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Metagenomic analyses of antibiotics resistance genes and their bacterial hosts in waste water samples collected from Al-Bierh wastewater treatment plant in Palestine

Musab Idreis Taha Hroub

Master Thesis

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Dedication

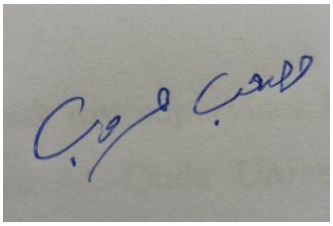
To my lovely wife and my kids, my parents, and dear sisters and brothers, for their encouragement and support. I would like to dedicate my work to every one stand with me in the hardest condition without let me down.

With love.

Declaration:

I certify that this thesis submitted for the degree of Master, is the result of my own research, except there otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Sign



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Abstract

Wastewater treatment plants (WWTPs) are considered as a hotspot for the proliferation and dissemination of antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARGs). In the West Bank, there are four working WWTPs in Jenin, Tulkarim, Ramallah, and Al-Bierh. Most of them have secondary treatment which depend on the activated sludge process except of Tulkarim plant which has only primary treatment. All of the effluents from those plants are released into the wadies. So, it has an adverse effect on both environment and human. In this study, Illumina high-throughput sequence analysis was used to determine the profile of ARB and ARGs in Al-Bierh WWTP. Raw waste water sample (influent) and secondary treated water sample (effluent) were collected over seasons, Summer (August) and Winter (February) 2018. DNA was extracted from each sample, quantified and used in DNA library preparation. The DNA was fragmented randomly to small fragments by transposome enzyme followed by enrichment in which two indices were added to each sample for barcoding. The DNA library was cleaned up to select the fragments of 300-500 bp size and sent for deep sequencing by Nextseq500 machine using 150-cycles mid output kit (single end read). The sequencing data was received as FASTAQ files and uploaded at galaxy platform *(https://usegalaxy.org/)* for bioinformatic analysis. The results showed a higher number of ARB (53 species) and a wide diversity of ARGs (400 subtypes) in February samples than August samples in which 30 ARB species and 253 ARGs subtypes were detected. There was a significant difference ($P<0.01$, $r= 0.9$) in the relative abundance of ARB bacteria and ARGs between the two seasons. The most abundant species found in both seasons and across the samples was *Acinetobacter baumannii* followed by *Escherichia coli* and *Klebsiella pneumoniae. Acinetobacter baumannii* commonly isolated from intensive care unit, and cause many diseases include respiratory, urinary, blood and skin infections. In addition, it has the ability to escape and resist antibiotics and classified by the WHO as a number one opportunistic and harmful bacteria. In this study, 107 Different antibiotics resistance genes conferring resistance to 12 antibiotic classes were detected. The most abundant antibiotic resistance group was macrolide and tetracycline. The removal efficiency of the top 10 ARB and ARGs was high ranged from 85-100%. Nonetheless, there is a concern of spreading and pre-filtration of ARB and ARGs in the WWTP which may be disposed to the environment through effluent and may threaten the public health and cause harm to the environment and humans. Therefore, we recommend to increase the awareness among locals about the effect of wastewater and accompanied pathogens on the human health and environment. Moreover, improving the sanitation and treatment systems should be a priority to policy makers to limit the burden of ARB and ARGs in treated waste water in Palestine.

Keywords: WWTPs, ARGs, ARB.

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List of Abbreviations

WWT	Wastewater treatment
WW	Wastewater
WWTPs	Wastewater treatment plants
BOD	Biological oxygen demand
UV	Ultraviolet
AOP	Advanced oxidation process
DO	Dissolved oxygen
COD	Chemical oxygen demand
TDS	Total dissolved solid
TSS	Total suspended solid
AS	Activated sludge
PCs	Pharmaceutical compounds
IW	Incoming wastewater
ARGs	Antibiotic resistance genes
ARB	Antibiotic resistance bacteria

MGEs	Mobile genetic elements
ESBL	Extended spectrum beta lactamases
MCM	Million cubic meters
PVDF	Polyvinylidene difluoride
°C	Celsius
gDNA	Genomic DNA
PCR	Polymerase Chain Reaction
bp	Base pair
NCBI	National Centre for Biotechnology Information
HGT	Horizontal gene transfer
UTIs	Urinary tract infections

Chapter one

Introduction

1.Introduction

1.1 background

Wastewater treatment (WWT) is a method of removal the pollutants and contaminants from wastewater (WW). The process consists of a biological, chemical, and physical pathways to remove contaminants. After treating of wastewater, it could be released safely to the environment. Sludge is a by-product of the treatment process. The sludge that comes from sewage called sewage sludge and needs further processing before use or elimination. It consists of two types, sludge and activated sludge, which contains a variety of organic and inorganic compounds. Sludge is consisting of a large percentage of water, and come from liquid wastewater that contains little amount of solid. There are two types of it, primary that come as a result of primary treatment, and secondary that result from secondary treatment process. The difference between sewage and sludge is that the former is a suspension of solid and water waste, in contrast, the sludge is solid separated from suspension in a liquid (Edris and Alalayah 2017).

World's need for water is increasing with the limited source of water, particularly in dried and semi-dried areas like Africa, South Asia, southern Europe, and the Middle East. Due to an increase in population number and urbanization, the need for wastewater treated plants (WWTPs) is become crucial to offer water that can be used as a source of water for agriculture (Gatica and Cytryn 2013). WWTPs have been used in the last decades to treat the water that results from human activities e.g., hospital, industry, domestic (including sewage), etc.

1.2 Wastewater Treatment Types

The WW treatment process includes three steps: physio-chemical as a primary step, biological as a secondary step, and tertiary process that executes special methods, e.g., advanced oxidative process. The main goal is to obtain high-quality water to be reused in a different application (Garrido-Cardenas, Polo-Lopez et al. 2017). As illustrated in Figure (1.1) (Modin, Persson et al. 2016).

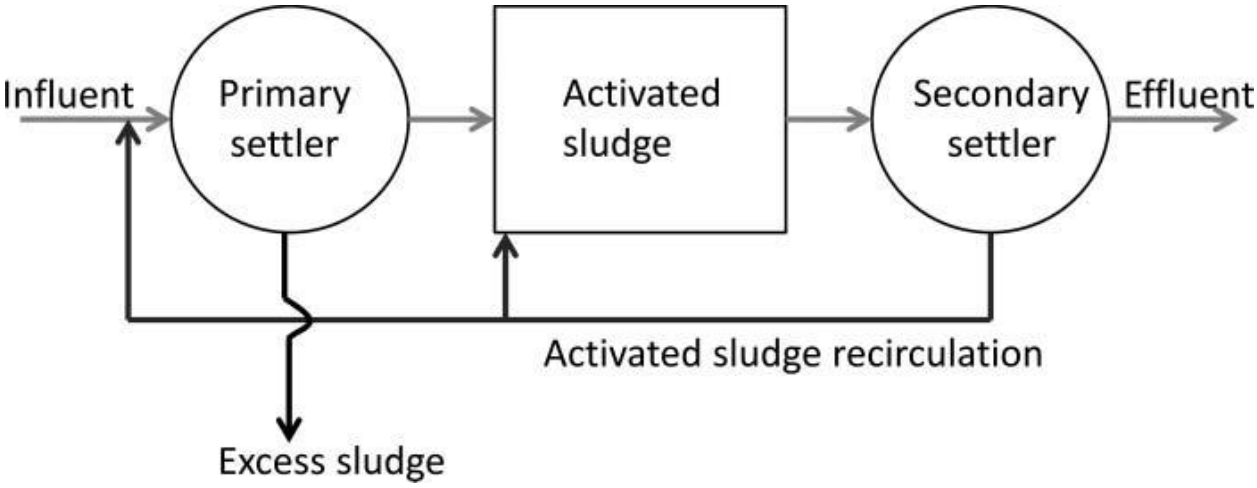


Figure 1.1: Flow process of WWTPs treatments

1.2.1 Primary treatment

In the first stage of WW treatment, the sewage flows through the large tank to settle down by gravity effect and the oil with grease is float on the surface which removed off. The sediment solid or grit is removed from the bottom and the scum washed off using water jets. Sludge is combined with these two previous components. Some of the light solid are suspended in water and called primary sludge, which treated later on to become bio solid (Sonune and Ghate 2004, Larsdotter 2006, Edris and Alalayah 2017).

1.2.2 Secondary treatment

In the second stage of treatment materials and organic substances are biologically degraded. This was done by using a community of microorganisms which reduce biological oxygen demand (BOD) by the oxidizing organic compound to carbon dioxide, water and oxidize ammonium. The main bacteria found is heterotrophic bacteria and protozoa in which the bacteria degrade the organic compounds and the protozoa graze the bacteria. The nutrients needed for microorganisms are obtained from the organic substances found in the WW. After microorganisms are fed, their density increased and by this effect, the process is done at the bottom of the water. The cleaned water is gathered and called secondary sludge or activated sludge. The further process needed to separate those microorganisms from the water before tertiary treatment. The organic substances in this sludge processed to finally get carbon dioxide (CO_2) by the action of aerobic fermentation, in contrast, anaerobic fermentation will produce methane. This will be useful as fuel for domestic uses like cooking. After the sludge move from the digester, 50% of its volume is removed (Larsdotter 2006, Edris and Alalayah 2017).

1.2.3 Disinfection

A process follows secondary treatment that treat waste water chemically or by radiation to further treating waste water. There are various methods used in the disinfection process, the following are the most commonly used one.

1.2.3.1 Chlorination

One of the most commonly used methods with its low cost and effective properties in tertiary treatment is chlorination. In this method chlorine gas, sodium hypochlorite, or calcium hypochlorite will be added to the water in the final step. However, there is a disadvantage to this method which is the production of toxic material, i.e., trichloromethanes and other chloramines (Naidoo and Olaniran 2013, Ferro, Polo-López et al. 2016).This method mainly used in Palestinian WWTPs (Abusharbak 2004).

1.2.3.2 Ultraviolet radiation

In this method, the action of the ultraviolet (UV) radiation will be on the genetic material of microorganisms such as DNA or RNA,that will inhibit the replication of the microorganisms. Wavelength used is 250-270 nm (Ferro, Polo-López et al. 2016). The UV radiation is mainly used due to its lower by-products toxic materials released to the environment. So, it does not affect human or aquatic life. It uses a mercury arc lamp to produce the UV waves. There are many factors to insure

more effective treatment including, UV light intensity, the quality of effluent, the length of the path from the lamp and exposure time. The mechanism of destruction of bacterial DNA is done by the formation of thymine dimers that will influence the cell replication and infection of the host. UV radiation will penetrate the cell wall of microorganisms and stop its replication. If it is given at a low dose the microorganisms will repair their DNA by repair pathway (Naidoo and Olaniran 2013).

1.2.3.3 Advanced oxidation process (AOP)

In this method, oxidation agents like H_2O_2, or O_3 are used to degrade substances. This method is based on the generation of hydroxyl radical(HO•) which has a total or partial ability to degrade organic matters (Ferro, Polo-López et al. 2016).

1.2.4 Tertiary treatment

To have high-quality water and enhance effluent quality, a third stage is needed by adding special matters or using special techniques. Another name for tertiary treatment is effluent polishing. It involves physical treatment that remove additional nutrient like nitrogen and phosphate, or sand filtration and carbon adsorption to ensure further removal of suspended solids and remained microorganisms like fecal coliforms, *Streptococci*, S*almonella* sp. And enteric viruses which not removed by previous treatments (Naidoo and Olaniran 2013, Edris and Alalayah 2017).

1.2.4.1 Nutrients removal

This process aims to remove nitrogen and phosphorus. Nitrogen removed by the nitrification-denitrification reaction. But ammonium is oxidized to nitrite under the aerobic condition and then converted to nitrate then finally to nitrogen gas N_2. On the contrary, phosphorus is removed from wastewater by mean of sedimentation with aluminum or iron to give ferric phosphate or aluminum phosphate as a final product. Also, it benefits in the removal of microorganisms from the previous section (secondary process) like fecal coliforms, streptococci, Salmonella spp., and enteric viruses (Larsdotter 2006, Naidoo and Olaniran 2013).

1.2.4.2 Filtration

Membrane filtration is one of the advanced methods used in this treatment by mean of removal of small pollutants. It divided into different types based on the size of the materials intended for be removed. First, media-coated filters which consist of several layers of media such as sand that can trap pollutant in the pores or by adherence of the pollutants to the surface of the media particles. Second,

the Pressure-driven membrane includes different types of filtration like microfiltration, ultrafiltration, nanofiltration, and reciprocal osmosis which remove micropollutants (Naidoo and Olaniran 2013).

1.2.4.3 Activated carbon

It commonly used in industrial WWT. It can remove soluble organic and inorganic materials like heavy metals, nitrates, and pharmaceutical compounds. This process is done when the WW move over the bed of activated carbon granules. Thermal activation will enhance the adsorption of these pollutants on carbon particles. The removal rate is affected by the type of materials found. The organic compound will lower the available adsorption site (Naidoo and Olaniran 2013).

1.3 Physical and Chemical Characteristics of Wastewater

There are many variables are used to determine the quality of water this include: phosphate, nitrate, suspended solid, nitrite and ammonia nitrogen, dissolved oxygen (DO), chemical oxygen demand (COD), BOD, pH, salinity and trace metals. Besides interspecies interactions like predation, competition, symbiosis, etc. These factors will select the abundance of microorganisms.

The presence of those pollutants in a higher concentration than normal will lead to serious problems that affect human and animal health, besides, cause a drawback in the treatment process (Lee, Kang et al. 2015).

The concentration of the hydrogen ion is crucial for wastewater, which shows the acid-base proprieties of wastewater. If the pH is less than 7, this will indicate the source of the septic condition in which it will be acidic and tends to be corrosive, also if the pH less than 5 or more than 10, this means that the waste is an industrial source. In both conditions, the waste is not suitable for the biological process because it destroys the biological treatment process.

Another important parameter is DO. It is needed for the respiration of microorganisms. The concentration of it depends on the temperature, atmospheric pressure, solubility, and salinity. In addition, BOD and COD determine how much oxygen demand needed by microorganisms when they process organic matter.

Solids have two important parameters which include total dissolved solids (TDS) and total suspended solids (TSS). Normally in the primary stage of treatment solids are removed but not dissolve one. In the second stage, the dissolve solids converted to settleable solid and removed by sedimentation tanks(Akpor and Muchie 2011).

1.4 Biological characteristics of wastewater

Sewage waste is a mixture of biological and non-biological matter. The former includes different types of pathogenic and nonpathogenic microbes like bacteria, viruses, fungi, protozoa. The second part contains various types of hazardous substances, e.g., pesticides, detergents, fats, oil, phenol and pharmaceutical compounds (PCs)(Sidhu, Vikram et al. 2017).

The human sewage microbes are a group of microorganisms that come from a human source like urine, stool, sweat, bathing and gastrointestinal tract, respiratory tract, urogenital tract, skin, and oral cavity. Some of these microorganisms are harmless to humans and benefit him, gut bacteria for example help in digestion and processing of some compounds like vitamins. The presence of those microbes in the environment will help indicates the pathogenic bacteria may co-existing along them (Cai, Ju et al. 2014).*Lachnospiraceae* group from the major *Clostridiales* group used as a fecal contamination indicator (McLellan, Newton et al. 2013).

A huge diversity of microorganisms is found living together in wastewater, sharing metabolic activities to allow the life of each other. Some of these organisms are nonpathogenic while some are highly pathogenic and others are an indicator for stool contamination. Table (1.1) shows the most common resistance bacteria found in wastewater(Ferro, Polo-López et al. 2016).In addition Table (1.2) show pathogens with their acute and chronic effects on man (Akpor and Muchie 2011).

Table 1.1: Main resistance bacteria that found in wastewater and associated diseases

Bacteria	**Family**	**Human disease**	**Detected in**
Clostridium difficile	Clostridiaceae	Antibiotic-associated diarrhea, pseudomembranous colitis, toxic megacolon, ileus, sepsis	Vegetables potentially exposed to contaminated water through irrigation wastewater treatment plants
Escherichia coli (0157)	Enterobacteriaceae	Gastrointestinal illness, hemorrhagic diarrhea and kidney failure	Cattle, sheep, turkey and domestic animals (occasionally) and soil

Bacteria	Family	Human disease	Detected in
Helicobacter pylori	Helicobacteraceae	Acute gastritis, gastric cancer, gastric carcinoma, gastric mucosa-associated lymphoid tissue lymphoma and peptic ulcers	Coastal waters, water biofilms
Klebsiella	Enterobacteriaceae	Pneumonia, urinary tract infections, septicemia and soft tissue infections	Faces of healthy animals and humans, drinking water
Legionella pneumophila	Legionellaceae	Legionnaires' disease (atypical pneumonia), respiratory infections	Rivers, different water subsystems
Salmonella enterica	Enterobacteriaceae	Mild self-limiting gastrointestinal illness, salmonellosis, typhoid fever	Contaminated irrigation water, river and seawater , urban wastewater
Shigella sonnei	Enterobacteriaceae	Shigellosis, acute gastroenteritis, pneumonia and bloody diarrhea	Recreational spray fountains, lakes, swimming pools and ground water sources

Table 1.2: Bacteria with acute and chronic effects on human health

Bacterial Agent	**Acute effects**	**Chronic or ultimate effects**
***E. coli* O157:H7**	Diarrhea	Adults: death (thrombocytopenia) Children: death (kidney failure)
Legionella pneumonia	Diarrhea	Elderly, death
Helicobacter pylori	Diarrhea	Gastritis Ulcers and stomach cancer
Vibrio cholerae	Diarrhea	Death
Campylobacter	Diarrhea	Death: Guillain-Barre syndrome
Yersinia	Diarrhea	Reactive fever
Salmonella	Diarrhea	Reactive fever
Cyanobacter	Diarrhea	Potential fever
Leptosporosis	Fever, Chills	Well's Disease

1.5 Bacterial structure in the treatment plant

Activated Sludge (AS) contains various types of microorganisms that live in harmony together. The diversity of microbes in AS is more than the incoming wastewater (IW) and contains uncultured types.

The stability of the bacterial community in the WW depends on three factors. First, the geographic place of presence and nutrition. Second, the chemical composition of the nutrient found in the wastewater. Third, the contamination with sewage that contains stool, as most of the bacterial species is related to sewage breakout. The chemical and biological components of the incoming sewage are an important factor to determine the type of bacteria found in the AS (Shchegolkova, Krasnov et al. 2016).

There are some bacteria at the phylum level found in both influent and AS includes *Proteobacteria* and *Bacteroidetes*. In contrast, *Firmicutes* and *Fusobacteria* found more in the influent. Also, there are some groups different between two samples from the major *Proteobacteria* phylum. This includes *Epsilonproteobacteria* that found more in the influent, but *alphaproteobacteria* and *betaproteobacteria* found more in AS. In addition, *Comamonadaceae*, *Flavobacteriaceae*, and *Campylobacteraceae* are found in influent and AS samples. While *Neisseriaceae* and *Moraxellaceae* majorly found in the influent sample. But *Nitrospiraceae* and *Chitinophagaceae* are specific to AS (Shanks, Newton et al. 2013, Lee, Kang et al. 2015).

Also, there are many bacterial families found in the influent sample that fed on the carbon include *Faecalibacterium, Gammaproteobacteria, Bacteroides, Parabacteroides* and *Lachnospiraceae*. The predominant bacterial taxonomy found in the sewage sample includes *Acinetobacter, Aeromonas,* and *Trichococcus* species, because these taxa will adapt themselves in the sewage since they found in low abundance in the freshwater. In contrast, some of the bacteria found in the sewer sample more than human feces include *Lactococcus* and *Enterobacteriaceae* (Vandewalle, Goetz et al. 2012).

The increased abundance of such bacteria causes heavy foaming in the AS. The elimination of the bacteria in the AS is determined by two factors. First, degradation potential, which in turn controlled by the number of the bacteria found. Second, metabolic pathways found in the bacteria and responsible for the degradation of pollutants (Shchegolkova, Krasnov et al. 2016).

1.6 Impact of the bacterial community structure of AS on the treatment process

The microbial community in the AS affects the process of WWTP by using microbes to process different kinds of compounds e.g., for nitrogen removal, there are ammonia-oxidizing bacteria, beside nitrite-oxidizing bacteria, and for phosphorus condensation, there are phosphate accumulating organisms. The abundance of the microbes is affected also by the seasons like *Archaea* are more prevalent in winter than summer. In contrast, *Eukaryota* is more abundant in summer than winter. The most abundant bacteria in WWTP are *Actinobacteria* followed by *Bacteroidetes, Chloroflexi* and *Firmicutes* (Ju, Guo et al. 2014).

Microbial community in the WWTP is not only affected the operation of the system but also the stability particularly when the diversity of the microorganisms is high. Also, the composition nutrient of wastewater affects the type and the structure of microbial community. Beside different systems lead

to a different microbial structure like *Proteobacteria* is high in WWTP that uses membrane bioreactor. Finally, the operational parameters like DO affect the ammonia oxidation activity.

Microbes monitoring in WW is much important and associated with health risk. One of the indicators of the quality of treated WW is *Escherichia coli* (*E. coli*) that indicates fecal contamination. This done by collecting 100 ml WW and culture. It could be done monthly. also monitoring *Clostridium* spores, *Enterococcus,* and *coliphages* is recommended depending upon the use of treated WW. Indeed, fecal indicator bacteria do not associate with pathogenic viruses, protozoa, and bacteria in sewage and environment (Ahmed, Staley et al. 2017).

1.7 Factors that affect the structure of the bacterial community

One of the physical parameters in WW that affect microbial community is temperature. It led to variation in the microbial community. The explanation is, microorganisms have different sensitivity and resistance under various temperatures. Low temperature decreases the activity of the microorganism and the treatment process too. Nonetheless, many bacteria adapt their selves upon different temperatures, e.g., *Methanosarcina mazei*-like microbes work under low temperature, but *Methanosaeta* at moderate temperature, in contrast, *Methanosarcina thermophile* work at high temperature (Chen, Lan et al. 2017).

Low or cold temperature affect microorganism growth due to low water availability and increased solute concentration. Bacterial adaptation to this crisis is a problem in WWTPs. In specific, nitrogen removal is inhibited when lower the temperature from 20 to 10 C̊. In contrast, organic compounds and nutrient removal are high in cold temperatures. So, the microorganisms that grow in much cold temperature will adapt themselves to this temperature and will be helpful to determine the bacterial taxa in this condition to manipulate the design of WWTP processes (González-Martínez, Sihvonen et al. 2018).

Many conditions affect the bacterial growth in WW, for example, *Acinetobacter spp.* utilize carbon sources and degrade different compound like fuel oil. These bacteria will be found mainly in the AS. Also *Aeromonas* and *Pseudomonas spp*. grow in mesophilic temperature with the aerobic and anaerobic condition and use carbohydrate compounds (Vandewalle, Goetz et al. 2012).

Many studies show that short-term temperature variation affects the morphology of the bacteria. In conclusion. the microbial activity, not the structure is affected by temperature variation (Chen, Lan et al. 2017).

1.8 Advantages and Disadvantages of WWTPs

1.8.1 Advantages of WWTPs use

Treatment of domestic and industrial wastewater is crucial for human health and the environment. The biological step (second step) in water treatment is important in the degradation of chemical toxins and xenobiotic. There is a different type of activated sludge (AS) depending on the type of organisms that have been found. This includes aerobic and anaerobic microorganisms like bacteria, archaea, fungi, and protozoa. They can neutralize organic compounds like toluene and benzopyrene (Shchegolkova, Krasnov et al. 2016).

WWTPs offer a new source of water that can be used for an application other than drinking. The agriculture sector especially can benefit from this in the case of low water supply and scarcity of resources to depend on. Another advantage of using TWW is the lesson of the discharge of TWW in the environment and result in the pollution of the ecosystem besides freshwater resources. Also, the irrigation of TWW will benefit the soil by enriching it with needed nutrients and fertilizers. This will enhance crops and plants grow and lower the application of fertilizer to the soil along with the cost to buy that fertilizer. In addition, many studies show that TWW irrigation will increase the organic matters in the soil along with many nutrients that benefit plant growth like iron, potassium, nitrogen, manganese, magnesium, calcium and others (Gatica and Cytryn 2013).

1.8.2 Disadvantages of WWTPs use

The WWTPs became a hotspot for the presence of microorganisms especially the antibiotic-resistance organisms that come from humans /animals and released into sewage through feces, urine, dead bodies, and manure. Moreover, WW that flow from hospitals and farming facilities could be the major source of antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB) that released in the environment (Baquero, Martínez et al. 2008). The major factor that affects the dissemination of ARB and ARGs is antibiotic usage. Worldwide 5-10% of the patients that enter the hospital acquire an infection as they stay(Nosocomial infection) (Schmieder and Edwards 2012).

The main route of transfer this resistance in the AS is by horizontal transfer of ARGs through mobile genetic elements (MGEs) such as plasmid, transposons, bacteriophages, insertion sequences and integron. There are many ARGs encode for the resistance of different antibiotics like fluoroquinolones, tetracycline, beta-lactam, sulfonamides, aminoglycosides, glycopeptides, phenicols, and trimethoprim. It was shown that MGEs were abundant in the sludge samples and this may be

important for the acquisition and mobility of various ARGs among the bacterial species(Guo, Li et al. 2017). There are five different mechanisms the bacteria resist the antibiotic, which are:. Affecting the influx and efflux of the antibiotic inside and outside bacterial cells. Also, affect the binding process between antibiotics and their target by either alter the target or by amplification of the target. Finally, inactivate the antibiotics by encoding a protein that bind and stop antibiotics action as illustrated in figure (1.2) (Schmieder and Edwards 2012).

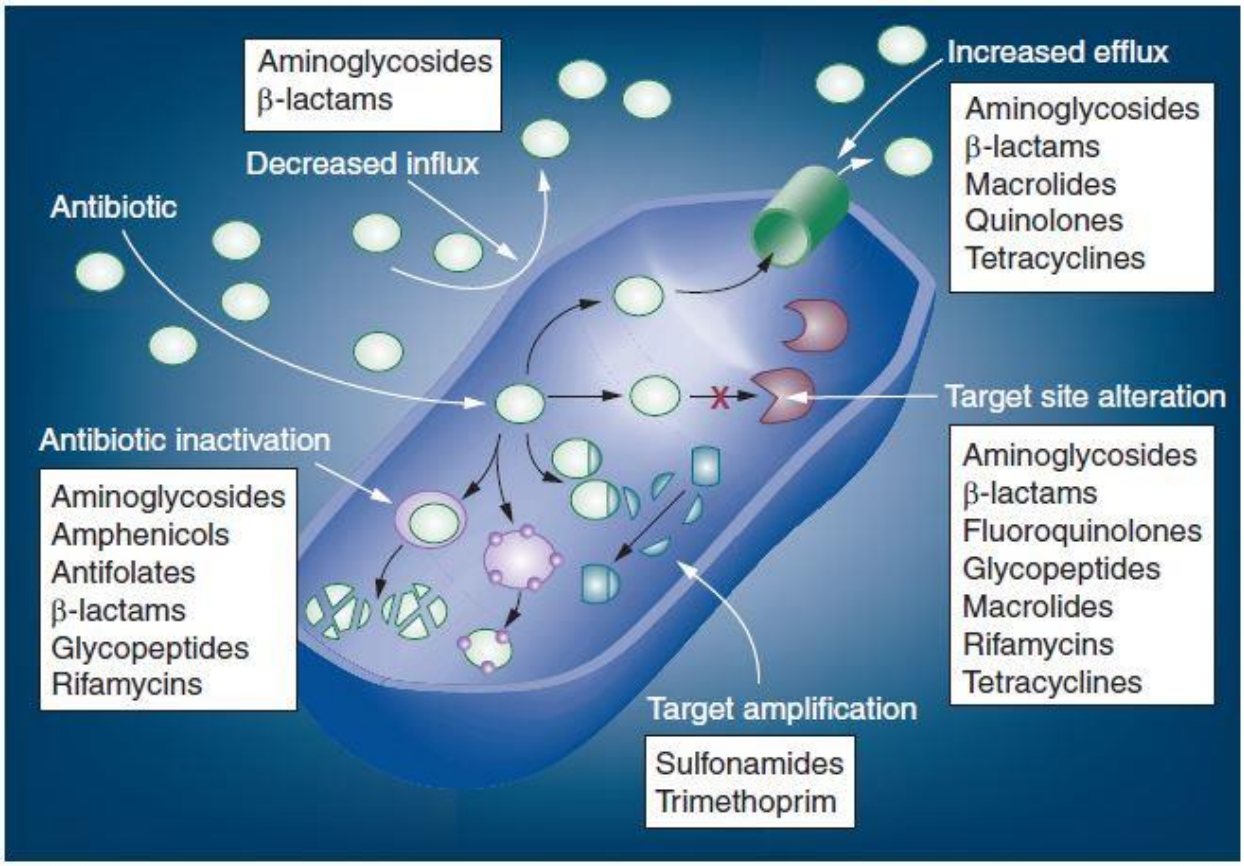


Figure 1.2: Resistance mechanisms of bacteria to antibiotics.

The spreading of ARGs and ARB in the environment could lead to antibiotic resistance in humans and threaten public health (Hu, Zhang et al. 2016). An infection caused by extended-spectrum B-lactamase (ESBL) producing *E. coli* has been raised in the developing countries (Nakayama, Tuyet Hoa et al. 2017).

The use of TWW for irrigation cause a change in the chemical and physical characteristics of the soil. Decrease the pH of the soil affects the solubility and the mobility of several compounds especially heavy metals which increase the uptake levels of these compounds by food crops (Khan, Cao et al. 2008). A study conducted to detect the presence of heavy metals in the soil irrigated with WW

compared with groundwater showed that the concentration of Cr+2 was the highest followed by Pb^{+2}, Cd^{+2}, Co^{+2}, Ni^{+2}, Cu^{+2}, Zn^{+2}, and Mn^{+2}. In addition, high levels of heavy metals in the leaf of food crops especially vegetables irrigated with WW have been reported (Mahmood and Malik 2013).

Also, there are different PCs found in waste water. the compounds that detected in the influent sample still found in the effluent samples but with lower concentration. The PCs that found in the primary sludge include ibuprofen, gemfibrozil, salicylic acid and caffeine compared to secondary sludge that have carbamazepine, 17_-ethinylestradiol, estriol and propranolol in higher concentration. The concern about these compounds that may enter the food crops through the roots of these plants if irrigated with this water, and the major compounds that enter in this way include carbamazepine, sulfamethoxazole , trimethoprim, ibuprofen and 17_-ethynilestradiol (Martín, Camacho-Muñoz et al. 2012).

1.9 Water status in the West Bank, Palestine

1.9.1 Availability

The limited surface resources and the variability in the rainfall lead to a low freshwater amount in the region, this will direct the arrow to the groundwater as a major source. The source of the water in the West bank is from mountain aquifer in the West Bank and extend to Israel. So, the limited groundwater leads to focus on infiltration through porous soil, and karstic rocks. The total balance in the West Bank is 679 MCM/ year (Mogheir, Zomlot et al. 2005).

1.9.2 Utilization

The available groundwater in Palestine is 1,209 MCM/year, and 1,046 goes for Israelis and only 259 for Palestinians. This implies the imbalance in water demand with the available one. The utilization of Palestinians per capita is 35-80 L/day and it is below WHO standards with 100L/capita/day. In contrast, Israeli consume 300 L/day. In addition. Israel uses 800 MCM/year of the Jordan river water (Mogheir, Zomlot et al. 2005).

1.9.3 Water consumption

The consumption of water from different sectors are gathered together and no separated like industrial and domestic uses. Palestinians consume about 127.4 MCM (Mogheir, Zomlot et al. 2005).

1.9.4 WWTPs in the West Bank

The Palestinian Water Authority works very hard to secure clean water or help in water management to supply for the Palestinian people. They are committed to provide a sanitized aquatic environment and protect public health. The treatment of WW is not only providing water for reuse but also enriches the groundwater quality and quantity (Samhan, Al-Sa`ed et al. 2010).

The wastewater management in the West Bank is low effective in sanitation, besides, inadequate wastewater treatment, and unhealthy disposing of untreated WW, besides the use of these WW for irrigation. There are four working treatment plants in the West Bank constructed under Israeli occupation and suffer from different issues including: overloaded, and not well maintained (Samhan, Al-Sa`ed et al. 2010).

Table (1.3) summarize those WWTPs in West Bank (Mogheir, Zomlot et al. 2005), and the map in figure (1.3) show the location of these WWTPs (Samhan, Al-Sa`ed et al. 2010). Most of the treatment process is secondary but there is some tertiary treatment in Gaza and in Ramallah. Also, primary treatment plant found in Tulkarim city which deposits its effluent in Wadi Zimer.

Table 1.3: General Characteristics of Municipalities Treatment Plants in the West Bank

Municipalities WWTP	**Type of Treatment**	**Population Served (Capita)**	**Effluent Quantity m^3/d**	**Effluent Disposal Method**
Al-Bireh	Screening	50,000	3200	Irrigation
	Aeration tanks			
	disinfection by UV radiation			
Ramallah	two aerated lagoons	47,500	1370	Wadi Bitunia
Jenin	Aerated lagoon	20,000	1500	Valleys
Tulkarim	Stabilization ponds	114,400	6742	Not available

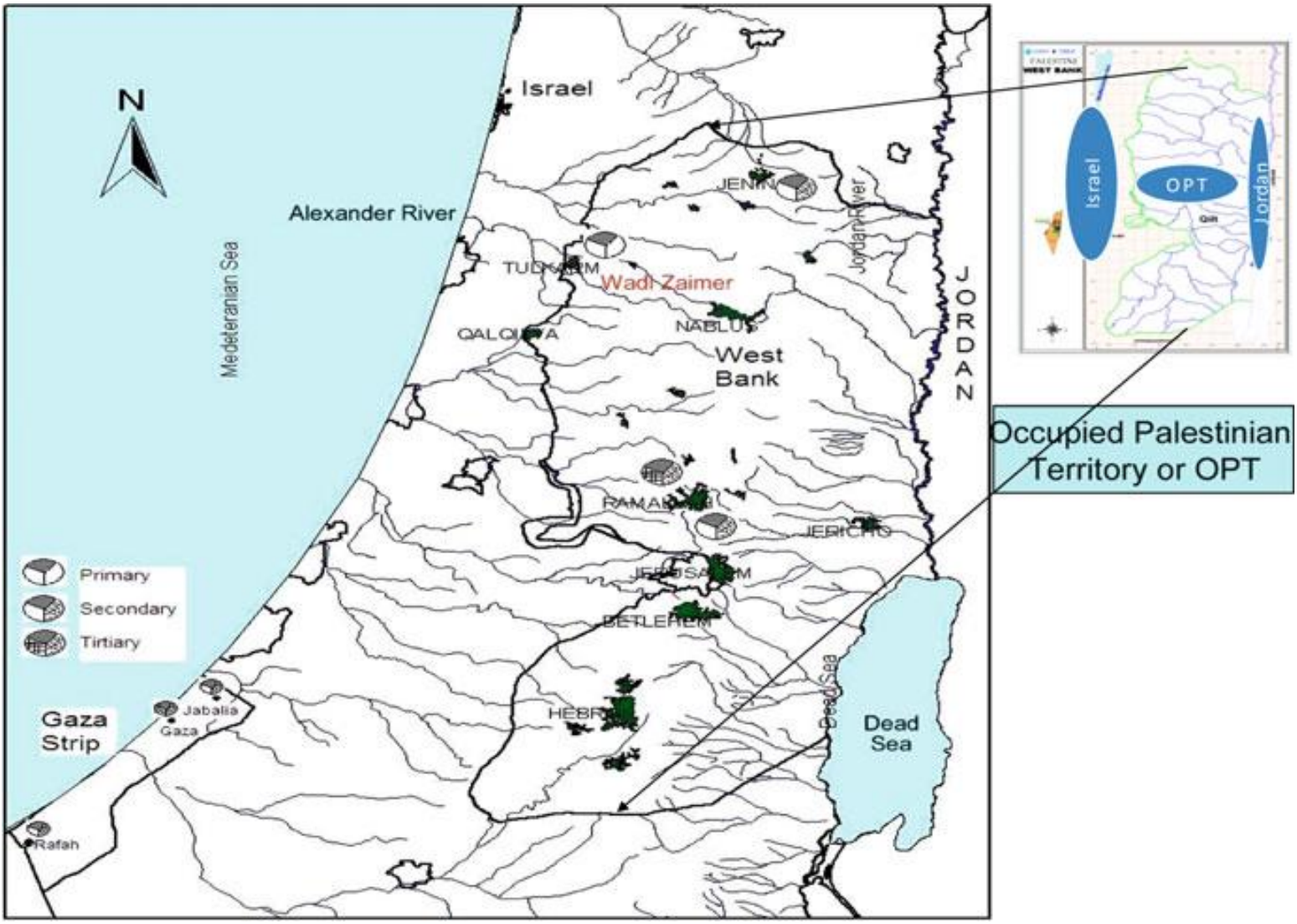


Figure 1.3: WWTPs location and receiving surface water bodies.

Recently it was reported that Al-Bireh plant is the only one working efficiently since it was reconstructed in the year 2000 while the others showed poor efficiency and quality (Samhan, Al-Sa`ed et al. 2010).

The need for WWTPs is a high priority due to the water crisis in this area and pollution. In the rural area there a concern of environmental threat because of discharging untreated WW and use it for agriculture in the absence of Palestinian laws that limit those practices. Also, surface and groundwater are threatening of pollution (Samhan, Al-Sa`ed et al. 2010).

Al-Bireh plant from its construction in 1997 and start working in 2000 still have higher efficiency of water treatment comparing to other plants (Abusharbak 2004).

1.10 Literature review

Worldwide, many kinds of research were conducted on wastewater treatment plants to detect different types of pathogens and the origin that come from or the source of the outbreak. A study conducted in Belgium assessed the differences in microbial composition in activated sludge from textile and municipal WWTPs and explained the observed differences by environmental factors, they found that *Proteobacteria* was the most dominant phylum found followed by *Bacteroidetes* in AS sample. When comparing the sample from municipal and textile WWTPs, *Bacteroidetes* and *Actinobacteria* found more in municipal WWTP compared to *Planctomycetes, Chloroflexi*, *Acidobacteria,* and *Chlorobi* which found more in textile WWTP.the difference is attributed to physical and chemical properties of WW (Meerbergen, Van Geel et al. 2016). In Hong Kong, a study examined the diversity and the disturbance of human pathogens from different sources includes influent, activated sludge, and effluents by a High-Throughput Shotgun Sequencing Technique, they revealed that *Firmicutes* was the most abundant phylum followed by *Proteobacteria , Actinobactria*, and *Bacteroidetes.*the abundance of the bacteria in the effluent sample was low compared to influent sample except *Clostridium* and *Mycobacterium* which found more in the effluent sample (Cai and Zhang 2013).

A study conducted in Michigan revealed that the concentration of tetracycline (*tetO* and *tetW*) beside sulfonamide (*sulI*) resistance genes and ARB were high in the final effluent of WWTP. Other studies found other significant resistance genes such as *ampC, vanA*, and *mecA* that resistance to ampicillin, vancomycin, and methicillin, respectively. Compared to the ground or freshwater, the reuse of WW for irrigation as the main source of water especially in dried and semi-dried areas in the world may affect the soil proprieties due to high microbial activity, biomass, and resistance. In addition, the treated wastewater introduce different MGEs like plasmid that propagate the resistance gene in the chromosomes of the native soil bacteria (Gatica and Cytryn 2013). It is reported that different clones of *E.coli* in WWTP effluents increase the resistance to amikacin, gentamicin, neomycin, ampicillin, and ciprofloxacin in river disposal area (Garrido-Cardenas, Polo-Lopez et al. 2017).

Hospitals WWTPs consider as the major hotspot of releasing and dissemination of ARGs and ARB into the environment. *Zoogloeal* organisms have found to resist antibiotics and measured in high concentrations in hospital wastewater (Ahn and Choi 2016). Also, the multidrug-resistant *Pseudomonas aeruginosa* found to be the primary pathogen in the discharge of hospitals WWTPs and responsible for this spread of ARB in the environment over a long period of time (Joyce, Pontes et al. 2016) . ARB found in the hospital WW are important values to prevent the dissemination of those pathogens in the environments (Ahn and Choi 2016).

Lee and colleagues (Lee, Kang et al. 2015) show more bacterial abundance in the AS sample than an influent sample. For example, *Alpha-* and *Betaproteobacteria* found majorly in AS, but *Moraxellaceae* and *Neisseriaceae* found in the influent sample.

Some bacteria found to be increased, or still in the aero tanks of WWTPs with same levels as in AS sample like *Comamonadaceae*, *Pseudomonadaceae*, *Verrucomicrobiaceae*, and *Flavobacteriaceae*, *Moraxellaceae.* These families are considered the major components of the AS in the WWTPs worldwide. These bacteria are important in the degradation of the organic compound. Also, there are many factors that play a roles in determine the type of the bacteria such as the type of the nutrients found in WW in the last degradation pathway like *Flavobacteriaceae* which increased in the presence of fatty acid, proteins and lipids in the final degradation pathway (Shchegolkova, Krasnov et al. 2016).

Another study revealed different types of phyla include found in WW include *Firmicutes, Bacteroidetes, Proteobacteria*, and families like *Acetobacteraceae, Bacillaceae, Pseudomonadaceae, Prevotellaceae* and *Veillonellaceae*. The majority of these bacteria are gram negative anaerobic and facultative anaerobes, However, *Acetobacteraceae* and *Pseudomonadaceae* were also detected which are aerobic ones (Silva-Bedoya, Sanchez Pinzon et al. 2016).

Ahmed and colleague (Ahmed, Staley et al. 2017) showed many bacterial genera in raw and secondary treated WW. *Pseudomonas spp.* is the most abundant one followed by *Arcobacte*r and *Bacteroides* that found in raw WW. In secondary WW, the same bacterial genera but fewer relative abundances were reported.

Also, another study revealed that the most abundant phylum in WW is *Firmicutes* followed by *Actinobacteria, Proteobacteria,* and *Bacteroidetes.* There are different phyla also detected include *Moraxella, Corynebacterium, Streptococcus, lactobacillus* that coming from the respiratory tract, oral cavity and vagina, respectively. In addition to *Faecalibacterium, Acinetobacter,* and *Ruminococcus* and *Dorea* that coming from the gut (Cai, Ju et al. 2014).

In the past, the culture-based method faces trouble in the analysis of the microbial community in the environmental sample, because it depends on the isolation, purification and the identification of microorganisms based on their morphology. Also, this method is restricted because it cannot culture 99% of the community found in the sample, this because the selectivity of the culture media to a specific type of bacteria, besides, there are different culture conditions and variables must be founded

to success the growth of the microorganisms. As a result, this method will despise the abundance and composition of the microbial community found in the sample (D. Hladilek, Gaines et al. 2016). In addition, the conventional culture-based method facing an obstacle in growing some bacterial species and time-consuming, besides some bacteria are uncultivable (Ahmed, Staley et al. 2017).

On the other hands, molecular methods are benefiting in the determination of microorganisms and biological activity of them under different situations. They have the ability to specifically determine the real time observation of the microorganisms and their metabolic pathway. Therefore, this method capable of determine the whole genomic DNA in the environmental sample without isolating or culturing each microorganism in the lab (Garrido-Cardenas, Polo-Lopez et al. 2017) and this benefit upon classical PCR method that depend only on specific primers to isolate ARGs and detect their abundance in the bacteria, besides, it is not useful in detecting novel ARGs like next-generation metagenomic analysis (Schmieder and Edwards 2012). Furthermore, the new next-generation sequencing (metagenomic) approach has the ability to detect the low abundance microorganisms by generating hundreds to thousands of sequences and give a clear picture of the microbial community found in the sample (Ahn and Choi 2016).

1.11 Study objectives:

The main objective: To investigate the ABR microbiota of WW samples (pre- and post-treatment) collected from Al-Bierh plant in Palestine.

Specific objectives:

1. To detect the presence of ARGs and related host bacteria in the treated WW.
2. To study the differences in ABR microbial contents of WW samples based on the season in the studied plant.
3. To evaluate Al-Bireh WWTP treatment efficiency.

Chapter two

Materials and Methods

2. Materials& methods

2.1 WWTP description

The water samples were collected from Al-Bireh plant that is located and serves Al-Bireh city. Al - Bireh plant was constructed in 1997 and start functioning in February 2000. It has a capacity of 6000 m^3/day of treated wastewater, and serve about 50000 capita in Al-Bireh town. This plant depends on the conventional activated sludge method as a secondary treatment process includes a low–loaded activated sludge stage which besides the removal of carbonaceous compounds (BOD removal) performs oxidation of nitrogen compounds (nitrification). The treatment process is performed in a unit composed of a mixing and distribution chamber for aeration tank feeding. No tertiary treatment process involved. However, the effluent output of this plant is released into the wadies. An overview of the plant structure is shown in figure (2.4) (Abusharbak 2004).

Figure 2.4: Overview of Al-Bierh plant

2.2 Sampling

Two different samples from Al-Bierh plant were collected. Raw (influent) sample (500 ml; one bottle) and treated (effluent) sample (500ml; one bottle) were collected in two different seasons; winter and summer in particular on February and August 2018. of influent and one of effluent from the plant in summer and winter were collected. The samples were composites by which every 5 meter a portion of water is collected in sterile bottles to finally have the whole sample, then, shipped within 48 hours in a cool box to the laboratory for preparation of DNA extraction.

2.3 Samples preparation

Influent samples (500 ml each) first divided into 50 ml sterile tubes (10 tubes), then centrifuged at maximum speed about 3220 rpm for 20 minutes in 5810 R centrifuge (Eppendorf, Germany) to obtain the pellet. The pellets were reconstituted with absolute ethanol in a ratio of 2:1 (sample to absolute ethanol) and kept in -20℃ to be used for DNA extraction (Ma, Li et al. 2017).

The effluent samples (500 ml each) were filtrated using Polyvinylidene difluoride (PVDF) membrane filter (Stericup 250 ml, Durapore, 0.45 um PVDF). The membrane then was removed carefully using sterile seizure and forcipes, transferred to sterile tubes and kept at -20℃ for DNA extraction.

2.4 DNA extraction and concentration

2.4.1 Influent samples

The frozen samples (10 tubes) were thawed at room temperature, homogenized separately by course vortex and finally collected in one tube. For DNA extraction, QIAamp® DNA Mini Kit (Qiagen GmbH, Germany) was used according to manufacture instructions of bacterial DNA extraction protocols, except in the final step 50 ul of elution buffer was added instead of 200 ul to obtain final volume sample equal to 50 ul.

2.4.2 Effluent samples

The filter paper first was thawed at room temperature and then carefully extracted from the tube and was cut into small pieces using sterile seizure. The DNA was extracted from filter pieces using the same kit and same procedures that mentioned above for influent samples (section 2.4.1).

2.4.3 Measurement of DNA concentration

To make sure that our DNA samples are enough for library preparation, the concentration of DNA sample was measured using the Qubit v4(Invitrogen, USA) machine. gDNA concentration was adjusted to 0.4 ng/ul to be accepted for library preparation.

2.5 Library preparation

For metagenomic analysis. The DNA library was prepared using the Nextera XT DNA library preparation kit (illumina®, USA). Briefly, the gDNA was normalized to have 5 ul DNA. First, Tagmentation was done using 5 ul of Tagmentation buffer (Amplicon Tagment Mix) with special Tagmentation mixture that contains transposomes and then incubated at 55℃ for 5 minutes using thermal cycler (T100™ Thermal cycler, BIO-RAD). The reaction was kept at 10℃ to stop the action of transposomes. The purpose of those transposomes was to obtain DNA fragments. Then, 5 ul of Neutralize Tagment Buffer added to the mix and incubated 5 minutes at room temperature, the final volume was 25 ul for the next step. Enrichment, in which two different index adapters were added (5 ul each) at both ends of the DNA fragment.

To attach those index adapters to the DNA, a 15 ul of Nextra PCR master mix were added to 35ul of Tagmented DNA to a final volume of 50 ul, the reaction mixture was subjected to a thermal cycler as followed: 72℃ for 3 minutes, 95℃ for 30 seconds, then 12 cycles of: 95℃ for 10 seconds, 55℃ for 30 seconds, 72℃ for 30 seconds, then 72℃ for 5 minutes, Hold at 10℃.

The obtained DNA library with fragments between 300-500 bp (double size selection). cleaning of the library was done. Briefly, 25 ul of the AMPure XP magnetic beads was added to 50 ul sample and incubated for 5 minutes at room temperature. The tubes were placed on a magnetic stand for 2 minutes. The supernatants were taken and transferred to new tubes and mixed with 40 ul of magnetic beads. The tubes were placed again on a magnetic stand until the mixture become clear. The supernatants in this step were discarded, the pellets were washed twice with freshly prepared 80% ethanol (EtOH). The supernatant was discarded again and the pellets were left on the magnetic stand for 15 minutes for air drying.

The tubes were removed from the magnetic stand and 25µl of elution buffer was added and then incubated for 2 minutes at room temperature followed by 2 minutes incubation in the magnetic stand until the mixture become clear. Finally, the supernatant (22ul) was transferred to new tubes and kept at 20C until further use. The quantity of the prepared DNA library was evaluated using Qubit machine.

2.6 DNA deep sequencing

Quality of library was evaluated by Tapstone machine, and the library was normalized to 4 nmol. Sequencing was done using the NextSeq 500/550 High Output Kit v2.5 (150 Cycles). The sequencing was done as single read. Output sequencing data was received as fastaq files.

2.7 Bioinformatics analysis

2.7.1 Analysis workflow

First, the fastaq files were converted to Fasta format using FASTQ to FASTA converter from FASTX-toolkit command on *usegalaxy.org*. Then the sequences files (FASTA) were uploaded to *https://usegalaxy.org.au*/ platform.

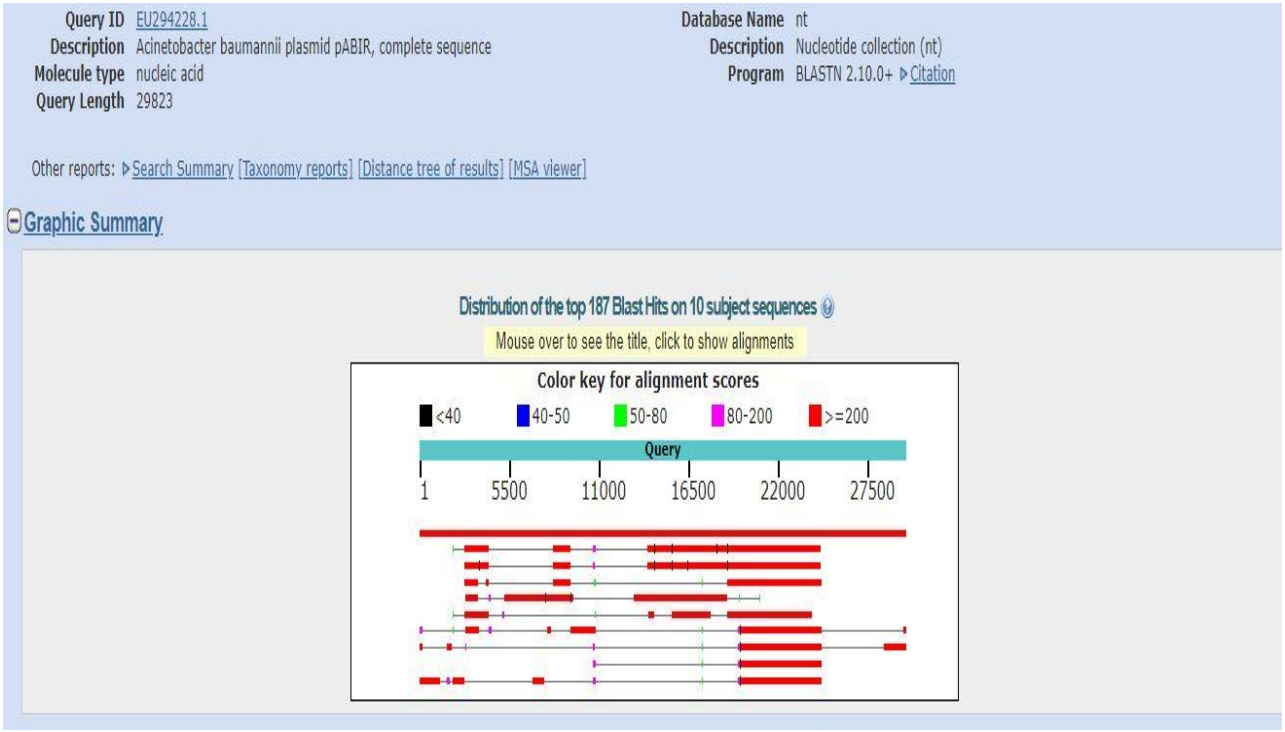
At the Australian galaxy, the annotation process was done using ABRicate command which perform mass screening of contigs for antimicrobial and virulence genes. The output results of this command include related reference sequence which indicated by accession number to each ARG.

After downloaded the results file of the ABRicate. SPSS v23 was used to measure the frequency of each ARGs. Then 'Cross Tab' command was applied to link the accession number to different types of ARGs.See appendix 1.

2.7.2 Identification of ARGs hosts by Blast analysis

A BLAST analysis was performed on *blast.ncbi.nlm.nih.gov* site.

The accession number accompanied with each ARGs from the previous analysis (section 2.7.1) uploaded and blast analysis was performed to identify the bacterial host. Each organism with identity and Query cover above 97% was included as doing elsewhere (Ravi, Ereqat et al. 2019) ,here an example of one analysis.



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Acinetobacter baumannii plasmid pABIR, complete sequence	55073	57861	100%	0.0	100.00%	EU294228.1
Acinetobacter johnsonii strain Acsw19 plasmid pAcsw19-2, complete sequence	10582	29795	44%	0.0	100.00%	CP043309.1
Acinetobacter nosocomialis strain AC1530 plasmid pAC1530, complete sequence	10567	31047	44%	0.0	99.95%	CP045561.1
plasmid1	10565	15229	26%	0.0	99.52%	CP026617.1
Acinetobacter baumannii strain DA33382 plasmid pDA33382-85, complete sequence	10554	27658	36%	0.0	99.97%	CP030109.1
Acinetobacter baumannii strain WCHAB005078 plasmid pOXA58_005078, complete sequence	9620	21061	32%	0.0	99.94%	CP027245.2
Acinetobacter wuhouensis strain WCHA60 plasmid p2_010060, complete sequence	9367	15618	27%	0.0	99.98%	CP031710.1
Acinetobacter sp. ACNIH2 plasmid pACI-c6b4, complete sequence	9361	12316	24%	0.0	99.96%	CP026417.1
Acinetobacter sp. ACNIH2 plasmid pACI-235c, complete sequence	9361	9886	17%	0.0	99.96%	CP026414.1
Acinetobacter baumannii strain KCRI-28 genome assembly, plasmid: pKCRI-28-1	9350	13637	27%	0.0	99.98%	LR026972.1

2.7.3 ARGs analysis

AMR gene family, drug class, and resistance mechanisms for each gene were analyzed using 'The Comprehensive Antibiotic Resistance Database', *https://card.mcmaster.ca/* which is a bioinformatic database of resistance genes, their products and associated phenotypes. The procedures done as follow:

1. In the main page of *https://card.mcmaster.ca/* 'Analyze' command was chosen.
2. Reference gene identifier (RGI) was selected.
3. The accession numbers obtained from the previous analysis (section 2.7.1) were uploaded, then 'submit' command to start the analysis.

Results

Search:

RGI Criteria	ARO Term	SNP	Detection Criteria	AMR Gene Family	Drug Class	Resistance Mechanism	% Identity of Matching Region	% Length of Reference Sequence
Strict	AAC(6')-Ib10		protein homolog model	AAC(6')	aminoglycoside antibiotic	antibiotic inactivation	96.67	103.45
Strict	cmlA5		protein homolog model	major facilitator superfamily (MFS) antibiotic efflux pump	phenicol antibiotic	antibiotic efflux	99.05	100.00

Previous 1 Next

Chapter three

Results

3. Results

Our study utilized a deep metagenomic sequencing approach to examine the ARGs present in influent and effluent samples collected from Al-Bireh plant over two different seasons. Table (3.4) showed the total number of raw reads (150 bp fragments produced in the library) for each sample. and table (3.5) show the number of reads for bacteria as represented in the krona pie at galaxy platform, see appendix 2. Difference was observed among the influent and effluent samples in two different seasons; Winter and Summer. Obviously, the amount of DNA in the Winter was more than the Summer.

Table 3.4: Total number of raw reads in WW samples obtained from Al-Bireh plants on Winter vs. Summer

Plant/Bireh	Influent (No. of reads)	Effluent (No. of reads)
Winter	$3.89*10^6$	150662
Summer	$0.39*10^6$	57598

Table 3.5: Total number of bacterial reads in WW samples obtained from Al-Bireh plant on Winter vs. Summer

Plant/Bireh	Influent (No. of reads)	Effluent (No. of reads)
Winter	$3.7 * 10^6$	95716
Summer	$0.37 * 10^6$	31788

3.1 Samples of Winter 2018:

3.1.1 Occurrence, abundance of ARGs and removal efficiency

3.1.1.1 ARGs abundance

In the influent sample, 400 ARGs subtypes were found and the top 10 ARGs comprised 44% of the total ARGs (table 3.6).The most abundant antimicrobial drug family was macrolide resistance which includes *msr(E)_4*, *mph(E)_1* and *mph(A)_1* genes, followed by tetracycline resistance group that includes *tet(Q)_1*, *tet(39)_1* and *tet(Q)_3* genes. Other families such as Beta-lactamase, sulfonamide, streptogramin and aminoglycoside were detected which include *cfxA2_1*, *sul1_10*, *erm(F)_1* and *aph(6)-Id_1*, respectively.

On the other hand, the effluent sample showed 88 ARGs subtypes, the top 10 ARGs comprised 67% of the total ARGs. The most abundant ARGs were also belong to macrolide class that includes *msr(E)_4*, *mph(E)_1*, *mph(G)_1* resistance gene. Tetracycline class have the second abundant genes which includes *tet(39)_1*, *oqxB_1* and *mef(C)_1* Followed by Sulfonamide which includes two resistance genes *sul1_10* and *sul2_1*. The last two genes *erm(F)_1* and *ant (3'')-Ia_1* were belong to streptogramin and aminoglycoside respectively. See appendix 3 for less abundant ARGs.

Table 3.6: Frequencies and percentages of top 10 ARGs in both influent & effluent WW.

Influent			Effluent		
Gene	**Frequency**	**%**	**Gene**	**Frequency**	**%**
msr(E)_4	1180	13.05	*msr(E)_4*	97	1.1
mph(E)_1	695	7.7	*mph(E)_1*	59	0.7
tet(Q)_1	576	6.4	*tet(39)_1*	37	0.4
cfxA2_1	286	3.2	*sul1_10*	16	0.2
tet(39)_1	248	2.7	*sul2_1*	15	0.17
sul1_10	195	2.2	*erm(F)_1*	14	0.15
erm(F)_1	151	1.7	*ant(3'')-Ia_1*	11	0.12
mph(A)_1	151	1.7	*oqxB_1*	11	0.12
tet(Q)_3	147	1.6	*mef(C)_1*	8	0.09
aph(6)-Id_1	140	1.5	*mph(G)_1*	8	0.09

3.1.1.2 Removal efficiency

The removal efficiency was calculated by the following equation:

$\frac{\text{influent frequency} - \text{effluent frequency}}{\text{influent frequency}} \times 100\%$ (Lai, Hess et al. 2018).

Tow genes *tet(Q)_3* and *aph(6)-Id_1*were completely removed after treatment. However, the removal efficiency for all genes ranged from 85-100% as shown in table (3.7).

Table 3.7: Removal efficiency of top 10 ARGs

Gene	Removal efficiency
tet(39)_1	85
erm(F)_1	91
msr(E)_4	92
mph(E)_1	92
sul1_10	92
mph(A)_1	99
cfxA2_1	99.6
tet(Q)_1	99.8
tet(Q)_3	100
aph(6)-Id_1	100

3.1.2 Identification of ARGs bacterial hosts and Removal efficiency

3.1.2.1 ARGs bacterial hosts

In the influent sample, 53 different bacterial species were found, the top 10 species comprised 74% of the total species abundance. Regarding phylum frequency, the most abundant phylum was *Proteobacteria (67%)* which includes, *Acinetobacter baumannii (A. baumannii), E. coli, Pseudomonas aeruginosa (P. aeruginosa) and Klebsiella pneumoniae (K. pneumoniae)* from the top 10. followed by *Firmicutes (8%)* which include *Streptococcus pneumoniae* from the top 10 and *Bacteroidetes (6%)* which includes, *Prevotella ruminicola*, *Prevotella denticola*, *Prevotella intermedia* and *Bacteroides fragilis* from the top 10. Uncultured bacterium represents 11.3% of the top 10.

On the other hand, in the effluent sample, only eight bacterial species were found. The most common phylum was *Proteobacteria (63%)* which includes the same bacterial species as influent sample except of *P. aeruginosa. Photobacterium damselae, Salmonella enteritidis* and *Salmonella enterica.* The second one is *Bacteroidetes (6%)* which includes only *Bacteroides fragilis* while uncultured bacterium *represent less than 1% of the* top 10 (table 3.8). Less abundant bacteria were described in appendix 3.

The percentages of each ARB were calculated from the number of specific reads of each bacterium divided by the total number of reads in the influent and effluent sample.

Table 3.8: Antibiotics resistance bacterial species and their abundance and percentages according to the number of reads

Influent			**Effluent**		
Bacteria	**abundance**	**%**	**Bacteria**	**abundance**	**%**
Acinetobacter baumannii	1553	19.6	*Acinetobacter baumannii*	134	1.7
Escherichia coli	933	11.8	*Uncultured bacterium*	59	0.7
Uncultured bacterium	893	11.3	*Escherichia coli*	28	0.4
Prevotella ruminicola	576	7.3	*Photobacterium damselae*	16	0.2
Pseudomonas aeruginosa	459	5.8	*Salmonella enteritidis*	15	0.19
Klebsiella pneumoniae	368	4.6	*Bacteroides fragilis*	14	0.18
Prevotella denticola	286	3.6	*Salmonella enterica*	14	0.18
Streptococcus pneumoniae	279	3.5	*Klebsiella pneumoniae*	6	0.08
Bacteroides fragilis	180	2.3			
Prevotella intermedia	147	1.9			

1.1.1.1 Removal efficiency of ARB

As shown in table (3.9), some of bacteria such as *Prevotella species* and *Streptococcus pneumoniae (S. pneumoniae)* were lost in the effluent sample and thus the removal efficiency was considered 100 %. However, the removal efficiency for the other bacteria was still high and ranged from 67-100 %.

Table 3.9: Removal efficiency of each organisms

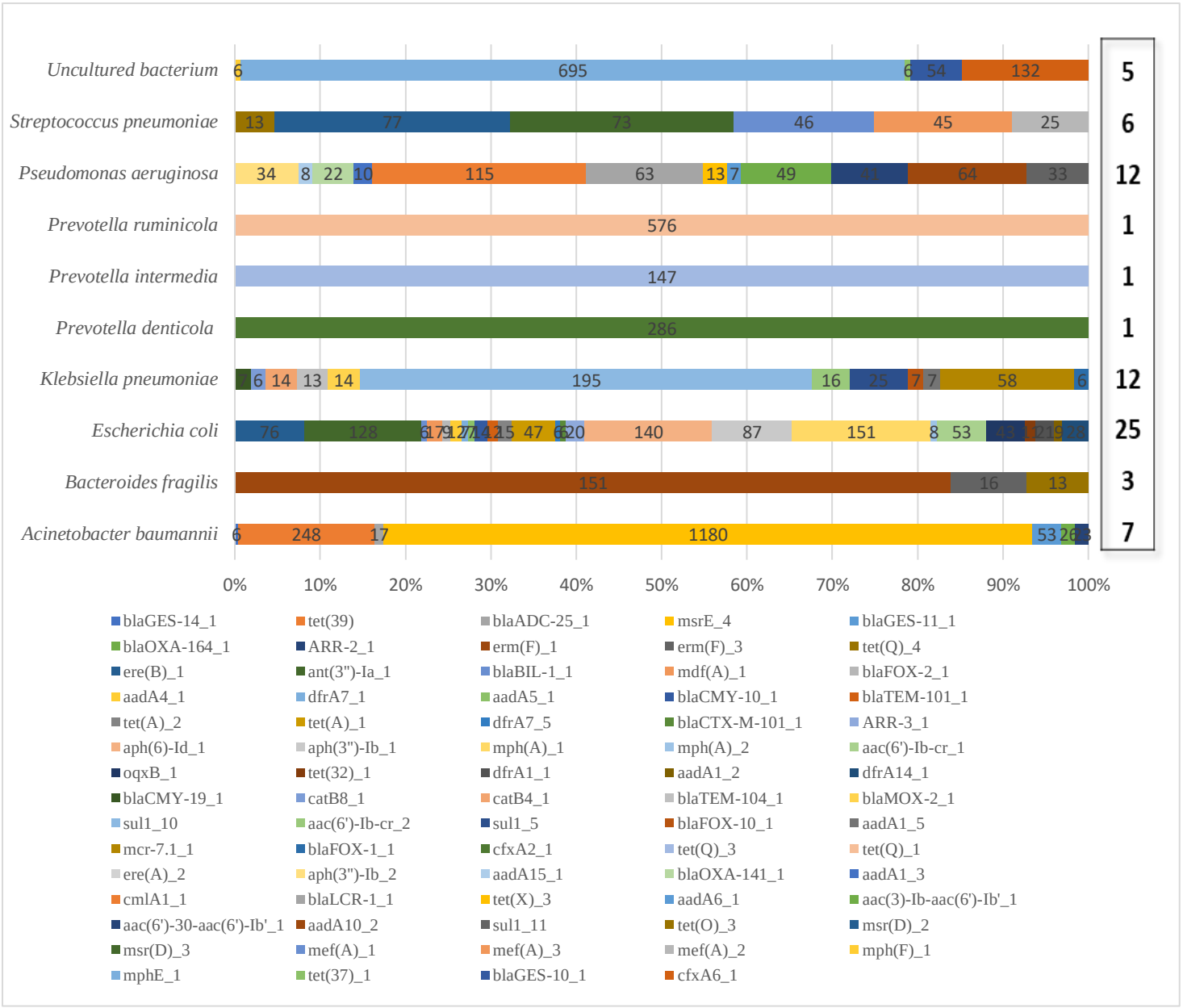
Bacteria	Removal efficiency
Salmonella enteritidis	67
Photobacterium damselae	89
Salmonella enterica	90
Acinetobacter baumannii	91
Bacteroides fragilis	92
Uncultured bacterium	93
Escherichia coli	97
Klebsiella pneumoniae	98
Prevotella denticola	100
Prevotella intermedia	100
Prevotella ruminicola	100
Pseudomonas aeruginosa	100
Streptococcus pneumoniae	100

3.1.3 ARGs and host bacteria

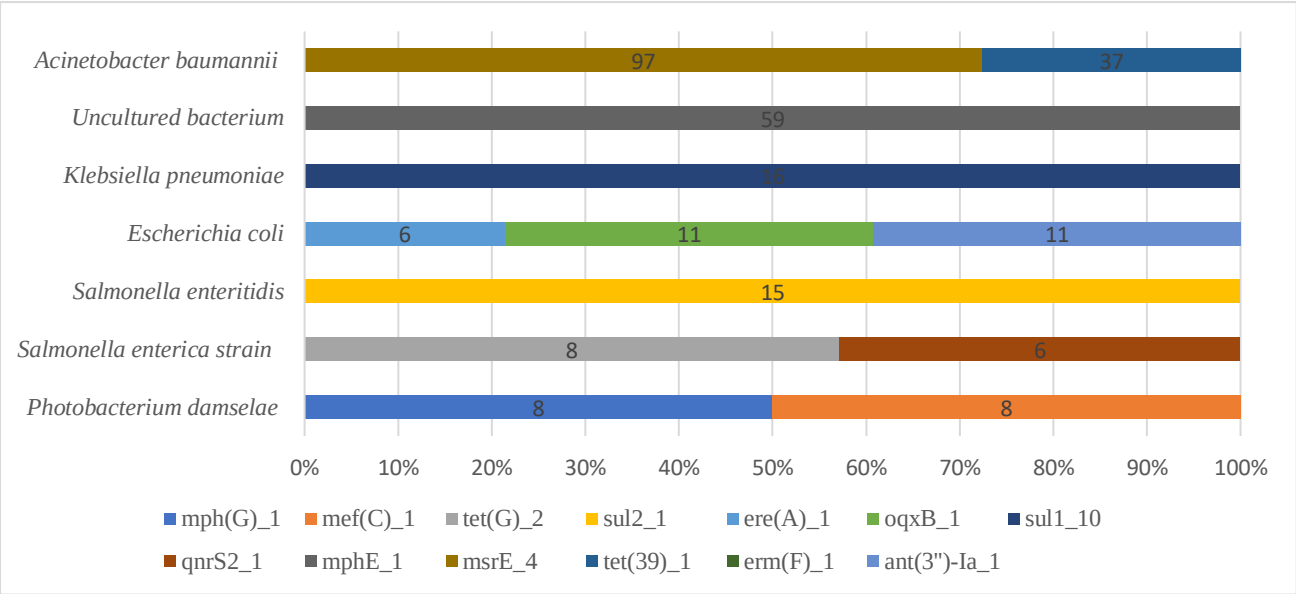
In our study, different bacterial species that harbor various ARGs types have been detected in the influent sample, the top 10 bacterial species were shown in Figure 3.5 (A). *S. pneumoniae* carried *msr(D)_2,3* as the most dominant genes which are a trimethoprim resistance gene while *P. aeruginosa* harbored *cmlA1_1* a tetracycline resistance gene. Moreover, Prevotella *intermedia* and *Prevotella ruminicola* carried tetracycline resistant genes *tetQ_1* and *tetQ_3*, i.e. respectively. In contrast, *Prevotella denticola* harbor a beta-lactamase resistance gene; *cfxA2_1*. *K. pneumoniae* carried *sul1_10* as the most frequent resistance gene which is a sulfonamide resistance gene. For *E. coli,* two dominant genes *mph(A)_1, aph(6)-Id_1* were found and considered as macrolide and aminoglycoside resistance respectively. A streptogramin resistance gene i.e *erm(F)-1* was detected in *Bacteroides fragilis.* Finally, the most frequent resistant gene in *A0 baumannii* was *msr(E)-4* a macrolide resistant gene.

On the other hand, in the effluent sample, *A. baumannii* still has *msr(E)-4* as a dominant gene like in influent sample but less frequent, see Figure 3.5 (B).

Unlike influent sample, *sul1_10* resistance gene with lower frequency was detected in *K. pneumoniae*. *E. coli* carried two genes with same frequency; *oqxB_1* and *ant (3'')-Ia_1* which were not detected in *E. coli* of influent sample. These two genes were classified as tetracycline and aminoglycoside resistance genes, respectively. *Salmonella enteritidis* carried one resistance gene i.e. *sul2_1*a sulfonamide resistance gene. *Salmonella enterica* carried *tet(G)_2* and *qnrS2*_1 gene which are tetracycline and quinolone resistance genes, respectively. Two genes *mph(G)_1* and *mef(C)_1* have the same frequency in *Photobacterium damselae* which are resistance for macrolide and tetracycline. For *mphE_1* was the dominant macrolide resistance gene found in both influent and effluent samples. See appendix 3 for the less abundant bacteria and related genes.


Uncultured bacterium
Streptococcus pneumoniae
Pseudomonas aeruginosa
Prevotella ruminicola
Prevotella intermedia
Prevotella denticola
Klebsiella pneumoniae
Escherichia coli
Bacteroides fragilis
Acinetobacter baumannii
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
blaGES-14_1
tet(39)
blaADC-25_1
msrE_4
blaGES-11_1
blaOXA-164_1
ARR-2_1
erm(F)_1
erm(F)_3
tet(Q)_4
ere(B)_1
ant(3")-Ia_1
blaBIL-1_1
mdf(A)_1
blaFOX-2_1
aadA4_1
dfrA7_1
aadA5_1
blaCMY-10_1
blaTEM-101_1
tet(A)_2
tet(A)_1
dfrA7_5
blaCTX-M-101_1
ARR-3_1
aph(6)-Id_1
aph(3")-Ib_1
mph(A)_1
mph(A)_2
aac(6')-Ib-cr_1
oqxB_1
tet(32)_1
dfrA1_1
aadA1_2
dfrA14_1
blaCMY-19_1
catB8_1
catB4_1
blaTEM-104_1
blaMOX-2_1
sul1_10
aac(6')-Ib-cr_2
sul1_5
blaFOX-10_1
aadA1_5
mcr-7.1_1
blaFOX-1_1
cfxA2_1
tet(Q)_3
tet(Q)_1
ere(A)_2
aph(3")-Ib_2
aadA15_1
blaOXA-141_1
aadA1_3
cmlA1_1
blaLCR-1_1
tet(X)_3
aadA6_1
aac(3)-Ib-aac(6')-Ib'_1
aac(6')-30-aac(6')-Ib'_1
aadA10_2
sul1_11
tet(O)_3
msr(D)_2
msr(D)_3
mef(A)_1
mef(A)_3
mef(A)_2
mph(F)_1
mphE_1
tet(37)_1
blaGES-10_1
cfxA6_1

A:



B:

Figure 3.5: ARGs and bacterial hosts of February sample. (A) influent sample, the number in right box indicate the number of ARGs harbored by each species, *E.coli* has the highest number (B) effluent sample, the number of harbored ARGs were decreased after treatment. Numbers on bars indicate the frequency of each resistance genes in the host bacteria.

1.2 Samples of Summer 2018:

1.2.1 ARGs abundance and removal efficiency

As shown in table (3.10), in the influent sample, 253 ARGs subtypes were found and the top 10 ARGs counted 38% of the total. Like in February sample the most abundant antimicrobial drug class was macrolide resistance which includes *msr(E)_4*, *mph(E)_1* and *mph(A)_1* genes followed by tetracycline resistance genes including *tet(39)_1* and *cmlA1_1*, aminoglycoside including *aph(6)-Id_1* and *ant(3")-Ia_1*.Sulfonamide and Trimethoprim which include *sul1_10 and msr(D)_2* genes, respectively.

On the other hand, in the effluent sample, 72 ARGs subtypes were found and the top 10 counted to 51% of the total. the most abundant ARGs family like in influent sample was macrolide including the same resistance genes beside *ere(A)_2* followed by Beta-lactamase (*blaLCR-1_1*, *blaOXA-10_1*

and *cfxA2_1)* and Sulfonamide including *sul1_10.* Moreover, two genes *tet(C)_2* and *ant(3")-Ia_1* belonged to tetracycline and aminoglycoside respectively. See appendix 4 for less abundant ARGs.

Table 3.10: ARGs abundance and percentages according to the number of reads

Influent			**Effluent**		
Gene	**Frequency**	**%**	**Gene**	**Frequency**	**%**
msr(E)_4	255	13.1	*msr(E)_4*	34	1.7
mph(E)_1	116	5.9	*mph(E)_1*	26	1.3
mph(A)_1	57	2.9	*mph(A)_1*	5	0.4
sul1_10	52	2.7	*sul1_10*	7	0.3
aph(6)-Id_1	40	2.1	*ere(A)_2*	5	0.3
tet(39)_1	40	2.1	*tet(C)_2*	4	0.2
cfxA2_1	35	1.8	*cfxA2_1*	3	0.15
ant(3")-Ia_1	31	1.6	*ant(3")-Ia_1*	3	0.15
cmlA1_1	27	1.4	*blaOXA-10_1*	3	0.15
msr(D)_2	27	1.4	*blaLCR-1_1*	3	0.15

The removal efficiency was also calculated as described above, only *aph(6)-Id_1* resistance gene which classified under aminoglycoside group have an absolute removal efficiency. Other genes still have high removal efficiency ranged from 78-100%. See appendix 4.

1.2.2 Identification of host bacteria

In the influent sample, 30 different bacterial species were found, the top 10 comprised 81% of the total bacterial species. At the phylum level, the most abundant one was *Proteobacteria (72%)* which

includes the same species that detected in February sample besides, *Aeromonas punctata*. The second most common phylum was *Firmicutes (*13%), then uncultured bacterium *(9.8%)* followed by *Bacteroidetes (2%)* which includes *Prevotella ruminicola, Prevotella denticola* shown in table 3.9. Please see appendix 2 for less abundant bacteria in the effluent sample, only two bacterial species were identified: *A. baumannii* and *K. pneumoniae* belonging to *Proteobacteria* (Table 3.11).

The removal efficiency was high like on February and ranged from 81- 100 %. See appendix 4.

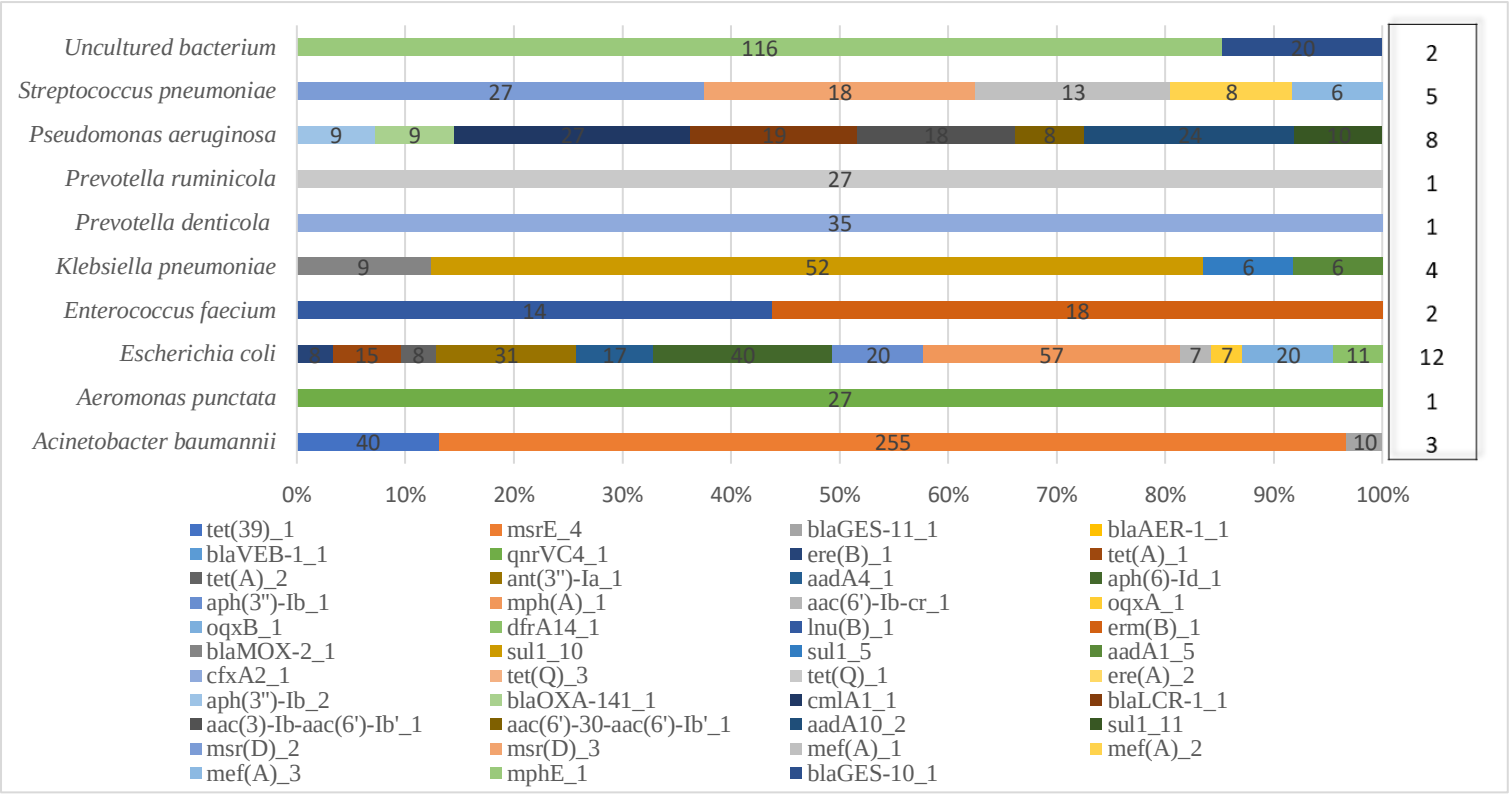
Table 3.11: Antibiotics resistance bacterial species abundance & percentages in both samples according to number of reads.

Influent			**Effluent**		
Bacteria	**abundance**	**%**	**Bacteria**	**abundance**	**%**
Acinetobacter baumannii	310	22.4	*Acinetobacter baumannii*	34	2.5
Escherichia coli	241	17.4	*Uncultured bacterium*	26	1.9
Uncultured bacterium	136	9.8	*Klebsiella pneumoniae*	7	0.5
Pseudomonas aeruginosa	124	8.9			
Klebsiella pneumoniae	73	5.3			
Streptococcus pneumoniae	72	5.2			
Prevotella denticola	35	2.5			
Enterococcus faecium	32	2.3			
Aeromonas punctata	27	1.9			
Prevotella ruminicola	27	1.9			

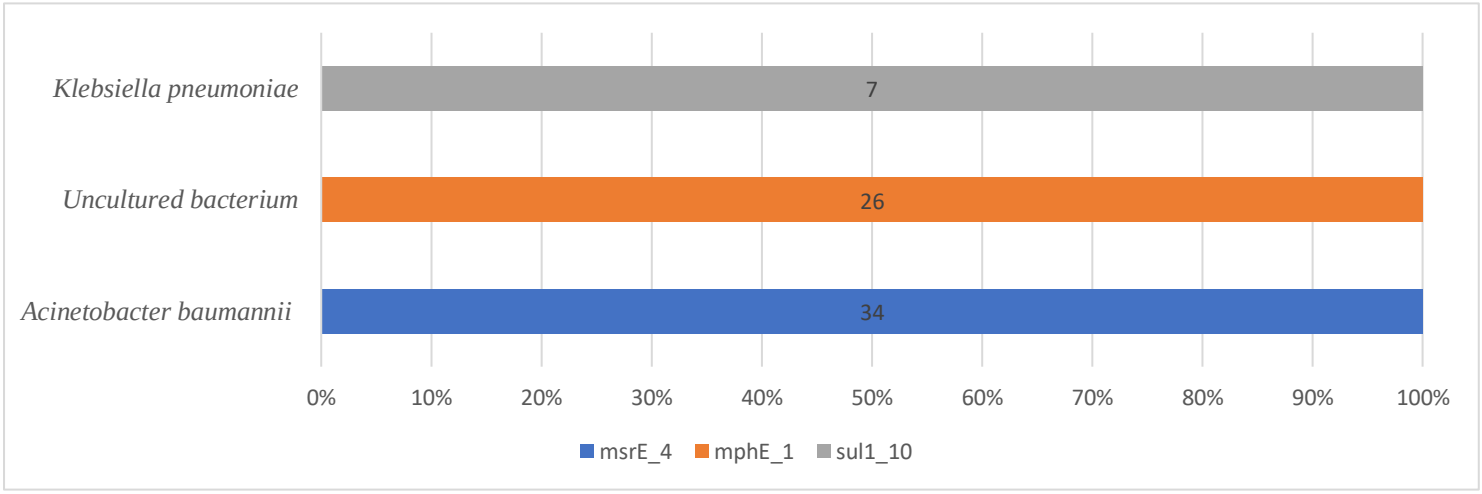
3.2.3 ARGs and host Bacterial species

As shown in Figure 3.6 (A), Different ARGs harbored by different bacterial species in the influent sample. *msr(D)_2,3* genes were detected *S. pneumoniae* and conferring resistance to a trimethoprim antibiotic class. Two genes (*cmlA1_1* and *aadA10_2)* conferring resistance to tetracycline and aminoglycoside, respectively, were detected in *P. aeruginosa*. In addition, a tetracycline resistant gene, *tetQ_1was* detected *in Prevotella ruminicola*. In contrast, *cfxA2_1* was detected in *Prevotella denticola* which is considered as beta-lactamase resistance gene. *sul1_10*, a sulfonamide resistance, was detected in *K. pneumoniae. lnu(B)_1* and *erm(B)_1* genes conferring resistance for Lincosamide and streptogramin antibiotics were detected in *Enterococcus faecium*. Two genes (*mph(A)_1, aph(6)-Id_1)* (showing resistance for macrolide and aminoglycoside) were detected in *E. coli. qnrVC4_1* a fluoroquinolone resistance gene, was found in *Aeromonas punctata*. Finally, *msr(E)-4* conferring resistance for macrolide was detected in *A. baumannii.*

In the effluent sample the most dominant ARGs still found in the host bacteria but with lower frequency as figure 3.6 (B) showed. See appendix 4 for less abundant bacteria and related genes.



A:



B:

Figure 3.6: ARGs detected in the ARB species host in August. (A) influent sample, the numbers in the right box indicated the number of harbored ARGs by each bacterial species, and *E.coli* has the highest number (B) effluent sample, the diversity of the ARGs was low . The numbers in the bar indicate the frequency of each resistance genes in the host bacteria.

3.3 ARGs families

As seen in table (3.12), there are 12 ARGs classification groups. The most diverse one that come with different resistance gene is beta-lactamase with 34 different genes. Followed by tetracycline and aminoglycoside which have 20 and 18 genes, respectively. The other group were ordered according to the number of genes.

Table 3.12: ARGs resistance group families and resistance mechanism according to 'The Comprehensive Antibiotic Resistance Database'

Class	**Gene**	**Resistance mechanism**
Beta-lactamase	*blaADC-25, blaCTX-M-101, penA, blaBIL-1, blaVEB-9, blaTEM-101, blaTEM-104, ampS, blaBRO-1, blaLCR-1, blaOXA-141, blaOXA-164, blaOXA-211, blaOXA-212, blaOXA-333, blaOXA-334, blaTRU-1, cfxA2, cfxA3, cfxA6, blaCMY-19, blaFOX-1, blaFOX-10, blaFOX-2, blaFOX-3, blaMOX-2, blaMOX-5, blaVCC-1, blaAER-1, blaOXA-1, blaGES-10, blaGES-11, blaGES-14, blaOXA-4*	Antibiotic inactivation
Tetracycline	*tet(32), tet(36), tet(37), tet(M), tet(O), tet(O/W), tetQ, tet(L), mdf(A), tet(A), mefC, , oqxA, oqxB, tet(G), tet(X), tetE, tet(39), tetA(P)*	Antibiotic inactivation, Antibiotic target protection, Antibiotic efflux
Aminoglycoside	*aac(3), aac(6'), aadA, aadA1, aadA10, aadA15, aadA17, aadA4, aadA5, aadA6, ant(3''), ant(3'')-Ia, ant(6), ant(6)-Ia,aph(3''), aph(3'')-Ib, aph(6)-Id, aac(6')-Ib-cr*	Antibiotic inactivation

Class	Gene	Resistance mechanism
Macrolide	*mphG, ere(A), ere(B), ere(D), mph(A), mph(F),mph(N), mphE, msrE,vat(B), erm(F), erm(35), erm(F), erm(G), erm(B)*	Antibiotic inactivation, antibiotic target protection
Chloramphenicol/florfenicol	*catB3, catB4, catB8, catQ, catB3, catB4, catB8, catQ, cmlA1, cmlB1*	Antibiotic inactivation
Trimethoprim	*dfrA1, dfrA14, dfrA14, dfrA7, mef(A)(mel), msr(D)(mel)*	Antibiotic target replacement
Quinolone	*qnrD1, qnrS2, qnrVC1, qnrVC4*	Antibiotic target protection
Lincosamide	*lnu(B), lnu(D)*	Antibiotic inactivation
Sulfonamide	*sul1, sul2*	Antibiotic target replacement
Rifamycin	*ARR-2,ARR-3*	Antibiotic inactivation
Peptides	*mcr-7.1*	Antibiotic target alteration

3.4 Seasons differences

3.4.1 Winter vs. Summer, influent sample

3.4.1.1 Bacterial differences

Figure (3.7) shows the differences according to number of reads of ARB in the influent sample between two the seasons Winter and Summer. Significance differences ($p < 0.01$, $r = 0.9$) was detected as the abundance of ARB in Winter was more than Summer sample.

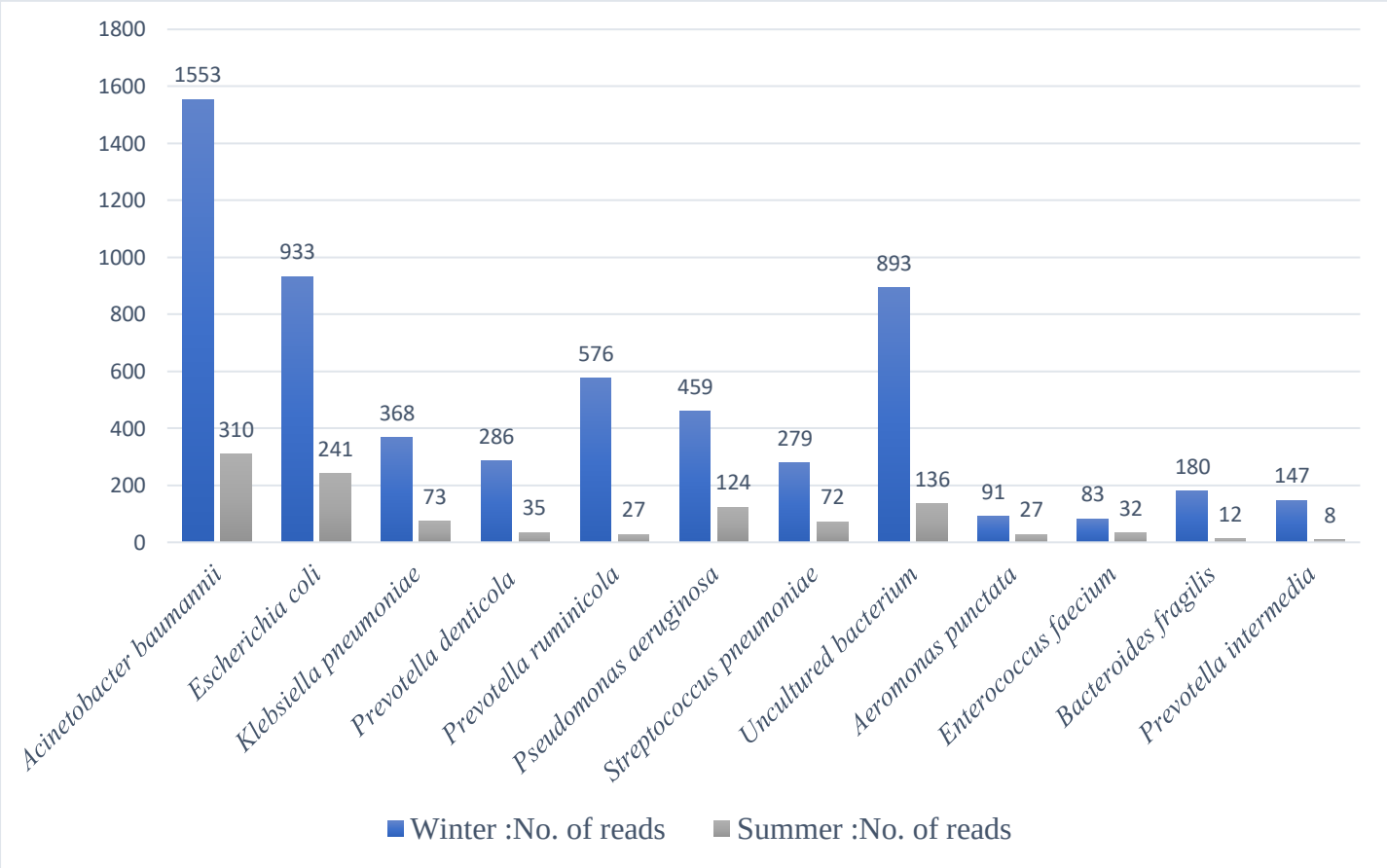


Figure 3.7: Differences of top 10 ARB species (No. of reads) between Winter and Summer

3.4.1.2 ARGs differences

Figure (3.8) shows the difference between ARGs number of reads in the influent samples. Significance differences ($p < 0.01$, $r = 0.89$) in the abundance of ARGs in which were more in the Winter than Summer sample.

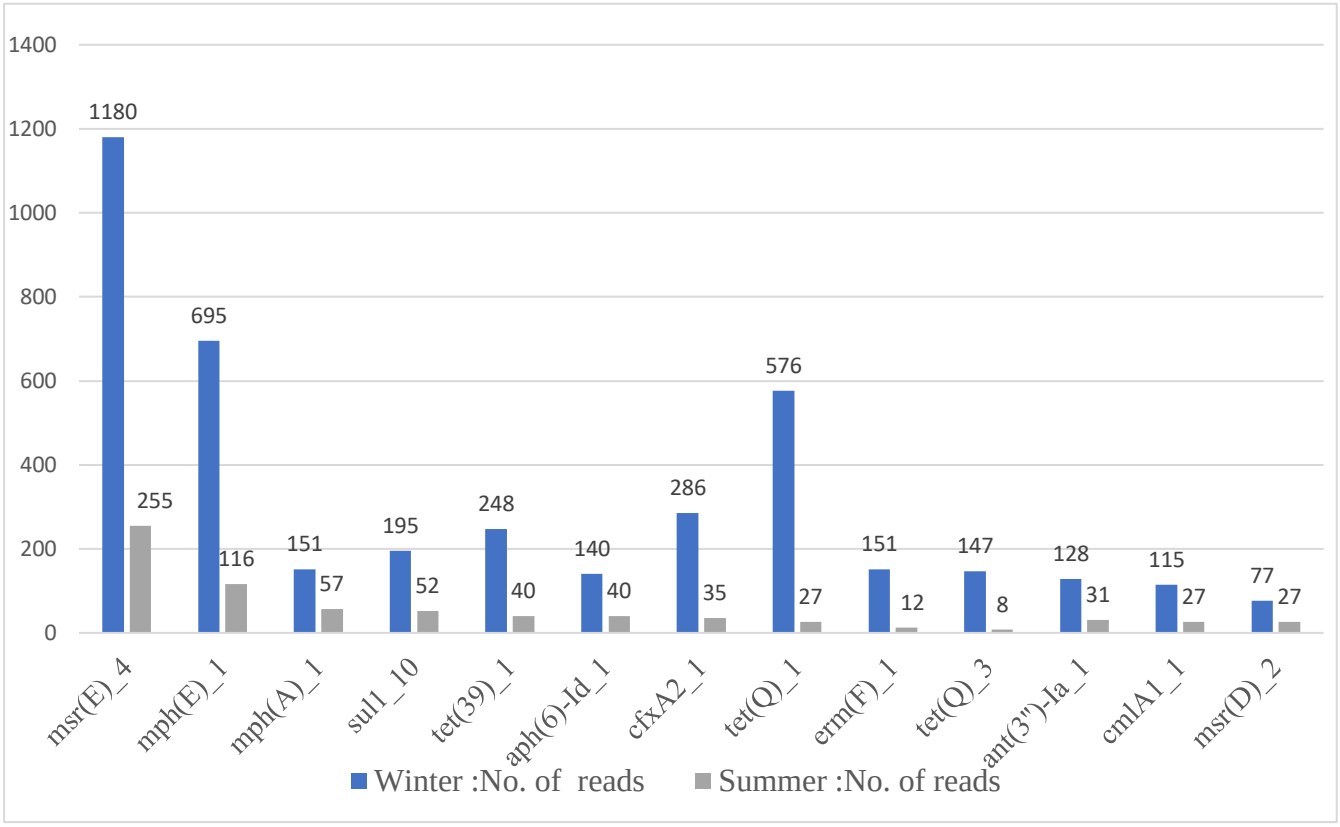


Figure 3.8: Difference of ARGs (No. of reads) between Winter and Summer

3.4.2 Winter vs. Summer, effluent sample

3.4.2.1 Bacterial differences

Figure (3.9) shows the difference according to the number of reads of bacterial species in the effluent sample between two seasons. In Summer only three species were found which were *A. baumannii* and *Uncultured bacterium* with lower abundance than Winter except *K. pneumoniae* which almost the same between two seasons. In Winter, three new species are raised, i.e. *Photobacterium damselae*, *Salmonella enterica*, and *Salmonella enteritidis.*

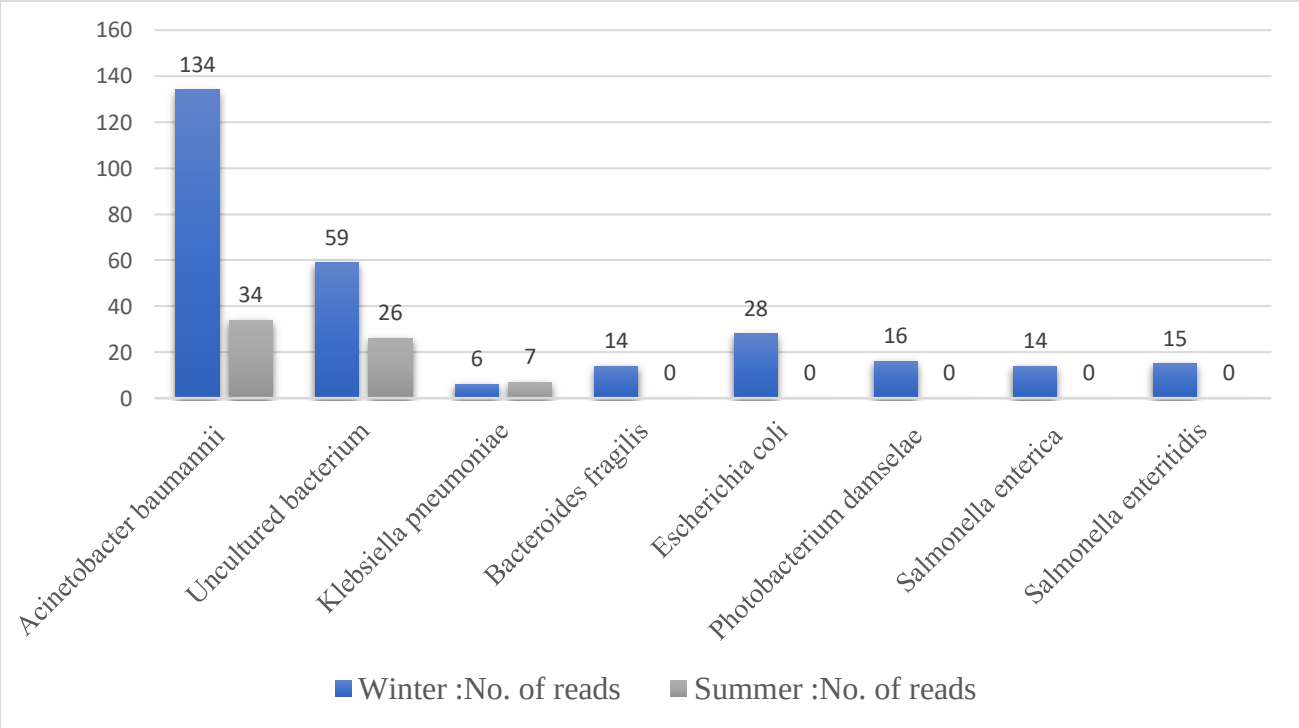


Figure 3.9: Difference of bacterial species (No. of reads) between Winter and Summer

3.4.2.2 ARGs differences

Figure (3.10) shows the difference according to the number of reads of ARGs between Winter and Summer. In the Winter sample show more abundance than summer sample.

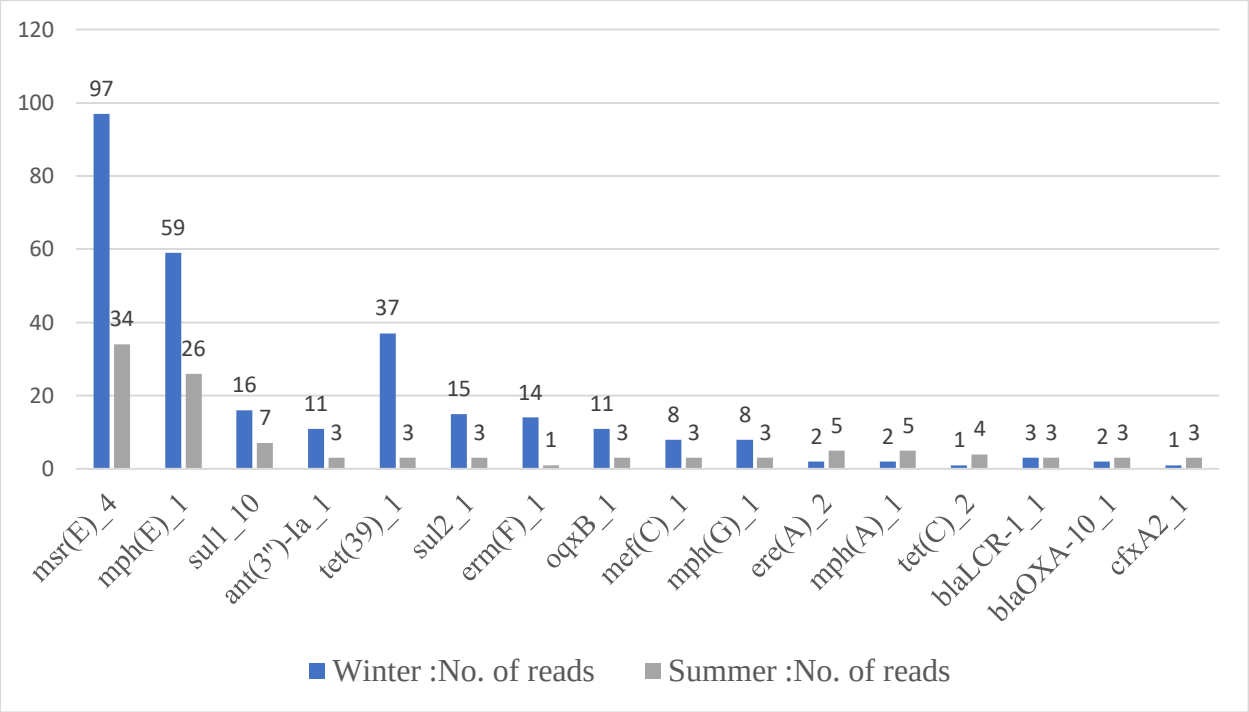


Figure 3.10: Differences of ARGs (No. of reads) on Winter compared to Summer

Chapter four

Discussion

4.Discussion

Antibiotics used for many ages to eliminate bacteria. These bacteria could acquire the resistance to antibiotics by horizontal gene transfer (HGT). This gives the reason for estimating the level of these genes in the environment, besides, activated sludge is a good source for these ARGs (Ziembińska-Buczyńska, Felis et al. 2015). The aim of this study was to document the presence of various types of ARB and ARGs in raw WW (influent) and treated WW (effluent) collected in two different seasons winter (February) and summer (August) from Al-Bierh WWTP. The sources of raw waste water that flow to this plant are houses, slaughterhouses and different hospitals in the area. In this study, a difference was observed in the amount of bacterial DNA - as represented by the total number of reads- between influent and effluent samples across the two seasons, In Winter, 3.7 million reads (DNA sequence) were detected in the influent sample compared to 95.7 thousand reads in the effluent sample. In contrast, in Summer, the total number of reads in the influent sample were 0.37 million reads compared to 31.7 thousand read for the effluent sample. The decreased number of reads after treatment may reflect to some extent the efficiency of the treatment plant. On the other hand, the reason for the high amount of bacterial DNA in Winter rather than Summer is due to rainfall water that carries most of microorganisms in the sewer pipeline and goes directly to AL-Bireh plant. Moreover, the prevalence of bacterial infection usually higher in Winter than Summer especially among vulnerable people such as children and elderly who do not have good immunity against these infections and may overuse broad spectrum antibiotics and thus developing of resistant bacteria. Also, most of bacteria that cause

gastrointestinal, respiratory track and urinary tract infections -and so the antibiotics-are released through feces and urine and other body fluids and finally to the sewage. In AL-Bireh plant, the treatment process depends basically on activated sludge which uses bacteria to degrade the contaminants and thus the microbial community in the AS are increased in Winter rather than in Summer.

4.1 Influent and effluent sample

4.1.1 Antibiotic resistance bacterial species

In Winter, 54 species of bacteria were detected in the influent sample versus 30 species of bacteria in the influent Summer sample, most of them were gram-negative bacteria in both influent and effluent samples. When comparing the bacterial community at species level, (assume α=0.01) there were significance differences in bacterial abundance among the two seasons Winter and Summer as shown in figure (7) ($p<0.01$, $r = 0.9$), the detected ARB are the most common causing disease and may survive at wide range temperature and pH. Ziembińska and colleagues (Ziembińska-Buczyńska, Felis et al. 2015) showed that the amount and diversity of bacteria in Winter were more than in Summer which explained by increasing the biomass of the reactor to maintain the effectiveness of waste water treatment under cold temperature, besides to the increase of antibiotics use during Winter which may increase the number of resistant bacteria. In our study, the top ten ARB (which had the highest abundance) were selected for the analysis. At the phylum level, the most abundant bacteria found in both seasons were *Proteobacteria* followed by *Firmicutes* and *Bacteroidetes*. Of the *Proteobacteria*, several species were identified such as: *A. baumannii*, *E. coli*, and *K.pneumonia*. However, these species were significantly more frequent in Winter than Summer. Our results are consistent with several other studies (Wagner and Loy 2002, Adrados, Sánchez et al. 2014, Gonzalez-Martinez, Rodriguez-Sanchez et al. 2015, Hu, Zhang et al. 2016) and inconsistent to Ju et al study (Cai, Ju et al. 2014) reported that the most abundant phylum in the influent sample was *Firmicutes* followed by *Proteobacteria , Actinobacteria*, and *Bacteroidetes*. Regarding to species frequencies, our results were in agreement with those reported by other studies (Shchegolkova, Krasnov et al. 2016, Hendriksen, Munk et al. 2019) which revealed that the most abundant bacterial species across the samples was *Acinetobacter* and disagreed with other results stated that the most frequent species were *Bacteroides*, *Escherichia, Streptococcus*, (Hendriksen, Munk et al. 2019) besides, (Ahmed, Staley et al. 2017) results reported that the most dominated species in raw WW samples (collected from four WWTPs) was *Pseudomonas*, followed by *Arcobacter* and *Bacteroides*. In our study, all the bacterial species

found were matched the pattern of human pathogens which cause different infections in respiratory tract, guts and urinary tract.

The effluent samples contain only 8 species in February sample and 3 species in Summer sample, most of them were *Proteobacteria* followed by *Bacteroidetes* with lower relative abundance than the influent samples. Our results inconsistent with (Jiao, Zhou et al. 2018) study showed that the most abundant phylum *Proteobacteria, which* was *less* frequent in Winter than Summer. *Acinetobacter* had the highest frequency in both seasons and this result different to what reported by Ahmad and colleagues (Ahmed, Staley et al. 2017) study who found the most frequent species in secondary treated WW sample was *Pseudomonas.*

In our study, across all samples, *A. baumannii* was the most abundant bacteria. This bacterium was reported as the worldwide nosocomial infection leading bacteria with high mortality and morbidity. It is mainly found in the intensive care units of hospitals that may cause many infections in the respiratory tract, bloodstream, urinary tract and wound infection (Lee, Lee et al. 2017, Kumburu, Sonda et al. 2019). This bacterium was classified as a multidrug-resistance that confer resistance against beta-lactams, fluoroquinolones, and aminoglycosides. The drug of choice to treat such bacteria is Colistin and tigecycline (Alekshun, 2007). The WHO stated that *A. baumannii* is one of the most danger ESKAPE microorganisms *(Enterococcus faecium, Staphylococcus aureus, K. pneumoniae, A. baumannii, P.aeruginosa, and Enterobacter species)* which have the ability to resist antibiotics drugs. The major resistance mechanisms of *A. baumannii* to resist antibiotics is beta-lactamases. However, this bacterium has the ability to enter an exogenous DNA as its genome harbors foreign DNA with high frequency, this explained by HGT. Tetracycline class antibiotic has proven successful treatment and good tolerability (Lee, Lee et al. 2017).

In this study we noticed that most of bacteria were efficiently removed after treatment of WW samples which could be attributed to the high performance of Al-Bierh plant as it based on using aeration tank and activated sludge as secondary process under the effect of oxygen. Moreover, it relied on the sedimentation process in which the pathogens were adsorbed in the biosolid phase (Ahmed, Staley et al. 2017). Most of the bacterial families found across the samples were characterized as gram-negative bacteria, and facultative aerobic except of *Acetobacteraceae* -including *A. baumannii* - and *Pseudomonadaceae* -including *P.aeruginosa* which were aerobic families (Silva-Bedoya, Sanchez Pinzon et al. 2016). A dramatic decrease in the anaerobic bacteria was noted after treatment which may be due to shifting from anaerobic to aerobic condition replacing the anaerobic bacteria with

facultative anaerobic ones with the aid of low temperature, and thus bacteria that live under oxygen condition will be proliferated and retained in the plant (Bengtsson-Palme, Hammarén et al. 2016).

4.1.2 ARGs

In the influent sample, 400 ARGs subtypes were found in Winter sample, the top 10 ARGs counted to 44% of the total ARGs while in Summer, 253 ARGs subtypes were found and the top 10 ARGs counted to 38% of the total. Assuming (α= 0.01) there was a significant difference ($p<0.01$, $r=0.89$) in ARGs abundance which was higher in Winter than Summer due to intensive use of antibiotics and this was in line with (Yang, Li et al. 2013) and disagreed to other studies conducted by (Du, Geng et al. 2014, Karkman, Johnson et al. 2016, Wen, Yang et al. 2016) who revealed that varied temperatures throughout different seasons did not have a significant effect on the ARGs abundance, beside, the abundance of the ARGs in the same season will differ between influent and effluent samples which was higher in the influent. Our findings were consistent with (Du, Geng et al. 2014) study and contrast to (Jiao, Zhou et al. 2018) study showed that abundance of ARGs decreased by one order of magnitude in Winter samples than in Summer. We noted that the most abundant resistant genes in the two seasons (as shown in tables 3.7 and 3.11) were *msr(E)*, *mph(E)* and *mph(A)*. These genes were reported as macrolide resistance genes especially to erythromycin antibiotics including azithromycin, clarithromycin, spiramycin. These drugs are used to treat respiratory infections and some UTIs that caused by *A. baumannii* (https://www.drugbank.ca/drugs/DB00199).

Our results were inconsistent to (Hu, Zhang et al. 2016) study revealed that tetracycline resistant genes were the most abundant genes in the influent sewage followed by Beta-lactamase, sulfonamide, streptogramin and aminoglycoside resistance genes. On the other hand, our results were in agreement with other studies (Szczepanowski, Linke et al. 2009, Christgen, Yang et al. 2015, Hendriksen, Munk et al. 2019, Pärnänen, Narciso-da-Rocha et al. 2019) showed that macrolides and tetracyclines were the most abundant resistance class. A study conducted in 2018, (Karkman, Do et al. 2018) revealed that *erm(F)* a macrolide resistance gene and *tetP(A)* and *tetP(B)* (tetracycline resistance genes) were the most abundant ARGs in the digested and dried sludge. In addition, Yang and colleagues (Yang, Li et al. 2013) showed that aminoglycoside and tetracycline resistant genes were the two most dominant genes in eight AS samples followed by sulfonamide and chloramphenicol. In our study, beta-lactamase resistant genes including *blaLCR-1_1*, *blaOXA-10_1* and *cfxA2_1* were more predominant in Summer samples than in Winter. One possible explanation that urinary tract infection is more prevalent in Summer and be treated by B-lactam antibiotics (Lee, Lee et al. 2017, Kumburu, Sonda et al. 2019).

The ARGs in effluent samples were classified under the same drug classes as in influent samples (shown in tables 3.7 and 3.11) but with different subtypes. In Winter sample, 88 ARGs subtypes found and the top 10 ARGs counted to 67% of the total, but in Summer sample, 72 ARGs subtypes were found and the top 10 counted to 51% of the total. The ARGs abundance differences between the two seasons may be attributed to the differences in the uses of antibiotics. Our findings differed from several studies (Naquin, Shrestha et al. 2015) showed that *ermB, sul1, tetA, tetX,* and *mecA* were the most abundant resistance genes in both influent and effluent which conferring resistant to erythromycin, sulfonamide, tetracycline, and methicillin, respectively. A study reported by (Zarei Baygi, Harb et al. 2019) was also found that the most abundant resistance genes were *sul1* and *intI1*, followed by *sul2, tetO, tetW, oxa-1, ermF, ermB,* and *ampC*. In addition, a study conducted by (Freeman, Yost et al. 2017) showed that *sul1, ermB, intI1, blaCTX-M, qnrS,* and *tetO* were the most abundant resistance genes in effluent samples. Finally, (Hu, Zhang et al. 2016) revealed Beta-lactamase as the most abundant resistance genes in the effluent samples. These reported variations in ARG composition across different AS samples that represented in different studies might be attributed to different factors: first, the different wastewater sources and treatment processes of WWTPs. secondly, the completeness of ARG databases, depth of metagenomic sequencing and alignment similarity which influence the results of ARG analysis (Liu et al., 2019). Finally, the selection pressure that favors ARG, beside the surrounding conditions that favor the host bacteria and HGT which allow the transfer of the gene in the bacterial community (Karkman, Johnson et al. 2016).

In this study, we observed that the removal efficiency of ARGs was high which was obvious in the differences of the relative abundances of ARGs between influent and effluent samples. Some of ARGs were completely removed from the influent samples such as *tet(Q)_3* and *aph(6)-Id_1* indicating removal of their bacterial hosts. However, the persistence of ARGs in effluent samples is still considered a form of pollution that may facilitate the spread of antibiotic resistant bacteria through HGT (Hu, Zhang et al. 2016).

4.1.3 Plasmid-associated ARGs

Plasmids play crucial role in acquisition of ARGs and allow transfer to a wide variety of microorganisms via horizontal gene transfer. Overall, 107 Different antibiotics resistance genes conferring resistance to 12 antibiotic classes were detected. This include; Beta-lactamase , aminoglycoside, , macrolide, quinolones, Lincosamide, phenicols, streptogramins, sulfonamides, peptides and tetracycline were most commonly reported as plasmid-associated genes (Carattoli 2009, Carattoli 2013, Rozwandowicz, Brouwer et al. 2018, Liu, Klümper et al. 2019, McMillan, Gupta et al.

2019). Other resistance genes such as *aac(6')-I, ant(3'')-Ia, ere(A), ARR-2, tet(M)* conferring resistance to aminoglycoside, macrolide, rifamycin and tetracycline were not plasmid associated genes (McMillan, Gupta et al. 2019). *mef(C)-1* reported to be found in the chromosome of the bacteria (Ziembińska-Buczyńska, 2015). In Winter samples, there were 16 different bacterial species (out of 53 species) that carry more than one resistance genes. In Summer samples there were 7 different bacterial species (out of 30 species) that carry more than one resistance genes. These results strongly indicate the presence of multidrug-resistance plasmids (Alekshun and Levy 2007, Partridge, Kwong et al. 2018). The spread of multidrug-resistant bacteria has become a worldwide public health concern which is associated with increased morbidity, increased risk of therapeutic failure and healthcare costs. Plasmids disseminate the ARGs between bacterial community through different mechanisms, one of them is conjugation. it consider the most important role in *Enterobacteriaceae* and *Enterococcaceae* (San Millan 2018). The plasmids control their replication out of the chromosomes in sophisticated way. Replicon is the element of replication of the parent plasmid that contain origin of replication and regulating factors. Also, many plasmids have an antitoxin-toxin mechanism which kill the daughter cells if it is not inherited the plasmid through cell division, this will promote the maintenance of plasmid genes (Carattoli, 2013). Plasmids exploit the host replication mechanism to replicate their own DNA and limit the replication of host plasmid. In some specific bacterial species, the plasmid can only maintain their DNA (Partridge, Kwong et al. 2018) .

To the best of our knowledge, this study is the first attempt to investigate the occurrence of AMR and ARGs in waste water samples collected from a treatment plant in Palestine. Even though, one limitation of this study that only one waste water treatment plant was investigated. Further, a single sample was analyzed from this site at two different seasons. However, our study provides a reliable base of evidence and accurately described and characterized the burden of AMR and ARGs in waste water samples which is essential to take public health actions.

Recommendations

We recommend to increase the awareness between the people about the effect of WW on the human health and environment, and improving treatment systems should be a priority to policy makers to limit the burden of ARB and ARGs in treated waste water in Palestine. Finally, the government must enforce a law about WW use violation.

Conclusion

Metagenomic shotgun analysis have an advantage upon classical PCR analysis in determination of unculturable bacteria and detection of novel ARGs without targeting specific bacterial genes. In this study, various types of pathogenic bacteria were found. Most of them were gram-negative and opportunistic pathogens, the most common one was *Acinetobacter baumannii*. The abundance of ARGs and related bacterial host were found to be higher in February than August which was probably due to the intensive use of antibiotics and the selection pressure of certain types of ARGs in the activated sludge community. The removal efficiency was high due to the sedimentation process that used in the treatment of waste water at Al-Bierh plant, however, our concern is the proliferation and dissemination of those ARB and ARGs to the environment through the effluent waste water that discharged at the wadies which certainly pollute the ground and fresh water, and the soil as well. The considerable amounts of antibiotics and ARGs that have not been eliminated after treatment can be taken up by the plants and crops and thus pose a public health problem. Therefore, we recommend to increase the awareness among locals about the effect of wastewater and accompanied pathogens on the human health and environment. Moreover, improving the sanitation and treatment systems should be a priority to policy makers to limit the burden of ARB and ARGs in treated waste water in Palestine.

References

Abusharbak, N. (2004). Development of Wastewater Treatment Tariff System: A Case Study of Al–Bireh Wastewater Treatment Plant. Master Master thesis, Birzeit University.

Adrados, B., O. Sánchez, C. Arias, E. Becares, L. Garrido, J. Mas, H. Brix and J. Morató (2014). "Microbial Communities from Different Types of Natural Wastewater Treatment Systems: Vertical and Horizontal Flow Constructed Wetlands and Biofilters." Water research **55C**: 304-312.

Ahmed, W., C. Staley, J. Sidhu, M. Sadowsky and S. Toze (2017). "Amplicon-based profiling of bacteria in raw and secondary treated wastewater from treatment plants across Australia." Appl Microbiol Biotechnol **101**(3): 1253-1266.

Ahn, Y. and J. Choi (2016). Bacterial Communities and Antibiotic Resistance Communities in a Full-Scale Hospital Wastewater Treatment Plant by High-Throughput Pyrosequencing.

Akpor, O. and M. Muchie (2011). Environmental and public health implications of wastewater quality.

Alekshun, M. and S. Levy (2007). "Alekshun MN, Levy SB.. Molecular mechanisms of antibacterial multidrug resistance. Cell 128: 1037-1050." Cell **128**: 1037-1050.

Baquero, F., J.-L. Martínez and R. Canton (2008). Antibiotics and Antibiotic Resistance in Water Environments.

Bengtsson-Palme, J., R. Hammarén, C. Pal, M. Östman, B. Björlenius, C.-F. Flach, J. Fick, E. Kristiansson, M. Tysklind and J. Larsson (2016). "Elucidating selection processes for antibiotic resistance in sewage treatment plants using metagenomics." Science of The Total Environment **572**.

Cai, L., F. Ju and T. Zhang (2014). "Tracking human sewage microbiome in a municipal wastewater treatment plant." Appl Microbiol Biotechnol **98**(7): 3317-3326.

Cai, L. and T. Zhang (2013). Detecting Human Bacterial Pathogens in Wastewater Treatment Plants by a High-Throughput Shotgun Sequencing Technique.

Carattoli, A. (2009). "Resistance Plasmid Families in <em>Enterobacteriaceae</em&gt." Antimicrobial Agents and Chemotherapy **53**(6): 2227.

Carattoli, A. (2013). "Plasmids and the spread of resistance." Int J Med Microbiol **303**(6-7): 298-304.

Chen, Y., S. Lan, L. Wang, S. Dong, H. Zhou, Z. Tan and X. Li (2017). "A review: Driving factors and regulation strategies of microbial community structure and dynamics in wastewater treatment systems." Chemosphere **174**: 173-182.

Christgen, B., Y. Yang, S. Zia, B. Li, D. C. Rodríguez, T. Zhang and D. Graham (2015). "Metagenomics Shows That Low-Energy Anaerobic−Aerobic Treatment Reactors Reduce Antibiotic Resistance Gene Levels from Domestic Wastewater." Environmental science & technology **49**: 2577–2584.

D. Hladilek, M., K. Gaines, J. Novak, D. A. Collard, D. Johnson and T. Canam (2016). Microbial community structure of a freshwater system receiving wastewater effluent.

Du, J., J. Geng, H. Ren, L. Ding, K. Xu and Y. Zhang (2014). "Variation of antibiotic resistance genes in municipal wastewater treatment plant with A2O-MBR system." Environmental science and pollution research international **22**.

Edris, G. and W. M. Alalayah (2017). "Sludge production from municipal wastewater treatment in sewage treatment plant AU - Demirbas, Ayhan." Energy Sources, Part A: Recovery, Utilization, and Environmental Effects **39**(10): 999-1006.

Ferro, G., M. Polo-López and P. Fernandez-Ibanez (2016). Conventional and New Processes for Urban Wastewater Disinfection: Effect on Emerging and Resistant Microorganisms.

Freeman, C., C. Yost, L. Scriver, K. Neudorf, L. Hansen and R. Jamieson (2017). "Antimicrobial resistance gene surveillance in the receiving waters of an upgraded wastewater treatment plant." FACETS **3**.

Garrido-Cardenas, J. A., M. I. Polo-Lopez and I. Oller-Alberola (2017). "Advanced microbial analysis for wastewater quality monitoring: metagenomics trend." Appl Microbiol Biotechnol **101**(20): 7445-7458.

Gatica, J. and E. Cytryn (2013). "Impact of treated wastewater irrigation on antibiotic resistance in the soil microbiome." Environmental science and pollution research international **20**(6): 3529-3538.

Gonzalez-Martinez, A., A. Rodriguez-Sanchez, B. Munoz-Palazon, M. J. Garcia-Ruiz, F. Osorio, M. C. van Loosdrecht and J. Gonzalez-Lopez (2015). "Microbial community analysis of a full-scale DEMON bioreactor." Bioprocess Biosyst Eng **38**(3): 499-508.

González-Martínez, A., M. Sihvonen, B. Muñoz Palazón, A. Rodriguez-Sanchez, A. Mikola and R. Vahala (2018). Microbial ecology of full-scale wastewater treatment systems in the Polar Arctic Circle: Archaea, Bacteria and Fungi.

Guo, J., J. Li, H. Chen, P. L Bond and Z. Yuan (2017). Metagenomic analysis reveals wastewater treatment plants as hotspots of antibiotic resistance genes and mobile genetic elements.

Hendriksen, R. S., P. Munk, P. Njage, B. van Bunnik, L. McNally, O. Lukjancenko, T. Röder, D. Nieuwenhuijse, S. K. Pedersen, J. Kjeldgaard, R. S. Kaas, P. T. L. C. Clausen, J. K. Vogt, P. Leekitcharoenphon, M. G. M. van de Schans, T. Zuidema, A. M. de Roda Husman, S. Rasmussen, B. Petersen, A. Bego, C. Rees, S. Cassar, K. Coventry, P. Collignon, F. Allerberger, T. O. Rahube, G. Oliveira, I. Ivanov, Y. Vuthy, T. Sopheak, C. K. Yost, C. Ke, H. Zheng, L. Baisheng, X. Jiao, P. Donado-Godoy, K. J. Coulibaly, M. Jergović, J. Hrenovic, R. Karpíšková, J. E. Villacis, M. Legesse, T. Eguale, A. Heikinheimo, L. Malania, A. Nitsche, A. Brinkmann, C. K. S. Saba, B. Kocsis, N. Solymosi, T. R. Thorsteinsdottir, A. M. Hatha, M. Alebouyeh, D. Morris, M. Cormican, L. O'Connor, J. Moran-Gilad, P. Alba, A. Battisti, Z. Shakenova, C. Kiiyukia, E. Ng'eno, L. Raka, J. Avsejenko, A. Bērziņš, V. Bartkevics, C. Penny, H. Rajandas, S. Parimannan, M. V. Haber, P. Pal, G.-J. Jeunen, N. Gemmell, K. Fashae, R. Holmstad, R. Hasan, S. Shakoor, M. L. Z. Rojas, D. Wasyl, G. Bosevska, M. Kochubovski, C. Radu, A. Gassama, V. Radosavljevic, S. Wuertz, R. Zuniga-Montanez, M. Y. F. Tay, D. Gavačová, K. Pastuchova, P. Truska, M. Trkov, K. Esterhuyse, K. Keddy, M. Cerdà-Cuéllar, S. Pathirage, L. Norrgren, S. Örn, D. G. J. Larsson, T. V. d. Heijden, H. H. Kumburu, B. Sanneh, P. Bidjada, B.-M. Njanpop-Lafourcade, S. C. Nikiema-Pessinaba, B. Levent, J. S. Meschke, N. K. Beck, C. D. Van, N. D. Phuc, D. M. N. Tran, G. Kwenda, D.-a. Tabo, A. L. Wester, S. Cuadros-Orellana, C. Amid, G. Cochrane, T. Sicheritz-Ponten, H. Schmitt, J. R. M. Alvarez, A. Aidara-Kane, S. J. Pamp, O. Lund, T. Hald, M. Woolhouse, M. P. Koopmans, H. Vigre, T. N. Petersen, F. M. Aarestrup and c.

The Global Sewage Surveillance project (2019). "Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage." Nature Communications **10**(1): 1124.

Hu, Q., X.-X. Zhang, S. Jia, K. Huang, J. Tang, P. Shi, L. Ye and H. Ren (2016). Metagenomic insights into ultraviolet disinfection effects on antibiotic resistome in biologically treated wastewater.

Jiao, Y. N., Z. C. Zhou, T. Chen, Y. Y. Wei, J. Zheng, R. X. Gao and H. Chen (2018). "Biomarkers of antibiotic resistance genes during seasonal changes in wastewater treatment systems." Environ Pollut **234**: 79-87.

Joyce, M., G. Pontes, P. Takita Serra, A. Balieiro, D. Castro, F. Pieri, J. Crainey, P. Nogueira and P. Orlandi (2016). Multidrug resistant Pseudomonas aeruginosa survey in a stream receiving effluents from ineffective wastewater hospital plants.

Ju, F., F. Guo, L. Ye, Y. Xia and T. Zhang (2014). Metagenomic analysis on seasonal microbial variations of activated sludge from a full-scale wastewater treatment plant over 4 years.

Karkman, A., T. T. Do, F. Walsh and M. P. J. Virta (2018). "Antibiotic-Resistance Genes in Waste Water." Trends in Microbiology **26**(3): 220-228.

Karkman, A., T. A. Johnson, C. Lyra, R. D. Stedtfeld, M. Tamminen, J. M. Tiedje and M. Virta (2016). "High-throughput quantification of antibiotic resistance genes from an urban wastewater treatment plant." FEMS Microbiology Ecology **92**(3).

Khan, S., Q. Cao, Y. M. Zheng, Y. Z. Huang and Y. G. Zhu (2008). "Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China." Environ Pollut **152**(3): 686-692.

Kumburu, H., T. Sonda, M. van Zwetselaar, P. Leekitcharoenphon, O. Lukjancenko, B. Mmbaga, M. Alifrangis, O. Lund, F. Aarestrup and G. Kibiki (2019). "Using WGS to identify antibiotic resistance genes and predict antimicrobial resistance phenotypes in MDR Acinetobacter baumannii in Tanzania." Journal of Antimicrobial Chemotherapy **74**: 1484-1493.

Lai, E., M. Hess and F. M. Mitloehner (2018). "Profiling of the Microbiome Associated With Nitrogen Removal During Vermifiltration of Wastewater From a Commercial Dairy." Frontiers in microbiology **9**: 1964-1964.

Larsdotter, K. (2006). Microalgae for Phosphorus Removal from Wastewater in a Nordic Climate Doctoral thesis, comprehensive summary, KTH.

Lee, C.-R., J. H. Lee, M. Park, K. S. Park, I. K. Bae, Y. B. Kim, C.-J. Cha, B. C. Jeong and S. H. Lee (2017). "Biology of Acinetobacter baumannii: Pathogenesis, Antibiotic Resistance Mechanisms, and Prospective Treatment Options." Frontiers in cellular and infection microbiology **7**: 55-55.

Lee, S.-H., H.-J. Kang and H.-D. Park (2015). Influence of influent wastewater communities on temporal variation of activated sludge communities.

Liu, Z., U. Klümper, Y. Liu, Y. Yang, Q. Wei, J. Lin, J.-D. Gu and M. Li (2019). "Metagenomic and metatranscriptomic analyses reveal activity and hosts of antibiotic resistance genes in activated sludge." Environment International **129**: 208-220.

Ma, L., A. Li, X. Yin and T. Zhang (2017). "The Prevalence of Integrons as the Carrier of Antibiotic Resistance Genes in Natural and Man-Made Environments." Environmental Science & Technology **51**.

Mahmood, A. and R. Malik (2013). Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan.

Martín, J., M. Camacho-Muñoz, J. Santos, I. Aparicio and E. Alonso (2012). "Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: Removal and ecotoxicological impact of wastewater discharges and sludge disposal." Journal of hazardous materials **239-240**: 40-47.

McLellan, S. L., R. J. Newton, J. L. Vandewalle, O. C. Shanks, S. M. Huse, A. M. Eren and M. L. Sogin (2013). "Sewage reflects the distribution of human faecal Lachnospiraceae." Environmental microbiology **15**(8): 2213-2227.

McMillan, E. A., S. K. Gupta, L. E. Williams, T. Jové, L. M. Hiott, T. A. Woodley, J. B. Barrett, C. R. Jackson, J. L. Wasilenko, M. Simmons, G. E. Tillman, M. McClelland and J. G. Frye (2019). "Antimicrobial Resistance Genes, Cassettes, and Plasmids Present in Salmonella enterica Associated With United States Food Animals." Frontiers in microbiology **10**: 832-832.

Meerbergen, K., M. Van Geel, M. Waud, K. Willems, R. Dewil, J. Van Impe, L. Appels and B. Lievens (2016). Assessing the composition of microbial communities in textile wastewater treatment plants in comparison with municipal wastewater treatment plants.

Modin, O., F. Persson, B.-M. Wilén and M. Hermansson (2016). Non-Oxidative Removal of Organics in the Activated Sludge Process.

Mogheir, Y., Z. Zomlot, T. Abu Hujair, A. Ahmad and D. Fatta (2005). Treated Wastewater Reuse in Palestine.

Naidoo, S. and A. Olaniran (2013). Treated Wastewater Effluent as a Source of Microbial Pollution of Surface Water Resources.

Nakayama, T., T. T. Tuyet Hoa, K. Harada, M. Warisaya, M. Asayama, A. Hinenoya, J. W. Lee, T. M. Phu, S. Ueda, Y. Sumimura, K. Hirata, N. T. Phuong and Y. Yamamoto (2017). "Water metagenomic analysis reveals low bacterial diversity and the presence of antimicrobial residues and resistance genes in a river containing wastewater from backyard aquacultures in the Mekong Delta, Vietnam." Environ Pollut **222**: 294-306.

Naquin, A., A. Shrestha, M. Sherpa, R. Nathaniel and R. Boopathy (2015). "Presence of antibiotic resistance genes in a sewage treatment plant in Thibodaux, Louisiana, USA." Bioresource technology **188**.

Pärnänen, K. M. M., C. Narciso-da-Rocha, D. Kneis, T. U. Berendonk, D. Cacace, T. T. Do, C. Elpers, D. Fatta-Kassinos, I. Henriques, T. Jaeger, A. Karkman, J. L. Martinez, S. G. Michael, I. Michael-Kordatou, K. O'Sullivan, S. Rodriguez-Mozaz, T. Schwartz, H. Sheng, H. Sørum, R. D. Stedtfeld, J. M. Tiedje, S. V. D. Giustina, F. Walsh, I. Vaz-Moreira, M. Virta and C. M. Manaia (2019). "Antibiotic resistance in European wastewater treatment plants mirrors the pattern of clinical antibiotic resistance prevalence." Science Advances **5**(3): eaau9124.

Partridge, S., S. Kwong, N. Firth and S. Jensen (2018). "Mobile Genetic Elements Associated with Antimicrobial Resistance." Clinical Microbiology Reviews **31**.

Ravi, A., S. Ereqat, A. Al-Jawabreh, Z. Abdeen, O. Abu Shamma, H. Hall, M. J. Pallen and A. Nasereddin (2019). "Metagenomic profiling of ticks: Identification of novel rickettsial genomes and detection of tick-borne canine parvovirus." PLoS neglected tropical diseases **13**(1): e0006805-e0006805.

Rozwandowicz, M., M. Brouwer, J. Fischer, J. Wagenaar, B. Gonzalez-Zorn, B. Guerra, D. Mevius and J. Hordijk (2018). "Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae." The Journal of antimicrobial chemotherapy **73**.

Samhan, S., R. Al-Sa`ed, K. Assaf, K. Friese, M. van Afferden, R. Muller, W. Tümpling, M. Ghanem, W. Ali and O. Zimmo (2010). Wastewater Management Overview in the Occupied Palestinian Territory**:** 229-248.

San Millan, A. (2018). "Evolution of Plasmid-Mediated Antibiotic Resistance in the Clinical Context." Trends in Microbiology **26**.

Schmieder, R. and R. Edwards (2012). "Schmieder R, Edwards R.. Insights into antibiotic resistance through metagenomic approaches. Fut Microbiol 7: 73-89." Future microbiology **7**: 73-89.

Shanks, O. C., R. J. Newton, C. A. Kelty, S. M. Huse, M. L. Sogin and S. L. McLellan (2013). "Comparison of the microbial community structures of untreated wastewaters from different geographic locales." Applied and environmental microbiology **79**(9): 2906-2913.

Shchegolkova, N. M., G. S. Krasnov, A. A. Belova, A. A. Dmitriev, S. L. Kharitonov, K. M. Klimina, N. V. Melnikova and A. V. Kudryavtseva (2016). "Microbial Community Structure of Activated Sludge in Treatment Plants with Different Wastewater Compositions." Front Microbiol **7**: 90.

Sidhu, C., S. Vikram and A. K. Pinnaka (2017). "Unraveling the Microbial Interactions and Metabolic Potentials in Pre- and Post-treated Sludge from a Wastewater Treatment Plant Using Metagenomic Studies." Frontiers in microbiology **8**: 1382-1382.

Silva-Bedoya, L., M. Sanchez Pinzon, G. Cadavid-Restrepo and C. Moreno (2016). Bacterial community analysis of an industrial wastewater treatment plant in Colombia with screening for lipid-degrading microorganisms.

Sonune, A. and R. Ghate (2004). Developments in Wastewater Treatment Methods.

Szczepanowski, R., B. Linke, I. Krahn, K.-H. Gartemann, T. Gützkow, W. Eichler, A. Pühler and A. Schlueter (2009). "Detection of 140 Clinically Relevant Antibiotic-Resistance Genes in the Plasmid Metagenome of Wastewater Treatment Plant Bacteria Showing Reduced Susceptibility to Selected Antibiotics." Microbiology (Reading, England) **155**: 2306-2319.

Vandewalle, J. L., G. W. Goetz, S. M. Huse, H. G. Morrison, M. L. Sogin, R. G. Hoffmann, K. Yan and S. L. McLellan (2012). "Acinetobacter, Aeromonas and Trichococcus populations dominate the microbial community within urban sewer infrastructure." Environmental microbiology **14**(9): 2538-2552.

Wagner, M. and A. Loy (2002). "Bacterial Community Composition and Function in Sewage Treatment Systems." Current opinion in biotechnology **13**: 218-227.

Wen, Q., L. Yang, R. Duan and Z. Chen (2016). "Monitoring and evaluation of antibiotic resistance genes in four municipal wastewater treatment plants in Harbin, Northeast China." Environmental Pollution **212**: 34-40.

Yang, Y., B. Li, F. Ju and T. Zhang (2013). "Exploring Variation of Antibiotic Resistance Genes in Activated Sludge over a Four-Year Period through a Metagenomic Approach." Environmental science & technology **47**.

Zarei Baygi, A., M. Harb, P. Wang, L. Stadler and A. Smith (2019). "Evaluating Antibiotic Resistance Gene Correlations with Antibiotic Exposure Conditions in Anaerobic Membrane Bioreactors." Environmental Science & Technology **53**.

Ziembińska-Buczyńska, A., E. Felis, J. Folkert, A. Meresta, D. Stawicka, A. Gnida and J. Surmacz-Górska (2015). "Detection of antibiotic resistance genes in wastewater treatment plant – molecular and classical approach / Wykrywanie genów oporności na antybiotyki w oczyszczalni ścieków – podejście klasyczne i biologii molekularnej." Archives of Environmental Protection **41**.

Appendix 1

1.1 the accession numbers and related ARGs detected in the Winter sample

Influent			Effluent		
Accession number	**ARGs**	**Frequency**	**Accession number**	**ARGs**	**Frequency**
A15097	*ere(B)*	76	AB571865	*mph(G)*	8
AB054980	*tetA(P)*	15		*mef(C)*	8
AB097942	*tet(X)*	13	AF133140	*tet(G)*	8
AB161450	*dfrA7*	7	AF542061	*sul2*	15
AB194410	*blaCMY-19*	7	AY183453	*ere(A)*	6
AB571865	*mph(G)*	49	DQ143913	*sul1*	16
	mef(c)	96	DQ485530	*qnrS2*	6
AF024602	*aph(3'')-Ib*	34	DQ839391	*mphE*	59
AF099140	*ere(A)*	33	EU294228	*msrE*	97
AF137361	*aadA5*	7	EU370913	*oqxB*	11
AF140629	*aadA6*	7	KT346360	*tet(39)*	37
AF227506	*catB8*	6	M14730	*erm(F)*	14
AF227520	*msr(D)*	73	X02340	*ant(3'')-Ia*	11
	mef(A)	45			
AF242872	*erm(B)*	6			
AF274302	*msr(D)*	77			
AF319779	*erm(35)*	6			
AF322577	*catB4*	14			
AF330699	*ant(6)-Ia*	8			
AF355189	*aac(3)-Ib-aac(6')-Ib'*	49			
AF381615	*blaCMY-10*	14			
AF472622	*cfxA3*	125			
AF495873	*blaTEM-101*	12			
AF504914	*cfxA2*	286			
AF515059	*penA*	7			
AF516719	*blaTEM-104*	13			
AF534183	*tet(A)*	9			
AF540889	*tet(37)*	6			
AF542061	*sul2*	45			
AJ009818	*catB3*	69			
AJ238249	*lnu(B)*	14			
AJ276453	*blaMOX-2*	14			
AJ313332	*tet(A)*	47			

Influent			Effluent		
Accession number	**ARGs**	**Frequency**	**Accession number**	**ARGs**	**Frequency**
AJ419170	*dfrA7*	6			
AJ514254	*tet(36)*	31			
AJ584652	*aac(6')-30-aac(6')-Ib'*	41			
AJ971089	*mef(A)*	46			
AM087405	*aadA10*	64			
AM183225	*sul2*	17			
AM260957	*mph(F)*	6			
AM296481	*cmlB1*	8			
AM889118	*tet(O/W)*	36			
APPZ0100	*OXA-2*	19			
AY040093	*blaTEM-1*	11			
AY046276	*tet(C)_2*	36			
AY144590	*aadA11_1*	17			
AY183453	*ere(A)_1*	14			
AY196695	*tet(A)_3*	8			
AY261378	*cphA1_3*	6			
AY485126	*tet(O/W)*	27			
AY665771	*aadA12_1*	48			
AY713504	*aadA13_1*	22			
AY928180	*lnu(C)_1*	23			
CP000645	*tetE*	7			
D16251	*mph(A)*	151			
DQ060146	*tet(W)*	70			
DQ143913	*sul1*	195			
DQ303918	*aac(6')-Ib-cr*	53			
DQ388123	*dfrA14*	8			
DQ393783	*aadA15*	8			
DQ485530	*qnrS2*	124			
DQ839391	*mphE*	695			
DQ914960	*sul1*	33			
EF016355	*ADC-25*	17			
EF452177	*lnu(D)*	18			
EF552405	*OXA-141*	22			
EF626943	*tet(32)*	12			
EF636461	*aac(6')-Ib-cr*	16			
EU046614	*blaTRU_1*	6			
EU294228	*msr€*	1180			
EU370913	*oqxB*	45			

Influent			Effluent		
Accession number	**ARGs**	**Frequency**	**Accession number**	**ARGs**	**Frequency**
EU436855	*qnrVC1*	6			
EU722333	*tet(32)*	11			
EU780013	*sul1*	25			
FJ228229	*qnrD1*	6			
FJ460181	*aadA17*	14			
FJ591049	*dfrA1*	21			
FJ591054	*aadA1*	9			
FJ820124	*blaGES-10*	54			
FJ854362	*blaGES-11*	53			
FJWZ0100	*OXA-1*	6			
GQ152600	*blaMOX-5*	11			
GQ342996	*cfxA6*	132			
GQ891757	*qnrVC4*	80			
GU014535	*tet(X)*	50			
GU207844	*blaGES-14*	6			
GU831575	*blaOXA-164*	26			
HG423652	*mef(A)*	100			
HM370393	*blaVEB-1*	44			
HQ141279	*ARR-2*	23			
HQ170510	*blaOXA-1*	82			
HQ398214	*blaCTX-M-101*	6			
J03306	*dfrA3*	6			
J03427	*OXA-10*	92			
JF806499	*ARR-3*	20			
JN861779	*blaOXA-211*	48			
JN861780	*blaOXA-212*	12			
JN899585	*erm(B)*	50			
JQ414041	*aadA1*	10			
JX049131	*blaFOX-10*	7			
JX185132	*aadA1*	7			
KF203107	*blaOXA-333*	31			
KF203108	*blaOXA-334*	9			
KF648874	*mph(N)*	16			
KF864551	*ant(6)-Ia*	33			
KF921535	*dfrA14*	28			
KP265721	*ere(D)*	52			
KT346360	*tet(39)_*	248			
KT818596	*blaVCC-1*	44			
KU721146	*blaOXA-464*	69			

Influent			Effluent		
Accession number	**ARGs**	**Frequency**	**Accession number**	**ARGs**	**Frequency**
KU721147	*blaOXA-465*	10			
KX827604	*blaOXA-427*	43			
L33696	*tet(Q)*	576			
M14730	erm(F)	151			
M15332	erm(G)	12			
M17808	*erm(F)*	16			
M18896	*tet(O)*	72			
M20925	*tet(O)*	18			
M22614	*cml_1*	8			
M28829	*aph(6)-Id*	140			
	aph(3'')-Ib	87			
M29725	*tet(L)*	12			
M37699	*tet(X)*	28			
M55620	*catQ*	20			
M64556	*cmlA1*	115			
MG267386	*mcr-7.1*	58			
NC_00312	*tet(C)_2*	17			
NC_01093	*tetB(P)_*	12			
NC010870	*aadA2_1*	12			
NZ_ABDU0	*tet(44)_*	7			
U14748	*AER-1*	6			
U18931	*erm(B)*	6			
U19459	*vat(B)*	13			
U36578	*mph(A)*	8			
U73497	*tet(Q)*	147			
U83667	*mef(A)*	25			
U86375	*erm(B)*	6			
V00359	*aph(3')-Ib*	35			
X00006	*tet(A)*	15			
X02340	*ant(3'')-Ia*	128			
X04555	*ant(2'')*	24			
X12868	*dfrA5_1*	11			
X15852	*aac(3)-I*	19			
X56809	*blaLCR-1*	63			
X58717	*tet(Q)*	121			
X74512	*blaBIL-1*	6			
X77455	*blaFOX-1*	6			
X80276	*ampS*	11			
X92947	*tet(M)*	14			

Influent			Effluent		
Accession number	**ARGs**	**Frequency**	**Accession number**	**ARGs**	**Frequency**
Y07780	*tet(O)*	13			
Y08743	*mdf(A)*	17			
Y10282	*blaFOX-2*	9			
Y11068	*blaFOX-3*	9			
Z21523	*tet(Q)*	13			
Z50802	*aadA4*	12			

1.2 Accession numbers and related ARGs in the Summer sample

Influent			Effluent		
Accession number	**ARGs**	**Frequency**	**Accession number**	**ARGs**	**Frequency**
A15097	*ere(B)*	8	DQ143913	*sul1*	7
AB571865	*mph(G)*	8	DQ839391	*mphE*	26
	mefC	18	EU294228	*msrE*	34
AF024602	*aph(3'')-Ib*	9			
AF099140	*ere(A)*	10			
AF227520	*msr(D)*	18			
	mef(A)	6			
AF274302	*msr(D)*	27			
AF355189	*aac(3)-Ib-aac(6')-Ib'*	18			
AF504914	*cfxA2*	35			
AF515059	*penA*	7			
AF534183	*tet(A)*	9			
AF542061	*sul2*	21			
AJ009818	*catB3*	11			
AJ238249	*lnu(B)*	14			
AJ276453	*blaMOX-2*	9			
AJ313332	*tet(A)*	15			
AJ584652	*aac(6')-30-aac(6')-Ib'*	8			
AJ971089	*mef(A)*	13			
AM087405	*aadA10*	24			
AM889118	*tet(O/W)*	7			
AY040093	*blaTEM-1*	6			
AY046276	*tet(C)_2*	22			
AY144590	*aadA11_1*	6			
AY183453	*ere(A)_1*	8			

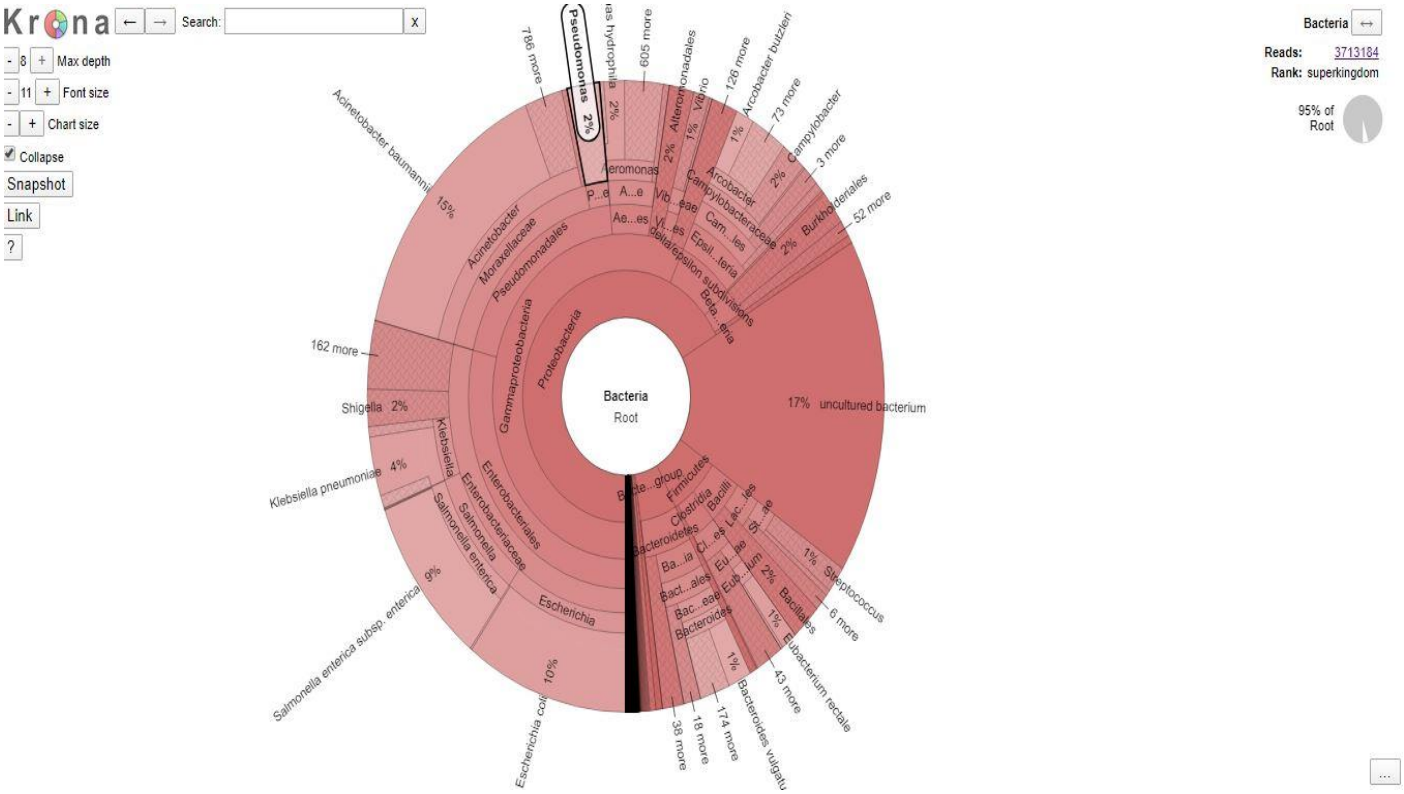
Influent			Effluent		
Accession number	**ARGs**	**frequency**	**Accession number**	**ARGs**	**frequency**
AY665771	*aadA12_1*	22			
D16251	*mph(A)*	57			
DQ060146	*tet(W)*	10			
DQ143913	*sul1*	52			
DQ303918	*aac(6')-Ib-cr*	7			
DQ485530	*qnrS2*	22			
DQ839391	*mphE*	116			
DQ914960	*sul1*	10			
EF552405	*blaOXA-141*	9			
EU294228	*msrE*	255			
EU370913	*oqxA*	7			
	oqxB	20			
EU780013	*sul1*	6			
FJ820124	*blaGES-10*	20			
FJ854362	*blaGES-11*	10			
GQ891757	*qnrVC4*	27			
HG423652	*mef(A)*	21			
HM370393	*blaVEB-1*	10			
J03427	*Alternat*	23			
JN899585	*erm(B)*	18			
JX185132	*aadA1*	6			
KF921535	*dfrA14*	11			
KP265721	*ere(D)*	10			
KT346360	*tet(39)*	40			
KU721146	*blaOXA-464*	8			
L33696	*tet(Q)*	27			
M14730	*erm(F)*	12			
M18896	*tet(O)*	17			
M28829	*aph(6)-Id*	40			
	aph(3'')-Ib	20			
M64556	*cmlA1*	27			
U14748	*AER-1*	9			
U73497	*tet(Q)*	8			
U83667	*mef(A)*	8			
X00006	*tet(A)*	8			
X02340	*ant(3'')-Ia*	31			
X04555	*ant(2'')*	6			
X56809	*blaLCR-1*	19			
X80276	*ampS*	6			
X92947	*tet(M)*	11			
Z50802	*aadA4*	17			
Z54180	*blaBRO-1*	6			

Appendix 2

2.1 Winter sample

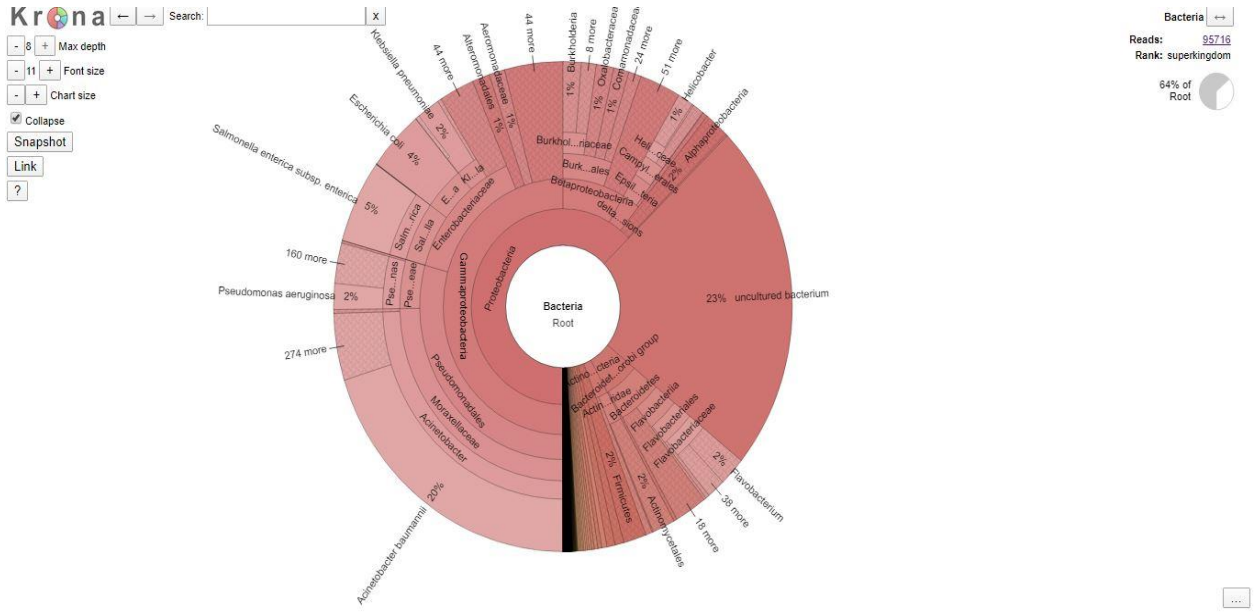
2.1.1 Number of reads of bacteria in influent sample

https://usegalaxy.org/datasets/bbd44e69cb8906b5b0749ef8eea6878e/display/?preview=True



2.1.2 Number of reads of bacteria effluent sample

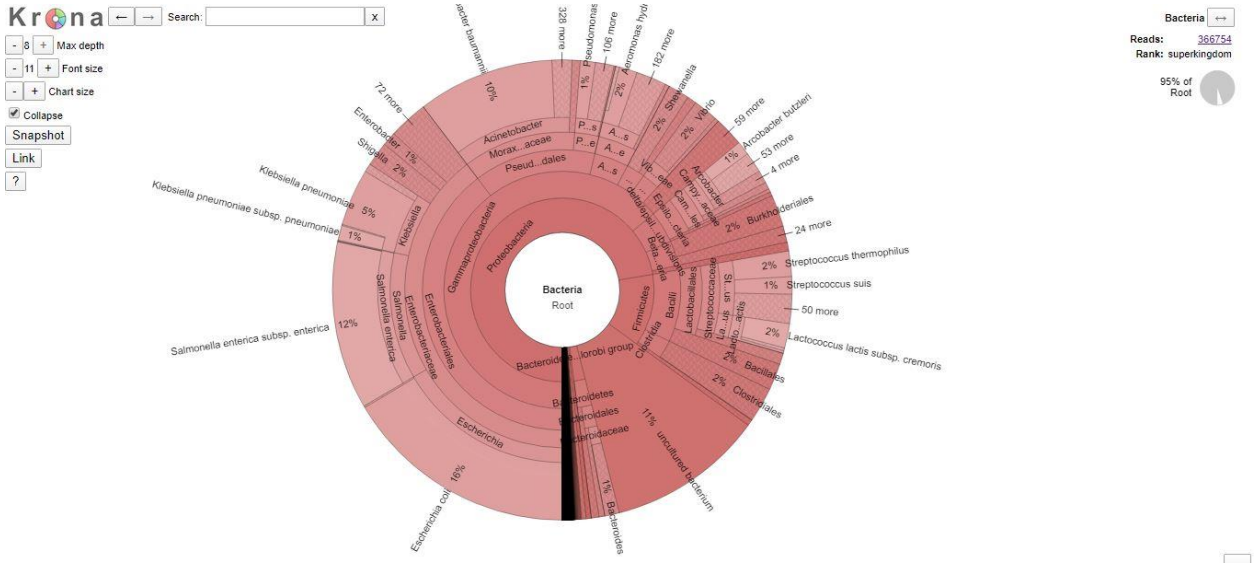
https://usegalaxy.org/datasets/bbd44e69cb8906b516b6b2a2ce63b2de/display/?preview=True



2.2 Summer sample

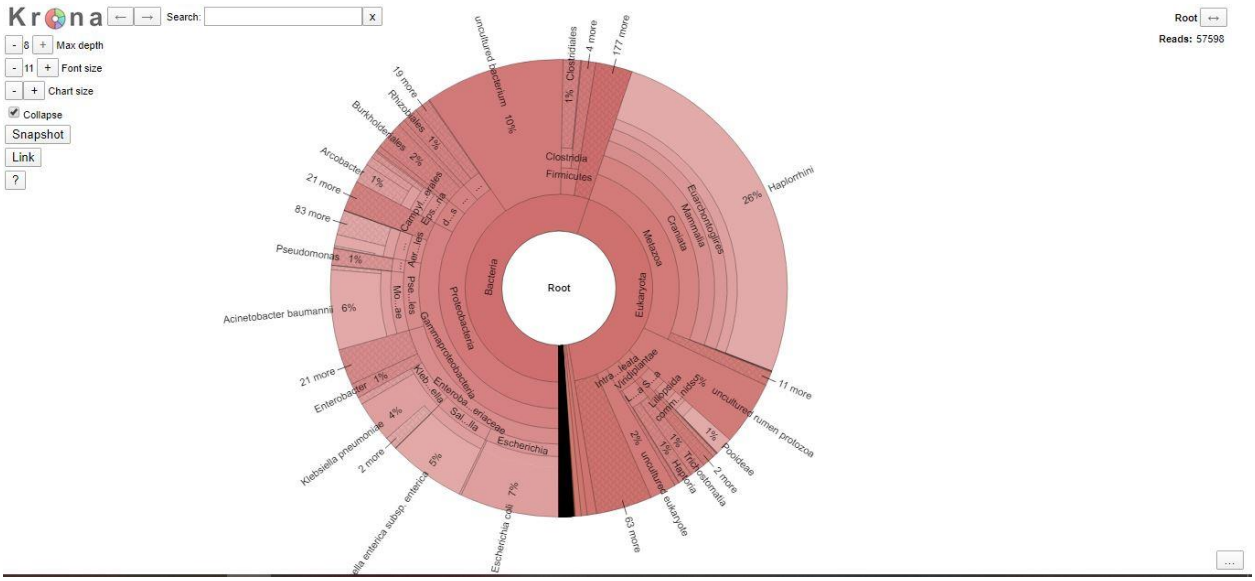
2.2.1 Number of reads of bacteria influent sample

https://usegalaxy.org/datasets/bbd44e69cb8906b555d38e744858711f/display/?preview=True



2.2.2 Number of reads of bacteria effluent sample

https://usegalaxy.org/datasets/bbd44e69cb8906b5be72b1580add2b08/display/?preview=True



Appendix 3

3.1 less abundant bacterial genera found in the influent winter sample

Bacterial species	Frequency	%
Photobacterium damselae	145	1.829191
Capnocytophaga ochracea	125	1.576889
Salmonella enterica	138	1.740886
B.thetaiotaomicron	121	1.526429
Campylobacter jejuni	105	1.324587
Acinetobacter johnsonii	100	1.261511
Streptococcus mitis	100	1.261511
Aeromonas punctata	91	1.147975
Aeromonas allosaccharophila	82	1.034439
Arcobacter butzleri	79	0.996594
Enterococcus faecium	83	1.047054
Bifidobacterium longum	70	0.883058
Salmonella typhimurium	69	0.870443
Riemerella	52	0.655986
Riemerella anatipestifer	50	0.630756
Salmonella enteritidis	45	0.56768
Aeromonas media	44	0.555065
Vibrio cholerae	44	0.555065
Enterobacter cloacae	43	0.54245
Clostridium perfringens	41	0.51722
Bacteroides sp. 139	37	0.466759
Bifidobacterium thermophilum	36	0.454144
Providencia stuartii	33	0.416299
Enterococcus faecalis	20	0.252302
S.mutans	18	0.227072
Streptococcus uberis	18	0.227072
Pasteurella trehalosi	17	0.214457
Exiguobacterium sp	16	0.201842
Aeromonas media strain ER.1.18	14	0.176612
S.pneumoniae	13	0.163996
Staphylococcus aureus	13	0.163996
B.sphaericus	12	0.151381
Streptococcus agalactiae	12	0.151381
Streptococcus salivarius	12	0.151381
Aeromonas sobria	11	0.138766
Klebsiella oxytoca	9	0.113536

Bacterial species	Frequency	%
Shigella sonnei	9	0.113536
Bordetella bronchiseptica	8	0.100921
Aeromonas salmonicida	7	0.088306
Neisseria meningitidis	7	0.088306
Aeromonas enteropelogenes	6	0.075691
Aeromonas hydrophila	6	0.075691
Vibrio cholerae strain VC627	6	0.075691

3.2 Less abundant ARGs in the influent & effluent of winter sample

Influent						Effluent		
ARGs	**Frequency**	**%**	**ARGs**	**Frequency**	**%**	**ARGs**	**Frequency**	**%**
cfxA6_1	132	1.459693	*blaOXA-85_1*	3	0.033175	*tet(G)_2*	8	0.088466
ant(3'')-Ia_1	128	1.415459	*blaSFO-1_1*	3	0.033175	*ere(A)_1*	6	0.06635
cfxA3_1	125	1.382285	*blaVEB-1_3*	3	0.033175	*qnrS2_1*	6	0.06635
qnrS2_1	124	1.371226	*cepA-29_1*	3	0.033175	*aph(6)-Id_1*	5	0.055291
tet(Q)_2	121	1.338052	*cfr(C)_1*	3	0.033175	*dfrA7_5*	4	0.044233
cmlA1_1	115	1.271702	*cphA1_4*	3	0.033175	*tet(G)_1*	4	0.044233
mef(A)_4	100	1.105828	*dfrA1_10*	3	0.033175	*blaADC-25_1*	3	0.033175
mef(C)_1	96	1.061595	*dfrA16_1*	3	0.033175	*blaLCR-1_1*	3	0.033175
blaOXA-10_1	92	1.017361	*erm(X)_1*	3	0.033175	*blaOXA-29_1*	3	0.033175
aph(3'')-Ib_1	87	0.96207	*mcr-2.2_1*	3	0.033175	*erm(F)_3*	3	0.033175
blaOXA-1_1	82	0.906779	*sul1_28*	3	0.033175	*sul2_10*	3	0.033175
qnrVC4_1	80	0.884662	*tet(34)_1*	3	0.033175	*tet(X)_1*	3	0.033175
msr(D)_2	77	0.851487	*tet(H)_1*	3	0.033175	*aac(2')-Ib_1*	2	0.022117
ere(B)_1	76	0.840429	*tet(O/W)_4*	3	0.033175	*aadA1_2*	2	0.022117
msr(D)_3	73	0.807254	*tet(W)_3*	3	0.033175	*aadA10_2*	2	0.022117
tet(O)_1	72	0.796196	*tetA(P)_3*	3	0.033175	*aadA11_1*	2	0.022117
tet(W)_1	70	0.774079	*aac(3)-IId_1*	2	0.022117	*aadA2_1*	2	0.022117
blaOXA-464_1	69	0.763021	*aac(6')-aph(2'')_1*	2	0.022117	*aph(3')-Ia_1*	2	0.022117
catB3_1	69	0.763021	*aac(6')-IIc_1*	2	0.022117	*ARR-2_1*	2	0.022117
aadA10_2	64	0.70773	*ant(2'')-Ia_10*	2	0.022117	*blaOXA-10_1*	2	0.022117
blaLCR-1_1	63	0.696671	*ant(6)-Ia_2*	2	0.022117	*blaTRU_1*	2	0.022117
mcr-7.1_1	58	0.64138	*ant(6)-Ib_1*	2	0.022117	*ere(A)_2*	2	0.022117
blaGES-10_1	54	0.597147	*aph(3')-VI_1*	2	0.022117	*floR_1*	2	0.022117
aac(6')-Ib-cr_1	53	0.586089	*blaACC-3_1*	2	0.022117	*mph(A)_1*	2	0.022117
blaGES-11_1	53	0.586089	*blaBES-1_1*	2	0.022117	*otr(A)_1*	2	0.022117

Influent						Effluent		
ARGs	**Frequency**	**%**	**ARGs**	**Frequency**	**%**	**ARGs**	**Frequency**	**%**
ere(D)_1	52	0.57503	*blaCARB-8_1*	2	0.022117	*sul1_11*	2	0.022117
erm(B)_1	50	0.552914	*blaCEPH-A3_1*	2	0.022117	*sul2_11*	2	0.022117
tet(X)_1	50	0.552914	*blaCTX-M-10_1*	2	0.022117	*tet(E)_3*	2	0.022117
aac(3)-Ib-aac(6')-Ib'_1	49	0.541856	*blaCTX-M-214_1*	2	0.022117		1	0.011058
mph(G)_1	49	0.541856	*blaDES-1_1*	2	0.022117	*aadA1_4*	1	0.011058
aadA12_1	48	0.530797	*blaFOX-4_1*	2	0.022117	*aadA1_5*	1	0.011058
blaOXA-211_1	48	0.530797	*blaFOX-5_1*	2	0.022117	*aadA10_1*	1	0.011058
tet(A)_1	47	0.519739	*blaMOX-3_1*	2	0.022117	*aadA12_1*	1	0.011058
mef(A)_1	46	0.508681	*blaMOX-4_1*	2	0.022117	*aadA6_1*	1	0.011058
mef(A)_3	45	0.497622	*blaNDM-10_1*	2	0.022117	*ant(6)-Ia_3*	1	0.011058
sul2_1	45	0.497622	*blaOXA-119_1*	2	0.022117	*aph(3'')-Ib_2*	1	0.011058
blaVCC-1_1	44	0.486564	*blaOXA-199_1*	2	0.022117	*aph(3')-Ib_1*	1	0.011058
blaVEB-1_1	44	0.486564	*blaOXA-224_1*	2	0.022117	*ARR-3_4*	1	0.011058
blaOXA-427_1	43	0.475506	*blaOXA-252_1*	2	0.022117	*blaCMY-10_1*	1	0.011058
oqxB_1	43	0.475506	*blaOXA-296_1*	2	0.022117	*blaCMY-8_1*	1	0.011058
aac(6')-30-aac(6')-Ib'_1	41	0.453389	*blaOXA-500_1*	2	0.022117	*blaIMP-5_1*	1	0.011058
tet(C)_2	36	0.398098	*blaSHV-100_1*	2	0.022117	*blaOXA-1_1*	1	0.011058
tet(O/W)_1	36	0.398098	*cat_2*	2	0.022117	*blaOXA-141_1*	1	0.011058
aph(3')-Ia_1	35	0.38704	*catA1_1*	2	0.022117	*blaOXA-164_1*	1	0.011058
aph(3'')-Ib_2	34	0.375981	*cphA4_1*	2	0.022117	*blaOXA-205_1*	1	0.011058
ant(6)-Ia_3	33	0.364923	*dfrA1_12*	2	0.022117	*blaOXA-21_1*	1	0.011058
ere(A)_2	33	0.364923	*dfrB5_1*	2	0.022117	*blaOXA-211_1*	1	0.011058
sul1_11	33	0.364923	*erm(A)_2*	2	0.022117	*blaOXA-296_1*	1	0.011058
blaOXA-333_1	31	0.342807	*erm(B)_15*	2	0.022117	*blaOXA-427_1*	1	0.011058
tet(36)_1	31	0.342807	*erm(F)_4*	2	0.022117	*blaOXA-464_1*	1	0.011058
dfrA14_1	28	0.309632	*erm(G)_2*	2	0.022117	*blaPAO_3*	1	0.011058
tet(X)_2	28	0.309632	*erm(X)_2*	2	0.022117	*blaTEM-104_1*	1	0.011058
tet(O/W)-1_1	27	0.298573	*fosA5_1*	2	0.022117	*catB3_1*	1	0.011058
blaOXA-164_1	26	0.287515	*lnu(F)_1*	2	0.022117	*catB8_1*	1	0.011058
mef(A)_2	25	0.276457	*mcr-3.12_1*	2	0.022117	*cfxA2_1*	1	0.011058
sul1_5	25	0.276457	*mcr-3.13_1*	2	0.022117	*cfxA6_1*	1	0.011058

Influent						Effluent		
ARGs	**Frequency**	**%**	**ARGs**	**Frequency**	**%**	**ARGs**	**Frequency**	**%**
ant(2'')-Ia_1	24	0.265399	*mcr-3.15_1*	2	0.022117	*cmlA1_1*	1	0.011058
ARR-2_1	23	0.25434	*mcr-4.1_1*	2	0.022117	*dfrA1_1*	1	0.011058
lnu(C)_1	23	0.25434	*mph(D)_1*	2	0.022117	*dfrA1_11*	1	0.011058
aadA13_1	22	0.243282	*oqxA_1*	2	0.022117	*dfrA18_1*	1	0.011058
blaOXA-141_1	22	0.243282	*qnrB10_2*	2	0.022117	*dfrA5_1*	1	0.011058
dfrA1_1	21	0.232224	*qnrB19_1*	2	0.022117	*ere(A)_6*	1	0.011058
ARR-3_1	20	0.221166	*qnrVC5_1*	2	0.022117	*erm(36)_1*	1	0.011058
catQ_1	20	0.221166	*qnrVC6_1*	2	0.022117	*mcr-4.1_1*	1	0.011058
aac(3)-Ia_1	19	0.210107	*spc_1*	2	0.022117	*mcr-7.1_1*	1	0.011058
blaOXA-280_1	19	0.210107	*sul1_35*	2	0.022117	*mef(A)_1*	1	0.011058
lnu(B)_1	19	0.210107	*sul1_36*	2	0.022117	*mef(A)_4*	1	0.011058
lnu(D)_1	18	0.199049	*tet(E)_2*	2	0.022117	*mph(F)_1*	1	0.011058
tet(O)_2	18	0.199049	*tet(G)_2*	2	0.022117	*msr(D)_2*	1	0.011058
aadA11_1	17	0.187991	*tet(M)_10*	2	0.022117	*ole(C)_1*	1	0.011058
blaADC-25_1	17	0.187991	*tet(M)_12*	2	0.022117	*qnrD1_1*	1	0.011058
mdf(A)_1	17	0.187991	*tet(W)_2*	2	0.022117	*qnrVC1_1*	1	0.011058
sul2_10	17	0.187991	*tetA(46)_1*	2	0.022117	*tet(A)_1*	1	0.011058
tet(C)_1	17	0.187991	*VanGXY_1*	2	0.022117	*tet(A)_2*	1	0.011058
aac(6')-Ib-cr_2	16	0.176932	*aac(3)-Ii_1*	1	0.011058	*tet(C)_2*	1	0.011058
erm(F)_3	16	0.176932	*aac(3)-IIa_1*	1	0.011058	*tet(O/W)_1*	1	0.011058
mph(N)_1	16	0.176932	*aac(6')-Ib-11_1*	1	0.011058	*tet(Q)_1*	1	0.011058
tet(A)_2	15	0.165874	*aadA2_2*	1	0.011058	*tet(Q)_2*	1	0.011058
tetA(P)_1	15	0.165874	*aadA24_1*	1	0.011058			
aadA17_1	14	0.154816	*aadA8_1*	1	0.011058			
blaCMY-10_1	14	0.154816	*ant(2'')-Ia_20*	1	0.011058			
blaMOX-2_1	14	0.154816	*ant(3'')-Ih-aac(6')-IId_1*	1	0.011058			
catB4_1	14	0.154816	*aph(2'')-Ig_1*	1	0.011058			
ere(A)_1	14	0.154816	*aph(3')-Ia_3*	1	0.011058			
tet(M)_1	14	0.154816	*aph(3')-IIa_1*	1	0.011058			
blaTEM-104_1	13	0.143758	*aph(6)-Id_3*	1	0.011058			
tet(O)_3	13	0.143758	*blaA_2*	1	0.011058			
tet(Q)_4	13	0.143758	*blaACI-1_1*	1	0.011058			
tet(X)_3	13	0.143758	*blaACT-6_1*	1	0.011058			
vat(B)_1	13	0.143758	*blaBEL-1_1*	1	0.011058			
aadA2_1	12	0.132699	*blaBRO-1_1*	1	0.011058			
aadA4_1	12	0.132699	*blaCMH-3_1*	1	0.011058			

Influent						Effluent		
ARGs	**Frequency**	**%**	**ARGs**	**Frequency**	**%**	**ARGs**	**Frequency**	**%**
blaOXA-212_1	12	0.132699	*blaCMY-100_1*	1	0.011058			
blaTEM-101_1	12	0.132699	*blaCMY-101_1*	1	0.011058			
erm(G)_1	12	0.132699	*blaCMY-107_1*	1	0.011058			
tet(32)_2	12	0.132699	*blaCMY-145_1*	1	0.011058			
tet(L)_2	12	0.132699	*blaCMY-26_1*	1	0.011058			
tetB(P)_1	12	0.132699	*blaCMY-70_1*	1	0.011058			
ampS_1	11	0.121641	*blaCMY-75_1*	1	0.011058			
blaMOX-5_1	11	0.121641	*blaCTX-M-100_1*	1	0.011058			
blaTEM-102_1	11	0.121641	*blaCTX-M-138_1*	1	0.011058			
dfrA5_1	11	0.121641	*blaCTX-M-177_1*	1	0.011058			
tet(32)_1	11	0.121641	*blaCTX-M-23_1*	1	0.011058			
aadA1_3	10	0.110583	*blaCTX-M-36_1*	1	0.011058			
blaOXA-490_1	10	0.110583	*blaDHA-13_1*	1	0.011058			
aadA1_2	9	0.099524	*blaFOX-7_1*	1	0.011058			
blaFOX-2_1	9	0.099524	*blaGES-21_1*	1	0.011058			
blaFOX-3_1	9	0.099524	*blaGES-6_1*	1	0.011058			
blaOXA-334_1	9	0.099524	*blaKPC-10_1*	1	0.011058			
blaOXA-347_1	9	0.099524	*blaLEN24_1*	1	0.011058			
tet(A)_6	9	0.099524	*blaMOX-7_1*	1	0.011058			
aadA15_1	8	0.088466	*blaNPS_1*	1	0.011058			
ant(6)-Ia_1	8	0.088466	*blaOCH-2_1*	1	0.011058			
cml_1	8	0.088466	*blaOXA-142_1*	1	0.011058			
cmlB1_1	8	0.088466	*blaOXA-160_1*	1	0.011058			
dfrA14_5	8	0.088466	*blaOXA-162_1*	1	0.011058			
mph(A)_2	8	0.088466	*blaOXA-181_1*	1	0.011058			
tet(A)_3	8	0.088466	*blaOXA-274_1*	1	0.011058			
aadA1_5	7	0.077408	*blaOXA-299_1*	1	0.011058			
aadA5_1	7	0.077408	*blaOXA-350_1*	1	0.011058			
aadA6_1	7	0.077408	*blaOXA-372_1*	1	0.011058			
blaCMY-19_1	7	0.077408	*blaOXA-417_1*	1	0.011058			
blaFOX-10_1	7	0.077408	*blaOXA-46_1*	1	0.011058			
blaOXA-209_1	7	0.077408	*blaOXA-47_1*	1	0.011058			
dfrA7_1	7	0.077408	*blaOXA-471_1*	1	0.011058			
penA_1	7	0.077408	*blaOXA-5_1*	1	0.011058			
tet(44)_1	7	0.077408	*blaOXY-1-1_1*	1	0.011058			
tet(E)_3	7	0.077408	*blaPAO_1*	1	0.011058			
blaAER-1_1	6	0.06635	*blaPER-1_1*	1	0.011058			
blaBIL-1_1	6	0.06635	*blaPLA-3A_1*	1	0.011058			
blaCTX-M-101_1	6	0.06635	*blaPLA-4A_1*	1	0.011058			
blaFOX-1_1	6	0.06635	*blaSHV-187_1*	1	0.011058			
blaGES-14_1	6	0.06635	*blaTEM-116_1*	1	0.011058			

Influent						Effluent		
ARGs	**Frequency**	**%**	**ARGs**	**Frequency**	**%**	**ARGs**	**Frequency**	**%**
blaOXA-129_1	6	0.06635	*blaTEM-219_1*	1	0.011058			
blaTRU_1	6	0.06635	*blaTEM-79_1*	1	0.011058			
catB8_1	6	0.06635	*blaTLA-1_1*	1	0.011058			
cphA1_3	6	0.06635	*blaVEB-3_1*	1	0.011058			
dfrA3_1	6	0.06635	*blaVEB-4_1*	1	0.011058			
dfrA7_5	6	0.06635	*catA2_1*	1	0.011058			
erm(35)_1	6	0.06635	*catB2_1*	1	0.011058			
erm(B)_10	6	0.06635	*catB9_1*	1	0.011058			
erm(B)_12	6	0.06635	*cepA_1*	1	0.011058			
erm(B)_6	6	0.06635	*cepA-44_1*	1	0.011058			
mph(F)_1	6	0.06635	*cfr(C)_2*	1	0.011058			
qnrD1_1	6	0.06635	*cfxA4_1*	1	0.011058			
qnrVC1_1	6	0.06635	*cfxA5_1*	1	0.011058			
tet(37)_1	6	0.06635	*cmlA1_2*	1	0.011058			
aadA16_1	5	0.055291	*cphA1_1*	1	0.011058			
ARR-3_4	5	0.055291	*cphA1_2*	1	0.011058			
blaEBR-1_1	5	0.055291	*cphA1_7*	1	0.011058			
blaGES-13_1	5	0.055291	*cphA5_1*	1	0.011058			
blaOXA-2_1	5	0.055291	*cphA6_1*	1	0.011058			
blaOXA-205_1	5	0.055291	*dfrA1_14*	1	0.011058			
blaOXA-21_1	5	0.055291	*dfrA1_16*	1	0.011058			
blaOXA-281_1	5	0.055291	*dfrA15_1*	1	0.011058			
blaOXA-304_1	5	0.055291	*dfrA17_1*	1	0.011058			
blaOXA-392_1	5	0.055291	*dfrA22_1*	1	0.011058			
mcr-5.2_1	5	0.055291	*dfrA25_1*	1	0.011058			
mef(B)_1	5	0.055291	*dfrG_1*	1	0.011058			
tet(40)_1	5	0.055291	*ere(A)_6*	1	0.011058			
tet(B)_1	5	0.055291	*erm(T)_1*	1	0.011058			
tet(M)_8	5	0.055291	*fosA2_1*	1	0.011058			
aac(6')-IIa_1	4	0.044233	*fosA6_1*	1	0.011058			
blaCARB-10_1	4	0.044233	*GENE*	1	0.011058			
blaCTX-M-102_1	4	0.044233	*imiH_1*	1	0.011058			
blaCTX-M-14b_1	4	0.044233	*imiS_1*	1	0.011058			
blaMOX-6_1	4	0.044233	*lnu(G)_1*	1	0.011058			
blaOXA-397_1	4	0.044233	*mcr-1.10_1*	1	0.011058			
blaOXA-491_1	4	0.044233	*mcr-2_1*	1	0.011058			
blaOXY-3-1_1	4	0.044233	*mcr-3.10_1*	1	0.011058			
catB1_1	4	0.044233	*mcr-3.14_1*	1	0.011058			
dfrA16_2	4	0.044233	*mcr-3.19_1*	1	0.011058			
erm(B)_18	4	0.044233	*mcr-3.7_1*	1	0.011058			
floR_1	4	0.044233	*mph(B)_1*	1	0.011058			
lsa(B)_1	4	0.044233	*qnrB14_1*	1	0.011058			

Influent						Effluent		
ARGs	**Frequency**	**%**	**ARGs**	**Frequency**	**%**	**ARGs**	**Frequency**	**%**
mcr-3.17_1	4	0.044233	*qnrB27_1*	1	0.011058			
sul2_11	4	0.044233	*qnrB32_2*	1	0.011058			
tet(O)_4	4	0.044233	*qnrB39_1*	1	0.011058			
tet(T)_1	4	0.044233	*qnrB4_1*	1	0.011058			
tet(W)_4	4	0.044233	*qnrC_1*	1	0.011058			
tetA(P)_2	4	0.044233	*qnrS1_1*	1	0.011058			
aac(6')-Ib-Suzhou_1	3	0.033175	*sul1_20*	1	0.011058			
aadA1_4	3	0.033175	*sul1_23*	1	0.011058			
aadA10_1	3	0.033175	*sul1_31*	1	0.011058			
ant(2'')-Ia_13	3	0.033175	*sul1_34*	1	0.011058			
aph(2'')-If_2	3	0.033175	*tet(D)_1*	1	0.011058			
aph(3')-III_1	3	0.033175	*tet(E)_1*	1	0.011058			
blaCARB-5_1	3	0.033175	*tet(G)_1*	1	0.011058			
blaCMY-1_1	3	0.033175	*tet(O/32/O)_7*	1	0.011058			
blaCMY-102_1	3	0.033175	*tet(S)_1*	1	0.011058			
blaFOX-9_1	3	0.033175	*VanC4XY_3*	1	0.011058			
blaOXA-101_1	3	0.033175	*VanHOX_1*	1	0.011058			
blaOXA-118_1	3	0.033175	*vat(F)_1*	1	0.011058			
blaOXA-192_1	3	0.033175						
blaOXA-20_1	3	0.033175						
blaOXA-373_1	3	0.033175						
blaOXA-420_1	3	0.033175						

3.3 bacterial species & related ARGs in the influent sample

Bacteria	**ARG**	**Frequency**
Acinetobacter johnsonii	*blaOXA-211_1*	48
	blaOXA-212_1	12
	blaOXA-333_1	31
	blaOXA-334_1	9
Aeromonas allosaccharophila	*blaOXA-1_1*	82
Aeromonas enteropelogenes	*blaTRU_1*	6
Aeromonas hydrophila	*blaAER-1_1*	6
Aeromonas media	*blaVEB-1*	44
	aadA17_1	14
Aeromonas punctata	*blaMOX-5_1*	11
	qnrVC4_1	80
Aeromonas salmonicida	*tetE_3*	7
Aeromonas sobria	*ampS_1*	11
Arcobacter butzleri	*blaOXA-464_1*	69
	blaOXA-490_1	10

Bacteria	ARG	Frequency
B.sphaericus	*erm(G)_1*	12
B.thetaiotaomicron	*tet(Q)_2*	121
Bacteroides sp. 139	*erm(35)_1*	6
	tet(36)_1	31
Bifidobacterium longum	*tet(W)_1*	70
Bifidobacterium thermophilum	*tet(O/W)_1*	36
Bordetella bronchiseptica	*cmlB1_1*	8
Campylobacter jejuni	*ant(6)-Ia_3*	33
	tet(O)_1	72
Capnocytophaga ochracea	*cfxA3_1*	125
Clostridium perfringens	*tetA(P)_1*	15
	catQ_1	20
	erm(B)_1	6
Enterobacter cloacae	*blaOXA-427_1*	43
Enterococcus faecalis	*erm(B)_1*	6
	tet(M)_1	14
Enterococcus faecium	*erm(B)_1*	50
	erm(B)_6	6
	lnu(B)_1	19
	ant(6)-Ia_1	8
Exiguobacterium sp	*mph(N)_1*	16
Klebsiella oxytoca	*blaFOX-3_1*	9
Neisseria meningitidis	*penA_1*	7
Pasteurella trehalosi	*sul2_10*	17
Photobacterium damselae	*mph(G)_1*	49
	mef(C)_1	96
Providencia stuartii	*ere(A)_2*	33
Riemerella anatipestifer strain 0511	*tet(X)_1*	50
Riemerella	*ere(D)_1*	52
S.mutans	*tet(O)_2*	18
Salmonella enterica subsp	*dfrA14_5*	8
	qnrD1_1	6
Salmonella enterica	*qnrS2_1*	124
Salmonella enteritidis	*sul2_1*	45
Salmonella typhimurium	*catB3_1*	69
Shigella sonnei	*tet(A)_6*	9
Staphylococcus aureus	*vat(B)_1*	13
Streptococcus agalactiae	*tet(L)_2*	12
Streptococcus mitis	*mef(A)_4*	100
Streptococcus salivarius	*tet(32)_2*	12
Streptococcus uberis	*lnu(D)_1*	18
Vibrio cholerae	*blaVCC-1_1*	44
	qnrVC1_1	6

Appendix 4

4.1 less abundance bacterial species in the influent summer sample

Bacteria	Frequency	%
Photobacterium damselae	26	1.877256
Salmonella enterica	22	1.588448
Salmonella enteritidis	21	1.516245
Streptococcus mitis	21	1.516245
Campylobacter jejuni	17	1.227437
Bacteroides fragilis	12	0.866426
Enterococcus faecalis	11	0.794224
Salmonella typhimurium	11	0.794224
Aeromonas media	10	0.722022
Bifidobacterium longum	10	0.722022
Providencia stuartii	10	0.722022
Riemerella	10	0.722022
Aeromonas hydrophila	9	0.649819
Shigella sonnei	9	0.649819
Arcobacter butzleri	8	0.577617
Prevotella intermedia	8	0.577617
Bifidobacterium thermophilum	7	0.505415
Neisseria meningitidis	7	0.505415
Aeromonas sobria	6	0.433213
Moraxella catarrhalis	6	0.433213

4.2 Less abundant ARGs in the influent & effluent of summer sample

Influent						Effluent		
ARG	**Frequency**	**%**	**ARG**	**Frequency**	**%**	**ARG**	**Frequency**	**%**
qnrVC4_1	27	1.381781	*aac(6')-IIc_1*	1	0.051177	*mef(C)_1*	3	0.153531
tet(Q)_1	27	1.381781	*aadA7_1*	1	0.051177	*mph(G)_1*	3	0.153531
aadA10_2	24	1.22825	*ant(2'')-Ia_10*	1	0.051177	*oqxB_1*	3	0.153531
blaOXA-10_1	23	1.177073	*ant(2'')-Ia_2*	1	0.051177	*sul1_11*	3	0.153531
aadA12_1	22	1.125896	*ant(2'')-Ia_20*	1	0.051177	*sul2_1*	3	0.153531
qnrS2_1	22	1.125896	*ant(3'')-Ih-aac(6')-IId_1*	1	0.051177	*tet(39)_1*	3	0.153531
tet(C)_2	22	1.125896	*aph(3')-III_1*	1	0.051177	*tet(A)_1*	3	0.153531
mef(A)_4	21	1.074719	*blaADC-25_1*	1	0.051177	*aadA10_2*	2	0.102354
sul2_1	21	1.074719	*blaBES-1_1*	1	0.051177	*aph(3'')-Ib_1*	2	0.102354
aph(3'')-Ib_1	20	1.023542	*blaBRO-2_1*	1	0.051177	*blaGES-11_1*	2	0.102354
blaGES-10_1	20	1.023542	*blaCARB-5_1*	1	0.051177	*blaMOX-2_1*	2	0.102354
oqxB_1	20	1.023542	*blaCEPH-A3_1*	1	0.051177	*blaMOX-6_1*	2	0.102354
blaLCR-1_1	19	0.972364	*blaCMH-3_1*	1	0.051177	*blaOXA-427_1*	2	0.102354
aac(3)-Ib-aac(6')-Ib'_1	18	0.921187	*blaCMY-1_1*	1	0.051177	*cfxA6_1*	2	0.102354
erm(B)_1	18	0.921187	*blaCMY-102_1*	1	0.051177	*mef(A)_4*	2	0.102354
mef(C)_1	18	0.921187	*blaCMY-114_1*	1	0.051177	*msr(D)_3*	2	0.102354
msr(D)_3	18	0.921187	*blaCMY-4_1*	1	0.051177	*qnrVC4_1*	2	0.102354
aadA4_1	17	0.87001	*blaCTX-M-101_1*	1	0.051177	*tet(A)_2*	2	0.102354
tet(O)_1	17	0.87001	*blaCTX-M-104_1*	1	0.051177	*tet(Q)_3*	2	0.102354
tet(A)_1	15	0.767656	*blaCTX-M-14b_1*	1	0.051177	*aac(3)-Ib-aac(6')-Ib'_1*	1	0.051177
lnu(B)_1	14	0.716479	*blaCTX-M-78_1*	1	0.051177	*aadA1_2*	1	0.051177
mef(A)_1	13	0.665302	*blaDES-1_1*	1	0.051177	*aadA12_1*	1	0.051177
erm(F)_1	12	0.614125	*blaFOX-1_1*	1	0.051177	*aadA2_1*	1	0.051177
catB3_1	11	0.562948	*blaFOX-2_1*	1	0.051177	*aadA2_2*	1	0.051177
dfrA14_1	11	0.562948	*blaGES-14_1*	1	0.051177	*aadA4_1*	1	0.051177
tet(M)_1	11	0.562948	*blaKPC-10_1*	1	0.051177	*ant(6)-Ia_1*	1	0.051177
blaGES-11_1	10	0.511771	*blaMOX-3_1*	1	0.051177	*ARR-2_1*	1	0.051177
blaVEB-1_1	10	0.511771	*blaMOX-7_1*	1	0.051177	*blaGES-10_1*	1	0.051177
ere(A)_2	10	0.511771	*blaOXA-118_1*	1	0.051177	*blaNPS_1*	1	0.051177
ere(D)_1	10	0.511771	*blaOXA-119_1*	1	0.051177	*blaOXA-205_1*	1	0.051177

Influent						Effluent		
ARG	**Frequency**	**%**	**ARG**	**Frequency**	**%**	**ARG**	**Frequency**	**%**
sul1_11	10	0.511771	*blaOXA-209_1*	1	0.051177	*blaOXA-46_1*	1	0.051177
tet(W)_1	10	0.511771	*blaOXA-21_1*	1	0.051177	*blaOXA-464_1*	1	0.051177
aph(3'')-Ib_2	9	0.460594	*blaOXA-211_1*	1	0.051177	*blaOXA-490_1*	1	0.051177
blaAER-1_1	9	0.460594	*blaOXA-224_1*	1	0.051177	*blaOXA-53_1*	1	0.051177
blaMOX-2_1	9	0.460594	*blaOXA-281_1*	1	0.051177	*blaTEM-101_1*	1	0.051177
blaOXA-141_1	9	0.460594	*blaOXA-308_1*	1	0.051177	*blaTEM-102_1*	1	0.051177
tet(A)_6	9	0.460594	*blaOXA-333_1*	1	0.051177	*blaTEM-104_1*	1	0.051177
aac(6')-30-aac(6')-Ib'_1	8	0.409417	*blaOXA-373_1*	1	0.051177	*blaVEB-1_1*	1	0.051177
blaOXA-464_1	8	0.409417	*blaOXA-46_1*	1	0.051177	*catB1_1*	1	0.051177
ere(A)_1	8	0.409417	*blaOXA-47_1*	1	0.051177	*catB8_1*	1	0.051177
ere(B)_1	8	0.409417	*blaPER-1_1*	1	0.051177	*catQ_1*	1	0.051177
mef(A)_2	8	0.409417	*blaPER-3_1*	1	0.051177	*cmlA1_1*	1	0.051177
mph(G)_1	8	0.409417	*blaSFO-1_1*	1	0.051177	*dfrA14_1*	1	0.051177
tet(A)_2	8	0.409417	*blaTEM-104_1*	1	0.051177	*dfrA16_1*	1	0.051177
tet(Q)_3	8	0.409417	*blaTEM-110_1*	1	0.051177	*dfrA16_2*	1	0.051177
aac(6')-Ib-cr_1	7	0.35824	*blaTEM-76_1*	1	0.051177	*dfrA5_1*	1	0.051177
oqxA_1	7	0.35824	*cat_2*	1	0.051177	*dfrA7_5*	1	0.051177
penA_1	7	0.35824	*catB2_1*	1	0.051177	*ere(A)_1*	1	0.051177
tet(O/W)_1	7	0.35824	*catB8_1*	1	0.051177	*erm(F)_1*	1	0.051177
aadA1_5	6	0.307062	*catP_1*	1	0.051177	*floR_1*	1	0.051177
aadA11_1	6	0.307062	*cmlA1_2*	1	0.051177	*fusB_1*	1	0.051177
ampS_1	6	0.307062	*cphA1_2*	1	0.051177	*mef(B)_1*	1	0.051177
ant(2'')-Ia_1	6	0.307062	*dfrA1_10*	1	0.051177	*mph(A)_2*	1	0.051177
blaBRO-1_1	6	0.307062	*dfrA10_1*	1	0.051177	*mph(F)_1*	1	0.051177
blaTEM-102_1	6	0.307062	*dfrA12_2*	1	0.051177	*msr(D)_2*	1	0.051177
mef(A)_3	6	0.307062	*dfrA15_1*	1	0.051177	*otr(C)_1*	1	0.051177
sul1_5	6	0.307062	*dfrA16_1*	1	0.051177	*qnrS2_1*	1	0.051177
aadA1_3	5	0.255885	*dfrA7_5*	1	0.051177	*sul1_5*	1	0.051177
aadA15_1	5	0.255885	*erm(35)_1*	1	0.051177	*sul2_10*	1	0.051177
ARR-3_1	5	0.255885	*erm(B)_12*	1	0.051177	*sul4_1*	1	0.051177
blaMOX-5_1	5	0.255885	*erm(F)_4*	1	0.051177	*tet(44)_1*	1	0.051177
blaMOX-6_1	5	0.255885	*erm(G)_2*	1	0.051177	*tet(C)_1*	1	0.051177
blaOXA-427_1	5	0.255885	*fosA_6*	1	0.051177			

Influent						Effluent		
ARG	**Frequency**	**%**	**ARG**	**Frequency**	**%**	**ARG**	**Frequency**	**%**
blaVCC-1_1	5	0.255885	*fosA_7*	1	0.051177			
cml_1	5	0.255885	*fosA2_1*	1	0.051177			
lnu(D)_1	5	0.255885	*fosA3_1*	1	0.051177			
mcr-7.1_1	5	0.255885	*fosA4_1*	1	0.051177			
mdf(A)_1	5	0.255885	*GENE*	1	0.051177			
sul2_10	5	0.255885	*lnu(F)_3*	1	0.051177			
tet(C)_1	5	0.255885	*lsa(C)_1*	1	0.051177			
tet(X)_1	5	0.255885	*mcr-3.17_1*	1	0.051177			
aadA2_1	4	0.204708	*mph(B)_1*	1	0.051177			
aadA24_1	4	0.204708	*mph(N)_1*	1	0.051177			
aadA5_1	4	0.204708	*qepA1_1*	1	0.051177			
ant(6)-Ia_3	4	0.204708	*qnrD1_1*	1	0.051177			
aph(3')-Ia_1	4	0.204708	*str_1*	1	0.051177			
blaOXA-1_1	4	0.204708	*sul1_28*	1	0.051177			
blaOXA-205_1	4	0.204708	*sul1_31*	1	0.051177			
blaOXA-392_1	4	0.204708	*sul1_34*	1	0.051177			
cfxA6_1	4	0.204708	*sul2_5*	1	0.051177			
dfrA1_1	4	0.204708	*sul3_2*	1	0.051177			
lnu(C)_1	4	0.204708	*tet(44)_1*	1	0.051177			
mph(A)_2	4	0.204708	*tet(B)_1*	1	0.051177			
qnrVC1_1	4	0.204708	*tet(B)_2*	1	0.051177			
sul2_11	4	0.204708	*tet(D)_1*	1	0.051177			
tet(L)_2	4	0.204708	*tet(G)_2*	1	0.051177			
tet(Q)_2	4	0.204708						
vat(B)_1	4	0.204708						
aac(6')-aph(2'')_1	3	0.153531						
aadA1_2	3	0.153531						
ARR-2_1	3	0.153531						
ARR-3_4	3	0.153531						
blaNPS_1	3	0.153531						
blaOXA-164_1	3	0.153531						
blaOXA-490_1	3	0.153531						
catQ_1	3	0.153531						
cfxA3_1	3	0.153531						
dfrA5_1	3	0.153531						
floR_1	3	0.153531						
lnu(F)_1	3	0.153531						
sul1_36	3	0.153531						
tet(32)_2	3	0.153531						
tet(36)_1	3	0.153531						
tet(A)_3	3	0.153531						

Influent						Effluent		
ARG	**Frequency**	**%**	**ARG**	**Frequency**	**%**	**ARG**	**Frequency**	**%**
tet(M)_12	3	0.153531						
tet(O/W)-1_1	3	0.153531						
aac(3)-Ib_1	2	0.102354						
aadA10_1	2	0.102354						
aadA13_1	2	0.102354						
aadA17_1	2	0.102354						
aadA6_1	2	0.102354						
ampH_1	2	0.102354						
ampH_2	2	0.102354						
blaBIL-1_1	2	0.102354						
blaCARB-4_1	2	0.102354						
blaCMY-10_1	2	0.102354						
blaDHA-13_1	2	0.102354						
blaGES-13_1	2	0.102354						
blaMOX-4_1	2	0.102354						
blaOXA-20_1	2	0.102354						
blaOXA-212_1	2	0.102354						
blaOXA-347_1	2	0.102354						
blaOXA-491_1	2	0.102354						
blaOXA-53_1	2	0.102354						
blaOXA-9_1	2	0.102354						
blaTEM-101_1	2	0.102354						
catA1_1	2	0.102354						
catB4_1	2	0.102354						
cmlB1_1	2	0.102354						
ere(A)_6	2	0.102354						
erm(B)_10	2	0.102354						
erm(G)_1	2	0.102354						
lsa(B)_1	2	0.102354						
mef(B)_1	2	0.102354						
mph(D)_1	2	0.102354						
mph(F)_1	2	0.102354						
sul1_35	2	0.102354						
tet(32)_1	2	0.102354						
tet(E)_3	2	0.102354						
tet(M)_2	2	0.102354						
tet(M)_8	2	0.102354						
tet(S)_1	2	0.102354						
tet(X)_2	2	0.102354						
tetB(P)_1	2	0.102354						
aac(3)-Ia_1	1	0.051177						

Influent						Effluent		
ARG	**Frequency**	**%**	**ARG**	**Frequency**	**%**	**ARG**	**Frequency**	**%**
aac(3)-Id_1	1	0.051177						
aac(3)-IIa_1	1	0.051177						
aac(3)-IId_1	1	0.051177						

4.3 Bacterial species & related ARGs in the influent sample

Bacteria	ARG	Frequency
Aeromonas sobria	*ampS_1*	6
Arcobacter butzleri	*blaOXA-464_1*	8
Bacteroides fragilis	*erm(F)_1*	12
Bifidobacterium longum	*tet(W)_1*	10
Bifidobacterium thermophilum	*tet(O/W)_1*	7
Campylobacter jejuni	*tet(O)_1*	17
Enterococcus faecalis	*tet(M)_1*	11
Moraxella catarrhalis	*blaBRO-1_1*	6
Neisseria meningitidis	*penA_1*	7
Photobacterium damselae	*mph(G)_1*	8
	mefC_1	18
Prevotella intermedia	*tet(Q)_3*	8
Providencia stuartii	*ere(A)_2*	10
Riemerella	*ere(D)_1*	10
Salmonella enterica	*qnrS2_1*	22
Salmonella enteritidis	*sul2_1*	21
Salmonella typhimurium	*catB3_1*	11
Shigella sonnei	*tet(A)_6*	9
Streptococcus mitis	*mef(A)_4*	21

4.4 Removal efficiency of top 10 bacterial host in August sample

Bacteria	Removal efficiency %
Acinetobacter baumannii	89
Escherichia coli	100
Uncultured bacterium	81

Bacteria	Removal efficiency %
Pseudomonas aeruginosa	100
Klebsiella pneumoniae	90
Streptococcus pneumoniae	100
Prevotella denticola	100
Enterococcus faecium	100
Aeromonas punctata	100
Prevotella ruminicola	100

4.5 Removal efficiency of top 10 ARGs in August sample

Gene	Removal efficiency %
msr(E)_4	87
mph(E)_1	78
mph(A)_1	91
sul1_10	87
aph(6)-Id_1	100
tet(39)_1	93
cfxA2_1	91
ant(3'')-Ia_1	90
cmlA1_1	96
msr(D)_2	96

معاينة البكتيريا والجينات المقاومة للمضادات الحيوية الموجودة في مياه الصرف الصحي من محطة معالجة مياه الصرف الصحي في البيرة في فلسطين: تحليل الميتاجينوم

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إشراف : د. سهير عريقات

ملخص

تعتبر محطات معالجة المياه العادمة (WWTPs) نقطة فعالة لتكاثر ونشر بكتيريا مقاومة المضادات الحيوية (ARB) وجينات مقاومة المضادات الحيوية . (ARGs)في الضفة الغربية ، توجد أربع محطات معالجة مياه الصرف الصحي العاملة في جنين وطولكرم ورام الله والبيرة. معظمهم لديهم علاج ثانوي يعتمد على عملية الحمأة المنشطة باستثناء معمل طولكرم الذي يحتوي على علاج أولي فقط. يتم إطلاق جميع النفايات السائلة من تلك النباتات في الوديان. لذلك ، له تأثير سلبي على كل من البيئة والبشر. في هذه الدراسة ، تم استخدام تحليل تسلسل Illumina عالي الإنتاجية لتحديد ملف تعريف ARB و ARGs في محطة البيره لتكرير المياه. تم جمع عينة من مياه الصرف الصحي الخام (الداخلة) وعينة المياه المعالجة الثانوية (النفايات السائلة) على مدار فصلين ، الصيف (أغسطس) والشتاء (فبراير) 2018. تم استخراج الحمض النووي من كل عينة ، واستخدامها في إعداد مكتبة الحمض النووي. تم تجزئة الحمض النووي بشكل عشوائي إلى أجزاء صغيرة بواسطة إنزيم transposome يليه التخصيب حيث تم إضافة مؤشرين إلى كل عينة من أجل الباركود. تم تنظيف مكتبة الحمض النووي لتحديد حجم قطع الحمض النووي بين 300-500 وأرسلت للتسلسل العميق بواسطة آلة Nextseq500 باستخدام مجموعة 150 دورة منتصف الإخراج (قراءة نهاية واحدة). تم استلام البيانات التسلسلية كملفات FASTAQوتحميلها على الموقع (https://usegalaxy.org/) لتحليل المعلومات البيولوجية. أظهرت النتائج وجود عدد أكبر من ARB(53 نوعًا) وتنوع كبير من ARGs (400 نوع فرعي) في عينات الشتاء مقارنة بعينات الصيف حيث تم اكتشاف 30 نوعًا من ARB و 253 نوعًا من . ARGsكان هناك اختلاف كبير($P < 0.01, r=0.9$)، في الوفرة النسبية للبكتيريا ARB و ARGs بين الموسمين. أكثر أنواع وفرة وجدت في كل من المواسم وعينات كان *Acinetobacter baumannii* تليها *E.coli* و *K.pneumonia*. تعزل *Acinetobacter baumanni* عادة من وحدة العناية المركزة ، وتسبب العديد من الأمراض وتشمل التهابات الجهاز التنفسي والبولي والدم والجلد. بالإضافة إلى ذلك ، لديها القدرة على الهروب من المضادات الحيوية ومقاومتها و تم تصنيفها من قبل منظمة الصحة العالمية باعتبارها البكتيريا الانتهازية والضارة رقم واحد. في هذه الدراسة ، تم الكشف عن 107 جينًا مقاومًا للمضادات الحيوية يمنح مقاومة لـ 12 صنف مضاد حيوي. كانت مجموعة المقاومة للمضادات الحيوية الأكثر وفرة هي الماكرولايد والتتراسيكلين. تراوحت كفاءة إزالة أفضل 10 ARB و ARGs بين 85-100 ٪. ومع ذلك ، هناك قلق من انتشار ARBو ARGs والترشيح المسبق لهما في محطة معالجة مياه الصرف الصحي والتي قد يتم التخلص منها على البيئة من خلال النفايات السائلة وقد تهدد الصحة العامة وتسبب ضررًا للبيئة والبشر. لذلك ، نوصي بزيادة الوعي بين السكان المحليين حول تأثير المياه العادمة ومسببات الأمراض المصاحبة لها على صحة الإنسان والبيئة. علاوة على ذلك ، ينبغي أن يكون تحسين أنظمة الصرف الصحي والمعالجة أولوية لصانعي السياسات للحد من عبء ARB و ARGs في مياه الصرف المعالجة في فلسطين.