1-1-2018

Quantification Of Antimicrobial Resistance Genes In Urban Agricultural Soil

Vidhya Bai Krishnoji Rao
Wayne State University,

QUANTIFICATION OF ANTIMICROBIAL RESISTANCE GENES IN URBAN AGRICULTURAL SOIL

By

VIDHYA BAI KRISHNOJI RAO

THESIS

Submitted to The Graduate School

Of Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

2018

MAJOR: NUTRITION AND FOOD SCIENCE

Approved By:

_____

Advisor Date

DEDICATION

I would like to thank my parents and my sister for their constant support and guidance throughout this journey.

ACKNOWLEDGEMENTS

The momentous occasion heralding the successful completion of my research work would be incomplete without mention of the kind support extended by many individuals in the success of my endeavor.

I would like to express my heartfelt gratitude to my advisor Dr. Yifan Zhang, Associate Professor of Food Microbiology, Department of Nutrition and Food Science, Wayne State University, Detroit. I thank her for all the guidance, inspiration and constant support rendered with complete dedication throughout my research work. All her valuable assistance and contributions have helped me fulfil my dream of pursuing masters and will be treasured in my heart forever.

I would like to extend my sincere gratitude to my committee members: Dr. Ahmad Heydari and Dr. Kevin Zhou for taking their time to be on my committee, providing me with their valuable comments and suggestions.

I would like to acknowledge all my collaborators for coordinating the project and providing soil samples for conducting the research.

I am highly indebted to Wayne State University for providing me with the infrastructure and giving an opportunity to pursue my research work in this highly reputed institution.

I am thankful to Graduate School of Wayne State University for the assistance provided throughout my studies.

I take the immense pleasure in thanking Thomas Michael Prychitko, Varun Tahlan, Abdullah Ibn Mafiz and Liyanage Nirasha Perera, for all their assistance to accomplish my research.

I am also grateful to my fellow lab members Kaitlyn Merz, Miguel Rivas, Eric Chang, Mohamad Damen and Nirosha Amarasekara for being such nice friends and assisting me throughout my research work.

I owe to my beloved parents, my mother Mrs. Geetha Bai R, my father Mr. Krishnoji Rao M and my sister Veena Bai K for their blessings, constant support, and encouragement throughout my life.

I will forever be thankful to my former college research advisor, Professor Dr. Sinosh Skariyachan. He has been helpful in providing immense advice many times during my undergraduate school career.

A special thanks to my Michigan friends for offering me their valuable advice and supporting me through this entire process.

Although there may be many more, who have remained unacknowledged in this humble note of gratitude, there are none who remain unappreciated.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ARB	: Antibiotic Resistant Bacteria
ARGs	: Antibiotic Resistance Genes
CDC	: Centers for Disease Control and Prevention
CFU	: Colony Forming Unit
DNA	: Deoxyribonucleic acid
ECOFF	: Epidemiological Cut OFF
ESBL	: Extended Spectrum Beta Lactamase
EUCAST	: European Committee on Antimicrobial Susceptibility Testing
HGT	: Horizontal Gene Transfer
MALDI-TOF	: Matrix Assisted Laser Desorption/Ionization – Time Of Flight
MBL	: Metallo Beta Lactamases
MDR	: Multi-Drug Resistant
MGE	: Mobile Genetic Elements
MLS	: Macrolide-Lincosamide-Streptogramin
MRSA	: Methicillin-Resistant *Staphylococcus aureus*
NDM	: New Delhi Metallo-β-Lactamases
PCR	: Polymerase Chain reaction
qPCR	: Quantitative Polymerase Chain Reaction
VBNC	: Viable But Non Culturable

CHAPTER 1

INTRODUCTION

1.1 Antibiotic Resistance as a Global Issue

Excessive use of antibiotics in human and veterinary settings has a direct correlation with biotic and abiotic factors like dissemination of heavy metals, pesticides, insecticides, plastics, physicochemical conditions etc., in the soil. These anthropogenic factors act as selective pressure upon Antibiotics Resistant Bacteria (ARB) which can promote towards the evolution of bacteria from simple ARB to complex untreatable “Superbugs”. Environmental areas that encompass anthropogenic pressure such as pharmaceutical manufacturing effluents, aquaculture facilities, municipal wastewater systems, chemical industry effluents and animal husbandry facilities are determined as hotspots and can be termed as “Hub of ARGs and ARBs” [1].

1.2 Antibiotic Resistance in the Environment

Soil consists of a repository of diverse microorganisms. Most of the natural antibiotics discovered are produced by soil microbes and hence it’s apparent for microbes to attain resistance to antibiotics. There are different classes of antibiotics such as beta-lactams, cephalosporins, carbapenems, sulfonamides etc. Environmental compartments that are subjected to anthropogenic pressure, such as pharmaceutical manufacturing effluents, municipal wastewater systems, aquaculture facilities and animal husbandry facilities are of the major concerns for drug resistance [2,3]. Treated or untreated effluents can contaminate the soil which can further contaminate the crops grown in such plots. Upon consumption by humans, it can further lead to the development of “superbugs” within the human gut microbiota and excretion of them in the form of feces can have a detrimental effect on healthy individuals. To keep our environment clean and safe for our

future generations, there is an urgent need to screen for ARGs in the soil of urban agricultural garden due to a gradual increase in community gardens (in this study - Metro Detroit Area). Soil harboring anthropogenic pollutants that can take centuries to degrade completely let out from industrial waste which can benefit microbes to be selective for certain resistance genes and can incorporate them in their extrachromosomal DNA via Mobile Genetic Elements (MGEs).

1.3 Role of Antibiotics for the Emerging Superbugs

Antibacterial drugs are chemotherapeutic agents that are a potent tool to fight against clinically relevant pathogenic bacteria at a specific concentration. The discovery of the antibiotic penicillin from the culture of fungus, *Penicillium notatum* in the year 1928 by Sir Alexander Fleming was a radical discovery for today's new classes of antibiotics [4]. By the 1950s, penicillin resistance became evident and was a substantial clinical problem [3]. In response, a wide range analog of beta-lactam class of antibiotics was discovered and deployed over the years due to abuse of antibiotics hence, bacteria attained resistance to these classes of antibiotics by a phenomenon called as a Beta-Lactamase cycle. For instance, the discovery of methicillin was in the year 1960 and the first case of methicillin-resistant *Staphylococcus aureus* (MRSA) was identified in the United Kingdom, the United States in the year 1962 and 1968 respectively [2,5]. New Delhi metallo-β-lactamases (NDMs) are the most recent additions to the class of Metallo Beta Lactamases (MBL). The emergence of this novel plasmid-encoded MBL family heralds a new era of antibiotic resistance due to their ability to hydrolyze almost all clinically available β-lactam class of antibiotics and rapid worldwide dissemination.

The term "superbug" can be defined as a bacterium that has acquired resistance to two or more classes of antibacterial drugs which can be a challenging task for medical practitioners. [6,7].

Unrestrictive and rampant use of antibiotics in developing countries without prescription has resulted in a remarkable increase in the infectious bacteria which are Multidrug Resistant (MDR) with constantly evolving new genes for survival by the mechanism of resistance to almost all known antibiotics [8]. Another contributing factor is the intensive use of antibiotics in animal husbandry posing a potential threat to the environment and humans. A recent report from the Centers for Disease Control and Prevention (CDC) evaluate that more than two million people are sickened every year due to antibiotic-resistant infections and resulting in nearly 23,000 deaths/year [9]. Over the last 30 years, there has been a drastic decrease in companies developing new antibiotics due to the multifactorial reasons that are generally attributed to finite commercial returns [6]. Hence, there is a demanding need to find out innovative approaches in identifying ARGs and establishment of standardized protocols to determine gene copy number to estimate the level of contamination of ARGs.

1.4 Antibiotic Resistance Genes and Antibiotic Resistance Bacteria in the Environment

A recently published article provided a strong foundation to determine bacterial load, indicator organisms in non – clinical settings like *Aeromonas spp., Escherichia coli, Enterococcus faecalis, Enterococcus faecium, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* carriers of potent ARGs like *intl-1, Tet-M, Sul-1, Sul-2,* bla_{TEM}, bla_{KPC}, bla_{NDM-1}, *qnrS,* bla_{CTX-M}, *aac-(6′)-Ib-cr, vanA, mecA , ermB and ermF* in soil microbiome [10]. In this study possible candidate like *intI-1, Sul2,* bla_{TEM} and TetM genes frequently occur in the environmental settings that are subjected to human activities [10]. Class 1 integron is commonly linked to ARG, and the abundance of this gene changes in response to environmental pressures. Class 1 integrons are often located on MGEs that can readily transfer between bacteria. The most common class 1 integron, *intI1* genes, are xeno-genetic assembled under selection pressures imposed by human activities

[10,29]. ARGs that are likely to exchange between species are typically associated with MGEs acts as vehicles such as integrons, plasmids and transposons. Although bacterial load and ARGs in clinically relevant settings are higher than those in environmental samples, it is still of great public health importance to characterize and quantify ARGs in environmental samples.

Most soil bacteria are Viable But Non Culturable (VBNC) and capable of persisting and spreading in the environment. Anthropogenic pressure/stressful conditions like low-temperatures, high antibiotics and other chemical contaminants enhances their ability of long term survival under stress and the ability to revive [11]. If these cells are present, the total number of viable bacteria in a sample could be underestimated by the traditional Colony Forming Unit (CFU) count method due to inherent non – culturability of VBNC cells [12]. For bacterial species causing human infections, non – detection of viable cells in quality control samples from the clinical samples, food industry, Waste Water Treatment plants, agricultural lands or water distribution systems may pose a serious risk to the public. Also, studies have shown that VBNC cells of *E. coli* was found in processed food [13,27] and those of *Salmonella typhimurium* were found in soil [14,15].

1.5 Tackling Antibiotic Resistance in the Environment

The rapid global urbanization and extensive anthropogenic activities has intensified the worldwide human health risks induced by ARGs. ARGs can replicate and disseminate independently in their host bacterial cells via Mobile Genetic Elements and have been recognized as emerging environmental pollutants [5,28]. Antibiotic resistance hotspots are found in environmental compartments that are subjected to anthropogenic pressure such as animal husbandry facilities, aquaculture facilities, pharmaceutical manufacturing effluents and municipal

wastewater systems [10]. Such hotspots are characterized by high bacterial loads concomitant with sub-therapeutic concentrations of antibiotics, providing ideal environment for ARGs and ARB.

A new era of work on the definition and standardization of protocols and methodologies for resistance testing in the environment should be established. This can include the implementation of advanced techniques like Next Generation Sequencing, probe based methods for quantification of resistance genes such as molecular beacons targeting the conserved regions identical to clinical isolates. Quantification data (gene copy number) and sequence comparison between diseased individual and environmental ARG would provide important information on the public health significance of environmental ARG. Implementing High Throughput Quantitative PCR (qPCR) for standardization of gene copy numbers by careful monitoring of primer sets, Good Laboratory Practices and precise DNA extraction techniques is needed.

1.6 Culturing and Non-Culturing Methods in the Detection of Antimicrobial Resistance

A wide array of antibiotic resistance detection techniques is available. The most widely used method in clinical microbiology is by measuring bacterial growth in the presence of antibiotic based on the phenotypic detection of antibiotic resistance. Such methods include agar dilution (the gold standard for the antibiogram), broth microdilution and microdilution, strips with an antibiotic gradient (E- test) and Disk Diffusion method. These conventional methods can take up to 24 hours to obtain the results. From the past decade scientists and engineers have focused on reducing the detection time with improved techniques, such as molecular techniques, microarrays, commercial methods, bioluminescence and chemiluminescence, colorimetric methods, immunochromatographic techniques, imaging methods, microfluids and bacterial lysis methods, nephelometry, Matrix Assisted Laser Desorption/Ionization – Time Of Flight (MALDI-TOF), mass spectrometry and flow cytometry [16]. Although these advanced techniques can be precise

and accurate, their disadvantages include probability of obtaining false positive and false negative errors that cannot be eliminated due to skill based errors, cross contamination, laboratory practices etc. Polymerase Chain Reaction (PCR) and real-time PCR, also known as quantitative PCR (qPCR), are the two major molecular techniques implemented to identify ARGs and ARBs. qPCR is accurate, sensitive, high throughput, yields results in a few hours, and allows for quantitative and qualitative determination of sample DNA.

This study was aimed to evaluate the level of ARGs contamination in the environment by qPCR. A recently published article provided a list of candidate genes and indicator bacteria occurring in the environmental settings that are subjected to intense human activities [10]. Most of the current databases like European Committee on Antimicrobial Susceptibility Testing (EUCAST) determine Epidemiological Cut OFF (ECOFF) value which do not relate to the therapeutic efficiency [10]. However, ECOFF estimates use databases in which the number of clinical isolates is several orders of magnitude higher than that of isolates of environmental origin. Thus, our study can serve as a groundwork and supplement above mentioned databases with data from environmental species and isolates.

1.7 Origin of Class 1 Integron and The Rise of Antibiotic Resistance

Environmental dissemination of ARGs has become an increasing concern for public health. Class 1 integrons are main players in the global problem of antibiotic resistance, because they can capture and express diverse resistance genes. They are often embedded in promiscuous plasmids and transposons, facilitating their lateral transfer into a wide range of pathogens [30]. In the environmental samples class 1 integron (*intI1*) exhibits considerable sequence diversity, whereas the clinical *intI1* has a consistent conserve sequence [29]. In this study ARGs belonging to the classes of tetracyclines (*Tet*M), β – Lactams (bla_{TEM}) and sulfonamides (*Sul2*) were target genes

due to their frequent occurrence in the environmental settings subjected to human activities [10]. Previous studies have claimed that class 1 integron is used as a proxy for anthropogenic pollution [10,29]. Studies have also shown a significant correlation between the clinical class 1 integron and gene cassettes encoding resistance to sulfonamides, tetracyclines and β – Lactams [29,30]. The prevalence of ARGs, ARBs with high level of MGEs in the environment can potentially increase the risk of gene dissemination and environmental pollution and threaten the public health. Our current study primarily focuses on the quantification of soil ARGs, the correlation between MGEs and ARGs, and predicting the possible biological contamination in soil. This study can serve as a foundation to bridge the findings between environmental ARGs and the clinical implications of environment pollutants.

CHAPTER 2

MATERIALS AND METHODS

2.1 Soil Sampling

Soil samples were collected during the summer of 2015 across three urban community gardens namely "E", "G", and "O" (**Figure 1)**. At each sampling spot a sample weighing approximately 350 – 450 grams was collected using a sterile soil sampler washed with 70% ethanol between samplings. Samples were sealed in sterile zip-lock bags, labelled accordingly, and transported to the laboratory on ice and stored at -20º C before analysis.

2.2 DNA Extraction

DNA from 43 soil samples (33 from Garden E, 5 from Garden G, and 5 from Garden O) weighing 0.25g was extracted using MoBio PowerSoil DNA kit (MO BIO, Carlsbad, CA) according to the manufacture's protocol. DNA concentrations were measured using spectrophotometry at a wavelength of 260 nm and calculated according to the formula: DNA ng/µl = Optical Density $(OD)_{260\ nm}$ X 100 X dilution factor and stored at -20° C before downstream analysis.

2.3 qPCR Primer Design

Primer sequences were either obtained from literature (16S rRNA, $bla_{TEM,}$ *intl-1*, and *Tet*M) or designed in this study (*sul*2) (**Table 1**). The reference gene sequences for *Sul-2* were retrieved from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Based on the conserved domain in the *sul2* gene, a comprehensive analysis and Multiple sequence alignment were carried out using CLUSTAL W Algorithm (https://www.ebi.ac.uk/Tools). qPCR primer set for *sul-2* gene was designed using Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/), where the

primer set met the general thumb rule with the percentage of G+C content was between 30 – 80 % mol, amplicon length of 106 – 200 bp. The specificity was manually verified using Basic Local Alignment Search Tool (BLAST). All primer sets were synthesized by Eurofins Genomics (Louisville, KY).

2.4 Establish Positive Control for qPCR

All PCR assay was conducted in 50 µl volume reaction using an Eppendorf thermal cycler (USA scientific, Orlando, FL). The PCR mixture consisted of 25 µL GoTaq Green Master Mix, 2 × (Promega, Madison, WI), 0.5 µM each of forward and reverse primer, 20 µL of nuclease-free water and 4 µL of template DNA. The temperature program was initially denatured at 95 ºC for 15min, followed by 35 cycles of denaturation at 95 ºC for 30 s, 30 s at different annealing temperatures (**Table 1**) and extension at 72 ºC for 30 s, with a final extension step for 10 min at 72 ºC. PCR products were analyzed by electrophoresis on a 1.5% agarose gel and visualized under UV light using the transilluminator (Bio-Rad Laboratories, Hercules, CA). Duplicate PCR reactions were performed for each sample to ensure reproducibility and sterile nuclease-free water was used as the negative control in every run.

After PCR amplification, gel slices of an agarose gel containing the desired PCR products were excised aseptically and purified using Purelink Quick Gel Extraction Kit (Invitrogen, Carlsbad, CA). The purified PCR product was ligated into a pCR 2.1 Topo-TA cloning vector (Invitrogen, Carlsbad, CA) and then cloned into chemically competent *E. coli* DH5α cells. Plasmids carrying the target genes were extracted with Purelink Plasmid Miniprep Kit (Invitrogen, Carlsbad, CA). Clones containing the desired gene of interest were selected. The gene of interest in the inserts was verified as the object of ARGs and 16S rRNA using the BLAST alignment tool

(https://blast.ncbi.nlm.nih.gov/). Clones with the desired gene of interest were chosen as the positive control for quantitative PCR as well as the standards for real-time PCR.

2.5 Quantitative Real Time PCR Assay Methods

The absolute copy number of 16S rRNA gene and other target genes (*intl-1, Sul-2, Tet-M and* bla_{TEM}) were quantified by Bio-Rad CFX96 based on the fluorescent dye SYBR-Green I (**Table 2**). The 16S rRNA gene was included to quantify the total bacterial population and to normalize the abundance of ARGs in the soil samples. A plasmid carrying target genes were used to generate calibration curves and their concentrations were measured with a spectrophotometer (Nano-Drop). With the known concentration and amplicon length of the target genes, the Gene Copy Numbers (GCN) were calculated directly from extracted plasmid DNA as described previously: Gene copies =

$$\left(\text{DNA concentration}\,\frac{\text{ng}}{\mu\text{l}}\right)\left(\frac{1\text{g}}{1000^3\text{ng}}\right)\left(\frac{1\text{ mol bp DNA}}{660\text{ ng DNA}}\right)\text{x}\left(\frac{6.023\text{ x }10^{23}\text{bp}}{\text{mol bp}}\right)\ x\ \left(\frac{1\text{ copy}}{\text{genome or plasmid size (bp)}}\right)$$

x (volume of template µl).

[19]. Eight-point calibration curves from a 10 – fold serial dilutions of a known copy number of the plasmid DNA were generated to produce a standard curve. Each qPCR reaction (10 µl) consisted of 5 µl Sso Advanced Universal SYBR Green Supermix (Bio-rad, Hercules, CA), 1 µM each primer, 2 µl of DNA template, 2 µl nuclease – free water. Amplification was conducted using Bio-Rad CFX96 (Bio-Rad, Hercules, CA) as follows: Initial denaturation 95 ºC-10 min, followed by 40 cycles of denaturation 95 ºC for 30 s, 30 s at the annealing temperatures (**Table 2**). All the qPCRs were performed in technical triplicates with negative control as *E. coli* 25922 strain and nuclease free water as no template control (**Figure 2**). Product specificity was confirmed by melt curve analysis (65 – 95 ºC), electrophoresed on a 1.5% agarose gel and visualized under UV light using the transilluminator (Bio-Rad Laboratories, Hercules, CA).

2.6 Statistical Analysis

All qPCR data were normalized among samples by dividing the copy numbers by 16S rRNA gene copy number, and subsequently multiplied by four to approximate the copies per cell (the average number of 16S rRNA genes per bacterial cell is estimated to be four based on the Ribosomal RNA Operon Copy Number Database. Pearson correlation coefficient was determined by SPSS V25.0 (IBM, Chicago, IL). Graphs were generated using Microsoft Excel V1708.0.

CHAPTER 3

RESULTS

3.1 Bacterial Load in Urban Agricultural Soil

Soil samples were collected from 3 gardens (33 from Garden E, 5 from Garden O, and 5 from Garden G) (five replicates for each sampling spot) and total of 43 samples. The abundance of soil bacteria as measured by 16S rRNA copy number that varied over four orders of magnitude (~5.2 x 10^8 to 6.6 x 10^{11} copies per gram of soil) (**Figure 3**). Among the 3 gardens bacterial contamination was high in garden "E" (6.79 x 10^{10} ± 3.2 x 10^{10} copies/gram of soil) followed by "G" (6.71 x 10^9 ± 2.52 x 10^{10} copies/gram of soil) and "O" (6.66 x 10^9 ± 2.53 x 10^{10} copies/gram of soil) (**Figure 4**). The clone libraries of 16S rRNA amplicons of 1504bp obtained from 3 gardens were constructed and sequenced successfully to confirm the gene identity.

DNA Sequencing was carried out in Eton Bioscience Laboratories, NJ and the sequenced 16S rRNA gene was subjected to a highly curated, annotated and user-friendly BLAST similarity search tool. BLAST searches of the GenBank database confirmed that all the 16S rRNA sequence matched the sequence identities greater than 90%. For sequences that exhibited more than 90% identity was considered for tree construction. Nucleotide sequences of 16S rRNA were aligned using Multiple Sequence Alignment (MSA) program CLUSTAL W. The Neighbor-Joining (NJ) trees were constructed using MEGA V7.0.26(http://megasoftware.net). The significance of the nodes was evaluated using bootstrap analysis with 1000 replicates (**Figure 5**). A phylogenetic tree of 16S rRNA sequence is shown in **Figure 5** where significance level $p < 0.05$ is observed in *Bacteroides spp.*

3.2 The Occurrence of ARGs in Soil Samples

Quantitative PCR was performed to examine the diversity and abundance of resistance genes (*intl-1, Sul-2, bla*$_{TEM}$ and *Tet-M*) in the soil samples across three different sampling spots (**Figure 2**). A total of 4 resistance genes and 16S rRNA gene were targeted and quantified based on the bacterial indicators to assess the antibiotic resistance status in the urban agricultural farms. There was no significant correlation between absolute abundance of ARGs and the estimated 16S rRNA gene (Data not shown). A significant correlation was seen upon normalization of *intI1* and *Sul2* to 16S rRNA gene (**Figure 6, Figure 7 and Table 3**). All 3 community gardens showing tetracycline resistance (91.3%) and sulfonamides (100%) are shown in **Table 4**. The diversity of ARGs in all 3 gardens was similar (**Table 4**). For example, Garden "E" confer resistance to more than two classes of antibiotics namely beta-lactams, *bla*$_{TEM}$ (96.9%); Tetracyclines, *Tet-M* (93.9%) and Sulfonamides, *Sul-2* (100%) (**Table 4** and **Figure 8**). The ARGs detected in all the 3 soil samples were abundant ranging from ~ 0.64 x 10^1 to ~3.58 x 10^4 gene copies per gram of soil. All the three ARGs genes detected in all the 3 gardens in soil samples conferred resistance to most commonly used antibiotics in animal husbandry sector namely Tetracyclines, Sulfonamides, and beta lactams. Tetracycline, sulfamethoxazole, and beta lactamase resistance genes found in all the 3 gardens with an absolute abundance of 7.87 x 10^3 copies gram^{-1}, 3.84 x 10^4 copies gram^{-1} and 1.22 x 10^4 copies gram^{-1} respectively (**Figure 9**). Significant correlation with $p < 0.05$ was observed between *bla*$_{TEM}$ and *Tet-M* gene (**Table 5**).

3.3 Distribution of Class 1 Integron in the Soil Samples

The total copy numbers of *intl-1* gene varied over five orders of magnitude (8.02 x 10^1 to 5.12 x 10^6 gene copies per gram) (**Figure 10 and Figure 2**). The abundance of *intl-1* gene was high in Garden O (5.12 x 10^6 copies per gram) followed by Gardens E and G (5.07 x 10^6 and 3.01

x 10^6 copies per gram respectively). *IntI-1* copy number per gram of sample is higher than normalized copy number per bacterial cell (**Figure 11).** Sample E-59 showed minimum intI-l GCN/ gram of sample and *intI-1*GCN/16S rRNA upon normalization (~8.02 x 10^1 copies/gram and ~1.23 x 10^{-5} copies/bacterial cell) (**Figure 12**). Sample "O-61" showed the highest amount of *intI-1* GCN/ gram of sample ~5.12 x 10^6 and Sample "E-31" displayed the highest amount of *intI-1* GCN/16S rRNA ~1.58 x 10^2 (**Figure 12**). A Pearson's correlation showed significance level of $p < 0.05$ between *intI-1* copies per 16S rRNA gene and *Sul-2* copies per 16S rRNA gene (**Table 3**). **Figure 6** and **Figure 7** depicts mean copy number of *intI1* and *Sul2* copies per 16S rRNA gene and copies per gram of soil sample respectively. Also, there was a significant correlation between *Sul-2* and bla_{TEM} gene copies per bacterial cell with $p < 0.01$ (**Table 5**). Therefore, MGEs like *intI-1* carrying sulfonamide class of resistance genes can be a potential indicator for the co-occurring ARGs which can have a clinical relevance like Extended Spectrum Beta-Lactamase (ESBL) genes due to selective pressure.

CHAPTER 4

DISCUSSION

4.1 Bacterial Communities in the Soil of Urban Agricultural Gardens.

Quantification of total bacteria in the soil sample was carried out by amplification of 16S rRNA gene using the universal primers. Garden "E" is in its proximity to hospitals, lakes and Waste Water treatment plant displayed abundance in bacterial community ~3.3 x 10^9 copies $gram^{-1}$ followed by sample "O" (~2.23 x 10^7 copies $gram^{-1}$) and sample "G" (~2.09 x 10^7 copies $gram^{-1}$) (**Figure 1 and Figure 4**). This suggests that environmental factors, location of the garden plays a crucial role in selection and co-selection of ARGs. Also, bacterial community may not be the only factor influencing the resistance profile. Anthropogenic factors can be a definitive criterion. Significant correlation was observed between *TetM* and bla_{TEM} ($p < 0.01$) (**Table 5**). A phylogenetic tree of 16S rRNA sequence is shown in **Figure 5** where significance level $p < 0.05$ is observed in *Bacteroides spp.* A survey conducted in 2002 states that *Bacteroides spp*. are becoming increasingly resistant to antibiotics particularly to Macrolide-Lincosamide-Streptogramin (MLS) and Tetracycline groups of antibiotics [23]. Conjugative transposons in *Bacteroides spp.* are responsible for most of the antibiotic resistance gene transfer within the species and between different genera [24]. Also, this study correlates the abundance contamination of bacterial community in the soil, sequence similarity and phylogenetic characterization of 16S rRNA gene to *Bacteroides spp.* which act as a potential fecal contaminated region and indicator microorganisms to carry MDR gene in low copy number due to their VBNC state in the environment. *Bacteroides* are abundant and are in a good position to transfer conjugative transposons to other microbes in the human and animal intestine. Conjugative transposons, plasmids and integrons that have the ability to transfer ARGs into other pathogenic bacteria via

HGT can increase the copy number of ARB and hence become clinically relevant. A recent study states that few bacterial groups and genetic determinants are used to assess the antibiotic resistance status in environmental settings [10].

4.2 The Occurrence of ARGs in Soil Samples

A very high level of ARGs were detected in all the samples. The diversity of ARGs per gram of soil was comparatively higher than normalized copy number per bacterial cell. ARGs conferring resistance to tetracyclines, β-lactams, and sulfonamides were abundant in all 3 gardens (**Figure 8**). A recent report in 2013 has stated that tetracyclines were the most sold antibiotic class for administering to food-producing species followed by penicillin and sulfonamides [20]. A study conducted in 1992, stated that 22% of the total annual production was for tetracyclines where less than half of its production was destined for the clinical use and the rest was added to the animal feed for the prophylactic control of disease and to stimulate weight gain [21]. Also, the stability and half-life of antibiotics in the environment should be considered too. Tetracycline antibiotics are stable in the environment and their activity remain unchanged upon human defecation when stored at room temperature.

4.3 Distribution of Clinical Class 1 Integron Integrase Gene in the Soil

Class I integron-integrase (*intI1*) gene was detected in all 3 gardens. Clinical class 1 integrons are prominent for their ability to acquire and disseminate antibiotic resistance genes as gene cassettes. Primers used to detect *intI*1 in the qPCR assay was based on the clinical variant of *intI*1 [13]. The absolute abundance of *intI1* amongst all the 3 gardens was approximately 5.12×10^6 copies $gram^{-1}$ (**Figure 11 and Figure 12**). These clinical class 1 integron could be a potential source for harboring *TetM, Sul2 and* bla_{TEM} gene in soil samples. A Pearson's correlation showed a significant correlation between bla_{TEM} and *sul2* $p < 0.01$. Also, correlation was observed between

intI1 and *Sul2* gene $p < 0.05$ (**Table 3**). Studies have focused on aerobic bacteria that are culturable and quantifiable. Anaerobes can also be a potential indicator organism for harboring ARGs facilitating human gut environment in the soil. A study conducted in 1984 suggested that presence of tetracycline resistance gene in the chromosome of *Bacteroides* [22]. The results strongly suggest that presence of clinical class 1 integron and ARGs are interdependent. Since most of the ARGs present on the extrachromosomal DNA like plasmid should be screened and then analysis in detecting ARGs should be carried out.

CHAPTER 5

CONCLUSION

Urban agricultural soil samples harbor high concentrations of tetracycline, sulfonamide, beta-lactams resistance genes and class 1 integron. This suggests that ARGs are common in the environment and they can be shaped by agricultural practices, history of land usage, and other human activities. The total abundance of class 1 integron was correlated with *sul-2* gene indicating a potential role of integrons in the propagation of ARGs in the urban agricultural farms. Also, a strong correlation was observed between *sul-2* and bla_{TEM} upon normalization to 16S rRNA gene. BLAST search and phylogenetic analysis of 16S rRNA sequencing results identified *Bacteroides spp.*, a common commensal bacterium in human and animal GI tract, suggesting possible fecal contamination in urban agricultural soil. This study collected much-needed information on the level of ARGs and MGEs in the environmental settings which will help researchers understand the possible mechanisms of the prevalence and persistence of ARG in the environment.

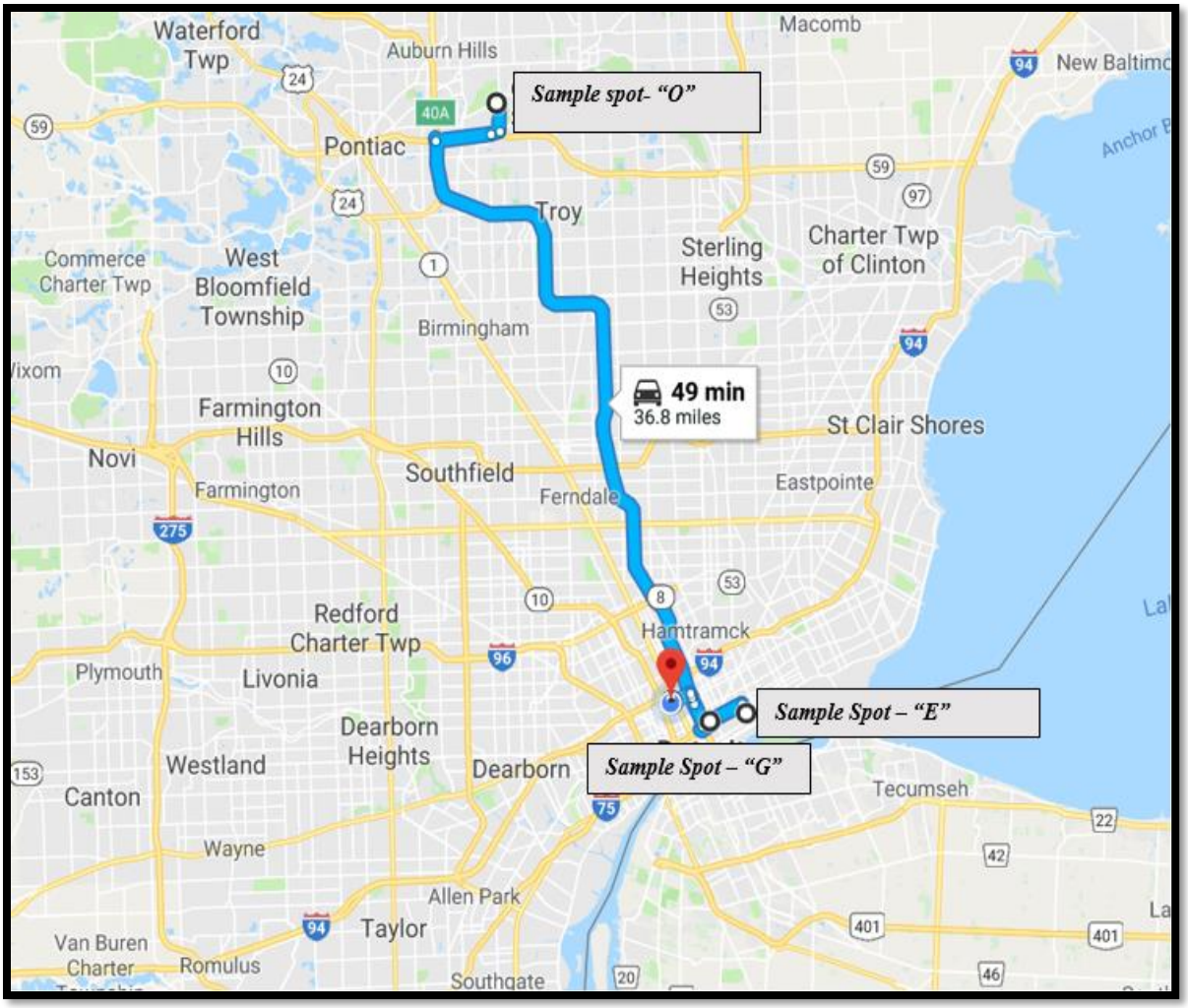

Figure 1: Route map showing the sampling spots of urban agricultural gardens "E", "G" and "O".

Table 1: PCR Primer Set Used In This Study

Target Genes	Primer set	Sequence	Reference	Annealing temperature T_a (ºC)	Amplicon length
Intl-1	*Intl-1F* *Intl-1R*	CGAACGAGTGGCGGAGGGTG TACCCGAGAGCTTGGCACCCA	Zhu et al., 2017	60	312 bp
Sul-2	*Sul-2F* *Sul-2R*	AACCGCCTTGTCCTTGATCC GACAGAAGCACCGGCAAATC	In this study	61	122 bp
Tet-M	*Tet-M F* *Tet-M R*	CATCATAGACACGCCAGGACATAT CGCCATCTTTTGCAGAAATCA	Zhu et al., 2017	60	101 bp
bla$_{TEM}$	*bla$_{TEM}$ F* *bla$_{TEM}$ R*	AGTATTCAACATTTCCGTGTCG GCTTAATCAGTGAGGCACCTATC	Maleki et al., 2018	61	850 bp
16S rRNA	27 - F 1492- R	AGAGTTTGATCMTGGCTCAG CGGTTACCTTGTTACGACTT	Jiang et al., 2006	50	1504 bp

Table 2: Description of The Primers and Protocols Used In Real-Time PCR Assays

Target Genes	Primer set	Sequence	Source	Amplicon length	Thermal cycling conditions	Calibration curve	R^2 Value
Intl-1	*Intl-1F* *Intl-1R*	CGAACGAGTGGCGGAGGGTG TACCCGAGAGCTTGGCACCCA	Zhu et al., 2017	312 bp	95 ºC for 10 min; 95 ºC for 30 s, 60 ºC for 30 s (40 cycles)	y = -4.035 logX + 42.852	96.2%
Sul-2	*Sul-2F* *Sul-2R*	AACCGCCTTGTCCTTGATCC GACAGAAGCACCGGCAAATC	In this Study	122 bp	95 ºC for 10 min; 95 ºC for 30 s, 61 ºC for 30 s (40 cycles)	y = -4.009 logX + 45.441	98.7%
Tet-M	*Tet-M F* *Tet-M R*	CATCATAGACACGCCAGGACATAT CGCCATCTTTTGCAGAAATCA	Zhu et al., 2017	101 bp	95 ºC for 10 min; 95 ºC for 30 s, 60 ºC for 30 s (40 cycles)	y = -3.547 logX + 40.538	99.7%
bla$_{TEM}$	*bla$_{TEM}$ F* *bla$_{TEM}$ R*	CGCCGCATACACTATTCTCAG GCTTCATTCAGCTCCGGTTC	-	239 bp	95ºC for 10 min; 95ºC for 30 s, 59ºC for 30 s (40 cycles)	y = -3.736 logX + 43.509	96.3%
16S rRNA	*16S rRNA-F* *16SrRNA-R*	GGGTTGCGCTCGTTGC ATGGYTGTCGTCAGCTCGTG	Zhu et al., 2017	60 bp	95ºC for 10 min; 95ºC for 30 s, 60ºC for 30 s (40 cycles)	y = -4.45 logX + 62.116	99.0%

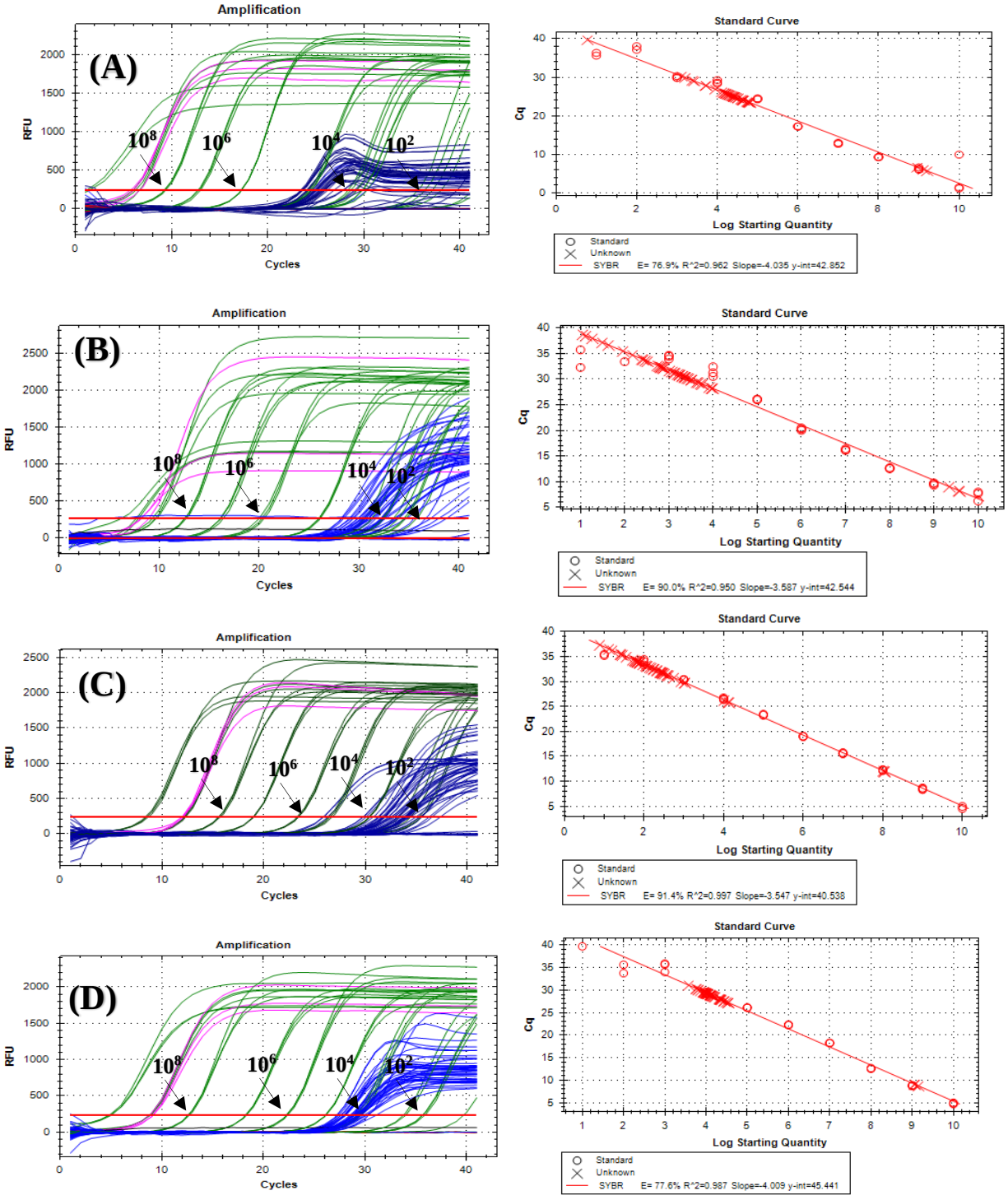

Figure 2: Quantification of MGE(*intI1*), ARGs (*bla*$_{TEM}$, *TetM*, *Sul2*) and their respective standard curves: qPCR amplification curves of *intl-1* (A), *bla*$_{TEM}$ (B), *Tet-M* (C), and *Sul-2* (D).

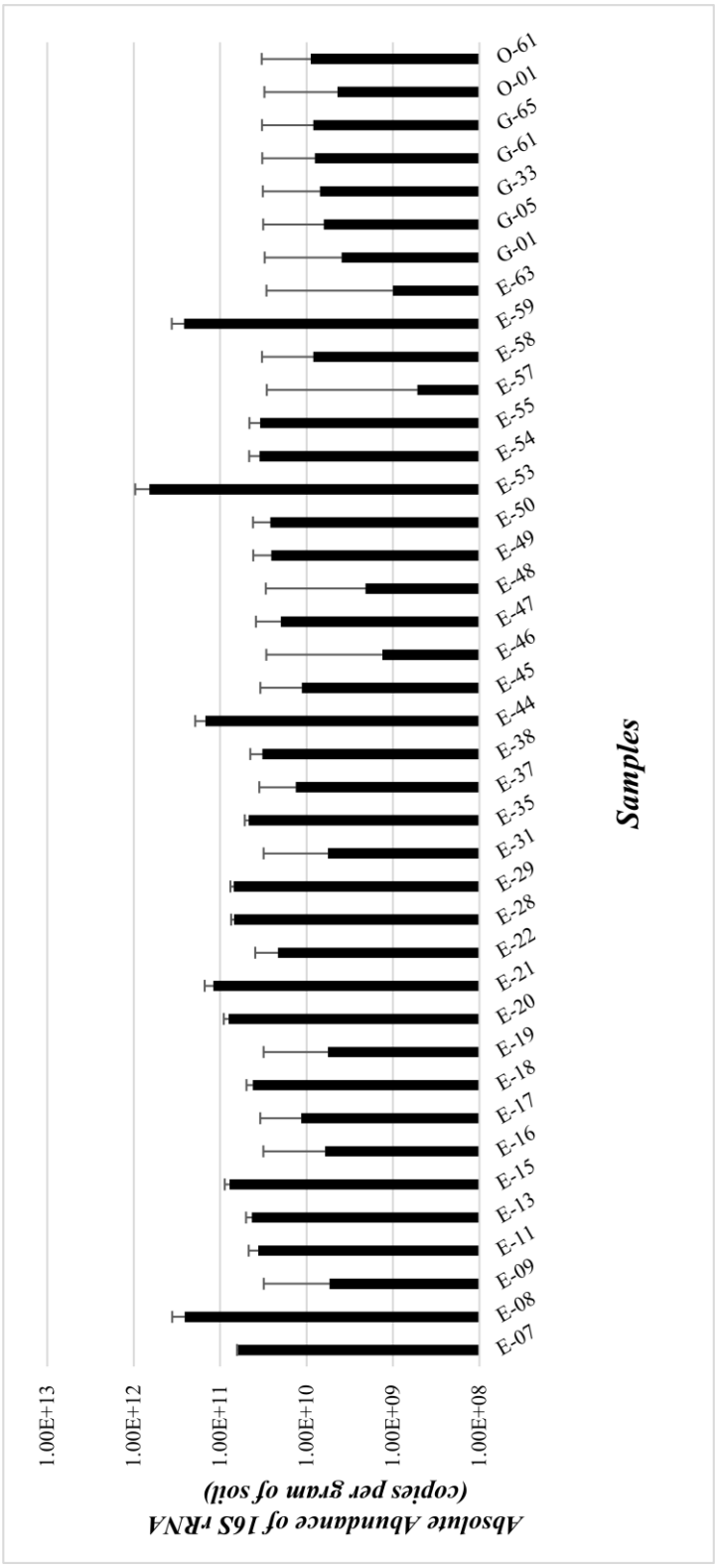

Figure 3: Absolute Abundance of 16S rRNA gene copies gram^{-1} of soil. Concentration of bacteria in all the 3 gardens (No. of samples in each garden: E-33, G-05 and O-05). Error bars represent Standard Deviation(SD).

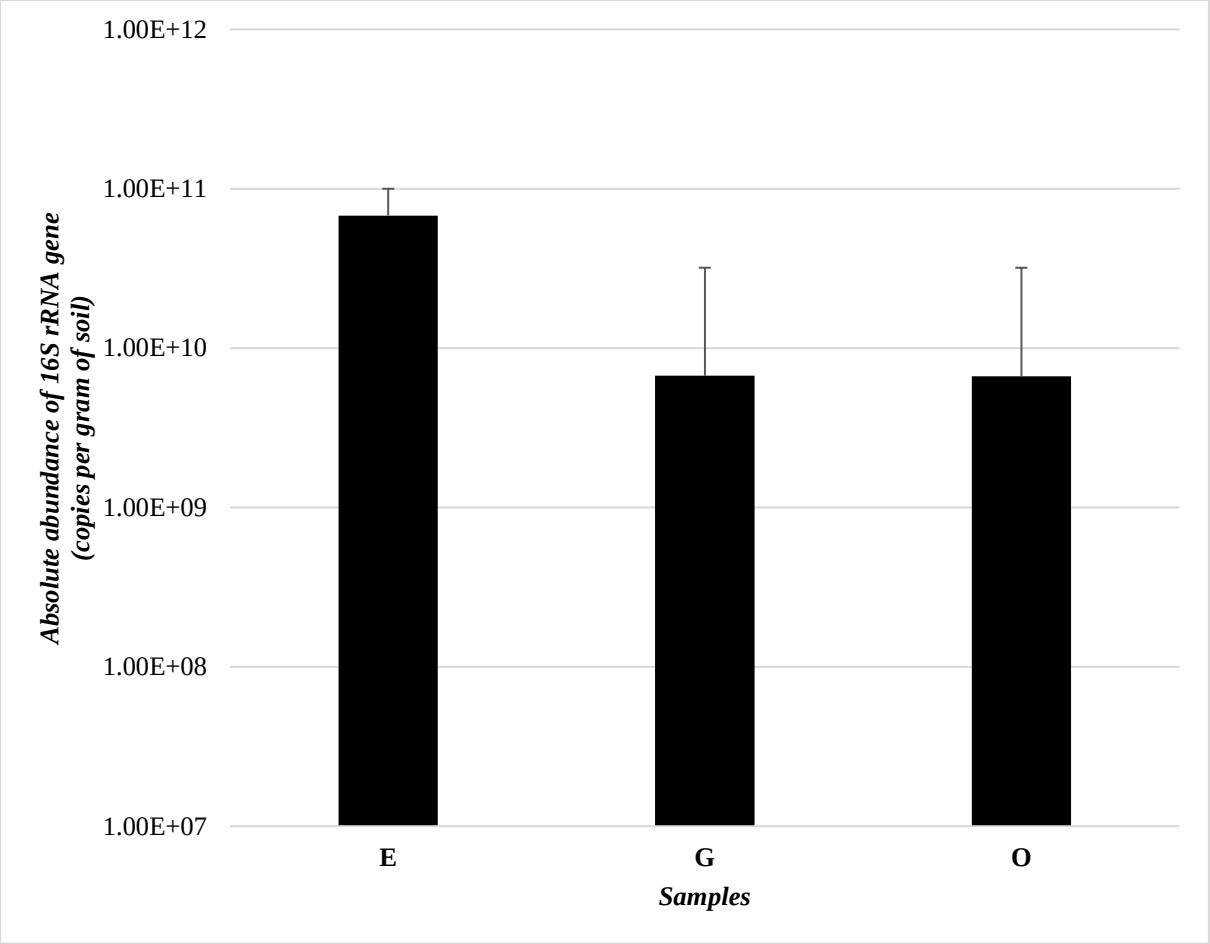

Figure 4: **Magnitude of Bacterial load within the 3 gardens** - Error bars represent Standard Deviation (SD). Number of samples in each garden: E – 33, G – 05, O- 05.

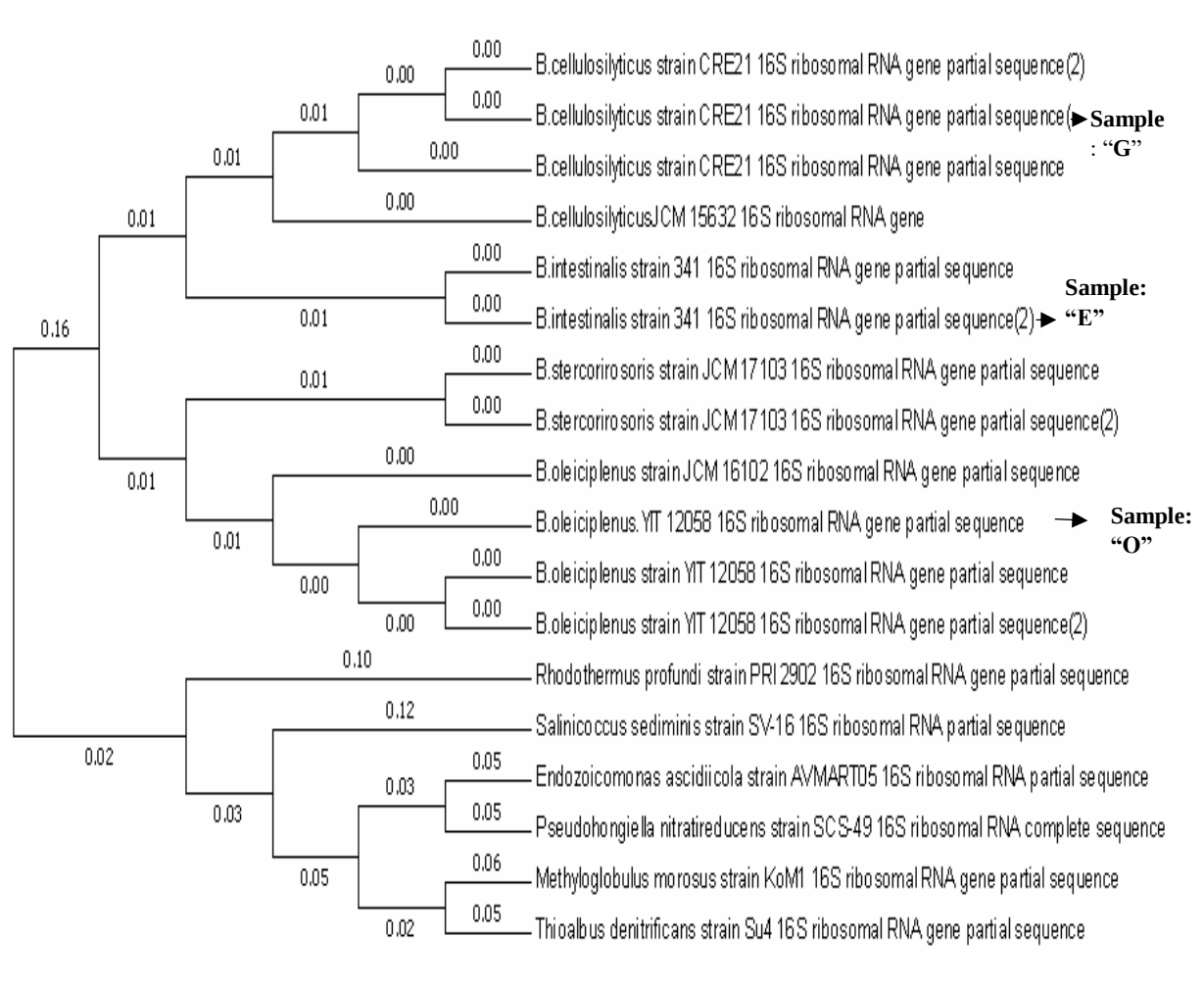

Figure 5: Neighbor-Joining (NJ) phylogenetic tree of 16S rRNA gene sequences (1504bp) detected in 3 gardens (E, G, O). Statistical significance $p < 0.05$ are indicated at nodes. The GenBank Accession numbers are: NR_112933.1, NR_113207.1, NR_113070.1, NR_112895.1, NR_041307.1, NR_042203.1, NR_042203.1, NR_145587.1, NR_146692.1, NR_118269.1, NR_116762.1, NR_153732.1, NR_122087.1, NR_146693.1, NR_146691.1.

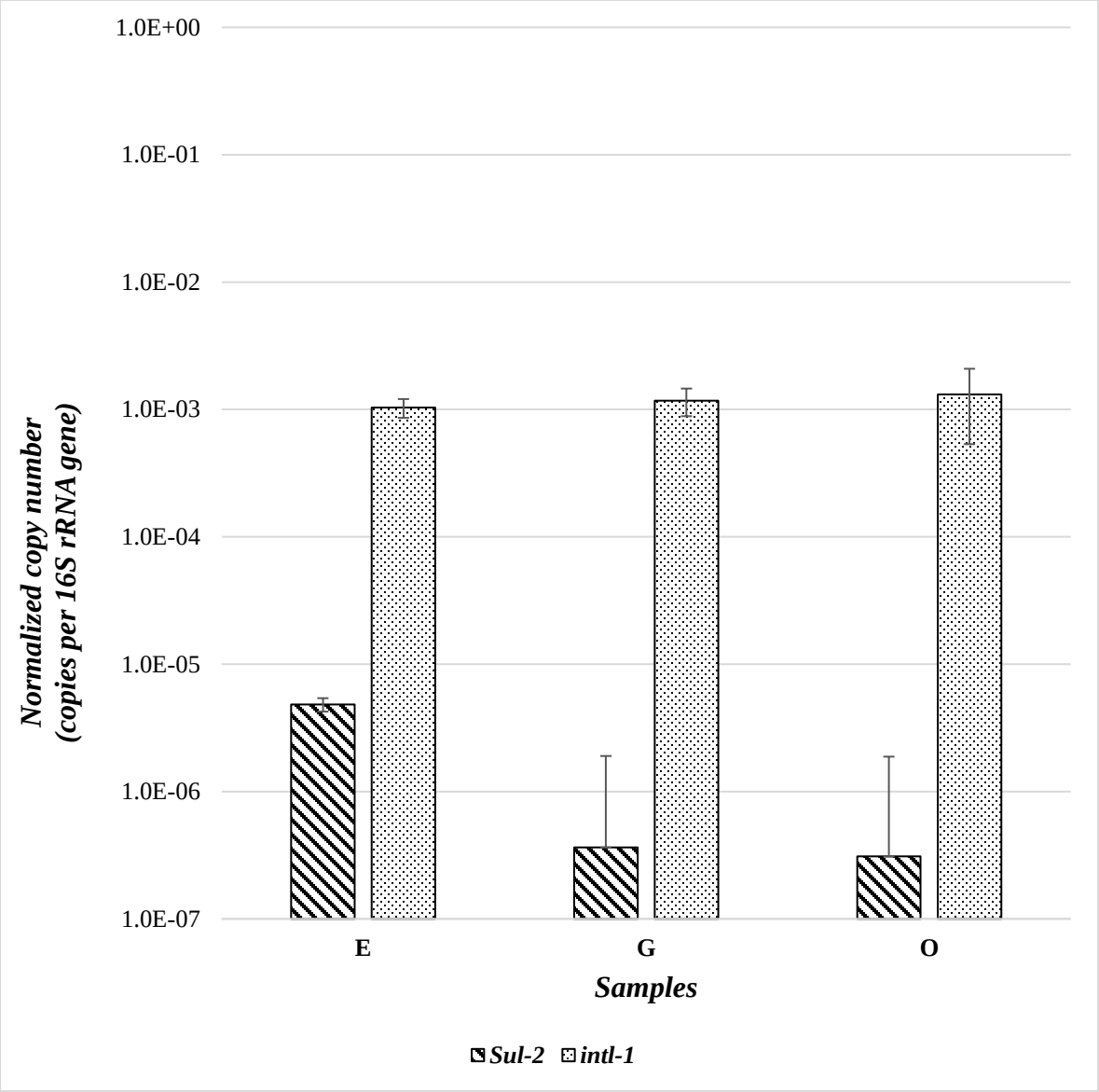

Figure 6: **Mean copy number of *intI1* and *Sul2* gene in soil samples** – Number of Samples: E - 33, G - 05, and O - 05. Error bars represent Standard Deviation(SD).

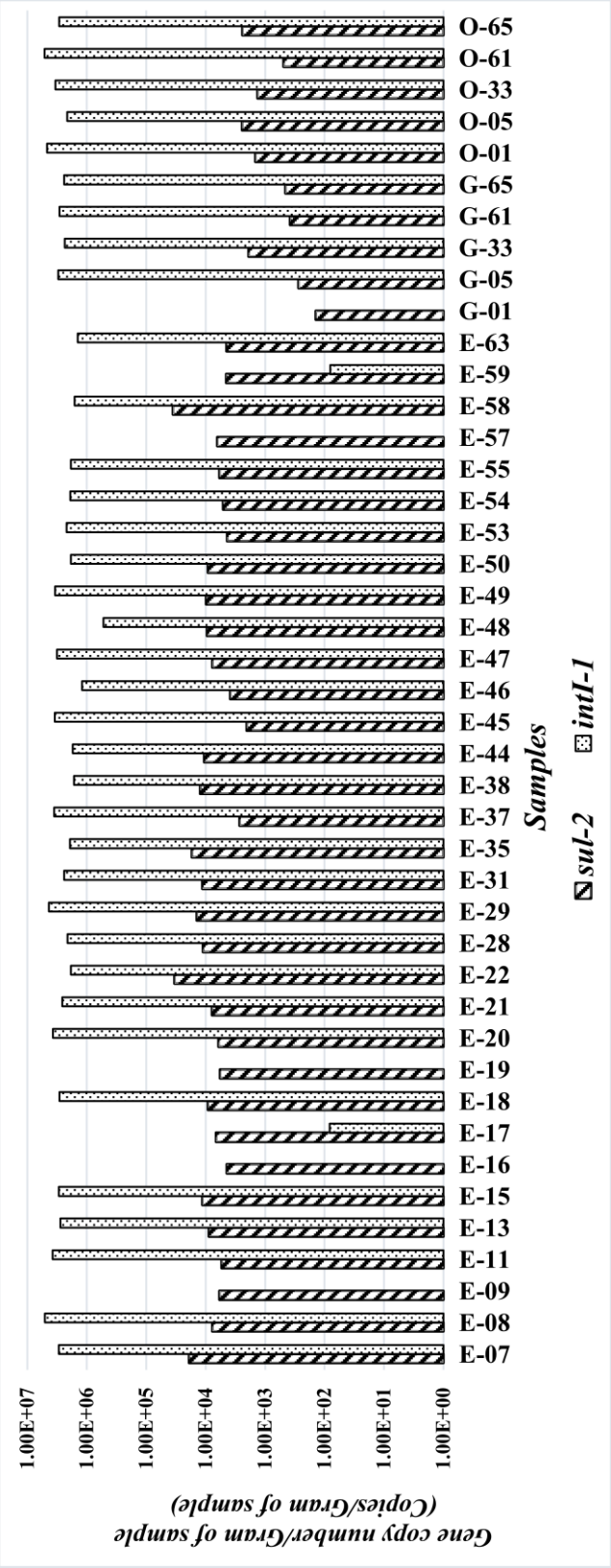

Figure 7: Absolute abundance (copies gram-1) of *intI1* and *sul2* gene in all 3 gardens – Abundance of *intI1* gene is directly proportional to *sul2* gene.

Table 3: Correlation of Normalized Abundance of ARGs

Correlations

		tetMcopies	intl1copies	sul2copies	blaTEM copies
tetMcopies	**Pearson Correlation**	1	.007	.300	.283
	Sig. (2-tailed)		.970	.051	.066
	N	43	35	43	43
intl1copies	**Pearson Correlation**	.007	1	**.371***	.205
	Sig. (2-tailed)	.970		**.028**	.237
	N	35	35	35	35
sul2copies	**Pearson Correlation**	.300	.371*	1	**.409****
	Sig. (2-tailed)	.051	.028		**.006**
	N	43	35	43	43
blaTEMcopies	**Pearson Correlation**	.283	.205	**.409****	1
	Sig. (2-tailed)	.066	.237	**.006**	
	N	43	35	43	43

***. Correlation is significant at the 0.05 level (2-tailed).**

****. Correlation is significant at the 0.01 level (2-tailed).**

Table 4: Frequency Table Showing Prevalence Of ARGs In Individual Soil Samples

Sample	*Tet-M*	*Intl-1*	*Sul-2*	*bla$_{TEM}$*
E-07	+	+	+	+
E-08	-	+	+	+
E-09	+	-	+	+
E-11	+	+	+	+
E-13	+	+	+	+
E-15	+	+	+	+
E-16	-	-	+	-
E-17	+	+	+	+
E-18	+	+	+	+
E-19	+	-	+	+
E-20	+	+	+	+
E-21	+	+	+	+
E-22	+	+	+	+
E-28	+	+	+	+
E-29	+	+	+	+
E-31	+	+	+	+
E-35	+	+	+	+
E-37	+	+	+	+
E-38	+	+	+	+
E-44	+	+	+	+
E-45	+	+	+	+
E-46	+	+	+	+
E-47	+	+	+	+
E-48	+	+	+	+
E-49	+	+	+	+
E-50	+	+	+	+
E-53	+	+	+	+
E-54	+	+	+	+
E-55	+	+	+	+
E-57	+	-	+	+
E-58	+	+	+	+
E-59	+	+	+	+
E-63	+	+	+	+
G-01	+	-	+	+
G-05	+	+	+	+
G-33	+	+	+	+
G-61	+	+	+	+

G-65	+	+	+	+
O-01	+	+	+	+
O-05	+	+	+	+
O-33	+	+	+	+
O-61	+	+	+	+
O-65	-	+	+	+
Frequency Index [a]	40/43	38/43	43/43	42/43

+: Present; -: Absent

[a] Frequency was calculated as the number of positive detection in total of 43 soil samples

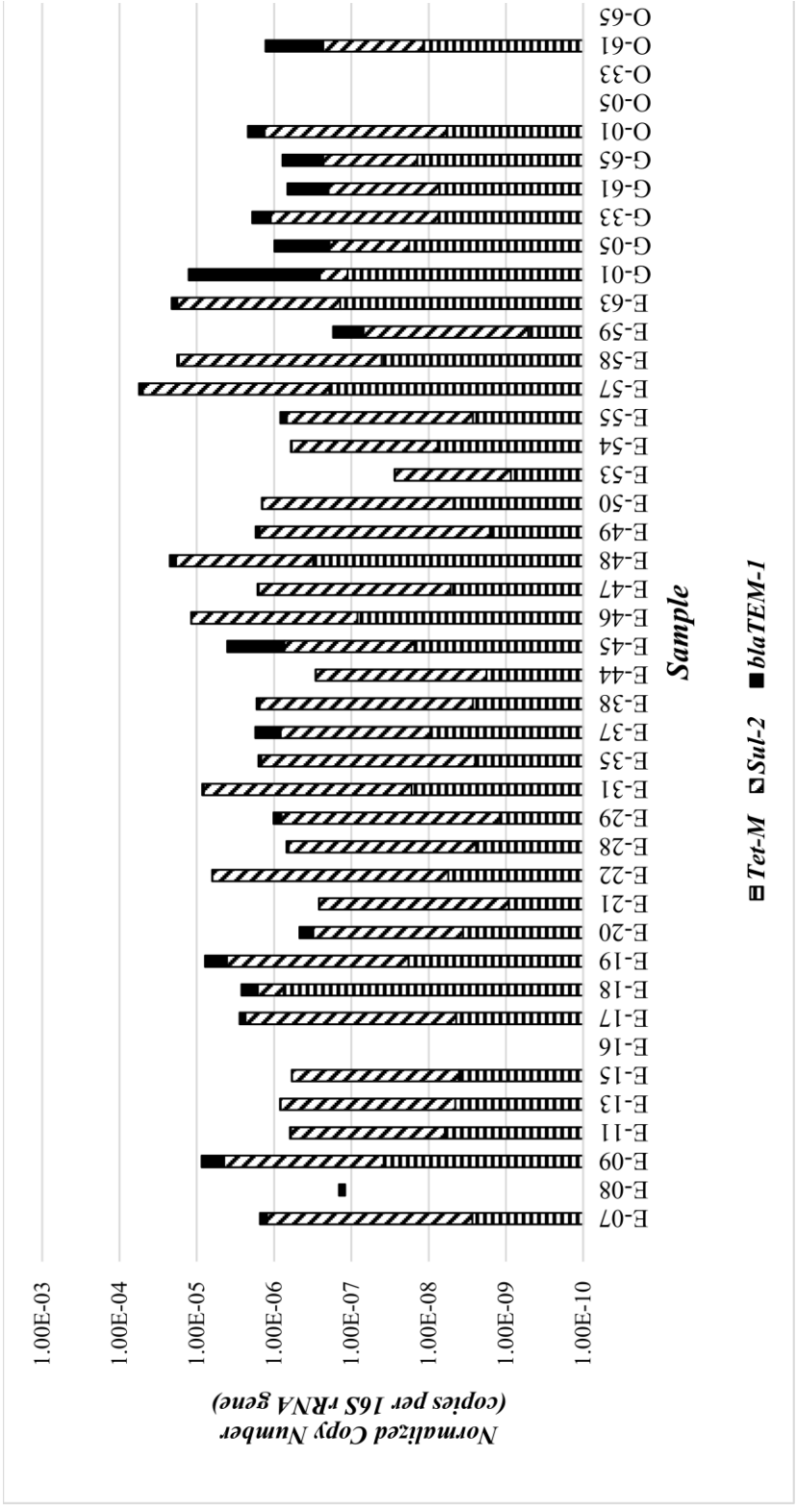

Figure 8: Abundance of ARGs profile in soil samples (copies per 16S rRNA). Gene Copy Number of ARGs normalized to 16S rRNA gene in three gardens. Classification of resistance genes based on the antibiotics to which they conferred resistance namely: Tetracyclines, β-lactams, Sulfonamides.

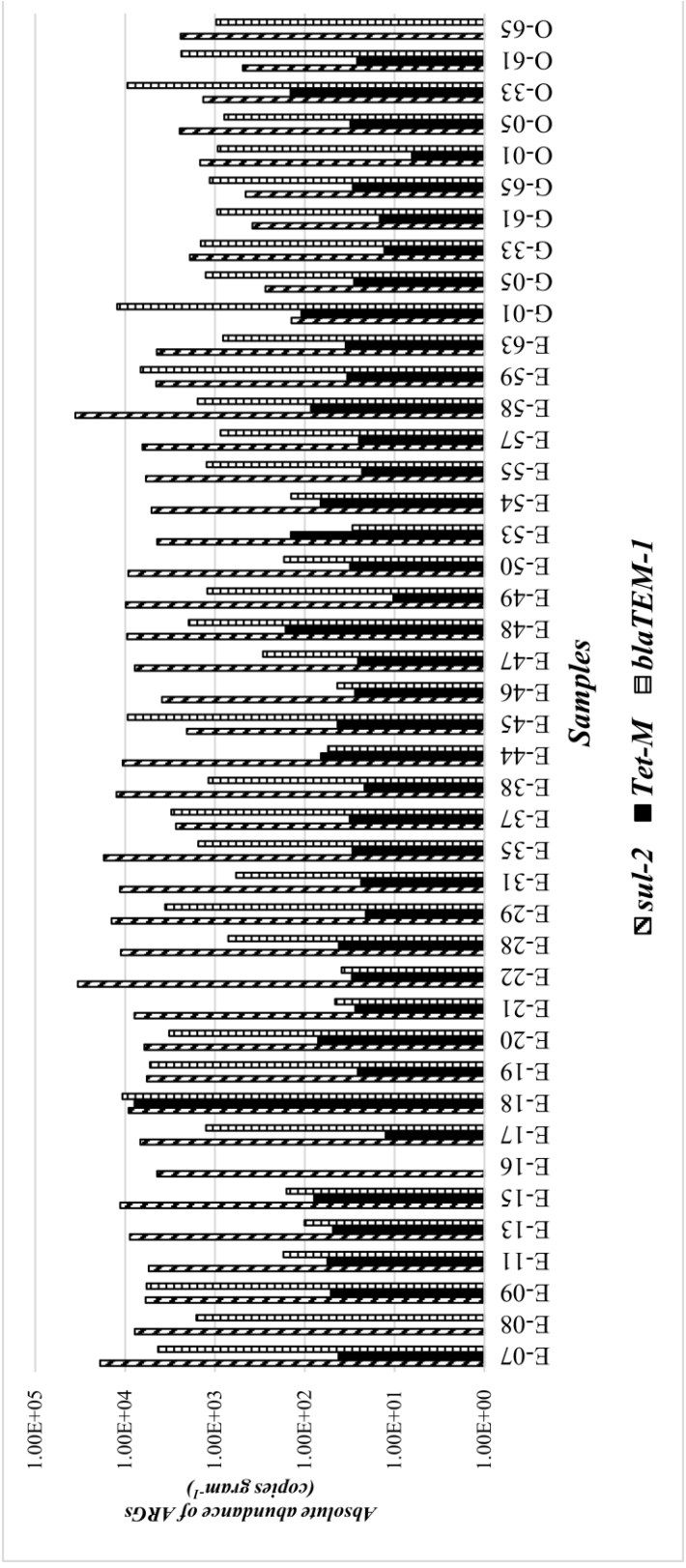

Figure 9: Absolute Abundance of Antibiotic Resistance Genes (Copies gram^{-1}) in all 3 gardens (E, G, O).

Table 5: Correlation of Total Absolute Abundance Resistance Genes per Gram of Sample

Correlations

		16S rRNA	ARGs Average	intl1	sul2	tetM	blatem
16S rRNA	**Pearson Correlation**	1	-.039	.068	-.015	-.003	-.099
	Sig. (2-tailed)		.805	.668	.925	.984	.538
	N	42	42	42	42	39	41
ARGs Average	**Pearson Correlation**	-.039	1	-.109	**.874****	**.348***	.268
	Sig. (2-tailed)	.805		.485	**.000**	**.028**	.087
	N	42	43	43	43	40	42
intl1	**Pearson Correlation**	.068	-.109	1	-.077	.073	-.118
	Sig. (2-tailed)	.668	.485		.625	.653	.457
	N	42	43	43	43	40	42
sul2	**Pearson Correlation**	-.015	.874**	-.077	1	.026	-.192
	Sig. (2-tailed)	.925	.000	.625		.872	.224
	N	42	43	43	43	40	42
tetM	**Pearson Correlation**	-.003	.348*	.073	.026	1	**.433****
	Sig. (2-tailed)	.984	.028	.653	.872		**.005**
	N	39	40	40	40	40	40
bla_{TEM}	**Pearson Correlation**	-.099	.268	-.118	-.192	.433**	1
	Sig. (2-tailed)	.538	.087	.457	.224	.005	
	N	41	42	42	42	40	42

****. Correlation is significant at the 0.01 level (2-tailed).**

***. Correlation is significant at the 0.05 level (2-tailed).**

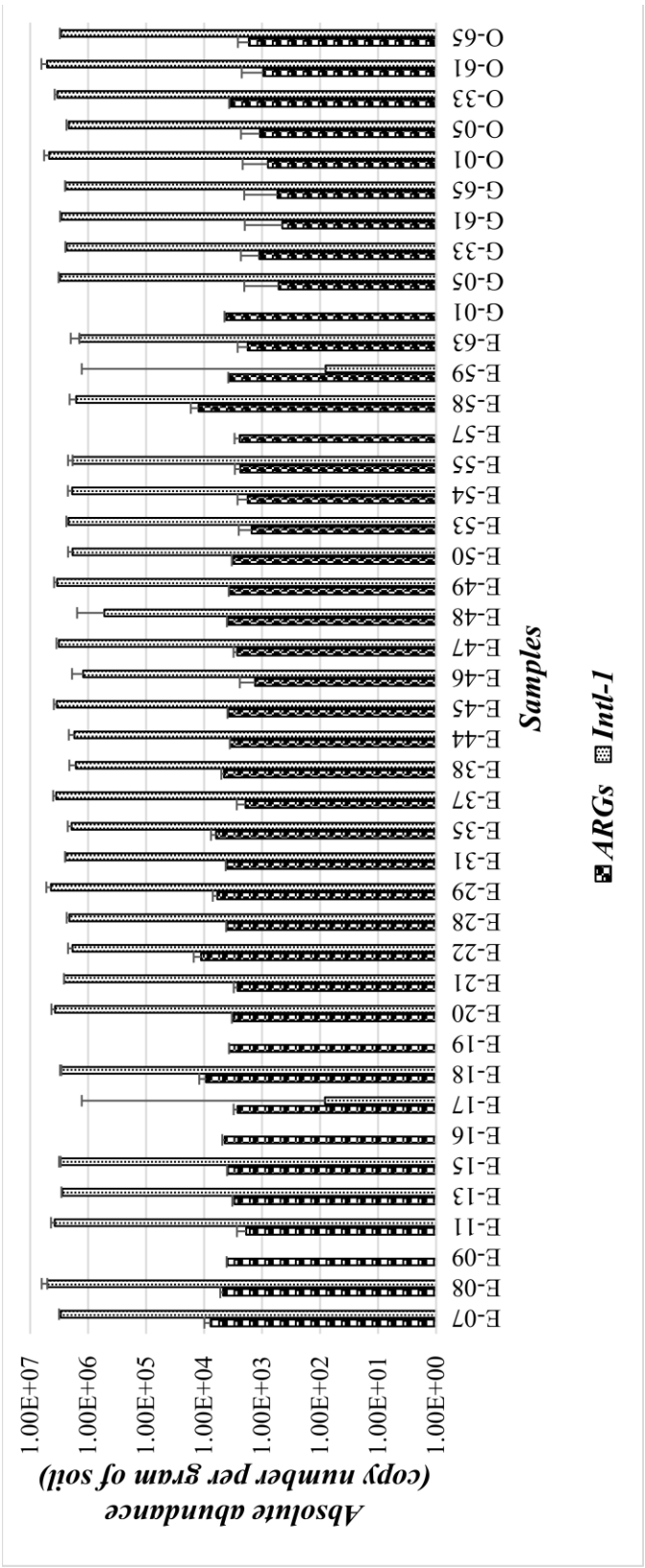

Figure 10: Distribution of Mobile Genetic Element *intI-1* and Antibiotic Resistance Genes (bla_{TEM}, *TetM and Sul2*) – Expressed as gene copies per gram of soil plotted on a logarithmic(log) scale. Error bars represent Standard Deviation(SD).

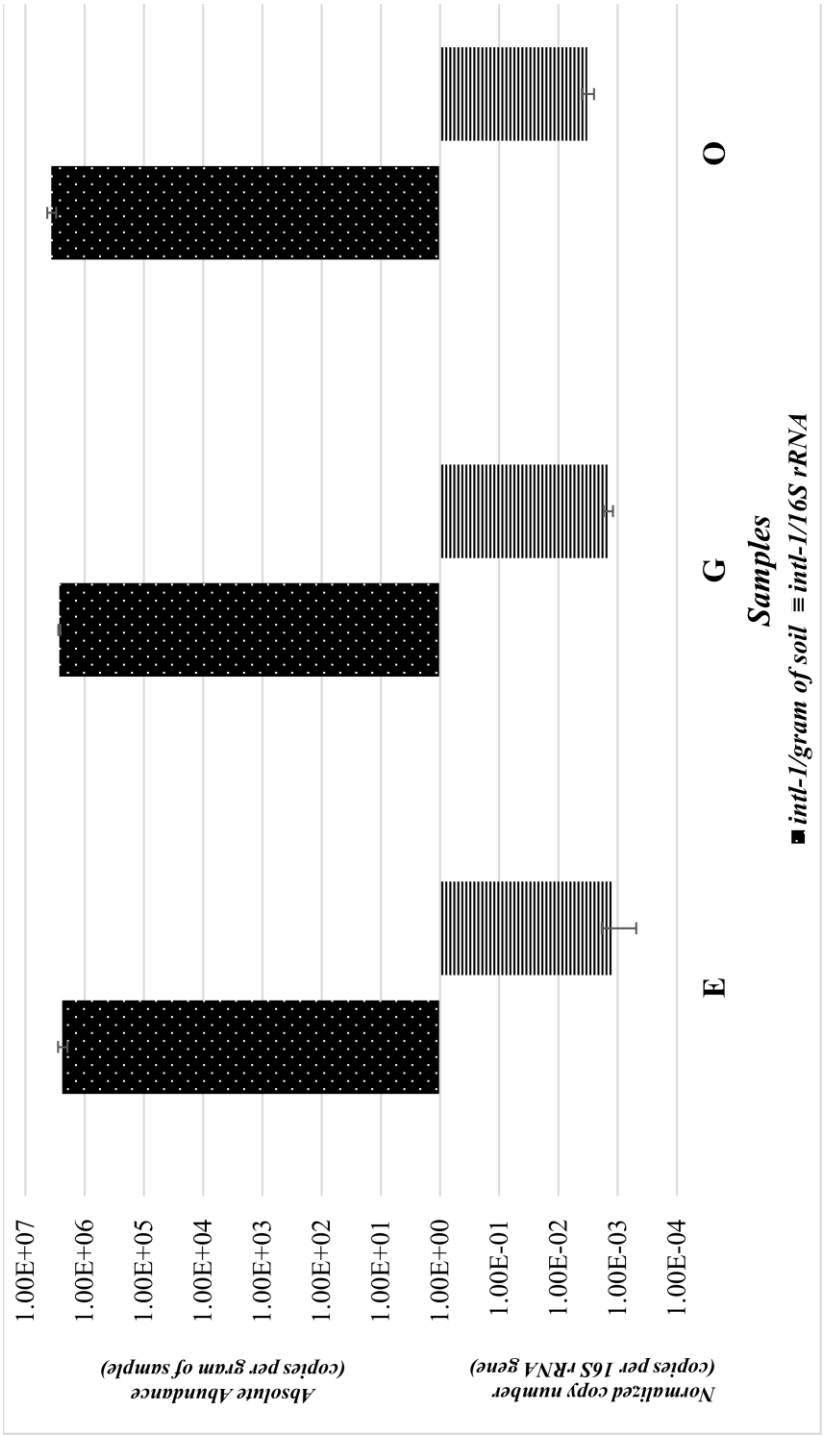

Figure 11: Comparison of absolute and normalized abundance of *intl-1 gene* per bacterial cell in all three gardens. Mean Distribution of *intI-1* gene in all three gardens was similar but varied upon normalization per 16S rRNA gene. Error bars represent Standard Deviation(SD). Number of samples in each garden: E – 33, G – 05, O-05

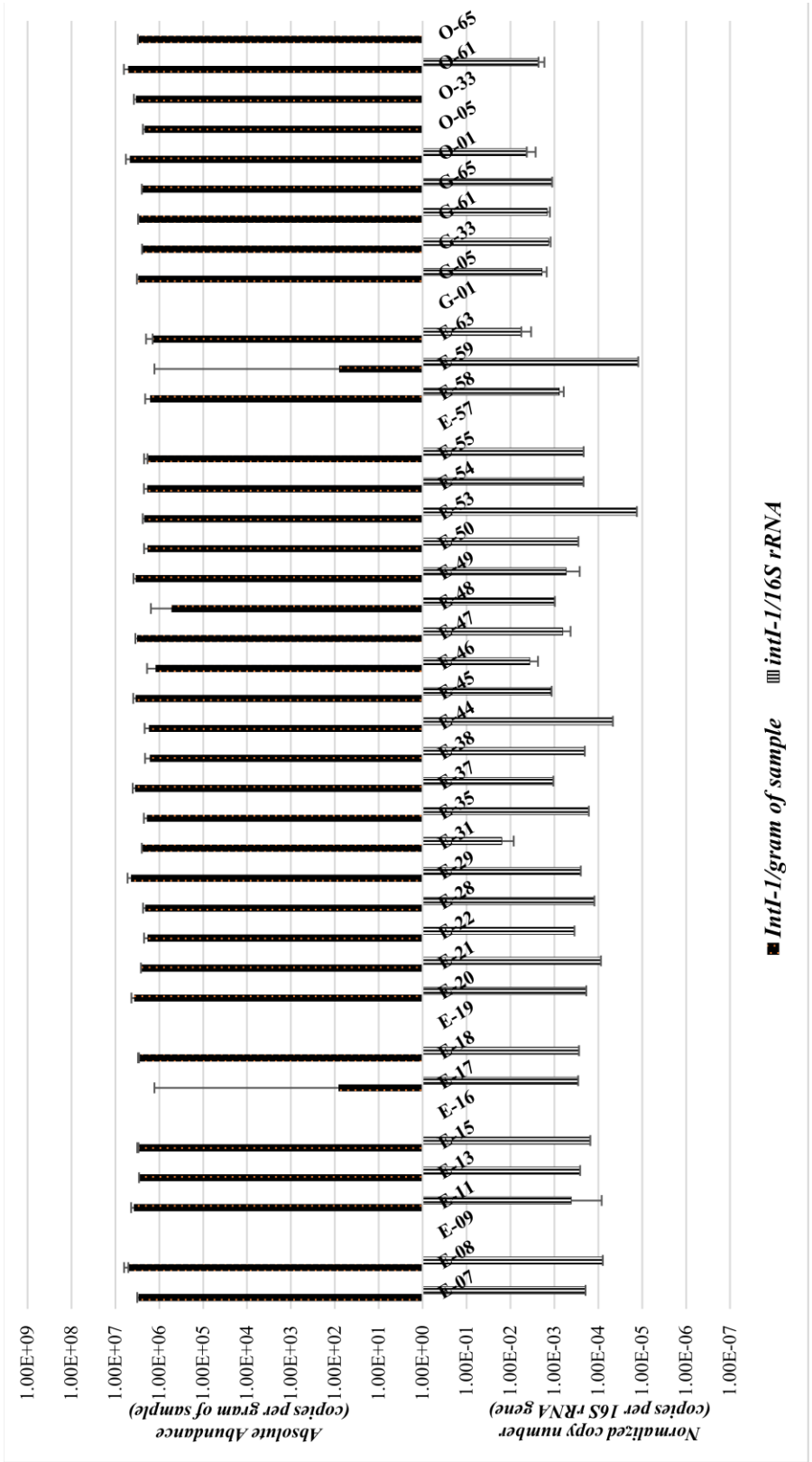

Figure 12: Comparison of absolute abundance of *intI-1* gene in the soil samples and normalized abundance by bacterial cells. Absolute abundance of *intI-1* per gram of soil sample are plotted on a positive Y axis (Log scale), Normalized gene copy number per bacterial cell is represented on the negative Y axis; Error bars represent Standard Deviation(SD).

REFERENCES

1. http://www.sciencemag.org/news/2012/08/soil-may-be-source-drug-resistant-bacteria.
2. Sengupta, S., Chattopadhyay, M. K., & Grossart, H.-P. (2013). The multifaceted roles of antibiotics and antibiotic resistance in nature. *Frontiers in Microbiology*, 4, 47.
3. Ventola, C. L. (2015). The Antibiotic Resistance Crisis: Part 1: Causes and Threats. *Pharmacy and Therapeutics*, 40(4), 277–283.
4. Fleming, A. (1929). Classics in infectious diseases: on the antibacterial action of cultures of a penicillium, with special reference to the use in the isolation of *B. influenzae*. *British Journal of Experimental Pathology*, 10, 226–236.
5. Centers for Disease Control and Prevention, Office of Infectious Disease. Antibiotic resistance threats in the United States, 2013. April 2013. Available at: http://www.cdc.gov/drugresistance/threat-report-2013. Accessed January 26, 2018.
6. Sciarretta, K., Røttingen, J. A., Opalska, A., Van Hengel, A. J., & Larsen, J. (2016). Economic incentives for antibacterial drug development: Literature review and considerations from the transatlantic task force on antimicrobial resistance. *Clinical Infectious Diseases, 63*(11), 1470-1474.
7. Kumarasamy, K. K., Toleman, M. A., Walsh, T. R., Bagaria, J., Butt, F., Balakrishnan, R., Woodford, N. (2010). The emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *The Lancet Infectious Diseases*, 10(9), 597–602.
8. Ghosh, T. S., Gupta, S. S., Nair, G. B., & Mande, S. S. (2013). *In Silico* Analysis of Antibiotic Resistance Genes in the Gut Microflora of Individuals from Diverse Geographies and Age-Groups. PLoS ONE, 8(12), e83823. http://doi.org/10.1371/journal.pone.0083823

9. United States Department of Agriculture, Office of Inspector General (2016) Resistance USDA's Response to Antibiotic Resistance: Audit Report 50601-0004-31: Washington, D.C. https://www.usda.gov/oig/webdocs/50601-0004-31.pdf. Accessed on: February 2, 2018.
10. Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., & Kreuzinger, N. (2015). Tackling antibiotic resistance: the environmental framework. *Nature Reviews Microbiology*, 13(5), 310.
11. Li, L., Mendis, N., Trigui, H., Oliver, J. D., & Faucher, S. P. (2014). The importance of the viable but non-culturable state in human bacterial pathogens. *Frontiers in Microbiology*, 5, 258.
12. Van Goethem, M. W., Pierneef, R., Bezuidt, O. K. I., Van De Peer, Y., Cowan, D. A., & Makhalanyane, T. P. (2018). A reservoir of "historical" antibiotic resistance genes in remote pristine Antarctic soils. *Microbiome*, 6, 40.
13. Zhu, Y. G., Zhao, Y., Li, B., Huang, C. L., Zhang, S. Y., Yu, S., & Su, J. Q. (2017). Continental-scale pollution of estuaries with antibiotic resistance genes. *Nature microbiology*, *2*, 16270.
14. Marti, E., & Balcázar, J. L. (2013). Real-Time PCR Assays for Quantification of *qnr* Genes in Environmental Water Samples and Chicken Feces. *Applied and Environmental Microbiology*, 79(5), 1743–1745.
15. Reissbrodt, R., Rienaecker, I., Romanova, J. M., Freestone, P. P. E., Haigh, R. D., Lyte, M., & Williams, P. H. (2002). Resuscitation of *Salmonella enterica* serovar Typhimurium and enterohemorrhagic *Escherichia coli* from the viable but nonculturable state by heat-stable enterobacterial autoinducer. *Applied and Environmental Microbiology,* 68(10), 4788-4794.

16. March-Rosselló, G. A. (2017). Rapid methods for detection of bacterial resistance to antibiotics. *Enfermedades infecciosas y microbiologia clinica (English ed.)*, *35*(3), 182-188.
17. Maleki, N., Tahanasab, Z., Mobasherizadeh, S., Rezaei, A., & Faghri, J. (2018). Prevalence of CTX-M and TEM β-lactamases in *Klebsiella pneumoniae* Isolates from Patients with Urinary Tract Infection, Al-Zahra Hospital, Isfahan, Iran. *Advanced Biomedical Research*, 7, 10.
18. Jiang H, Dong H, Zhang G, Yu B, Chapman LR, Fields MW (June 2006). Microbial diversity in water and sediment of Lake Chaka, an athalassohaline lake in northwestern China. *Applied and Environmental Microbiology*. 72 (6): 3832–45.
19. Ritalahti, K. M., Amos, B. K., Sung, Y., Wu, Q., Koenigsberg, S. S., & Löffler, F. E. (2006). Quantitative PCR targeting 16S rRNA and reductive dehalogenase genes simultaneously monitors multiple *Dehalococcoides* strains. *Applied and environmental microbiology*, 72(4), 2765-2774.
20. European Medicines Agency (2013). Sales of Veterinary Antimicrobial Agents in 25 EU/EEA Countries in 2011. Report EMA/236501/2013. London: European Medicines Agency.
21. Johnson, R., & Adams, J. (1992). The ecology and evolution of tetracycline resistance. *Trends in ecology & evolution*, *7*(9), 295-299.
22. Guiney, D. G., Hasegawa, P., & Davis, C. E. (1984). Expression in *Escherichia coli* of cryptic tetracycline resistance genes from *Bacteroides* R plasmids. *Plasmid*, *11*(3), 248-252.
23. Shoemaker, N. B., Vlamakis, H., Hayes, K., & Salyers, A. A. (2001). Evidence for Extensive Resistance Gene Transfer among *Bacteroides spp*. and among Bacteroides and Other Genera in the Human Colon. *Applied and environmental microbiology*, 67(2), 561-568.

24. Whittle, G., Shoemaker, N. B., & Salyers, A. A. (2002). The role of Bacteroides conjugative transposons in the dissemination of antibiotic resistance genes. *Cellular and Molecular Life Sciences CMLS*, *59*(12), 2044-2054.

25. Wu, D., Huang, X. H., Sun, J. Z., Graham, D. W., & Xie, B. (2017). Antibiotic resistance genes and associated microbial community conditions in aging landfill systems. *Environmental Science & Technology*, *51*(21), 12859-12867.

26. Koczura, R., Mokracka, J., Taraszewska, A., & Łopacinska, N. (2016). Abundance of class 1 integron-integrase and sulfonamide resistance genes in river water and sediment is affected by anthropogenic pressure and environmental factors. *Microbial ecology*, 72(4), 909-916.

27. Makino, S. I., Kii, T., Asakura, H., Shirahata, T., Ikeda, T., Takeshi, K., & Itoh, K. (2000). Does Enterohemorrhagic *Escherichia coli* O157: H7 Enter the Viable but Nonculturable State in Salted Salmon Roe? *Applied and environmental microbiology*, 66(12), 5536-5539.

28. Ouyang, W. Y., Huang, F. Y., Zhao, Y., Li, H., & Su, J. Q. (2015). Increased levels of antibiotic resistance in urban stream of Jiulongjiang River, China. *Applied microbiology and biotechnology*, *99*(13), 5697-5707.

29. Gillings, M. R., Gaze, W. H., Pruden, A., Smalla, K., Tiedje, J. M., & Zhu, Y. G. (2015). Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. *The ISME journal*, *9*(6), 1269.

30. Gillings, M., Boucher, Y., Labbate, M., Holmes, A., Krishnan, S., Holley, M., & Stokes, H. W. (2008). The evolution of class 1 integrons and the rise of antibiotic resistance. Journal of bacteriology, 190(14), 5095-5100.

ABSTRACT

QUANTIFICATION OF ANTIMICROBIAL RESISTANCE GENES IN URBAN AGRICULTURAL SOIL

by

VIDHYA BAI KRISHNOJI RAO

August 2018

Advisor: Dr. Yifan Zhang

Major: Nutrition and Food Science

Degree: Master of Science

The increased dissemination of antibiotic resistance genes and their acquisition by clinically relevant microbes in the environmental setting is becoming a global alarming issue. Environmental areas that encompass anthropogenic pressure such as pharmaceutical manufacturing effluents, aquaculture facilities, municipal wastewater systems, chemical industry effluents and animal husbandry facilities are hotspots of ARGs and ARBs. The main objective of the present study was to investigate the prevalence, identification, and quantification of class 1 integron (*intI1*) and common antibiotic resistance genes (*Sul2*, *TetM*, bla_{TEM}) in urban agricultural soil. Quantitative PCR was implemented to determine the abundance of ARGs in the soil. Standardization of *intI1* gene copy number (10^6 copies $gram^{-1}$) and ARGs (*Sul2*, *TetM*, bla_{TEM}) was performed and the absolute abundance of resistance genes was normalized by bacterial cell. Correlation between *intI1* and *Sul2* gene with significance level of $p < 0.05$ was observed. This study suggests that ARGs are common in the environment including urban agricultural soil that receives no animal wastes or wastewater. Mobile genetic elements (MGE) may play an important role in spreading ARGs in the environment.

AUTOBIOGRAPHICAL STATEMENT

EDUCATION:

Master of Science in Nutrition and Food Science

Wayne State University, Detroit, MI, USA. (August 2016-May 2018)

Bachelor of Engineering in Biotechnology,

Dayananda Sagar College of Engineering- Bangalore, Karnataka, India.

(Affiliated to Visvesvaraya Technological University, Belgaum) (August 2011- May 2015)

AWARDS:

Recipient of Graduate Match Funding Fall 2017 and Winter 2018 – Wayne State University

PROFESSIONAL APPOINTMENTS:

Student Assistant, Under Dr. Yifan Zhang, (September – Dec 2017)

Wayne State University, Detroit, MI, USA.

Instructional/Non-instructional Assistant, Under Dr. Yifan Zhang, (May - August 2017)

Wayne State University, Detroit, MI, USA.