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Prevalence And Antimicrobial Resistance Of E. Coli And Enterococcus Species In Detroit Urban Agriculture

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**PREVALENCE AND ANTIMICROBIAL RESISTANCE OF *E. COLI* AND
ENTEROCOCCUS SPECIES IN DETROIT URBAN AGRICULTURE**

by

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THESIS

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Of Wayne State University,

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Approved By:

Advisor

Date

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DEDICATION

To those who perceive the truth beyond the enchanted world

ACKNOWLEDGMENTS

When I walk down my memory lane towards the destination of my thesis referred to as” *Prevalence and antimicrobial resistance of E. coli and Enterococcus species in Detroit Urban Agriculture*” I always stop here and wish to express my deepest sense of gratitude to my advisor, Dr. Yifan Zhang, for her invaluable guidance and priceless advice given to me to make this research a success. Without Dr. Zhang’s assistance and contributions, I may not have been able to complete my graduate studies at Wayne State University. All her contributions will be treasured in my mind forever.

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CHAPTER 1

INTRODUCTION

1.1 Urban Agriculture

Urban agriculture is defined as the cultivating crops and livestock farming within and the periphery of the cities and towns for food production and other uses [1, 2]. It includes individual gardens, community gardens shared by several gardeners, non-profit gardens that provide community services and large scale profit farms [3, 4]. Urban gardens generates economic, social and environmental benefits to the cities by creating new jobs, generating revenue from locally grown food, ensuring food security and improving wellness of residents and assembling green environment by assuring the ecological balance [3, 5].

It is estimated that urban agriculture is practiced by more than 0.2 billion people worldwide ensuring the food security for nearly 0.8 billion urban population [6]. United States Department of Agriculture (USDA) claims that there is an increasing demand for locally harvested food among the consumers specially in local cafeterias and farmers markets [3]. In 1991, 33% of the United Sates farms were located in the metropolitan areas and they have been attributed more than 30% of the crops and farm animal production in the country. According to the estimates the average annual food production of the United States urban farms are more than \$35 million [7].

Detroit in the state of Michigan has a remarkable history for urban farming. According to the history the city itself has started as a farmland. In the early twentieth century, backyard

gardening was popular in Detroit because of the migration of African-American sharecroppers [4]. The abandoned land areas due to the recent decline of the automobile industry has been re-creating a perfect opportunity in Detroit for urban farming. There are around 30,000 acers of vacant lands available in Detroit and approximately 4800 acers are owned by the government which affords easy access to urban farming [3, 8].

1.2 Fresh Produce Consumption

Fresh fruits and vegetables play a vital role in a balanced and healthy diet. They are considered as important sources of essential nutrients, phytochemicals and dietary fibers [9]. When it comes to the long-term health benefits, the consumption of fresh fruits and vegetables are encouraged in many countries to combat against some chronic health conditions such as cancer and cardiovascular diseases [10]. The consumption of fresh fruits and vegetables more than 400g per day is recommended to improve the health of a person [11].

In the United States, from 1994 to 2004 the import cost of fresh produce has doubled to \$12.7 billion [12]. Therefore, it is evident that the fresh produce consumption has increased in the past decade. Since these fruits and vegetables are usually consumed in raw or minimally processed way, they can be considered as vehicles for transmitting human pathogens [10]. From 1990 to 2005 there were around 713 foodborne illness outbreaks that were linked with fresh produce [11]. Lettuce, sprouts, berries and melon are the most frequently reported food items that accompanied with the foodborne illness outbreaks. Out of these outbreaks, more than 50% were associated with bacteria pathogens [13].

Active and passive surveillance data indicate that in the United States alone, more than 30 major foodborne pathogens are identified per year. These pathogens cause 9.4 million cases of foodborne illnesses that lead to 55,961 medications and 1,351 deaths [14]. The average cost per each case lies in-between \$ 1068-1626 resulting accumulated annual cost of illness \$ 51.0 - 77.7 billion [15].

1.3 Antimicrobial Resistance in Agriculture

Antimicrobial agents are the substances produced from one microorganism that can kill or inhibit the growth of other microorganism [16]. In USA, approximately 40 different antimicrobial agents belonging to at least 14 different classes are being used in agriculture as growth promoters and to control and treat the animal and plant diseases [16, 17]. Out of them more than 80% of these antimicrobials are primarily used as growth promotive and prophylactic agents [18]. Streptomycin, oxytetracycline, gentamicin and oxolinic acid are the most frequently applying antimicrobials in agriculture where streptomycin and oxytetracycline are the only two antimicrobials registered by the United States Environment Protection Agency (USEPA) for plant base agriculture as remedy to diseases [16, 19]. According to the regulations twelve different types of fruits, ornamental fruit crops and vegetables including apple, pear, bean celery, tomato, potato, palm, rose and etc. are allowed to use streptomycin as disease controlling agent and four fruit crops including apple, nectarine, peach and pear are register under oxytetracycline [19].

The emergence of antimicrobial-resistant bacteria in agricultural systems is one of the major concerns today [20]. Excessive use of antimicrobials in agriculture, use of animal

manure as a fertilizer and soil conditioner and irrigation with treated waste water act as major contributors in agricultural soil for the emergence of these new antimicrobial resistance phenotypes [18, 21-23]. Studies have shown that use of animal manure and treated waste water introduce large number of antimicrobial-resistant bacteria, antimicrobial residue and antimicrobial resistance genes into the agricultural soil [22-24]. A study conducted in USA by collecting 43 soil samples from two farms has shown that the use of pig manure was result in 0.7 $\mu\text{g}/\text{Kg}$ of sulfamethazine in farm soil [23]. In addition, several tetracycline and sulfonamide resistance genes were detected in agricultural soil treated with pig manure in Germany [24].

There are two major mechanisms that allow bacteria to acquire antimicrobial resistance from the environment. The vertical spreading of antimicrobial resistance which is known as clonal dissemination and horizontal gene transfer between different bacterial isolates, species, genera as well as different kingdoms which is known as the trans-kingdom transfer from plant to bacteria are the first and second mechanisms respectively [21, 25].

The key concern is that fresh fruits and vegetables can serve as vehicles for transmitting antimicrobial-resistant bacteria from agricultural environment to human. Moreover the diseases caused by these bacteria in human host is also resilient to the conventional antimicrobials [18]. Therefore, the emergence of new antimicrobial-resistant bacteria can be identified as imminent threat to the world health and has become one of the key challenges faced by the agricultural industry today. The world health organization has also recognized the emergence of new antimicrobial-resistant bacteria as a critical challenge for the human health. According to their reports, in the United States there are around two

million people infected by antimicrobial-resistant pathogens in every year and more than 10,000 people die from the infection [26].

1.4 *E. coli* and *Enterococcus* Species

Escherichia coli is a Gram-negative, rod shaped, facultatively anaerobic bacteria commonly found in the intestine of the warm-blooded animals. The majority of *E. coli* are harmless and they play an important role as gut microflora in humans. But pathogenic *E. coli* can cause common gastrointestinal problems including diarrhea vomiting upon the ingestion of contaminated food or water.

Escherichia coli O157:H7 is considered as one of the major foodborne outbreak causing pathogens in the United States. This bacterium is capable of producing Shiga toxins and categorized under Enterohemorrhagic *E. coli* (EHEC). It causes non-bloody diarrhea, hemorrhagic colitis and hemolytic uremic syndrome in infected patients [27, 28]. However, most of the *E. coli* O157:H7 outbreaks are combined with animal and dairy products, while the outbreaks caused by consumption of contaminated fresh produce is also being reported. From 1990 to 2004 pathogenic *E. coli* has caused 27 foodborne outbreaks linked with seed sprouts, fresh fruits and leafy vegetables [29]. On September 2006, *E. coli* O157:H7 outbreak has been reported in 26 states including 205 confirmed cases due to consumption of contaminated pre-packed spinach [28].

Antimicrobial-resistant *E. coli* are prevalent in the environment that can acquire antimicrobial resistance via different mechanisms. A study conducted by collecting samples from animal and plant origin shows that pathogenic *E. coli* including *E. coli*,

O157:H7 were resistant to ampicillin, tetracycline, sulfamethoxazole, streptomycin, kanamycin, chloramphenicol and cephalothin. Further, the study identified that these bacteria exhibit integron-mediated resistance gene transfer [30]. Irrigation water may be a good source of spreading the antimicrobial resistance. A study conducted by collecting irrigation water from Rio Grande river shows *E. coli* were resistant to cephalothin (18%), ampicillin (11%), tetracycline (9%), streptomycin (4%), kanamycin (2%) and gentamicin (0.3%) and several *E. coli* isolates contained class 1 and 2 integron-specific sequences [31].

Along with *E. coli*, the Gram positive, facultative anaerobic non-spore-forming bacteria, *Enterococcus* also has a significant impact on human health [32]. Enterococci are one of the leading bacteria genus that cause nosocomial infections. The most common infections are urinary tract infections, bacteremia, and endocarditis [33, 34]. Enterococci are ubiquitous in the environment and they are mostly found in soil, water, food and the gastrointestinal tract of animals and human. This genus includes more than 40 *Enterococcus* species and among them *E. faecalis* and *E. faecium* are the most common in clinical samples [32, 35]. Presence of these two bacteria along with *E. durans* and *E. hirae* is an indication of sewage contamination [32]. Beside these species *E. casseliflavus*, *E. mundtii* and *E. gallinarum* are also prevalent in soil, water and vegetables [34].

Enterococcus species are intrinsically resistant to lots of antimicrobials and these bacteria can acquire antimicrobial resistance from the environment. Many studies have shown that *E. faecium* and *E. faecalis* isolated from non-clinical origins are resistant to different antimicrobials including ampicillin, penicillin, tetracycline, erythromycin,

chloramphenicol, gentamicin, rifampicin, lincomycin, vancomycin and fusidic acid [17, 21, 34, 36]. *Enterococcus* species acquire resistance to aminopenicillins, fluoroquinolones and rifampin via mutation in the chromosomal gene. Moreover, they can exchange resistance gene through conjugation via conjugative transposons or conjugative plasmids with broad host range. Examples for conjugational antimicrobial resistance are resistance to tetracyclines, chloramphenicol, macrolides and glycopeptide [33].

Objective:

Detroit is famous for urban farming from the 19th century and currently it has more than 200 urban farms [8]. Due to the decline of the automobile industry the abandoned land areas are now being converted in to urban farms. Therefore, the safety and sustainability of this agricultural system must be ensured to produce quality foods. Nevertheless, still there are limited data available on the soil quality and the biological contaminants such as foodborne bacterial pathogens and their resistance to antimicrobials in these urban farms. The present study was aimed to investigate the prevalence and antimicrobial resistance of *E. coli* and *Enterococcus* species in soil and vegetables in urban gardens located around the metro Detroit area.

CHAPTER 2

MATERIALS AND METHODS

2.1 Soil and Vegetable Sample Collection

During the summer 2015, a total of 19 soil samples and 48 vegetable samples including 21 leafy greens and 27 root vegetables which are ready to be harvested were collected from three urban gardens (E, O and G) in Detroit, MI. Leek, kale, chard, collard greens, mustard greens, scallion, arugula, potato, squash, carrot and turnip were present among the collected vegetable samples. At each sampling point a sample with an approximate mass of 300-400 g was collected into sterile zip-lock bags and stored into a cooler filled with ice.

2.2 Isolation and Purification of Bacterial Strains

E. coli and *Enterococcus* species were isolated and purified from soil and vegetable samples as shown in Figure 1.

2.3 DNA Extraction

E. coli and *Enterococcus* DNA was extracted using a boiling method. Depending on the colony size, 1-3 colonies for *E. coli* and 3-5 colonies for *Enterococcus* were mixed with 200 μ L of sterilized distilled water and homogenized by overtaxing. The DNA was denatured in the water bath at 95 °C for of 15 minutes. Thereafter, the sample was centrifuged at 14,000 rpm (Thermo Electron LED GmpH, Osterode, Germany) for 5 minutes. The supernatant was transferred into new tubes and stored at -20 °C for subsequent use.

2.4 PCR Identification of Bacteria Strains

PCR was performed to identify the isolates of *E. coli* and *Enterococcus* species. Five microliters of genomic DNA were added to the reaction mixture as template. In PCR, Eco1 (5' GACCTCGGTTTAGTTCACAGA 3') and Eco2 (5' CACACGTGACGCTGACCA 3') primers were used to identify *E. coli* targeting the *malb* promoter gene with 518bp product size [37]. *Enterococcus* species were identified by targeting the *tuf* gene. The gene was amplified using Ent1 (5' TACTGACAAACCATTTCATGATG 3') and Ent2 (5' AACTTTCGTCACCAACGCGAAC 3') primers with 112pb product size [38]. *E. coli* ATCC 25922 and *E. faecalis* JH2-2 were used as the positive control for *E. coli* and *Enterococcus* respectively. After the PCR reaction, the product was electrophoresed on a 1.5% agarose gel and visualized under UV light using the transilluminator (Bio-Rad Laboratories, Hercules, CA).

2.5 Disc Diffusion Test

Thirty-four isolates of *E. coli* and 55 isolates of *Enterococcus* from unique samples were selected for the disc diffusion test. This assay was performed to analyze the antimicrobial susceptibility profile of *E. coli* and *Enterococcus* as described by the Clinical and Laboratory Standards Institute (CLSI) [39]. Briefly, 0.5 McFarland solution was prepared using overnight bacterial cultures on the tryptic soy agar plates (Becton, Dickinson and company, Sparks, MD). Using a cotton swab the bacteria suspension in 0.5 McFarland solution was spread on the entire Mueller-Hinton agar plates (Oxoid Ltd., Basingstoke, Hampshire, England). Antimicrobial discs were applied on to the Mueller-Hinton agar

plates using a dispenser. All bacteria were examined for their susceptibility to ampicillin, chloramphenicol, ciprofloxacin, streptomycin, and tetracycline (Becton, Dickinson and company, Sparks, MD). In addition, ceftriaxone and trimethoprim/sulfamethoxazole were tested for *E. coli* and erythromycin and vancomycin were tested for *Enterococcus* species. The diameter of the zone of inhibition was measured and interpreted according to the CLSI guidelines after 16-18 hours of incubation at 37 °C. *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as the standard strains for quality control.

2.6 Molecular Identification of Bacteria Species

2.6.1 PCR Based Amplification and 16S rDNA Sequencing

All 55 *Enterococcus* isolates were screened for *E. faecalis* using FL1 (5' ACTTATGTGACTAACTTAACC 3') and FL2 (5' TAATGGTGAATCTTGGTTTGG 3') primers with 360bp product size targeting the *sodA* gene [32, 40]. In the PCR assay, *E. faecalis* JH2-2 was used as the positive control. PCR negative samples for *E. faecalis* were subjected to the 16S rDNA sequencing by amplifying the 16S rRNA gene. For 16S sequencing *Enterococcus* DNA was extracted using QIAamp® DNA mini kit (Qiagen, Hilden, Germany). The primers used for the 16S rRNA gene amplification was 27F (5' AGAGTTTGATCCTGGCTCAG 3') and 1492R (5' TACCTTGTTACGACTT 3') with product size of 1164bp [41, 42]. The DNA sequencing was carried out in Eton Bioscience Laboratories, NJ and the resulted DNA sequences were analyzed using website for rDNA sequence data (<https://rdp.cme.msu.edu/>).

2.6.2 Pulsed-field Gel Electrophoresis (PFGE) on *E. coli*

PFGE was performed following the standardized PulseNet protocol for *E. coli* O157:H7 with few modifications [43]. Briefly, *E. coli* was grown on tryptic soy agar plates at 37 °C for 18 hours and the cell suspension was prepared in TE buffer (10 mM Tris-HCl, and 1 mM EDTA, pH 8.0) by adjusting the cell density to 0.50-0.54 using Dade Microscan Turbidity Meter (Siemens Healthcare Diagnostic Inc, CA). Genomic DNA was prepared by mixing 200 µL of standardized cell suspension, 10µL of 20 mg/mL proteinase K stock solution (Sigma aldrich, St. Louis, MO) and 200 µL of 1% melted Megabase agarose (Bio-Red, Hercules, CA).

Plugs were then incubated in cell lysis buffer (50mM Tris HCl: 50mM EDTA, pH 8.0 and 1% Sarcosyl) supplemented with proteinase K stock at final concentration of 0.1mg/ml in a shaker (180 rpm) at 54 °C for 3 hours. After the incubation plugs were washed with distilled water and TE buffer and digested with 20U of *XbaI* (New England Biolabs, Ipswich, MA) at 37 °C overnight. Plugs were loaded on 1% Megabase agarose in 0.5x Tris-Borate EDTA buffer and electrophoresis was performed on a CHEF-DR III apparatus (Bio-Rad Laboratories). The following parameters were used, initial switch time, 2.2s; final switch time, 54.2s; run time, 20 hours; angle, 120°; gradient, 6 V/cm; temperature, 14 °C; ramping factor: linear. The resulting PFGE patterns were analyzed using the BioNumerics software program (ver. 6.5; Applied Maths, Austin, TX, USA) and *Salmonella* serotype *Braenderup* H9812 was used to normalize the gel image. The Dice similarity coefficient and the unweighed pair group method with arithmetic means (UPGMA), with 1.5% of position tolerance and 1% optimization were used for the clustering analysis.

CHAPTER 3

RESULTS

3.1 Prevalence of E. coli and Enterococcus Species in Soil and Vegetables

A total of 45 *E. coli* and 83 *Enterococcus* were isolated from soil samples and 100 *E. coli* and 186 *Enterococcus* were isolated from vegetable samples (Table 1). In soil samples, *E. coli* prevalence was higher in garden G (80%) and E (77.8%), but none of the soil samples collected from garden O were contaminated with *E. coli*. All the soil samples collected from garden G and O were contaminated with *Enterococcus* species and seven out of nine samples collected from garden E carried *Enterococcus* (Figure 2 and Table 1). Vegetable samples collected from garden E were highly contaminated with *E. coli* (77.2%) in comparison to garden G and O. However, all three gardens showed high contamination of *Enterococcus* species in vegetable samples (Figure 1).

3.2 Antimicrobial Resistance of E. coli and Enterococcus Species in Soil and Vegetables

A total of 18 *E. coli* (52.9%) isolates and 44 *Enterococcus* (80%) isolates were resistant to at least one antimicrobial agent. Among the seven antimicrobials that were tested, *E. coli* showed resistance to ampicillin only in both soil and vegetables, with prevalence rate of 23.5% and 36.8% respectively. *E. coli* isolated from garden E showed ampicillin resistance in both soil and vegetable samples, but garden G exhibited ampicillin resistance in vegetable only (Table 2). Streptomycin resistance was common in 64.7% of *Enterococcus* isolated from soil, followed by 11.8% to erythromycin, 5.9% to ciprofloxacin and 5.9% to tetracycline. Streptomycin resistance was also the most prominent in *Enterococcus* in

vegetables with prevalence of 78.9% among the three gardens. Further, resistance to ampicillin (31.6%), ciprofloxacin (7.9%), erythromycin (7.9%) and tetracycline (7.9%) was also observed in *Enterococcus* of vegetable origin (Table 2).

3.3 *Enterococcus* Species Distribution and Antimicrobial Resistance

PCR and 16S rDNA sequencing on unique *Enterococcus* isolates revealed that *E. mundtii* (29.4%) was the most abundant in soil, followed by *E. casseliflavus* (11.8%), *E. durans* (11.8%), *E. faecalis* (11.8%) and *E. faecium* (5.9%) as shown in Figure 3. Five out of 6 isolates of *E. mundtii* showed resistance to streptomycin and both *E. casseliflavus* were found to be resistant to streptomycin (100%) and one isolate was resistant to ciprofloxacin. *E. faecalis* isolated from garden E exhibited multidrug resistance being resistant to erythromycin, streptomycin and tetracycline (Figure 4).

In contrast, the vast majority of *Enterococcus* from vegetables was identified as *E. faecalis* (81.6%). Further, *E. faecium* (7.9%) and *E. mundtii* (2.6%) were also present in the vegetable samples (Figure 3). *E. faecalis* showed higher resistant to streptomycin (80.1%) and ampicillin (38.7%) compared to erythromycin (6.5%) and tetracycline (3.2%). All three *E. faecium* isolates were resistant to ciprofloxacin and streptomycin and two isolates (66.7%) were resistant to tetracycline. Two *E. faecium* strains isolated from vegetable samples collected from garden G showed multi-drug resistance being resistant to ciprofloxacin, streptomycin and tetracycline. Moreover, one of these multi-drug resistant *E. faecium* also showed resistant to erythromycin (Table 3).

Garden variation was observed in the distribution of *Enterococcus* species in soil and vegetables. *E. mundtii* (28.6%) and *E. faecalis* (28.6%) were highly abundant in soil samples compared to other *Enterococcus* species in garden E. However, all the vegetable samples were 100% dominated by *E. faecalis*. *E. mundtii* (60%) and *E. casseliflavus* (40%) were the only two species dominated in the soil sample collected from garden G. However, the vegetable samples collected from garden G were positive for *E. faecium* (50%). In garden O, *E. durans* (40%) was prevalent in soil samples and 14 out of 17 (82.3%) vegetable samples were contaminated with *E. faecalis* (Table 4).

3.4 Clonal Relatedness among *E. coli*

PFGE technique was used to identify the similarity among the *E. coli* isolates. According to the dendrogram twenty-five unique patterns were identified among 30 typeable isolates of *E. coli* from three urban gardens. *XbaI* restriction enzyme digestion produced 15 – 22 fragments and the overall similarity among the isolates was 53.4%. Two soil samples from garden G, G_S_61C and G_S_65D were identical clones. Both isolates were intermediately resistant to ampicillin and 65D showed intermediate resistance to streptomycin. In vegetable samples, two sets of identical clones were found among the leek samples collected from garden E. E_V_12C, 13B and 18C isolates were identical to each other and E_V_15E, 16A and 17B isolates were also identical clones. E_V_12C, 13B and 18C showed same antimicrobial susceptibility profiles being resistance to ampicillin. Moreover, E_V_15E and 16A were resistant to ampicillin and 17B showed intermediate resistance. Identical PFGE patterns were identified either in soil or vegetable within the same garden,

but no common clones were shared by isolates from soil and vegetable samples or from different gardens (Figure 5).

CHAPTER 4

DISCUSSION

The study on *E. coli* and *Enterococcus* species has been mainly concentrated to the research work based on clinical samples, animal originated foods and environmental samples such as water, soil and plants [34, 36, 44]. Although, there have been a few studies related to isolating these bacteria from vegetables, most of them were associated with outbreak investigations [17, 28, 45]. Therefore, the current investigation was conducted as a preliminary study to acquire basic understanding of microbiological quality and antimicrobial resistance of *E. coli* and *Enterococcus* present in soil and vegetables collected from Detroit urban gardens.

The current study was able to isolate *E. coli* and *Enterococcus* species from soil and vegetable samples collected from urban gardens. The abundance of these bacteria in soil and vegetables may depend on the history of the particular land, past and present exposure to manure, airborne transmission of pathogens, etc. [29, 46, 47]. Garden O is an organic farm, it uses a special organic fertilizer known as alfalfa meal as a nitrogen source [48, 49]. This fertilizer acts as a rapid decomposer and it enhances the prevalence of nonpathogenic bacteria in soil. Therefore, the pathogenic and natural soil bacteria would engage in a competition for growth. The addition of alfalfa meal could be a reason for the less prevalence of pathogenic *E. coli* and *Enterococcus* species in the soil samples collected from garden O.

Compared to *E. coli*, *Enterococcus* are widespread in the environment. Present study results also demonstrate that *Enterococcus* are prominent in the soil and vegetable samples. The soil samples were dominated by *E. mundtii*, *E. casseliflavus*, *E. durans*, *E. faecalis* and *E. faecium*. However, vegetable sample were highly contaminated with *E. faecalis* and *E. faecium*. Since these two species are considered as major fecal enterococci, their occurrence in vegetables may serve as an indication of sewage contamination in the agricultural practice [32]. In addition, *Enterococcus* species abundance in vegetable was considerably higher in comparison to soil and then would create food safety issues in urban agriculture. The vegetables might serve as carriers for transmitting foodborne pathogens and cause foodborne illnesses. Further, if the initial bacteria count is high, these two species can survive cooking and food processing which enhance the food safety related problems [36].

The present study provided the evidences for the presence of antimicrobial-resistant *E. coli* and *Enterococcus* species in soil and vegetables. *E. coli* that were isolated from soil and vegetables exhibited resistance to ampicillin only. Resistance to ciprofloxacin, erythromycin, streptomycin and tetracycline were common in *Enterococcus* isolated from soil and vegetable samples. In addition to that ampicillin resistance was found in vegetable samples. The present study has detected relatively high streptomycin resistance in *Enterococcus* species isolated from soil and vegetables compared to other antimicrobials. Although streptomycin has been approved by USEPA for agricultural use, none of the three gardens studied has used streptomycin as antimicrobial agent to treat or control plant diseases. Therefore, chances are low that bacteria would develop resistance to streptomycin

due to antimicrobial selection. However, presence of antimicrobial residue and antimicrobial resistance genes in urban garden soil may be possible reasons for the higher prevalence of streptomycin-resistant bacteria in soil and vegetables compared to other antimicrobial agents. Other than that, there is a possibility of having heavy metals and pesticide residue in the garden soil which creates a selective pressure on bacteria to acquire antimicrobial resistance.

There are plenty of studies showing that *E. coli* and *Enterococcus* species are resistant to various antimicrobials in environmental samples including fresh vegetables [30, 34, 35]. Many studies claim that the substantial diversity of bacteria in nutrient rich soil and mobile elements might have increased the prevalence of antimicrobial-resistant bacteria in soil via resistance gene transfer. According to the current knowledge, bacterial gene transfer occurs through conjugation, transformation and transduction in the agricultural settings. Most studies have shown that antimicrobial-resistant bacteria actively secrete antimicrobials to the nutrient rich soil which eventually become antimicrobial residue, tend to enhance the resistance genes transfer from resistant bacteria to non-resistant bacteria [25, 50].

Furthermore, many studies have shown that agricultural practices such as the use of animal manure, pesticide and presence of heavy metals in soil that create a selective pressure on bacteria might have a significant impact on increasing the abundance of antimicrobial-resistant bacteria in soil [25, 46, 51, 52]. Recent publications show that integrons, a cluster of multiple resistance genes, present in the bacteria inhabited in contaminated soils contain heavy metal and disinfectant resistance genes which also can disseminated in the environment upgrading the antimicrobial resistance. Studies shows that specially *E. coli*

could acquire antimicrobial resistance through integron-mediated resistance gene transfer [25, 31]. Moreover, bacteria could acquire antimicrobial resistance from plant through a mechanism known as trans-kingdom transfer [25].

Since urban agriculture is a complex and multivariate environment with lots of confounding variables, one or more above mentioned process or combinations of several mechanisms might play an important role in the dissemination of resistance genes. The present study indicates that bacteria isolated from vegetable samples exhibit resistance to diverse types of antimicrobials compared to bacteria isolated from soil and two *E. faecium* strains isolated from vegetable samples also showed multi-drug resistance. Therefore, it is evident that fresh vegetables grown in urban agriculture are an important reservoir of antimicrobial resistance.

PFGE results demonstrated a diverse genetic background of *E. coli* suggesting a widespread distribution of bacteria in urban agriculture. Nevertheless, common PFGE patterns identified in *E. coli* with similar antimicrobial resistance profiles indicates clonal distribution of the bacteria in the urban agriculture. Therefore, it is important to evaluate the potential factors that contribute to the resistance gene dissemination in these agricultural environments.

Further studies are recommended to conform the results of this preliminary study and more gardens should be included to get a general overview on microbiological contamination and antimicrobial resistance in Detroit urban agriculture. In addition, the relationship between bacteria and their antimicrobial resistance with physical, chemical and biological

contaminants such as heavy metals, pesticides, antimicrobial residues and non-pathogenic soil bacteria present in the urban agricultural environment should be taken into consideration in future studies.

CHAPTER 5

CONCLUSION

Urban agriculture is a good source for providing healthy and inexpensive food. The preset study was designed to evaluate the food safety issue associated with urban agriculture. The results suggest that vegetables are contaminated with common foodborne bacteria such as *E. coli* and *Enterococcus* species. Further, these bacteria showed resistance to common antimicrobials such as ampicillin, ciprofloxacin, erythromycin streptomycin, and tetracycline. The data suggest that urban agricultural soil and vegetables can serve as a reservoir of foodborne bacteria and antimicrobial resistance which is a potential public health concern to food consumers and urban gardeners.

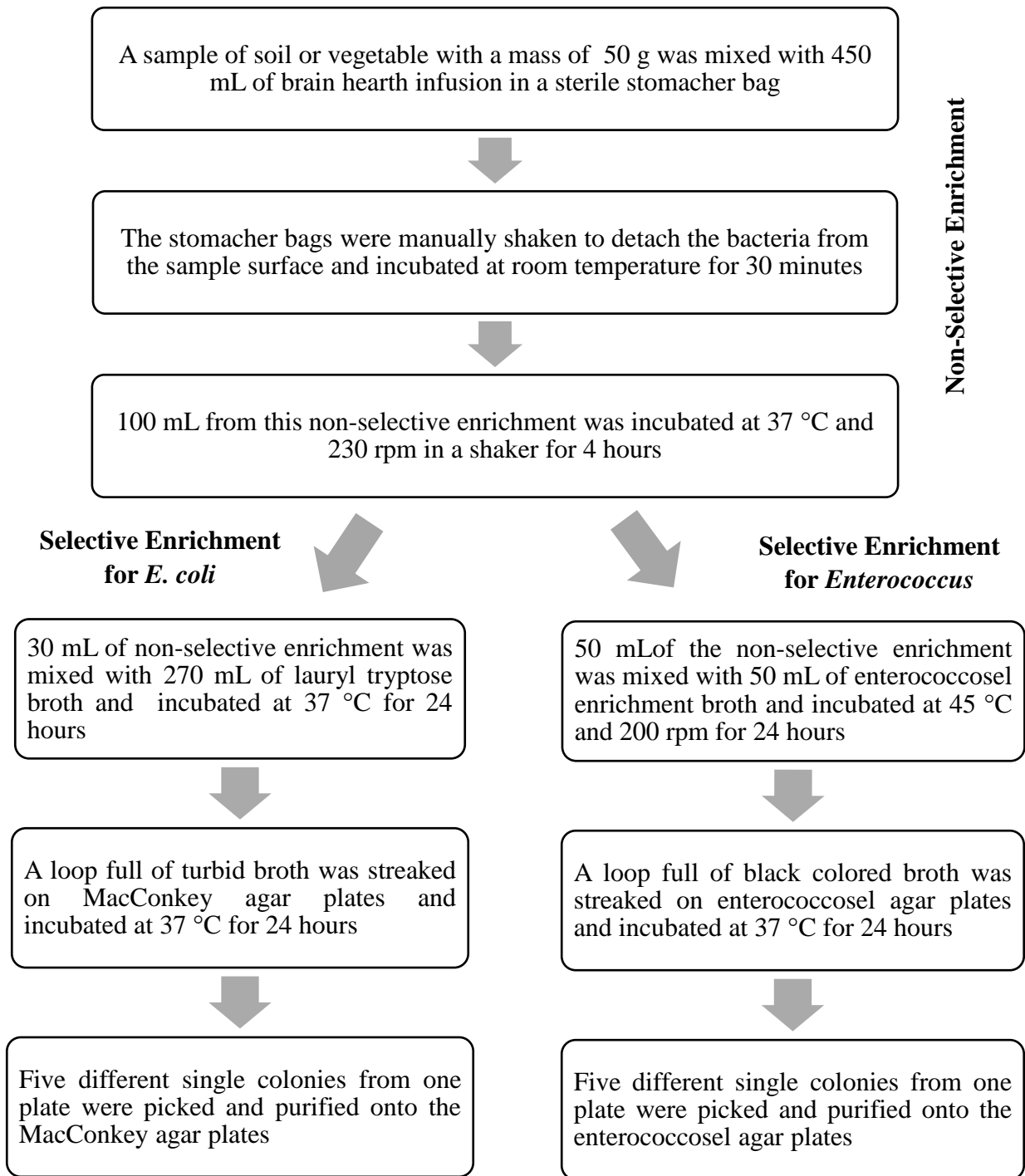


Figure 1: Isolation and purification of bacterial strains from soil and vegetable samples

Table 1: Prevalence of *E. coli* and *Enterococcus* species in soil and vegetables

Garden	Soil				Vegetables			
	<i>E. coli</i>		<i>Enterococcus</i>		<i>E. coli</i>		<i>Enterococcus</i>	
	No. of + Samples /Total	No. of Isolates	No. of + Samples /Total	No. of Isolates	No. of + Samples /Total	No. of Isolates	No. of + Samples /Total	No. of Isolates
E	7/9	32	7/9	35	17/22	73	17/22	85
G	4/5	13	5/5	23	1/5	5	4/5	20
O	0/5	0	5/5	25	5/21	22	17/21	81
Total	11/19	45	17/19	83	23/48	100	38/48	186

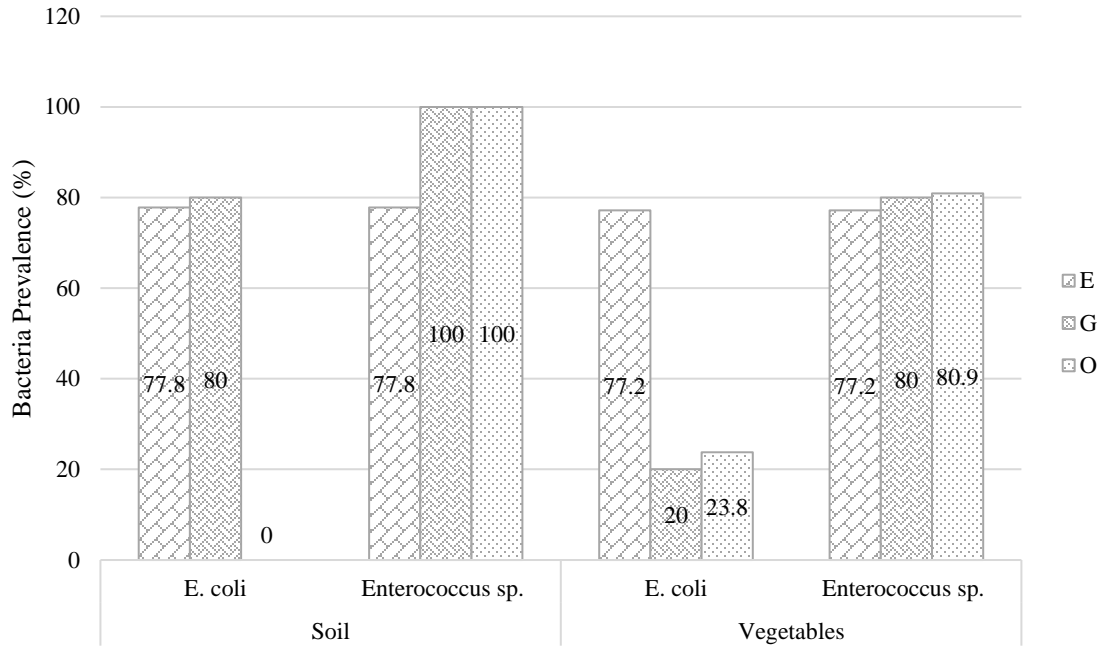


Figure 2: Prevalence of *E. coli* and *Enterococcus* species in soil and vegetables among the three gardens. In garden E, n=9 (Soil), n=22 (Vegetables); G, n=5 (Soil), n=5 (Vegetables); O, n=5 (Soil), n=21 (Vegetables)

Table 2: Antimicrobial resistance of *E. coli* and *Enterococcus* species in soil and vegetables

Bacteria	Source	Antimicrobial Resistance (%)	Antimicrobial Resistance Phenotypes		
			E	G	O
<i>E. coli</i>	Soil	4/17 (23.5)	Ampicillin ^r	-	-
	Vegetables	14/38 (36.8)	Ampicillin ^r	Ampicillin ^r	-
<i>Enterococcus</i>	Soil	11/17 (64.7)	Streptomycin ^r	Streptomycin ^r	Streptomycin ^r
		2/17 (11.8)	Erythromycin ^r	-	-
		1/17 (5.9)	-	Ciprofloxacin ^r	-
		1/17 (5.9)	Tetracycline ^r	-	-
	Vegetables	30/38 (78.9)	Streptomycin ^r	Streptomycin ^r	Streptomycin ^r
		12/38 (31.6)	Ampicillin ^r	-	-
		3/38 (7.9)	-	Ciprofloxacin ^r	Ciprofloxacin ^r
		3/38 (7.9)	Erythromycin ^r	Erythromycin ^r	Erythromycin ^r
		3/38 (7.9)	Tetracycline ^r	Tetracycline ^r	-

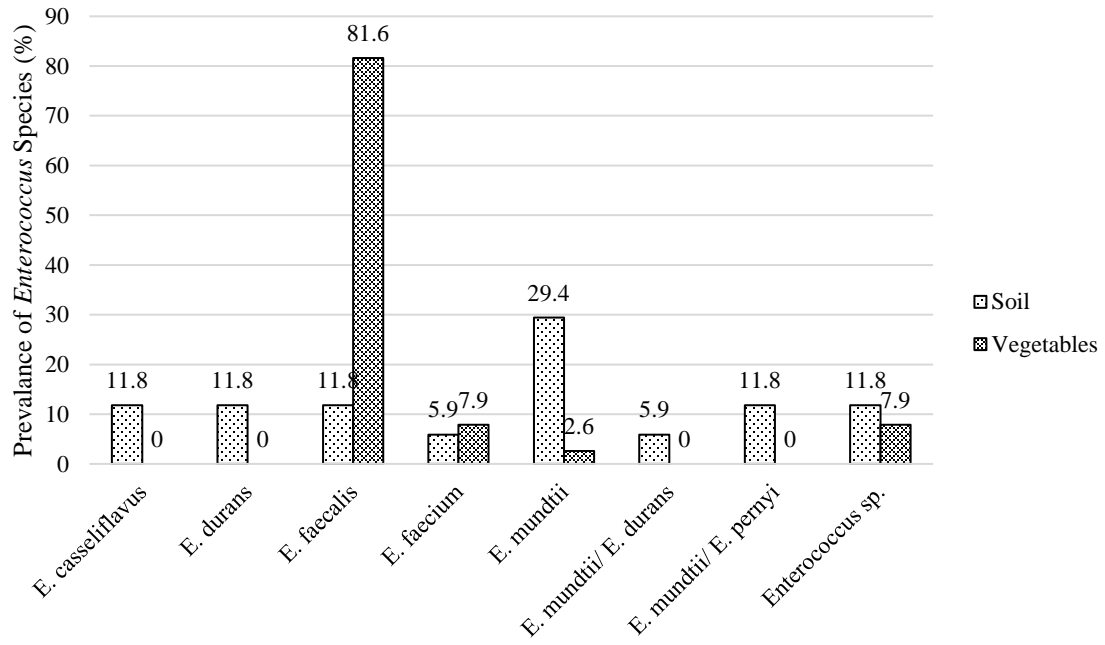


Figure 3: Prevalence of *Enterococcus* species in soil and vegetables. n= 55

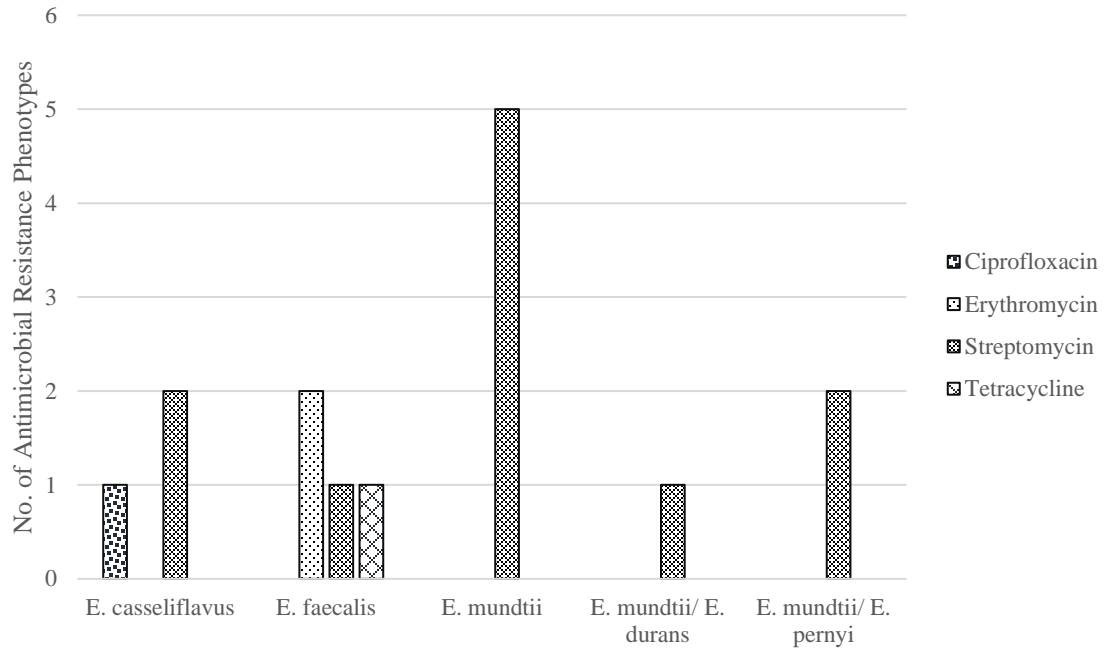


Figure 4: Antimicrobial resistance of *Enterococcus* species isolated from soil. n=17

Table 3: Antimicrobial resistance of *Enterococcus* species isolated from vegetables

Identity of the Bacteria	Number of Isolates	Antimicrobial Resistance (%)						
		Ampicillin	Chloramphenicol	Ciprofloxacin	Erythromycin	Streptomycin	Tetracycline	Vancomycin
<i>E. faecalis</i>	31	12/31 (38.7)	0	0	2/31 (6.5)	25/31 (80.1)	1/31 (3.2)	0
<i>E. faecium</i>	3	0	0	3/3 (100)	1/3 (33.3)	3/3 (100)	2/3 (66.7)	0
<i>E. mundtii</i>	1	0	0	0	0	1/1 (100)	0	0
<i>Enterococcus</i> sp.	3	0	0	0	0	1/3 (33.3)	0	0
Total	38	12/38 (32.4)	0	3/38 (7.9)	3/38 (7.9)	30/38 (78.9)	3/38 (7.9)	0

Table 4: Distribution of *Enterococcus* species in the three gardens

Garden	Sample Type	Number of Isolates	<i>Enterococcus</i> Species Abundance (%)							<i>Enterococcus</i> sp.
			<i>E. casseliflavus</i>	<i>E. durans</i>	<i>E. faecalis</i>	<i>E. faecium</i>	<i>E. mundtii</i>	<i>E. mundtii/E. durans</i>	<i>E. mundtii/E. pernyi</i>	
E	Soil	7	0	0	2/7 (28.6)	1/7 (14.3)	2/7 (28.6)	1/7 (14.3)	1/7 (14.3)	0
	Vegetables	17	0	0	17/17 (100)	0	0	0	0	0
G	Soil	5	2/5 (40)	0	0	0	3/5 (60)	0	0	0
	Vegetables	4	0	0	0	2/4 (50)	0	0	0	2/4 (50)
O	Soil	5	0	2/5 (40)	0	0	1/5 (20)	0	1/5 (20)	1/5 (20)
	Vegetables	17	0	0	14/17 (82.3)	1/17 (5.9)	1/17 (5.9)	0	0	1/17 (5.9)
Total		55	2/55 (3.6)	2/55 (3.6)	33/55 (60)	4/55 (7.3)	7/55 (12.7)	1/55 (1.8)	2/55 (3.6)	4/55 (7.3)

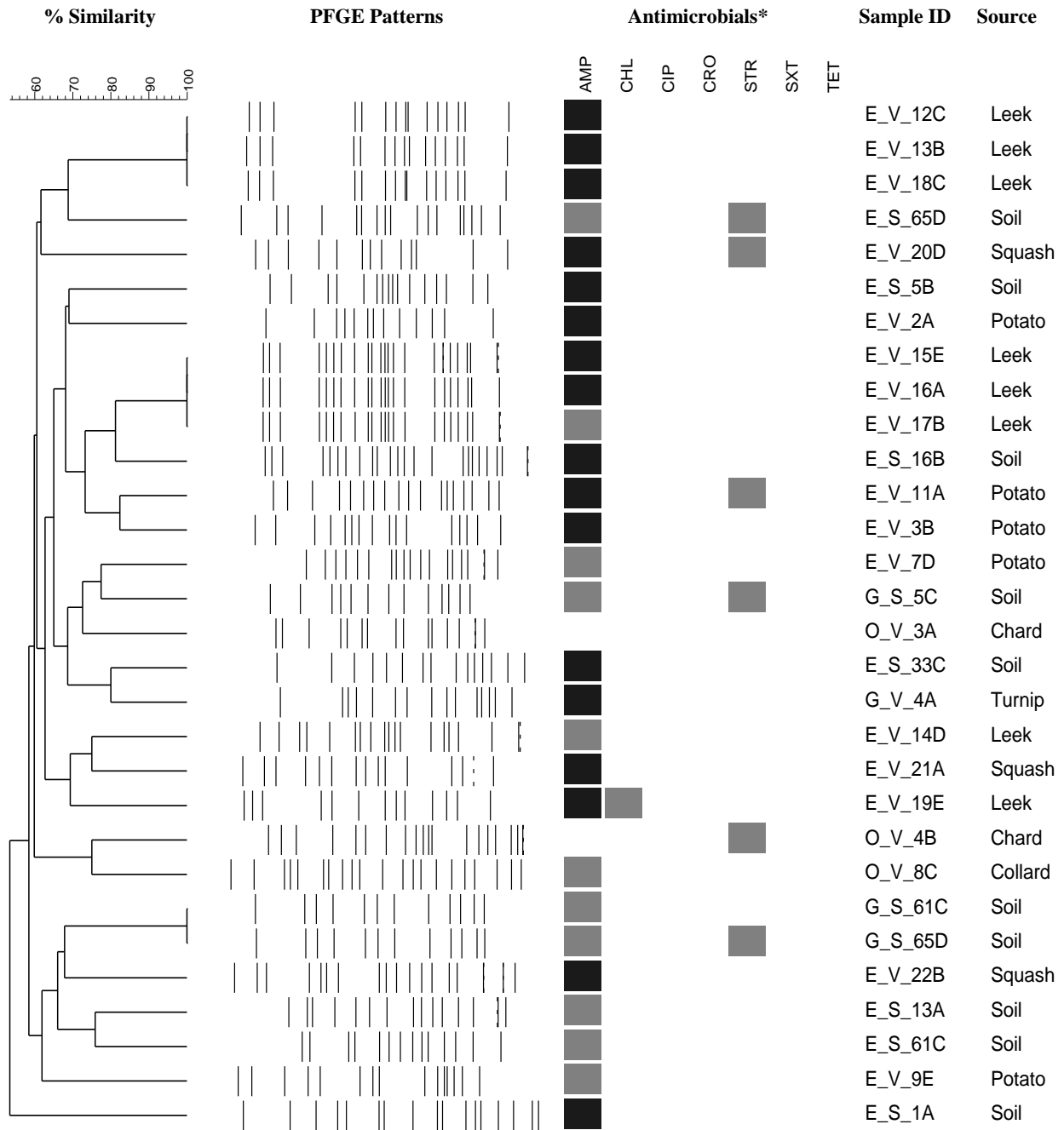


Figure 5: PFGE dendrogram representing the genetic similarity of 30 typeable isolates of *E. coli* based on the *Xba*I digestion and their antimicrobial susceptibility profiles. * Black, resistant; grey, intermediate; white, susceptible. AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; CRO, ceftriaxone; STR, streptomycin; SXT, sulfamethoxazole-trimethoprim; TET, tetracycline.

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ABSTRACT**PREVALENCE AND ANTIMICROBIAL RESISTANCE OF *E. COLI* AND
ENTEROCOCCUS SPECIES IN DETROIT URBAN AGRICULTURE**

By

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May, 2017

Advisor: Dr. Yifan Zhang**Major:** Nutrition and Food Science**Degree:** Master of Science

Urban farming is gaining popularity around the world as a sustainable agricultural system for providing healthy and inexpensive food. However, there are limited data available on the microbial safety related to this sector of agriculture. The objective of the present study was to examine the prevalence and antimicrobial susceptibilities of *E. coli* and *Enterococcus* in soil and vegetables associated with urban agriculture.

A total of 19 soil samples and 48 vegetable samples, including 21 leafy greens and 27 root vegetables, were collected from three urban gardens in Detroit. *E. coli* and *Enterococcus* were isolated and identified by PCR. *Enterococcus* species were determined on unique isolates by PCR and 16S rDNA sequencing. The disc diffusion test was used to examine the antimicrobial susceptibility profile of all unique isolates of *E. coli* and *Enterococcus*. Pulsed-field gel electrophoresis (PFGE) was performed to distinguish the bacteria at the molecular level.

Out of 19 soil samples, 11 (57.9%) carried *E. coli* and 17 (89.5%) contained *Enterococcus*. Of 48 vegetable samples, 23 (48%) were positive for *E. coli* and 38 (79%) for *Enterococcus*. *Enterococcus mundtii* (29.4%) was the most abundant in soil, followed by *Enterococcus casseliflavus* (11.8%), *Enterococcus durans* (11.8%) and *Enterococcus faecalis* (11.8%). In contrast, the vast majority of *Enterococcus* from vegetables was identified as *E. faecalis* (81.6%).

E. coli showed resistance to ampicillin only in both soil and vegetables, with a prevalence rate of 23.5% and 36.8% respectively. Streptomycin resistance was observed in 64.7% of *Enterococcus* isolated from soil followed by 11.8% erythromycin. Antimicrobial-resistant phenotypes in *Enterococcus* in vegetables were more diverse than soil. Streptomycin resistance was the most prominent with a prevalence of 78.9%. Further, resistance to ampicillin (31.6%), ciprofloxacin (7.9%), erythromycin (7.9%) and tetracycline (7.9%) was observed in *Enterococcus* of vegetable origin. Two *E. faecium* strains (3.6%) showed multi-drug resistance and both were isolated from vegetables and *E. faecalis* (1.8%) isolated from soil also showed multidrug-resistant. No statistical difference was observed in antimicrobial resistance prevalence between isolates of soil and vegetable origin.

PFGE results demonstrate a diverse population of *E. coli* and no unique patterns identified in isolates from soil or vegetables. The identification of common PFGE patterns suggests the clonal distribution of *E. coli* in urban gardens.

In conclusion, common foodborne bacteria are prevalent in the urban agricultural system and may serve as a vital source of food contamination and antimicrobial resistance.

Antimicrobial resistance also confers *E. coli* and *Enterococcus* a fitness advantage to persist in the environment.

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- **Perera, L. N.**, Mafiz, A. I and Zhang, Y. Prevalence and Antibiotic Resistance of *E. coli* and *Enterococcus* in Urban Agriculture. International Association for Food Protection Annual Meeting, 2017; 2017 July 9 - 12; Tampa, FL (Accepted for an Oral Presentation)