

Deanship of Graduate Studies

Al-Quds University



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

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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

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Thesis approval

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in waste water samples collected from Al-Bierh wastewater treatment plant in
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Dedication

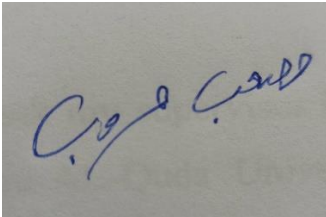
To my lovely wife and my kids, my parents, and dear sisters and brothers, for their encouragement and support. I would like to dedicate my work to every one stand with me in the hardest condition without let me down.

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

A handwritten signature in blue ink, written in Arabic script, which reads "Musab Idreis Taha Hroub". The signature is written on a light-colored background.

Musab Idreis Taha Hroub

Date: December 21, 2019

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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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