 6,783 (0.52%)	Fusobacteria - 5,289 (0.47%)	Euryarchaeota - 8,039 (0.68%)	unclassified (derived from Bacteria) - 12,962 (1.47%)
	Cyanobacteria - 2,234 (0.22%)	Euryarchaeota - 4,657 (0.38%)	unclassified (derived from Bacteria) - 6,348 (0.42%)	Chloroflexi - 11,122 (1.19%)	Cyanobacteria - 2,207 (0.21%)	Fusobacteria - 4,586 (0.35%)	unclassified (derived from Viruses) - 3,608 (0.32%)	unclassified (derived from Bacteria) - 5,439 (0.46%)	Euryarchaeota - 9,225 (1.05%)
	unclassified (derived from Viruses) - 2,085 (0.20%)	Fusobacteria - 4,149 (0.34%)	Chloroflexi - 5,968 (0.40%)	Planctomycetes - 9,586 (1.03%)	unclassified (derived from Viruses) - 2,135 (0.21%)	unclassified (derived from Bacteria) - 4,343 (0.34%)	Fibrobacteres - 3,144 (0.28%)	Fusobacteria - 4,645 (0.39%)	Planctomycetes - 8,307 (0.94%)
	unclassified (derived from Eukaryota) - 2,070 (0.20%)	unclassified (derived from Viruses) - 2,987 (0.24%)	Fusobacteria - 5,738 (0.38%)	Cyanobacteria - 8,282 (0.89%)	unclassified (derived from Bacteria) - 1,979 (0.19%)	unclassified (derived from Eukaryota) - 4,259 (0.33%)	Cyanobacteria - 2,969 (0.26%)	unclassified (derived from Viruses) - 3,424 (0.29%)	Chloroflexi - 8,205 (0.93%)
	Chloroflexi - 1,871 (0.18%)	Cyanobacteria - 2,511 (0.20%)	Cyanobacteria - 5,202 (0.35%)	unclassified (derived from Viruses) - 6,516 (0.70%)	Chlorobi - 1,884 (0.18%)	Cyanobacteria - 3,482 (0.27%)	unclassified (derived from Bacteria) - 2,714 (0.24%)	Spirochaetes - 3,218 (0.27%)	Cyanobacteria - 7,453 (0.85%)
	unclassified (derived from Bacteria) - 1,869 (0.18%)	Spirochaetes - 2,269 (0.18%)	Euryarchaeota - 5,067 (0.34%)	Euryarchaeota - 6,465 (0.69%)	Spirochaetes - 1,665 (0.16%)	Fibrobacteres - 3,241 (0.25%)	Chloroflexi - 2,617 (0.23%)	Cyanobacteria - 3,197 (0.27%)	Nitrospirae - 6,081 (0.69%)
	Spirochaetes - 1,854 (0.18%)	Fibrobacteres - 2,118 (0.17%)	Planctomycetes - 4,017 (0.27%)	Nitrospirae - 5,417 (0.58%)	Fibrobacteres - 1,614 (0.16%)	Chlorobi - 2,937 (0.23%)	Spirochaetes - 2,602 (0.23%)	Chlorobi - 2,305 (0.20%)	unclassified (derived from Viruses) - 5,747 (0.65%)
	Lentisphaerae - 1,786 (0.17%)	Chloroflexi - 2,087 (0.17%)	Spirochaetes - 3,211 (0.21%)	unclassified (derived from Eukaryota) - 4,899 (0.53%)	unclassified (derived from Eukaryota) - 1,554 (0.15%)	Spirochaetes - 2,813 (0.22%)	unclassified (derived from Eukaryota) - 2,397 (0.21%)	Fibrobacteres - 2,190 (0.19%)	unclassified (derived from unclassified sequences) - 3,632 (0.41%)
	Chlorobi - 1,718 (0.17%)	Chlorobi - 2,004 (0.16%)	unclassified (derived from Viruses) - 3,178 (0.21%)	Acidobacteria - 4,082 (0.44%)	Euryarchaeota - 1,398 (0.14%)	unclassified (derived from Viruses) - 2,372 (0.18%)	Euryarchaeota - 2,205 (0.19%)	Chloroflexi - 1,995 (0.17%)	Spirochaetes - 3,436 (0.39%)

Table A.8.- Phylum data of the WWTP samples.

	SNTAB_1_1	SNTC_1_1	SFC_1_1	UTS_1_1	SNTAB_2_1	SNTC_2_1	SNTAB_3_1	SNTC_3_1	UTS_3_1
Class	Bacteroidia - 214,724 (23.56%)	Gammaproteobacteria - 234,076 (21.58%)	Betaproteobacteria - 268,032 (20.35%)	Betaproteobacteria - 207,161 (26.29%)	Gammaproteobacteria - 227,474 (24.94%)	Bacteroidia - 235,131 (20.59%)	Bacteroidia - 218,170 (21.84%)	Bacteroidia - 227,849 (21.95%)	Betaproteobacteria - 179,265 (23.73%)
	Gammaproteobacteria - 162,929 (17.87%)	Bacteroidia - 227,121 (20.93%)	Bacteroidia - 262,103 (19.90%)	Alphaproteobacteria - 86,843 (11.02%)	Bacteroidia - 181,030 (19.85%)	Gammaproteobacteria - 207,251 (18.14%)	Gammaproteobacteria - 167,153 (16.74%)	Betaproteobacteria - 206,600 (19.91%)	Bacteroidia - 101,343 (13.42%)
	Betaproteobacteria - 129,962 (14.26%)	Betaproteobacteria - 223,124 (20.57%)	Gammaproteobacteria - 235,106 (17.85%)	Gammaproteobacteria - 80,035 (10.16%)	Betaproteobacteria - 136,456 (14.96%)	Betaproteobacteria - 202,614 (17.74%)	Betaproteobacteria - 150,332 (15.05%)	Gammaproteobacteria - 182,984 (17.63%)	Gammaproteobacteria - 82,258 (10.89%)
	Clostridia - 107,589 (11.80%)	Clostridia - 99,676 (9.19%)	Clostridia - 129,672 (9.85%)	Bacteroidia - 78,760 (10.00%)	Clostridia - 79,908 (8.76%)	Clostridia - 114,893 (10.06%)	Clostridia - 131,509 (13.17%)	Clostridia - 110,587 (10.66%)	Alphaproteobacteria - 75,846 (10.04%)
	Flavobacteria - 71,455 (7.84%)	Flavobacteria - 56,149 (5.18%)	Epsilonproteobacteria - 90,729 (6.89%)	Clostridia - 51,790 (6.57%)	Epsilonproteobacteria - 78,690 (8.63%)	Epsilonproteobacteria - 103,515 (9.06%)	Flavobacteria - 74,066 (7.42%)	Flavobacteria - 65,799 (6.34%)	Clostridia - 54,802 (7.25%)
	Epsilonproteobacteria - 68,156 (7.48%)	Epsilonproteobacteria - 53,404 (4.92%)	Flavobacteria - 69,869 (5.31%)	Deltaproteobacteria - 51,298 (6.51%)	Flavobacteria - 77,155 (8.46%)	Flavobacteria - 75,144 (6.58%)	Epsilonproteobacteria - 73,013 (7.31%)	Epsilonproteobacteria - 57,618 (5.55%)	Deltaproteobacteria - 51,666 (6.84%)
	Alphaproteobacteria - 40,289 (4.42%)	Deltaproteobacteria - 44,844 (4.13%)	Deltaproteobacteria - 68,899 (5.23%)	Actinobacteria (class) - 33,896 (4.30%)	Alphaproteobacteria - 29,247 (3.21%)	Deltaproteobacteria - 62,795 (5.50%)	Alphaproteobacteria - 45,869 (4.59%)	Deltaproteobacteria - 46,037 (4.44%)	Actinobacteria (class) - 23,691 (3.14%)
	Deltaproteobacteria - 24,694 (2.71%)	Alphaproteobacteria - 35,989 (3.32%)	Alphaproteobacteria - 41,057 (3.12%)	Flavobacteria - 21,823 (2.77%)	Deltaproteobacteria - 28,455 (3.12%)	Alphaproteobacteria - 26,258 (2.30%)	Deltaproteobacteria - 31,289 (3.13%)	Alphaproteobacteria - 27,002 (2.60%)	Epsilonproteobacteria - 22,500 (2.98%)
	Sphingobacteria - 11,420 (1.25%)	Bacilli - 15,332 (1.41%)	Bacilli - 19,000 (1.44%)	Epsilonproteobacteria - 15,272 (1.94%)	Bacilli - 10,075 (1.10%)	Bacilli - 14,160 (1.24%)	Bacilli - 17,398 (1.74%)	Bacilli - 14,224 (1.37%)	Flavobacteria - 17,908 (2.37%)
	Bacilli - 11,264 (1.24%)	Negativicutes - 12,904 (1.19%)	Actinobacteria (class) - 16,220 (1.23%)	Sphingobacteria - 13,139 (1.67%)	Sphingobacteria - 8,924 (0.98%)	Sphingobacteria - 10,300 (0.90%)	Actinobacteria (class) - 9,350 (0.94%)	Actinobacteria (class) - 10,205 (0.98%)	unclassified (derived from Bacteria) - 12,962 (1.72%)
	Cytophagia - 9,637 (1.06%)	Sphingobacteria - 11,055 (1.02%)	Sphingobacteria - 13,913 (1.06%)	unclassified (derived from Bacteria) - 12,983 (1.65%)	Cytophagia - 6,244 (0.68%)	Actinobacteria (class) - 8,734 (0.76%)	Sphingobacteria - 7,626 (0.76%)	Sphingobacteria - 8,785 (0.85%)	Bacilli - 12,556 (1.66%)
	Negativicutes - 8,134 (0.89%)	Actinobacteria (class) - 10,027 (0.92%)	Cytophagia - 9,775 (0.74%)	Bacilli - 12,631 (1.60%)	Actinobacteria (class) - 5,114 (0.56%)	Cytophagia - 8,176 (0.72%)	Negativicutes - 7,517 (0.75%)	Negativicutes - 8,235 (0.79%)	Sphingobacteria - 9,722 (1.29%)
	Actinobacteria (class) - 6,576 (0.72%)	Cytophagia - 5,826 (0.54%)	Negativicutes - 7,518 (0.57%)	Planctomycetacia - 9,586 (1.22%)	Negativicutes - 4,125 (0.45%)	Negativicutes - 7,033 (0.62%)	Cytophagia - 7,267 (0.73%)	Methanomicrobia - 6,606 (0.64%)	Negativicutes - 8,429 (1.12%)
	Verrucomicrobiae - 5,231 (0.57%)	Verrucomicrobiae - 5,217 (0.48%)	Verrucomicrobiae - 7,087 (0.54%)	Cytophagia - 9,034 (1.15%)	Fusobacteria (class) - 3,658 (0.40%)	Methanomicrobia - 5,206 (0.46%)	Opitutae - 6,850 (0.69%)	Cytophagia - 6,386 (0.62%)	Planctomycetacia - 8,307 (1.10%)

Table A.9.- Class data of the WWTP samples.

	SNTAB_1_1	SNTC_1_1	SFC_1_1	UTS_1_1	SNTAB_2_1	SNTC_2_1	SNTAB_3_1	SNTC_3_1	UTS_3_1
Order	Bacteroidales - 214,724 (25.75%)	Bacteroidales - 227,121 (23.09%)	Bacteroidales - 262,103 (21.99%)	Burkholderiales - 123,891 (17.95%)	Bacteroidales - 181,030 (21.61%)	Bacteroidales - 235,131 (22.54%)	Bacteroidales - 218,170 (23.90%)	Bacteroidales - 227,849 (24.16%)	Burkholderiales - 109,904 (16.50%)
	Clostridiales - 104,511 (12.53%)	Burkholderiales - 122,471 (12.45%)	Burkholderiales - 136,695 (11.47%)	Bacteroidales - 78,760 (11.41%)	Pseudomonadales - 152,063 (18.15%)	Pseudomonadales - 116,493 (11.16%)	Clostridiales - 127,547 (13.98%)	Burkholderiales - 112,940 (11.98%)	Bacteroidales - 101,343 (15.21%)
	Pseudomonadales - 85,760 (10.28%)	Pseudomonadales - 122,166 (12.42%)	Clostridiales - 124,943 (10.48%)	Clostridiales - 47,486 (6.88%)	Clostridiales - 77,538 (9.25%)	Burkholderiales - 111,911 (10.73%)	Pseudomonadales - 84,399 (9.25%)	Clostridiales - 106,578 (11.30%)	Clostridiales - 49,857 (7.48%)
	Flavobacteriales - 67,475 (8.09%)	Clostridiales - 96,030 (9.76%)	Pseudomonadales - 120,924 (10.14%)	Rhizobiales - 24,985 (3.62%)	Flavobacteriales - 72,813 (8.69%)	Clostridiales - 110,804 (10.62%)	Burkholderiales - 78,080 (8.56%)	Pseudomonadales - 84,563 (8.97%)	Rhizobiales - 23,507 (3.53%)
	Burkholderiales - 63,567 (7.62%)	Flavobacteriales - 53,148 (5.40%)	Campylobacterales - 80,797 (6.78%)	Rhodocyclales - 24,292 (3.52%)	Campylobacterales - 70,775 (8.45%)	Campylobacterales - 92,945 (8.91%)	Flavobacteriales - 69,710 (7.64%)	Flavobacteriales - 61,886 (6.56%)	Campylobacterales - 19,899 (2.99%)
	Campylobacterales - 61,018 (7.32%)	Campylobacterales - 47,340 (4.81%)	Flavobacteriales - 65,868 (5.52%)	Actinomycetales - 23,290 (3.37%)	Burkholderiales - 69,214 (8.26%)	Flavobacteriales - 71,065 (6.81%)	Campylobacterales - 65,096 (7.13%)	Campylobacterales - 50,932 (5.40%)	Rhodocyclales - 19,541 (2.93%)
	Rhodocyclales - 25,700 (3.08%)	Rhodocyclales - 36,490 (3.71%)	Rhodocyclales - 48,984 (4.11%)	Flavobacteriales - 19,960 (2.89%)	Rhodocyclales - 26,211 (3.13%)	Rhodocyclales - 35,312 (3.38%)	Rhodocyclales - 28,044 (3.07%)	Rhodocyclales - 36,123 (3.83%)	Actinomycetales - 17,133 (2.57%)
	Aeromonadales - 20,135 (2.41%)	Aeromonadales - 28,734 (2.92%)	Aeromonadales - 27,422 (2.30%)	Myxococcales - 16,085 (2.33%)	Alteromonadales - 17,216 (2.05%)	Aeromonadales - 18,605 (1.78%)	Aeromonadales - 19,121 (2.10%)	Aeromonadales - 27,243 (2.89%)	Flavobacteriales - 16,394 (2.46%)
	Rhizobiales - 14,373 (1.72%)	Enterobacteriales - 28,006 (2.85%)	Enterobacteriales - 26,524 (2.22%)	Sphingomonadales - 16,010 (2.32%)	Aeromonadales - 13,481 (1.61%)	Desulfobacterales - 17,579 (1.68%)	Enterobacteriales - 15,657 (1.72%)	Enterobacteriales - 20,586 (2.18%)	Pseudomonadales - 16,317 (2.45%)
	Enterobacteriales - 14,053 (1.69%)	Selenomonadales - 12,904 (1.31%)	Myxococcales - 24,327 (2.04%)	Pseudomonadales - 15,895 (2.30%)	Desulfobacterales - 11,876 (1.42%)	Enterobacteriales - 15,908 (1.52%)	Rhizobiales - 14,373 (1.57%)	Desulfuromonadales - 10,016 (1.06%)	Enterobacteriales - 15,380 (2.31%)
	Sphingobacteriales - 11,420 (1.37%)	Sphingobacteriales - 11,055 (1.12%)	Sphingobacteriales - 13,913 (1.17%)	Bdellovibrionales - 13,687 (1.98%)	Enterobacteriales - 11,331 (1.35%)	Alteromonadales - 11,367 (1.09%)	Rhodobacterales - 11,098 (1.22%)	Desulfobacterales - 9,866 (1.05%)	Bdellovibrionales - 15,186 (2.28%)
	Cytophagales - 9,637 (1.16%)	Desulfobacterales - 11,050 (1.12%)	Lactobacillales - 11,690 (0.98%)	Campylobacterales - 13,415 (1.94%)	Rhizobiales - 9,767 (1.17%)	Bdellovibrionales - 11,121 (1.07%)	Desulfobacterales - 9,116 (1.00%)	Sphingobacteriales - 8,785 (0.93%)	unclassified (derived from Bacteria) - 12,962 (1.95%)
	Selenomonadales - 8,134 (0.98%)	Neisseriales - 10,295 (1.05%)	Rhizobiales - 11,245 (0.94%)	Sphingobacteriales - 13,139 (1.90%)	Sphingobacteriales - 8,924 (1.07%)	Sphingobacteriales - 10,300 (0.99%)	Bacillales - 8,342 (0.91%)	Selenomonadales - 8,235 (0.87%)	Rhodobacterales - 11,189 (1.68%)
	Alteromonadales - 7,611 (0.91%)	Rhizobiales - 9,964 (1.01%)	Actinomycetales - 10,236 (0.86%)	Enterobacteriales - 13,073 (1.89%)	Rhodobacterales - 6,933 (0.83%)	Desulfuromonadales - 9,657 (0.93%)	Lactobacillales - 8,132 (0.89%)	Lactobacillales - 7,908 (0.84%)	Sphingomonadales - 10,442 (1.57%)

Table A.10.- Order data of the WWTP samples.

	SNTAB_1_1	SNTC_1_1	SFC_1_1	UTS_1_1	SNTAB_2_1	SNTC_2_1	SNTAB_3_1	SNTC_3_1	UTS_3_1
Family	Bacteroidaceae - 108,684 (15.44%)	Bacteroidaceae - 109,300 (12.95%)	Bacteroidaceae - 129,791 (12.74%)	Comamonadaceae - 56,090 (9.54%)	Pseudomonadaceae - 112,317 (15.61%)	Bacteroidaceae - 110,345 (12.45%)	Bacteroidaceae - 107,675 (14.08%)	Bacteroidaceae - 109,937 (13.83%)	Bacteroidaceae - 56,340 (9.89%)
	Moraxellaceae - 49,159 (6.99%)	Comamonadaceae - 71,913 (8.52%)	Comamonadaceae - 70,398 (6.91%)	Bacteroidaceae - 44,189 (7.51%)	Bacteroidaceae - 87,120 (12.11%)	Campylobacteraceae - 75,201 (8.48%)	Moraxellaceae - 50,847 (6.65%)	Comamonadaceae - 63,342 (7.97%)	Comamonadaceae - 53,743 (9.43%)
	Campylobacteraceae - 48,713 (6.92%)	Pseudomonadaceae - 69,517 (8.24%)	Pseudomonadaceae - 68,877 (6.76%)	Rhodocyclaceae - 24,292 (4.13%)	Campylobacteraceae - 56,531 (7.86%)	Pseudomonadaceae - 74,490 (8.40%)	Campylobacteraceae - 50,715 (6.63%)	Moraxellaceae - 46,592 (5.86%)	Rhodocyclaceae - 19,541 (3.43%)
	Ruminococcaceae - 36,317 (5.16%)	Moraxellaceae - 51,850 (6.14%)	Campylobacteraceae - 64,165 (6.30%)	Burkholderiaceae - 18,774 (3.19%)	Moraxellaceae - 38,647 (5.37%)	Comamonadaceae - 61,123 (6.89%)	Comamonadaceae - 42,433 (5.55%)	Campylobacteraceae - 40,183 (5.06%)	Burkholderiaceae - 16,593 (2.91%)
	Flavobacteriaceae - 36,192 (5.14%)	Rhodocyclaceae - 36,490 (4.32%)	Moraxellaceae - 51,347 (5.04%)	Flavobacteriaceae - 13,474 (2.29%)	Flavobacteriaceae - 37,508 (5.21%)	Moraxellaceae - 41,055 (4.63%)	Ruminococcaceae - 41,221 (5.39%)	Pseudomonadaceae - 37,317 (4.69%)	Campylobacteraceae - 15,385 (2.70%)
	Pseudomonadaceae - 35,846 (5.09%)	Campylobacteraceae - 36,299 (4.30%)	Rhodocyclaceae - 48,984 (4.81%)	Enterobacteriaceae - 13,073 (2.22%)	Comamonadaceae - 36,299 (5.04%)	Flavobacteriaceae - 38,046 (4.29%)	Pseudomonadaceae - 32,749 (4.28%)	Rhodocyclaceae - 36,123 (4.54%)	Enterobacteriaceae - 15,380 (2.70%)
	Comamonadaceae - 33,742 (4.80%)	Prevotellaceae - 33,815 (4.01%)	Flavobacteriaceae - 38,169 (3.75%)	unclassified (derived from Bacteria) - 12,983 (2.21%)	Ruminococcaceae - 29,855 (4.15%)	Ruminococcaceae - 36,058 (4.07%)	Flavobacteriaceae - 31,596 (4.13%)	Ruminococcaceae - 33,876 (4.26%)	Prevotellaceae - 14,125 (2.48%)
	Rhodocyclaceae - 25,700 (3.65%)	Ruminococcaceae - 31,862 (3.78%)	Ruminococcaceae - 37,913 (3.72%)	Ruminococcaceae - 12,356 (2.10%)	Rhodocyclaceae - 26,211 (3.64%)	Rhodocyclaceae - 35,312 (3.98%)	Rhodocyclaceae - 28,044 (3.67%)	Prevotellaceae - 31,609 (3.98%)	unclassified (derived from Bacteria) - 12,962 (2.27%)
	Prevotellaceae - 24,782 (3.52%)	Flavobacteriaceae - 28,977 (3.43%)	Prevotellaceae - 37,799 (3.71%)	Prevotellaceae - 11,892 (2.02%)	Porphyromonadaceae - 19,562 (2.72%)	Prevotellaceae - 31,703 (3.58%)	Prevotellaceae - 26,610 (3.48%)	Flavobacteriaceae - 30,432 (3.83%)	Ruminococcaceae - 12,463 (2.19%)
	Aeromonadaceae - 19,405 (2.76%)	Aeromonadaceae - 28,052 (3.32%)	Aeromonadaceae - 26,552 (2.61%)	Bdellovibrionaceae - 9,928 (1.69%)	Prevotellaceae - 19,098 (2.65%)	Porphyromonadaceae - 23,142 (2.61%)	unclassified (derived from Flavobacteriales) - 19,259 (2.52%)	Aeromonadaceae - 26,626 (3.35%)	Bdellovibrionaceae - 11,053 (1.94%)
	Porphyromonadaceae - 18,054 (2.57%)	Enterobacteriaceae - 28,006 (3.32%)	Enterobacteriaceae - 26,523 (2.60%)	Pseudomonadaceae - 9,852 (1.67%)	unclassified (derived from Flavobacteriales) - 15,566 (2.16%)	Aeromonadaceae - 17,904 (2.02%)	Porphyromonadaceae - 19,093 (2.50%)	Enterobacteriaceae - 20,586 (2.59%)	Flavobacteriaceae - 11,014 (1.93%)
	unclassified (derived from Flavobacteriales) - 14,312 (2.03%)	Porphyromonadaceae - 17,143 (2.03%)	Porphyromonadaceae - 19,142 (1.88%)	Campylobacteraceae - 9,845 (1.67%)	Shewanellaceae - 13,912 (1.93%)	Enterobacteriaceae - 15,908 (1.79%)	Aeromonadaceae - 18,626 (2.44%)	Porphyromonadaceae - 18,069 (2.27%)	Pseudomonadaceae - 9,055 (1.59%)
	Enterobacteriaceae - 14,053 (2.00%)	Rikenellaceae - 14,869 (1.76%)	Clostridiaceae - 17,707 (1.74%)	Sphingomonadaceae - 9,832 (1.67%)	Aeromonadaceae - 13,126 (1.82%)	Rikenellaceae - 14,843 (1.67%)	Lachnospiraceae - 17,061 (2.23%)	Rikenellaceae - 15,494 (1.95%)	Rhodobacteraceae - 8,968 (1.57%)
	Rikenellaceae - 13,908 (1.98%)	Clostridiaceae - 12,774 (1.51%)	Rikenellaceae - 16,880 (1.66%)	unclassified (derived from Burkholderiales) - 9,620 (1.64%)	Rikenellaceae - 11,875 (1.65%)	unclassified (derived from Flavobacteriales) - 14,214 (1.60%)	Enterobacteriaceae - 15,657 (2.05%)	unclassified (derived from Flavobacteriales) - 14,589 (1.84%)	Aeromonadaceae - 7,944 (1.39%)

Table A.11.- Family data of the WWTP samples.

	SNTAB_1_1	SNTC_1_1	SFC_1_1	UTS_1_1	SNTAB_2_1	SNTC_2_1	SNTAB_3_1	SNTC_3_1	UTS_3_1
Genus	Bacteroides - 108,684 (17.55%)	Bacteroides - 109,300 (15.21%)	Bacteroides - 129,791 (14.55%)	Bacteroides - 44,189 (8.81%)	Pseudomonas - 101,399 (15.77%)	Bacteroides - 110,345 (14.10%)	Bacteroides - 107,675 (15.96%)	Bacteroides - 109,937 (15.98%)	Bacteroides - 56,340 (11.60%)
	Arcobacter - 39,129 (6.32%)	Pseudomonas - 58,264 (8.11%)	Pseudomonas - 60,105 (6.74%)	Dechloromonas - 15,473 (3.09%)	Bacteroides - 87,120 (13.55%)	Arcobacter - 62,031 (7.93%)	Acinetobacter - 42,345 (6.28%)	Acinetobacter - 38,823 (5.64%)	Prevotella - 14,125 (2.91%)
	Acinetobacter - 37,961 (6.13%)	Acinetobacter - 41,633 (5.79%)	Arcobacter - 52,861 (5.92%)	unclassified (derived from Bacteria) - 12,082 (2.41%)	Arcobacter - 45,775 (7.12%)	Pseudomonas - 60,538 (7.74%)	Arcobacter - 41,022 (6.08%)	Prevotella - 31,608 (4.59%)	Dechloromonas - 12,316 (2.54%)
	Pseudomonas - 28,342 (4.58%)	Prevotella - 33,809 (4.71%)	Acinetobacter - 41,844 (4.69%)	Prevotella - 11,892 (2.37%)	Acinetobacter - 33,589 (5.22%)	Acinetobacter - 36,582 (4.67%)	Prevotella - 26,609 (3.94%)	Arcobacter - 30,858 (4.48%)	unclassified (derived from Bacteria) - 11,645 (2.40%)
	Prevotella - 24,780 (4.00%)	Arcobacter - 27,480 (3.82%)	Prevotella - 37,794 (4.24%)	Bdellovibrio - 9,928 (1.98%)	Prevotella - 19,095 (2.97%)	Prevotella - 31,701 (4.05%)	Pseudomonas - 24,924 (3.69%)	Pseudomonas - 29,223 (4.25%)	Arcobacter - 11,343 (2.34%)
	Faecalibacterium - 18,254 (2.95%)	Aeromonas - 17,012 (2.37%)	Dechloromonas - 24,418 (2.74%)	Pseudomonas - 7,183 (1.43%)	Flavobacterium - 18,830 (2.93%)	Flavobacterium - 18,256 (2.33%)	Faecalibacterium - 21,442 (3.18%)	Faecalibacterium - 16,851 (2.45%)	Bdellovibrio - 11,053 (2.28%)
	unclassified (derived from Flavobacteriales) - 14,308 (2.31%)	Faecalibacterium - 15,710 (2.19%)	Faecalibacterium - 17,257 (1.93%)	Burkholderia - 7,175 (1.43%)	Faecalibacterium - 16,802 (2.61%)	Faecalibacterium - 18,101 (2.31%)	unclassified (derived from Flavobacteriales) - 19,254 (2.85%)	Aeromonas - 15,863 (2.31%)	Clostridium - 7,139 (1.47%)
	Alistipes - 13,908 (2.25%)	Alistipes - 14,865 (2.07%)	Clostridium - 17,043 (1.91%)	Arcobacter - 6,738 (1.34%)	unclassified (derived from Flavobacteriales) - 15,560 (2.42%)	Paludibacter - 16,964 (2.17%)	Alistipes - 14,539 (2.15%)	Alistipes - 15,494 (2.25%)	Alistipes - 6,357 (1.31%)
	Flavobacterium - 13,006 (2.10%)	Dechloromonas - 14,569 (2.03%)	Aeromonas - 16,973 (1.90%)	Clostridium - 6,478 (1.29%)	Paludibacter - 14,278 (2.22%)	Alistipes - 14,842 (1.90%)	Clostridium - 14,331 (2.12%)	unclassified (derived from Flavobacteriales) - 14,585 (2.12%)	Faecalibacterium - 6,345 (1.31%)
	Paludibacter - 11,795 (1.91%)	Clostridium - 12,186 (1.70%)	Alistipes - 16,880 (1.89%)	Faecalibacterium - 5,886 (1.17%)	Shewanella - 13,912 (2.16%)	unclassified (derived from Flavobacteriales) - 14,200 (1.81%)	Paludibacter - 13,018 (1.93%)	Clostridium - 13,137 (1.91%)	Pseudomonas - 5,932 (1.22%)
	Aeromonas - 11,735 (1.90%)	unclassified (derived from Flavobacteriales) - 11,363 (1.58%)	unclassified (derived from Flavobacteriales) - 13,702 (1.54%)	Acidovorax - 5,769 (1.15%)	Alistipes - 11,875 (1.85%)	Dechloromonas - 13,758 (1.76%)	Flavobacterium - 12,399 (1.84%)	Flavobacterium - 12,684 (1.84%)	Acidovorax - 5,869 (1.21%)
	Clostridium - 11,548 (1.87%)	Acidovorax - 11,123 (1.55%)	Ruminococcus - 12,232 (1.37%)	Polaromonas - 5,684 (1.13%)	Desulfobulbus - 9,518 (1.48%)	Clostridium - 13,175 (1.68%)	Eubacterium - 11,853 (1.76%)	Dechloromonas - 12,253 (1.78%)	Acinetobacter - 5,841 (1.20%)
	Ruminococcus - 10,437 (1.69%)	Paludibacter - 10,944 (1.52%)	Paludibacter - 12,045 (1.35%)	Polynucleobacter - 5,276 (1.05%)	Dechloromonas - 8,322 (1.29%)	Desulfobulbus - 13,056 (1.67%)	Ruminococcus - 11,581 (1.72%)	Paludibacter - 11,765 (1.71%)	Desulfovibrio - 5,500 (1.13%)
	Eubacterium - 9,358 (1.51%)	Flavobacterium - 10,526 (1.47%)	Flavobacterium - 11,001 (1.23%)	Chitinophaga - 5,178 (1.03%)	Aeromonas - 8,253 (1.28%)	Ruminococcus - 10,407 (1.33%)	Aeromonas - 10,882 (1.61%)	Eubacterium - 10,072 (1.46%)	

Table A.12.- Genus data of the WWTP samples.

Appendix 4.- Antibiotic Resistance Genes Data in CWW Samples:

Resistance Genes	CWW_1_1	CWW_1_2	CWW_1_3	CWW_2_1	CWW_3_1	CWW_4_3	CWW_5_1	CWW_6_3	Total
aac(3)-Ia	✓	✓	✓	✓	✓	✓	✓	✓	8
aac(3)-Ib	✓		✓	✓	✓	✓	✓	✓	7
aac(3)-Id	✓	✓	✓	✓	✓	✓	✓	✓	8
aac(3)-IIa	✓					✓	✓	✓	4
aac(3)-IId		✓	✓	✓	✓		✓		5
aac(6')-29b					✓		✓	✓	3
aac(6')-aph(2")	✓	✓	✓	✓	✓	✓	✓	✓	8
aac(6')-Ia					✓				1
aac(6')-IIa					✓	✓	✓	✓	4
aac(6')-Ib3	✓	✓	✓	✓			✓	✓	6
aac(6')-Ic	✓	✓	✓	✓	✓	✓	✓	✓	8
aac(6')-Im		✓							1
aac(6')-Ip			✓						1
aac(6')-Iz			✓						1
aadA1		✓	✓	✓	✓	✓	✓	✓	7
aadA10					✓	✓	✓	✓	4
aadA11	✓	✓	✓	✓	✓	✓	✓	✓	8
aadA13					✓		✓		2
aadA15	✓								1
aadA16							✓		1
aadA2		✓	✓		✓	✓	✓	✓	6
aadA24			✓						1

Resistance Genes	CWW_1_1	CWW_1_2	CWW_1_3	CWW_2_1	CWW_3_1	CWW_4_3	CWW_5_1	CWW_6_3	Total
aadA3						✓	✓		2
aadA4	✓		✓						2
aadA5	✓	✓	✓	✓	✓	✓	✓	✓	8
aadA7		✓					✓	✓	3
ant(2")	✓	✓	✓	✓	✓	✓	✓	✓	8
ant(3")						✓			1
ant(6)-Ia	✓	✓	✓	✓	✓	✓	✓	✓	8
ant(6)-Ib						✓	✓	✓	3
ant(9)-Ia	✓								1
aph(2")-Ib				✓					1
aph(3')-Ia	✓	✓	✓	✓	✓	✓	✓	✓	8
aph(3")-Ib	✓	✓	✓	✓	✓	✓	✓	✓	8
aph(3')-Ib				✓	✓				2
aph(3')-IIa	✓						✓		2
aph(3')-IIc					✓		✓		2
aph(3')-III		✓	✓	✓	✓	✓	✓	✓	7
aph(3')-VI	✓		✓	✓				✓	4
aph(3')-VIa		✓			✓	✓			3
aph(6)-Ic		✓							1
aph(6)-Id	✓	✓	✓	✓	✓	✓	✓	✓	8
str				✓		✓			2

Table A.13.- Aminoglycoside Resistance Genes present on of the CWW samples.

Resistance Genes	CWW_1_1	CWW_1_2	CWW_1_3	CWW_2_1	CWW_3_1	CWW_4_3	CWW_5_1	CWW_6_3	Total
ampH_1	✓					✓			2
ampS_1						✓	✓		2
blaACI-1			✓			✓			2
blaAER-1	✓	✓	✓	✓	✓	✓	✓	✓	8
blaBEL-1				✓	✓				2
blaCARB-1				✓					1
blaDES-1				✓					1
blaEBR-1				✓	✓				2
blaFOX-2								✓	1
blaGES-5	✓	✓	✓	✓	✓	✓	✓	✓	8
blaIMP-13	✓	✓	✓	✓	✓	✓	✓	✓	8
blaIMP-58			✓						1
blaLCR-1	✓	✓		✓	✓				4
blaMOX-2			✓		✓	✓		✓	4
blaMOX-3							✓		1
blaMOX-4	✓	✓							2
blaNPS-1			✓	✓	✓	✓	✓		5
blaOCH-4					✓				1
blaOKP-B-8						✓	✓		2
blaOXA-1				✓	✓	✓			3
blaOXA-2	✓	✓	✓	✓	✓	✓	✓	✓	8
blaOXA-4			✓					✓	2
blaOXA-5							✓		1
blaOXA-9	✓	✓		✓	✓	✓	✓	✓	7
blaOXA-10	✓	✓	✓	✓	✓	✓	✓	✓	8
blaOXA-18					✓				1
blaOXA-20	✓				✓		✓	✓	4
blaOXA-21					✓				1
blaOXA-58	✓	✓	✓	✓	✓	✓	✓	✓	8
blaOXA-72	✓	✓	✓	✓	✓	✓		✓	7
blaOXA-118							✓	✓	2
blaOXA-119	✓	✓	✓			✓	✓	✓	6
blaOXA-129	✓	✓	✓	✓	✓	✓	✓		7

Resistance Genes	CWW_1_1	CWW_1_2	CWW_1_3	CWW_2_1	CWW_3_1	CWW_4_3	CWW_5_1	CWW_6_3	Total
blaOXA-198				✓					1
blaOXA-205				✓	✓		✓		3
blaOXA-209					✓				1
blaOXA-211		✓		✓					2
blaOXA-257			✓						1
blaOXA-275				✓					1
blaOXA-280	✓		✓		✓	✓	✓		5
blaOXA-281								✓	1
blaOXA-296	✓		✓	✓	✓	✓	✓		6
blaOXA-299	✓								1
blaOXA-300	✓								1
blaOXA-347	✓	✓	✓	✓	✓	✓	✓	✓	8
blaOXA-372	✓	✓	✓	✓	✓	✓	✓	✓	8
blaOXA-392	✓						✓		2
blaOXA-427	✓	✓	✓	✓	✓	✓	✓	✓	8
blaOXA-437							✓		1
blaOXA-464	✓	✓	✓	✓	✓	✓	✓	✓	8
blaOXA-490	✓	✓							2
blaPER-1							✓		1
blaSHV-27		✓							1
blaSHV-67							✓		1
blaSHV-150								✓	1
blaTEM-1		✓	✓	✓		✓			4
blaTEM-116					✓				1
blaTLA-1						✓	✓		2
blaVEB-1	✓	✓	✓	✓	✓	✓	✓	✓	8
blaVIM-48					✓	✓			2
blaZ								✓	1
cepA	✓	✓	✓		✓	✓		✓	6
CfxA			✓	✓	✓	✓	✓	✓	6
cfxA3		✓							1
cfxA5	✓								1
cfxA6	✓	✓	✓	✓	✓		✓	✓	7
cphA		✓	✓		✓	✓	✓		5
imiH								✓	1

Table A.14.- Betalactam Resistance Genes present on of the CWW samples.

Resistance Genes	CWW_1_1	CWW_1_2	CWW_1_3	CWW_2_1	CWW_3_1	CWW_4_3	CWW_5_1	CWW_6_3	Total
arr-2						✓		✓	2
arr-6				✓			✓		2
cat	✓	✓	✓	✓	✓	✓	✓	✓	8
catA1	✓		✓	✓	✓	✓	✓	✓	7
catA2			✓						1
catB3	✓		✓	✓	✓	✓	✓		6
catP			✓						1
catQ	✓			✓		✓			3
catS		✓	✓				✓	✓	4
cmlA1	✓	✓	✓	✓	✓	✓	✓	✓	8
cmlB1			✓						1
floR	✓	✓	✓	✓	✓	✓		✓	7
cfr(B)			✓						1
cfr(C)	✓	✓	✓	✓	✓		✓	✓	7
lsa(E)	✓	✓	✓	✓	✓	✓	✓	✓	8
mdf(A)	✓	✓	✓	✓	✓	✓	✓	✓	8
msr(D)					✓	✓	✓	✓	4
msr(E)	✓	✓	✓	✓	✓	✓	✓	✓	8
dfrA1				✓	✓				2
dfrA5								✓	1
dfrA7			✓						1
dfrA12			✓						1
dfrA14	✓		✓	✓	✓	✓	✓	✓	7
dfrA15		✓		✓					2
dfrA32	✓				✓				2
dfrB1							✓		1
ereA	✓	✓	✓	✓	✓	✓	✓	✓	8
ereB			✓		✓	✓	✓	✓	5
ereD	✓	✓	✓	✓	✓	✓	✓	✓	8
mef(A)/msr(D)	✓	✓	✓	✓	✓	✓	✓	✓	8
mef(B)	✓			✓	✓	✓	✓	✓	6
mef(C)	✓	✓	✓	✓	✓	✓	✓	✓	8
mph(A)	✓	✓		✓	✓	✓	✓	✓	7
mph(E)	✓	✓	✓	✓	✓	✓	✓	✓	8
mph(G)	✓	✓	✓	✓	✓	✓	✓	✓	8
erm(42)				✓	✓		✓	✓	4
erm(A)		✓	✓		✓		✓		4
erm(B)	✓	✓	✓	✓	✓	✓	✓	✓	8
erm(F)	✓	✓	✓	✓	✓	✓	✓	✓	8
erm(G)	✓	✓	✓	✓	✓	✓	✓	✓	8

Resistance Genes	CWW_1_1	CWW_1_2	CWW_1_3	CWW_2_1	CWW_3_1	CWW_4_3	CWW_5_1	CWW_6_3	Total
fosA				✓	✓	✓	✓	✓	5
fosA5	✓								1
lnu(B)			✓	✓	✓	✓	✓		5
lnu(C)	✓	✓	✓	✓	✓	✓	✓	✓	8
lnu(F)			✓	✓	✓	✓	✓	✓	6
mcr-3.12							✓		1
mcr-3.17	✓					✓	✓		3
mcr-5.1			✓		✓				2
mcr-5.2						✓			1
nimA		✓		✓					2
nimD		✓		✓	✓			✓	4
nimE	✓			✓		✓		✓	4
nimJ			✓						1
oqxA	✓	✓	✓		✓		✓	✓	6
oqxB	✓	✓	✓		✓	✓	✓	✓	7
qnrS2	✓	✓	✓	✓	✓	✓	✓	✓	8
qnrVC4	✓	✓		✓	✓	✓	✓	✓	7
sul1	✓	✓	✓	✓	✓	✓	✓	✓	8
sul2	✓	✓	✓	✓	✓	✓	✓	✓	8
sul4						✓	✓		2
tet(32)	✓	✓	✓	✓	✓	✓	✓	✓	8
tet(36)	✓		✓	✓	✓	✓	✓	✓	7
tet(39)	✓	✓	✓	✓	✓	✓	✓	✓	8
tet(40)	✓	✓	✓	✓	✓	✓	✓	✓	8
tet(44)	✓	✓	✓	✓	✓	✓	✓	✓	8
tet(A)	✓	✓	✓	✓	✓	✓	✓	✓	8
tet(B)			✓	✓		✓			3
tet(C)	✓	✓	✓	✓	✓	✓	✓	✓	8
tet(E)	✓	✓	✓		✓	✓	✓		6
tet(G)			✓	✓		✓	✓		4
tet(M)	✓	✓	✓	✓	✓	✓	✓	✓	8
tet(O)	✓	✓	✓	✓	✓		✓	✓	7
tet(O/32/O)					✓	✓	✓	✓	4
tet(P)/tetA(P)						✓			1
tet(Q)	✓	✓	✓	✓	✓	✓	✓	✓	8
tet(W)	✓	✓	✓	✓	✓	✓	✓	✓	8
tet(X)	✓	✓	✓	✓	✓	✓	✓	✓	8
VanGXY	✓	✓	✓	✓	✓	✓	✓		7
VanHBX	✓			✓	✓	✓			4

Table A.15.- Rest of the Resistance Genes present on of the CWW samples.

Resistance Genes	CWW_1_1	CWW_1_2	CWW_1_3	CWW_2_1	CWW_3_1	CWW_4_3	CWW_5_1	CWW_6_3	Always present	Mean	Total	Always present %
Aminoglicoside resistance genes	19	20	22	20	24	23	28	23	11	22.22	43	25.58
Betalactam resistance genes	28	25	27	29	34	30	32	26	11	28.74	68	16.18
Rifamycin resistance genes	0	0	0	1	0	1	1	1	0	0.5	2	0.00
Chloranphenicol resistance genes	6	4	9	6	5	6	5	5	2	5.75	10	20.00
Diaminopyrimidines resistance genes	2	1	3	3	3	1	2	2	0	2.125	8	0.00
Macrolides resistance genes	8	7	7	8	9	9	9	9	6	8.25	9	66.67
MLSb phenotype resistance genes	3	4	4	4	5	3	5	4	3	4	5	60.00
Lincosamides resistance genes	1	1	3	3	3	3	3	2	1	2.375	3	33.33
Polypeptide resistance genes	1	0	1	0	1	2	2	0	0	0.875	4	0.00
Nitroimidazoles resistance genes	1	2	1	3	1	1	0	2	0	1.375	4	0.00
Fluoroquinolones resistance genes	4	4	3	2	4	3	4	4	1	3.5	4	25.00
Sulfonamides resistance genes	2	2	2	2	2	3	3	2	2	2.25	3	66.67
Tetracyclines resistance genes	13	12	15	14	14	16	15	13	10	14	17	58.82
Glycopeptides resistance genes	2	1	1	2	2	2	1	0	0	1.375	2	0.00
Mixed resistance genes	4	4	5	4	5	4	5	5	3	4.5	6	50.00
Other resistance genes	1	0	0	1	1	1	1	1	0	0.75	2	0.00
Total	95	87	103	102	113	108	116	99	50	102.9	190	26.32

Table A.16.- Data about the Resistance Genes present on of the CWW samples.

Appendix 5 .- Antibiotic Resistance Genes Data in WWTP Samples:

Resistance Genes	SNTAB_1_1	SNTC_1_1	SFC_1_1	UTS_1_1	SNTAB_2_1	SNTC_2_1	SNTAB_3_1	SNTC_3_1	UTS_3_1	
aac(3)-Ia			✓		✓		✓	✓		4
aac(3)-Id	✓									1
aac(3)-IIa									✓	1
aac(3)-IId		✓								1
aac(6')-Ia		✓	✓							2
aac(6')-Ib3	✓	✓				✓		✓		4
aac(6')-Ib-Hangzhou					✓					1
aadA1	✓		✓	✓			✓	✓	✓	6
aadA10		✓	✓	✓	✓	✓	✓	✓	✓	8
aadA11		✓		✓		✓	✓	✓	✓	6
aadA13	✓	✓	✓			✓	✓	✓		6
aadA2	✓	✓			✓		✓	✓		5
aadA24				✓						1
aadA3			✓				✓	✓		3
aadA4					✓		✓			2
aadA5	✓	✓	✓		✓	✓		✓	✓	7

Resistance Genes	SNTAB_1_1	SNTC_1_1	SFC_1_1	UTS_1_1	SNTAB_2_1	SNTC_2_1	SNTAB_3_1	SNTC_3_1	UTS_3_1	
aadA6	✓		✓		✓				✓	4
aadA7	✓	✓						✓		3
aadA8b						✓				1
aadD/ANT(4')-Ia			✓							1
ant(2")	✓	✓				✓	✓	✓		5
ant(3")					✓	✓		✓	✓	4
ant(6)-Ia	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
ant(6)-Ib		✓	✓				✓			3
aph(3')-Ia	✓	✓			✓					3
aph(3")-Ib	✓	✓	✓	✓		✓	✓	✓	✓	8
aph(3')-III	✓	✓	✓	✓	✓	✓	✓	✓		8
aph(3')-VI									✓	1
aph(6)-Id	✓	✓	✓	✓	✓	✓	✓	✓	✓	9

Table A.17.- Aminoglycoside Resistance Genes present on of the WWTP samples.

Resistance Genes	SNTAB_1_1	SNTC_1_1	SFC_1_1	UTS_1_1	SNTAB_2_1	SNTC_2_1	SNTAB_3_1	SNTC_3_1	UTS_3_1	
ampH_1							✓			1
ampS_1			✓	✓	✓					3
blaACI-1			✓						✓	2
blaAER-1	✓	✓	✓	✓	✓	✓	✓	✓		8
blaBEL-1			✓		✓		✓			3
blaBRO-2			✓		✓		✓	✓		4
blaCARB-5							✓			1
blaCARB-10				✓	✓					2
blaCMY-1			✓							1
blaDES-1									✓	1
blaEBR-1		✓	✓				✓			3
blaFOX-3							✓			1
blaFOX-4					✓			✓		2
blaGES-5	✓	✓	✓		✓	✓	✓	✓		7
blaIMP-5				✓						1
blaIMP-13				✓					✓	2
blaIMP-22					✓					1
blaIMP-70									✓	1
blaKHM-1							✓			1
blaLCR-1		✓			✓	✓	✓	✓		5
blaMOX-2		✓	✓					✓		3
blaMOX-5	✓			✓						2
blaMOX-6		✓								1
blaNPS-1	✓		✓		✓					3
blaOXA-1	✓				✓					2
blaOXA-2	✓	✓	✓		✓	✓	✓	✓		7
blaOXA-4									✓	1
blaOXA-5			✓	✓					✓	3
blaOXA-7			✓							1
blaOXA-9		✓			✓			✓		3
blaOXA-10	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
blaOXA-20	✓	✓	✓	✓	✓	✓	✓	✓		8
blaOXA-58		✓	✓				✓	✓		4
blaOXA-118			✓	✓						2
blaOXA-119	✓		✓		✓	✓	✓	✓		6

Resistance Genes	SNTAB_1_1	SNTC_1_1	SFC_1_1	UTS_1_1	SNTAB_2_1	SNTC_2_1	SNTAB_3_1	SNTC_3_1	UTS_3_1	
blaOXA-129	✓	✓	✓		✓	✓	✓	✓		7
blaOXA-205		✓	✓						✓	3
blaOXA-209	✓	✓			✓	✓	✓	✓		6
blaOXA-211	✓	✓	✓		✓	✓	✓			6
blaOXA-280								✓	✓	2
blaOXA-282			✓							1
blaOXA-296	✓	✓	✓	✓	✓	✓	✓	✓		8
blaOXA-333					✓					1
blaOXA-347	✓	✓	✓		✓	✓	✓	✓		7
blaOXA-372	✓	✓	✓		✓	✓	✓	✓		7
blaOXA-392		✓	✓			✓	✓	✓		5
blaOXA-415				✓						1
blaOXA-427	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
blaOXA-464		✓	✓			✓			✓	4
blaOXA-490		✓		✓			✓			3
blaOXA-491					✓		✓			2
blaOXA-534				✓						1
blaOXA-549		✓			✓					2
blaOXA-551					✓					1
blaPER-2					✓					1
blaSHV-148									✓	1
blaSHV-150		✓								1
blaTEM-1			✓						✓	2
blaTLA-1			✓							1
blaVEB-1	✓	✓	✓		✓	✓	✓	✓		7
blaVEB-2	✓									1
blaVEB-3			✓							1
blaVEB-6							✓			1
cepA				✓		✓				2
cfiA4			✓							1
CfxA	✓	✓	✓	✓			✓	✓	✓	7
cfxA3					✓	✓				2
cfxA6	✓	✓	✓		✓	✓	✓	✓	✓	8
cphA	✓	✓						✓		3

Table A.18.- Betalactam Resistance Genes present on of the WWTP samples.

Resistance Genes	SNTAB_1_1	SNTC_1_1	SFC_1_1	UTS_1_1	SNTAB_2_1	SNTC_2_1	SNTAB_3_1	SNTC_3_1	UTS_3_1	
cat	✓	✓	✓		✓	✓	✓	✓		7
catA1	✓									1
catA2					✓					1
catB3		✓	✓		✓	✓		✓	✓	6
catP	✓									1
catQ	✓	✓	✓		✓			✓	✓	6
catS		✓				✓				2
cmlA1	✓	✓	✓		✓	✓	✓	✓		7
cmlA4			✓							1
cfr(C)					✓		✓			2
lsa(E)	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
mdf(A)									✓	1
msr(D)	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
msr(E)	✓	✓	✓	✓	✓	✓	✓	✓		8
dfrA1	✓	✓			✓					3
dfrA3		✓	✓		✓		✓			4
dfrA7					✓					1
dfrA14		✓	✓			✓	✓	✓		5
dfrA15			✓							1
dfrA16						✓				1
dfrB1					✓		✓			2
dfrB3				✓						1
dfrB4								✓		1
ereA	✓	✓	✓		✓	✓	✓	✓	✓	8
ereB		✓	✓				✓			3
ereD	✓	✓	✓		✓	✓	✓	✓		7
mef(A)/msr(D)	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
mef(B)	✓	✓	✓	✓	✓	✓	✓	✓		8
mef(C)	✓	✓	✓	✓	✓	✓	✓	✓		8
mph(A)		✓	✓		✓	✓	✓	✓		6
mph(B)	✓									1
mph(E)	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
mph(G)	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
mph(N)	✓	✓	✓		✓	✓	✓	✓		7
erm(A)		✓								1
erm(B)	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
erm(F)	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
erm(G)	✓	✓	✓		✓	✓	✓	✓	✓	8

Resistance Genes	SNTAB_1_1	SNTC_1_1	SFC_1_1	UTS_1_1	SNTAB_2_1	SNTC_2_1	SNTAB_3_1	SNTC_3_1	UTS_3_1	
erm(Q)					✓					1
fosA		✓	✓		✓		✓	✓		5
lnu(B)	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
lnu(C)	✓	✓	✓	✓	✓	✓		✓	✓	8
lnu(D)						✓	✓			2
lnu(F)	✓	✓	✓		✓	✓	✓	✓	✓	8
mcr-3.12					✓					1
mcr-3.17		✓	✓					✓		3
mcr-3.3									✓	1
mcr-3.6	✓						✓			2
mcr-5.1	✓	✓	✓		✓	✓	✓	✓		7
nimE	✓									1
oqxA		✓	✓				✓	✓		4
oqxB		✓	✓		✓			✓		4
qnrB19		✓						✓		2
qnrD1		✓								1
qnrS2	✓	✓	✓	✓	✓	✓	✓	✓		8
qnrVC4		✓	✓		✓		✓			4
sul1	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
sul2	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
sul4	✓	✓			✓	✓				4
tet(32)	✓	✓	✓		✓	✓	✓	✓	✓	8
tet(36)	✓	✓	✓		✓	✓	✓	✓		7
tet(39)	✓	✓	✓	✓	✓	✓	✓	✓		8
tet(40)	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
tet(44)	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
tet(A)	✓	✓	✓	✓	✓	✓	✓	✓		8
tet(B)			✓							1
tet(C)	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
tet(E)	✓	✓	✓		✓	✓	✓	✓		7
tet(G)		✓			✓		✓			3
tet(M)	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
tet(O)	✓		✓	✓	✓	✓	✓	✓	✓	8
tet(O/32/O)		✓		✓	✓					3
tet(P)/tetA(P)	✓						✓			2
tet(Q)	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
tet(W)	✓	✓	✓	✓	✓	✓	✓	✓	✓	9
tet(X)	✓	✓	✓	✓	✓	✓	✓	✓	✓	9

Table A.19.- Rest of the Resistance Genes present on of the WWTP samples.

Resistance Genes	SNTAB_1_1	SNTC_1_1	SFC_1_1	UTS_1_1	SNTAB_2_1	SNTC_2_1	SNTAB_3_1	SNTC_3_1	UTS_3_1	Always Present	Mean	Total	Always present %
Aminoglicoside resistance genes	14	16	14	8	12	12	14	16	11	2	13	29	6.90
Betalactam resistance genes	21	28	35	17	31	20	29	24	15	2	24.44	69	2.90
Chloranphenicol resistance genes	5	5	5	0	5	4	2	4	2	0	3.556	9	0.00
Diaminopyrimidines resistance genes	1	3	3	1	4	2	3	2	0	0	2.111	9	0.00
Macrolides resistance genes	9	9	9	5	8	8	9	8	4	3	7.667	11	27.27
MLSb phenotype resistance genes	3	4	3	2	3	3	3	3	3	2	3	5	40.00
Lincosamides resistance genes	3	3	3	2	3	4	3	3	3	1	3	4	25.00
Polypeptide resistance genes	2	2	2	0	2	1	2	2	1	0	1.556	5	0.00
Nitroimidazoles resistance genes	1	0	0	0	0	0	0	0	0	0	0.111	1	0.00
Fluoroquinolones resistance genes	1	6	4	1	3	1	3	4	0	0	2.556	6	0.00
Sulfonamides resistance genes	3	3	2	2	3	3	2	2	2	2	2.444	3	66.67
Tetracyclines resistance genes	14	14	14	11	15	13	15	13	9	7	13.11	17	41.18
Mixed resistance genes	3	3	3	3	4	3	4	3	3	2	3.222	5	40.00
Other resistance genes	0	1	1	0	1	0	1	1	0	0	0.556	1	0.00
Total	80	98	99	52	96	75	91	86	53	21	81.11	174	12.07

Table A.20.- Data about the Resistance Genes present on of the WWTP samples.