ANTIBIOTIC RESISTANCE AND BACTERIAL MICROBIOME IN LETTUCE-SOIL SYSTEMS

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Crop and Soil Sciences - Environmental Toxicology – Doctor of Philosophy
Environmental Science and Policy – Dual Major

2020

ABSTRACT

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Food safety challenges from emerging contaminants such as antibiotics and antibiotic resistance genes (ARGs) have received increasing attention due to rapid increases in their abundance in agroecosystems. This is particularly true in soil-vegetable systems as microbiomes and antibiotic resistomes of vegetables are important to their quality and safety and could be influenced by crop production with contaminated soil and water. Additionally, the food safety of vegetables may also drive consumers' preference and demand for certain food products (especially for labeled products such as USDA Organic, Raised Without Antibiotics, etc.). Using a soil-lettuce (*Lactuca sativa*) model system, the first study in this dissertation assessed how irrigation with antibiotics-contaminated water via overhead or soil-surface irrigation could influence bacterial communities and ARG profiles in lettuce shoots, roots, and soil, using 16S rRNA amplicon sequencing and high throughput qPCR techniques, respectively. The overall abundance and diversity of ARGs and bacteria associated with soil-surface irrigated lettuce shoots were lower than those under overhead irrigation, indicating soil-surface irrigation may have lower risks of producing food crops with high abundance of ARGs. ARG profiles and bacterial communities were sensitive to pharmaceutical exposure, but no consistent patterns of changes were observed. The second study examined the fate and transport of selected antibiotics through bulk soil, rhizosphere soil, and lettuce roots and shoots under soil-surface irrigation. Root concentration factors based on the antibiotic concentrations in bulk soil (RCF_{bs}) were significantly higher than those based on antibiotic concentrations in rhizosphere soil (RCF_{rs}) for

ciprofloxacin, lincomycin, oxytetracycline, sulfamethoxazole, and tetracycline, similar for trimethoprim and tylosin, and lower for monensin. The third study investigated bacterial community assembly and ARG profiles in lettuce shoots, roots, rhizosphere soil, and bulk soil upon exposure to antibiotics. Bacterial communities were driven by stochastic processes upon exposure to low level antibiotics, and were more resilient in roots and rhizosphere soil than in bulk soil and shoots. The fourth study explored the importance of demographics, food-relevant habits, and foodborne disease perception to consumers' buy and pay preferences to labeled products by using conventional statistical and novel machine learning methods to analyze survey data. Consumers' willingness to buy or to pay more for certain labeled food products is dependent on certain demographic traits (e.g., urban living) and food-relevant habits (e.g., cooking fresh produce). Machine learning methods achieved sufficient prediction accuracy scores for estimating consumers' willingness to buy or to pay for labeled products, and thus could be useful tools for evaluating survey data and facilitating the development of strategies promoting healthy food production and consumption.

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This dissertation is dedicated to my parents, Jun Liu and Wenmin Shen.
Thank you for always supporting and believing in me

ACKNOWLEDGMENTS

I want to thank my major advisor, Dr. Wei Zhang, for guidance and support throughout my Ph.D. journey. His knowledge, time, enthusiasm, commitment, and encouragement were greatly appreciated. I am especially grateful for his support in difficult times when I felt stressed in course work, research, and future career directions. Furthermore, I am thankful for his open mind supporting me in learning to code and to develop an interdisciplinary class project into one of my dissertation chapters. Finally, I appreciate his advice on how to be a good researcher. Also, I want to thank the other members of my guidance committee, Dr. Hui Li, Dr. Elliot Ryser, and Dr. Ashley Shade, for their valuable input and assistance.

I want to thank the Department of Plant, Soil and Microbial Sciences, the Environmental Science and Policy Program, the Institute of Integrative toxicology, and College of Agriculture and Natural Resources at Michigan State University for providing excellent training, curriculum, fellowships, and conference travel grants. Thanks to the administrative staff for helping me through my Ph.D. program.

I want to acknowledge my colleagues for insightful discussions and helps: Drs. James M. Tiedje, Feng Gao, Robert D. Stedtfeld, Joseph Hamm, Yuanbo Li, Xun Qian, Yingjie Zhang, Zeyou Chen, Wei Sun, Sangho Jeon, Cheng-hua Liu, Ya-hui Chuang, and Xueping Guo along with Jianzhou He, Geoff Rhodes, and others I have worked with. Thanks to my parents Jun Liu and Wenmin Shen, for their never-ending praise, support, and love. Additionally, I would like to thank my friends in the U.S., China, Canada, and around the world for interesting conversations.

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KEY TO ABBREVIATIONS

ARB: antibiotic resistant bacteria

ARGs: antibiotic resistance genes

CDC: United States Centers for Disease Control and Prevention

DNA: deoxyribonucleic acid

HPCC: high performance computing center

MDR: multidrug resistance genes

MGEs: mobile genetic elements

MLSB: macrolide-lincosamide-streptogramin B

MSU: Michigan State University

OTU: operational taxonomic unit

PCoA: principle coordinate analysis

qPCR: quantitative polymerase chain reaction

rRNA: ribosomal ribonucleic acid

USDA: United States Department of Agriculture

WHO: World Health Organization

CHAPTER I

Introduction and Objectives

Introduction

New food safety challenges in vegetable production are being brought about by emerging contaminants such as antibiotics, and antibiotic resistance genes (ARGs), as a result of their rapid proliferation in agroecosystems. The imprudent use of antibiotics in human healthcare and animal production has led to trace level of antibiotics in the environment including reclaimed water [1-3]. For example, sulfamethoxazole and trimethoprim were detected at levels up to 22 and 3.1 µg/L in wastewater effluents [4-6]. Vegetables and soils can be exposed to low levels of pharmaceuticals when irrigated with reclaimed waters. Pharmaceuticals (especially antibiotics) may be considered deterministic factors in shaping the microbiome of vegetable production systems as many pharmaceuticals are bioactive to microorganisms. Several studies have shown that exposure to pharmaceuticals and heavy metals from animal manures, wastewaters, or biosolids could change ARG profiles and bacterial communities in soil and water environments [7-12]. Antibiotics and ARGs may also interact with typical microbial pathogens (e.g., *Salmonella*) to collectively impact the safety of vegetables. Since food consumption can result in direct exposure to antibiotics, ARGs, and foodborne pathogens, it is important to understand the changes in bacterial communities and ARG profiles due to their potential influence on food safety and ultimately human health.

Crop irrigation with reclaimed water is increasingly practiced worldwide to meet water demand [13]. However, it was reported that there was higher incidence *Escherichia* coli in lettuce irrigated with overhead sprinklers than with soil-surface irrigation [14]. Thus, it is important to investigate how irrigation methods may influence the microbiome and ARGs in vegetables. Additionally, in soil-plant systems bulk soil, rhizosphere soil, plant roots and shoots may play a critical role in regulating the uptake and accumulation of antibiotics as well as changes in

bacterial microbiome and ARG profiles upon exposure to anthropogenic antibiotics. Thus, it is essential to study the plant uptake of antibiotics, bacterial microbiome, and ARGs in the continuum of bulk soil, rhizosphere soil, roots, and shoots.

The perceived safety of vegetables can drive consumer preferences and demand. Studies have been performed on consumers' willingness to buy and pay more for organic labeled products and other branded products based on demographics and other relevant survey questions [15, 16]. However, few studies have examined the purchasing preferences of consumers to various product labels (e.g., *Raised Without Antibiotics*, *No Medically Important Antibiotics, No Growth Promoting Hormones*, *Cage Free, USDA Organic, Locally Raised, Generic Brand,* and *Major Brand*), as related to food-relevant habits and foodborne disease perceptions of the consumers. Therefore, it is critical that consumers' preferences to labeled products are assessed so that consumer-oriented strategies for mitigating antibiotic resistance can be developed.

The successful launch and progress of the Human Microbiome Project and the Earth Microbiome Project have sparked researchers' enthusiasm to understanding microbial ecology in humans, environment, animals, and plants [17, 18]. Along with the progress in understanding microbiomes, next-generation sequencing technologies have advanced tremendously with increases in speed, read length, efficiency, and a rapid decrease in per-base cost [19]. As a result, the sizes and dimensions of sequencing raw data output have increased exponentially. Data analyses need to be highly efficient and reproducible to meet cutting-edge analytical techniques. The work in this dissertation used high throughput qPCR and 16S rRNA amplicon sequencing in addition to liquid chromatograph mass spectrometry in tandem (LC-MS/MS) and culture-dependent isolation, in combination with computational tools and methods (i.e., R, Python, and machine learning).

Objectives

This dissertation has four main objectives:

1. Characterize bacterial communities and ARGs in a soil-lettuce system upon exposure to antibiotics via overhead and soil-surface irrigations.
2. Investigate the uptake and accumulation of antibiotics in lettuce through the continuum of bulk soil, rhizosphere soil, roots and shoots.
3. Assess the changes in bacterial communities and ARGs profiles in distinct niches of soil-plant systems including bulk soil, rhizosphere soil, roots, and shoots.
4. Determine the consumers' purchasing preferences for various labeled food products as influenced by demographics, food-relevant habits, and foodborne disease perceptions.

The following chapters address the four objectives of my research. Objective 1 is addressed in Chapter II, Objective 2 in Chapter III, Objective 3 in Chapter IV, and Objective 4 in Chapter V. This dissertation ends with Chapter VI that summarizes the findings and identifies future research directions.