Assessment of Microbial Risk Factors Associated with Irrigation Water use on Small Alabama Farms

by

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Abstract

Fresh produce is often associated with foodborne outbreaks as these products easily become contaminated with foodborne pathogens from the environment and poor handling practices. Approximately 46% of foodborne illnesses in the US are attributed to produce contamination according to the CDC. At farm level there are several factors or contamination routes for produce, and water can represent a high risk of contamination. To reduce the food safety issues, The Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) set regulations to protect the safety of produce during growing, harvesting, packing, and holding produce for human consumption. Some farms are PSR exempt due to their small size and may be at risk of produce contamination, therefore, monitoring and establishing food safety standards for exempt farms is important to guarantee produce safety. The objectives of this study were 1) evaluate the microbial quality of agricultural water used for irrigation in PSR exempt Alabama farms, 2) identify significant differences between three approved generic *E. coli* enumeration methods and, 3) recognize food safety/handling practices implemented among Alabama produce growers. Agricultural water from 5 locations were evaluated throughout Alabama during 2019-2020 (n = 30). Each location was sampled 3 times per growing season (2 seasons). Generic *E. coli* were enumerated using the methods EPA 1103.1(mTEC), EPA 1604 (MI) and Hach 10029 (mColiBlue24). The highest population of generic *E. coli* was 59 CFU/100 mL and there was no detectable generic *E. coli* in the ground water sources, only in the surface water. No significant difference was found between the three evaluated methods ($P > 0.05$). Next, a survey with 10 yes-no questions was developed and administered both base paper based and electronically using Qualtrics Software. The survey responses indicated that more awareness on produce safety is

needed among small farm growers from Alabama, as well more accessible educational materials and tools.

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List of Abbreviations

AAES	Alabama Agricultural Experimental Stations
AL	Alabama
APC	Aerobic Plate Count
APHIS	Animal and Plant Health Inspection Services
BSAAO	Biological Soil Amendments of animal origin
CDC	Center for Diseases Control and Prevention
CFU	Colony Forming Unit
E. coli	*Escherichia coli*
EPA	U.S Environmental Protection Agency
ERS	Economic Research Service
ERS	Economic Research Service
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FIB	Fecal Indicator Bacteria
FSMA	Food Safety Modernization Act
FY	Fiscal Year
GAP	Good Agricultural Practices
GHP	God Handling Practices
GM	Geometric Mean
HUS	Hemolytic uremic syndrome
LOD	Limit of Detection
MBP	Manure Borne Pathogens
ND	None detected
PBS	Phosphate Buffered Saline
PSR	Produce Safety Rule
RACs	Raw agricultural commodities

RCR	Rarely consumed raw
RTE	Ready to eat
SD	Standard Deviation
STV	Statistical Threshold Value
TC	Total coliforms
US	United States
USDA	U.S Department of Agriculture
UV	Ultraviolet
WHO	World Health Organization

1. Introduction

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) suggest the consumption of at least 400 grams of fruits and vegetables per day as part of a healthy diet, associated with the prevention of chronic diseases such as heart disease, cancer, diabetes, and obesity. Also, incorporating fruits and vegetables can prevent and relieve several micronutrient deficiencies (World Health Organization, 2003). Today, consumers have easy access to nutritional information and make more informed decisions concerning what they eat (MINTEL 2018).This is shown in recent years by an increase in fruit and vegetable consumption by consumer (Hanning et al., 2009; Uyttendaele et al., 2015; Chatziprodromidou et al., 2018).

The food system is very complex, and even with modern technologies and resources, there are still challenges, such as food safety gaps. The CDC estimates that 48 million people are sickened each year in US, resulting in 120,000 hospitalizations and 3,000 deaths from foodborne diseases (CDC 2020). In relation to food categories, it was found that produce, in comparison to others, represented 46% of illnesses and 23% of deaths from 1998 -2008 just in the United States (Painter et al., 2013). Narrowing down the information, it is reported that the most common foodborne pathogens in fresh produce include *Campylobacter* spp., *Staphylococcus aureus, Clostridium* spp., *Listeria monocytogenes, Salmonella* spp., *Escherichia coli* O157: H7, *Shigella* spp., and *Yersinia* spp. (Steele and Odumeru 2004; Yeni et al., 2016; Riggio et al., 2019).

Produce can become contaminated at any point in the food chain. There are several routes of contamination with water a possible source. Therefore, monitoring and establishing microbiological standards for agricultural water is important to guarantee the safety of fruits and

vegetables and to reduce the effects on human health, economics, and social impacts. The United States Environmental Protection Agency (US EPA) is the entity responsible for regulating water standards in the U.S. It has different regulations for water based on its use, including drinking water, ground water, oceans and coastal waters, wastewater, animal feeding operations, mountaintop mining, ocean and coastal waters and others.

In 2015 The Food and Drug Administration (FDA), through the Food Safety Modernization Act (FSMA) established the Produce Safety Rule (PSR). It included standards for growing, harvesting, packaging, and holding produce for human consumption. The PSR provides science- based minimum standards for the safe growing, harvesting, packaging, and holding of fruits and vegetables for human consumption, which until then, there were no regulations.

The objectives for this review are (1) to recognize important topics on foodborne outbreaks due to produce and routes of contamination of foodborne pathogens in fresh produce and their impact in food safety. (2) To get a deeper understanding of different factors that can affect the biological quality of water used for irrigation. (3) To understand three different enumeration methods for generic *E. coli*.

2. Literature Review

2.1. Agriculture in Alabama and irrigation water

According to the last census conducted by the United States Department of Agriculture (USDA) in 2017, Alabama had 40,592 farms all over the state including crops and livestock. From those, about 1,490 farms were classified as vegetables harvested for sale (excluding potatoes, sweet potatoes, and ginseng) and 1,684 as land in orchards (USDA 2017). In 2020, only 283 produce farms in Alabama were certified with the USDA GAP (Good Agricultural Practices) and GHP (Good Handling Practices) (USDA 2021). This can represent challenges for produce safety due to gaps between the farmers and food safety practices.

Fresh produce can be contaminated at any point in the food chain. Irrigation water can be one source of contamination. Waterborne diseases cause about 2.2 million deaths per year worldwide with common pathogens including Hepatitis viruses, Norovirus, *Crystosporidium, Giargia, Salmonella* typhymurium, *Vibrio cholerae*, *Escherichia coli* O157:H7 and *Campylobacter jejuni* (Ramirez- Castillo et al., 2015).

According to the CDC in 2017, a total of 6,930 deaths were attributed to pathogens transmitted by water, with 91% associated with *Legionella, Pseudomonas,* and nontuberculous mycobacterial infection. The remaining were associated with *Campylobacter, Crystosporidium, E. coli, Giardia,* Hepatitis A, *Salmonella* non typhoidal and *Shigella* . These pathogens not only can be transmitted through water but across different routes including contaminated foods.

The Food and Drug Administration defines agricultural water as “water that is intended to, or likely to, contact the harvestable portion of covered produce or food-contact surfaces” (FDA, 2018). Irrigation water is considered the water used for an irrigation system to sustain plant growth in

agricultural and horticultural practices. Water in agriculture represents about 80% of use from the hydric resources worldwide. In developed countries, water for irrigation represent 60% while in developing countries can represent the 90% (Velasco- Muñoz et al., 2018).

Water for irrigation represents 80% of the total water consumption in the United States (USDA ERS, 2019). In 2015, the use of water in Alabama was 9,250 million gallons of fresh water per day with 223 million gallons used for irrigation. Irrigation water can come from different sources, with the USDA classifying water into surface water and ground water. In 2015, 124 million gallons of surface water and 98.9 million gallons per day for irrigation in Alabama (Dieter 2018).

Monitoring foodborne pathogens or indicator microorganisms in water for irrigation and establishing microbiological standards are important to guarantee produce safety. This can be challenging because there are many variables that need to be considered, such as the method of irrigation, method of sampling, crop type, temperature of the environment, temperature of the water, timing of application, type of water (ground, surface, or municipal water) and others. The Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) provides guidelines for agricultural water and establishes science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption.

2.2. Foodborne outbreaks due to produce

Produce can become contaminated with chemical, biological, or physical agents at any point in the food chain. Fresh produce and other types of food that do not receive any antimicrobial treatment before eating are more likely to be a source of pathogens. In fact, the number of outbreaks associated with fresh produce have risen in the last few years, with an estimated increase from 14.8% (1998) to 22.8% (2007) just in the United States (Wadamori et al., 2017). In the

European Union in 2010, 10% of verified foodborne outbreaks were related to the consumption of fruits, vegetables, berries, and juices (Van et al., 2013). More recent literature reports that from 2010 to 2017, 1797 outbreaks occurred in the United States, 85 of which were multistate outbreaks and of those 228 (12.7%) were associated with fresh produce (Carstens et al., 2019).

The most recent surveillance for foodborne disease outbreaks reported from the CDC 2020 show that of 841 foodborne disease outbreaks, 521 illnesses implicated fruits. In addition, the most common etiological agents were bacteria, causing 198 of these outbreaks. Norovirus was the most common cause of confirmed single-etiology outbreak representing 35% of the outbreaks. *Salmonella* was the most common etiologic agent in fruits and vegetables, and the most common serotypes were Newport and Braenderup, recovered from products including papaya, melon, leafy greens, mango, romaine lettuce and watermelon (CDC 2020). It is reported that from 2010 to 2017 about 85 outbreaks were associated with fresh produce and the most common pathogens reported were Hepatitis A, *Cyclospora cayetanensis*, *Salmonella enterica*, *E. coli* and *Listeria monocytogenes* (Carstens et al., 2019).

Consumer demand, globalization, and the international trade have led to a more complex food system. Globalization of food supplies contribute to the availability of fresh produce but can represent challenges for regulating food safety practices. Each country has its own practices and regulations contributing to more diverse foodborne pathogen populations (Zhang et al., 2018). The risks increase when produce comes from countries with lower safety standards, especially developing countries which often find it difficult to meet food safety requirements from developed countries (Callejon et al., 2015; Carstens et al.,2019; Riggio et al., 2019).

Consumers are potentially exposed to a variety of foodborne pathogens, increasing the probability that outbreaks will be widespread or multistate and chances that an outbreak will be detected

(Gradl 2019). Foodborne outbreaks cause significant impacts in the economy. It is reported that in the United States in 2013, food safety incidents represented an impact of around $7 billion (Hussain and Dawson, 2013).

According to the CDC, it is estimated that each year in the US, approximately 48 million people contract a foodborne illness. The Economic Research Service (ERS) estimates that 15 pathogens (*Campylobacter* spp., *Clostridium perfringens, Cryptosporidium* spp., *Cyclospora cayetanensis*, *Listeria monocytogenes*, Norovirus, *Salmonella* non-typhoidal species, *Shigella* spp., STEC O157, STEC non-O157, *Toxoplasma gondii*, *Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio* other non- cholera species and *Yersinia enterocolitica)* are responsible for 95% of foodborne illness representing $15.5 billion in economic burden annually. Of this economic burden pathogens: *Salmonella* (non- typhoidal species), *Toxoplasma gondii*, *Listeria monocytogenes*, Norovirus and *Campylobacter*, account for 90% of the amount (Hoffman et al., 2015).

There is significant evidence implicating water irrigation in spreading of foodborne diseases. However, confirming the "cause-effect" relationship is challenging because the same pathogenic strain must be isolated from the patient, produce and irrigation sources (Pachepsky et al., 2011). If there is not enough evidence, then researchers make hypothesis and inferences as described in Figure 2.2.1.

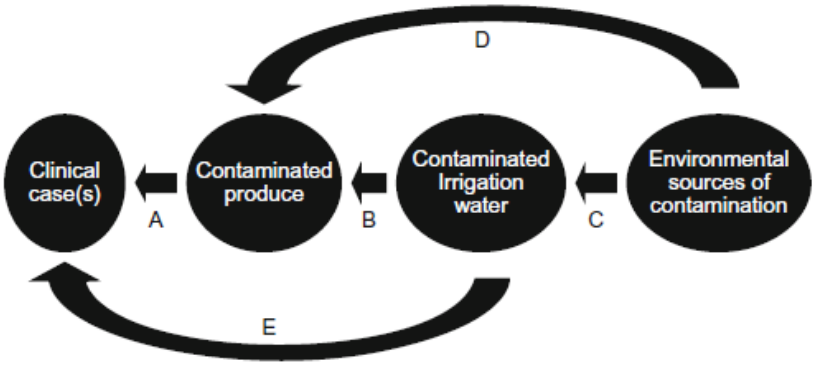

Figure 2.2.1 Irrigation water as a source of pathogenic contamination, inference diagram

Source: Pachepsky et al., 2011.

2.3. Relevant outbreaks associated to fresh produce in the United States

Over the past years, many outbreaks linked to produce have occurred in the United States leading deaths and economic losses. The CDC reports from 1998 to 2013: 972 raw produce outbreaks resulting in 34,674 outbreaks- associated illnesses, 2315 hospitalizations and 72 deaths. For this review, some relevant outbreaks are listed.

In 2006 there was an outbreak associated with spinach where a strain of *E. coli* O157:H7 was responsible for infecting 199 people leading to three deaths. This outbreak was multistate, involving over 26 states (CDC, 2006). Another outbreak related to *E. coli* was reported by the CDC in 2010 where shredded romaine lettuce was a source of contamination from *E. coli* O145 causing 31 illnesses with 5 states involved (CDC, 2010). Cantaloupe is a fruit that has been associated with outbreaks and in 2011 cantaloupes caused 147 listeriosis cases leading 33 deaths. The strain involved was *Listeria monocytogenes* (CDC, 2011).

There have been recent outbreaks associated with produce as well. In 2019 a total of 167 people from 27 states became sick by eating romaine lettuce contaminated with *E. coli* O157:H7 (CDC, 2020). *E. coli* O157:H7 was involved in other outbreak in 2020, 40 people became sick by eating leafy greens, 4 of them developed hemolytic uremic syndrome (HUS) (CDC, 2020).

2.4. Routes of produce contamination

The path from farm to fork for produce travels through different conditions and environments. To reduce illnesses and foodborne outbreaks, it is important to understand the potential routes of produce contamination, produce safety challenges, ways to reduce the incidence of pathogens and their survival and growth on produce and approaches to eliminate or reduce the level of contaminants. Throughout the food supply chain produce can become contaminated by different hazards usually identified as chemical, physical, and biological. Hazards like pesticides and mycotoxins are categorized as chemical. Physical hazards include sand, metal pieces and dust. Biological hazards include foodborne pathogens like bacteria, viruses, or parasites (Hussain and Gooneratne, 2017).

The type of produce can determine potential risks of contaminations. For example, wax covers, and low pH can resist microbial contamination. Produce with high moisture and nutrient content or natural openings can be an easy target for microbial pathogens (Yeni et al., 2016). Fresh produce is usually consumed raw with no treatment for the elimination of pathogens before consumption, which increases the potential risk of contamination for consumers (Yeni et al., 2016; Gradl 2019).

Produce contamination involves many variables, differences in the type of produce and individual farm. Agricultural water and soils are common variables found to be reservoirs and transmission routes of pathogens during the preharvest stage due to the survival of pathogens (Iwu and Okoh,

2019). Other factors that need to be considered include topography, land- use, climate, atmospheric disposition, irrigation water, manure compost, fecal contamination by animals, employees, and others. Researchers Maffei et al., (2016) had identified some of the sources and routes for produce contamination during preharvest and postharvest which are summarized in figure 2.4.1

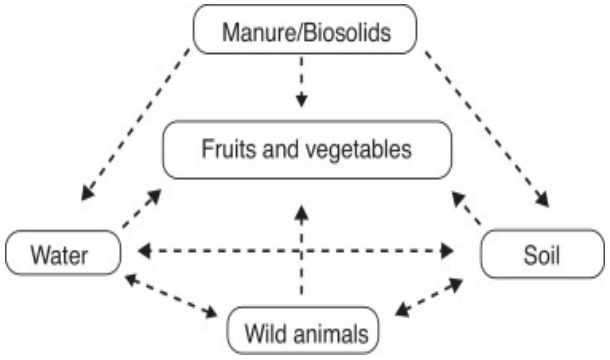

Figure 2.4.1 Sources and routes for produce contamination during preharvest and postharvest.

Source: Maffei et al., 2016

Beuchat and Ryu (1997) suggested that fresh fruits and vegetables can become contaminated during preharvest and postharvest through various sources of contamination (Table 2.4.1). The FSMA Produce Safety Rules identified 6 key parameters as the main sources of contamination for fresh produce: Agricultural water, Biological Soil Amendments, Sprouts, Domesticated and Wild Animals, Worker Training and Health and Hygiene, and Equipment, Tools and Buildings. Those parameters will be discussed in the next pages.

Table 2.4.1 Causes of microbial contamination.

Stage	Sources of pathogenic microorganism on fresh fruits and vegetables
Preharvest	Feces
	Soil
	Irrigation Water
	Water used to apply fungicides and insecticides
	Green or inadequately composted manure
	Air (dust)
	Wild and domestic animals
	Insects
	Human Handling
Postharvest	Feces
	Human handling (workers and consumers)
	Harvesting equipment
	Transport containers
	Wild and domestic animals
	Insects
	Air
	Wash and rinse water
	Sorting packaging, cutting and further processing equipment
	Ice
	Transport vehicles
	Improper storage (temperature, physical, environment
	Improper packaging
	Cross- contamination
	Improper display temperature
	Improper handling after wholesale or retail purchase

Source: Beuchat and Ryu, 1997

2.5. Food Safety Modernization Act

The Food Safety Modernization Act from the Food and Drug Administration was signed into law in January 2011 intending to allow the FDA to protect public health by helping to ensure the safety and security of the food supply. FSMA shifted the focus from responding to foodborne outbreaks to a prevention model. Provisions allow the enforcement of regulations to achieve higher rates of compliance using risk- based safety standards and provide improved responses to contain problems when they occur. The FDA has approved seven major rules regarding the

implementation of FSMA and two that are proposed. These rules intend to increase the safety among different points in the supply chain for animal and human food.

1. FSMA Final Rule on Accredited Third- Party Certification
2. FSMA Final Rule for Preventive Controls for Human Food
3. FSMA Final Rule for Preventive Controls for Animal Food
4. FSMA Final Rule on Foreign Supplier Verification Programs (FSVP) for Importers of Food for Humans and Animals
5. FSMA Final Rule for Mitigation Strategies to Protect Food Against Intentional Adulteration
6. FSMA Final Rule on Sanitary Transportation of Human and Animal Food
7. FSMA Final Rule on Produce Safety
8. FSMA Proposed Rule on Laboratory Accreditation
9. FSMA Proposed Rule for Food Traceability

For this research I am focusing on the FSMA Final Rule on Produce Safety: Standards for the growing, harvesting, packing, and holding of produce for human consumption.

2.6. Final Rule on Produce Safety

The Produce Safety Rule is the first regulation for agricultural water in the US. The rule is part of the agency's ongoing efforts to implement the FDA Food Safety Modernization Act, with the final rule effective January 2016. The main objective of this regulation is to minimize the risk of serious adverse health consequences or death from consumption of contaminated produce. It is important to mention that the standards outlined in the PSR do not apply to produce that are rarely consumed raw (RCR), personal on farm consumption or produce that is not considered as an agricultural commodity. Produce receiving any treatment or processing that reduces the presence

of microorganisms can be qualified for an exemption from the rule. This, rule does apply to both domestic and imported produce.

The PSR does not apply to all farms. Farms with an average annual value of produce sold during the previous 3-year period of $25,000 or less, are exempt from the proposed rules. Farms that meet the following requirements can also qualify for exemption and modified requirements; 1) less than $500,000 in food sales per year during the 3 previous years 2) farm's sales to be qualified end-users (consumer of the food, restaurant or retail food establishment in the same State or same Indian reservation) must exceed sales to others . However, those farms are required to include their name and complete business address on the label or display the same information at the point of purchase.

The PSR considers five major routes of contamination in the following areas: worker training and health hygiene, agricultural water, biological soil amendments, domesticated and wild animals, equipment, tools, and buildings and sprouts. Those key factors are discussed in the following pages. All these requirements are effective after the publication of the regulation with different compliance dates based on farm size. Consult the PSR for more details.

2.6.1. Biological soil amendments

One common source of pathogens is from manure or compost application, especially raw manure, or improperly treated compost. Organic amendments are solid or liquid, include compost, animal manures, slurries, crop residues, digestates from the anaerobic treatment of waste, biosolids and others. These organic amendments are typically used in agricultural soil as fertilizers but are not necessarily composted or anaerobically digested (Urra et al., 2019).

The use of organic amendments can be beneficial to soil health and increase crop productivity, but also can represent a risk for environmental and human health. Organic amendments can be a source of harmful contaminants for humans such as heavy metals, organic pollutants, and emerging contaminants. Amendments derived from raw, unstable animal products or biosolids can contain bacteria (including antibiotic resistant bacteria and antibiotic resistant genes), parasites, viruses, and fungi (Urra et al., 2019). In the United States, 90% of poultry litter is applied to agricultural lands as Biological Soil Amendments of Animal Origin (BSAAO). A study by Sharma and collaborators indicates that there is a higher extended survival of *E. coli* in poultry litter in comparison to other animals' manure (Sharma et al., 2019). Consumer driven food trends are becoming more popular, and the production and consumption of organic products is one of them. Organic agriculture is based on ecological principles, and biological soil amendments of animal origin (BSAAOs) are used to improve soil fertility. BSAAOs can be a source of pathogens like *E. coli,* O157:H7, *Salmonella* spp*, Listeria* spp*, Yersinia enterocolitica,* and *Campylobacter* (Ramos et al., 2019; Sharma et al., 2019; Alegbeleye and Sant'Ana, 2020). In fact, many studies have compared the microbiological quality of vegetables from organic and traditional produce and most of them indicate that there is a higher incidence of *E. coli* in organic products (Maffei et al., 2016). A study in Brazil comparing iceberg lettuce from organic, hydroponic, and conventional systems reported that there was a higher population of *E. coli* in the organic lettuce (Maffei et al., 2016).

Pathogens from animal waste or manure applications are referred to as manure borne pathogens (MBP). Pathogens can contaminate produce by being released from the waste and then transported to water sources through soils. The rate and efficiency of transportation depends on the number of pathogens shed, release rate, flow conditions, precipitation, and proximity to water source (Alegbeleye and Sant'Ana, 2020). MBP contaminate watersources through different phases. It is

estimated that microorganisms can migrate into soils by convection/advection and dispersion. Spreading of the pathogen depends on the soil characteristics, which it has been demonstrated that *Salmonella enterica* can leach through sandy soils (Mantha et al., 2017). The FMSA FDA Produce Safety Rule has guidelines related to biological soil amendments of animal origin waste in the Subpart F, which classifies the biological soil amendments as raw manure, stabilized compost, and biological soil amendments of animal origin. For raw manure there is still ongoing research to determine the number of days needed between applications to minimize the risk of contamination. The FDA suggests following the USDA's National Organic Program standards which call for a 120-day interval between application of the manure for crops in contact with the soil and 90 days for crops without direct contact with the soil. For stabilized compost, there are microbial standards to set limits for the following pathogens *Listeria monocytogenes, Salmonella* spp. fecal coliforms and *E. coli* O157:H7 (FDA, 2016).

2.6.2. Domesticated and wild animals

Animals are considered another vector for produce contamination. Some microorganisms such as *E. coli* are part of the microbiota of animals, but when shed in the environment, can represent a risk of contamination. Insects like flies can be vectors for cross transmission Insects who feed directly from plants can be riskier because they provide direct routes for internalizing pathogens to plants in the field. Damages to plant tissues in plants by insects or other animals allow the ingress to pathogens (Alegbeleye et al., 2018).

Pathogens transmitted from animals are known as zoonotic foodborne pathogens. Some of these pathogens include *E. coli, Salmonella* spp*, Shigella* spp*, Campylobacter* spp and others. It has been found that ants and cockroaches can be a source of human parasites and can transmit foodborne pathogens like *Salmonella* Typhimurium*, Entamoeba histolytica, Klebsiella pneumoniae,*

Mycobacyerium leprae, Shigella and *Staphylococcus aureus* (Sarwar 2015). Some studies have reported cases where zoonotic foodborne pathogens have contaminated produce, including *Campylobacter jejuni* (host: poultry, wild birds, and mammals) related to peas, *E. coli* O157: H7 (host: domestic ruminants, deer, wild avian, flies, bugs) associated with lettuce, spinach, sprouts, apple juice and others. Also, *Listeria monocytogenes* (host: present in cattle) has contaminated produce like cantaloupe, cabbage, and sprouts (Jay-Russell, 2013).

The management of produce safety from wild animals is complex, especially in open crop fields and orchards (Jay-Russell, 2013). A report from Wildlife Services (WL) from 2012 showed that a survey by the National Statistics Service reported wildlife damage in agriculture was about $944 million in 2001. The field crop losses totaled $619 million and losses from vegetables, fruits and nuts represented $146 million (USDA APHIS 2012). In June 2019, the USDA, Animal and Plant Health Inspection Services (APHIS) reported that in the 2018 fiscal year (FY) $80 million was used to help the management from wildlife damage, 49% was used to prevent or reduce wildlife hazards to human health and safety and 25% on protecting agriculture (Gandhi et al., 2019).

The spread of zoonotic foodborne pathogens can be through direct deposition from fecal material or by other agricultural factors like water, worker boots and others. Riskier crops like leafy greens and strawberries because they grew on the ground and are more susceptible to fecal contamination by wild animals and domestic animals. The ability of zoonotic pathogens to survive in the plant environment depends on the plant surface, biofilm as well physical characteristics from the plant that can promote bacterial growth like netted melons which have uneven surfaces. The dose that pathogens for attachment and internalization in plants it is usually low, therefore, it can be challenging to get rid of the microorganism by just washing the produce (Jay- Russell 2013). Some studies have demonstrated that pathogens can penetrate stomata (pores found in epidermis of

leaves), roots, internalized in plant tissues or just reside on leaf surfaces from the plants (Erickson 2012). *Salmonella* and *E. coli* O157: H7 can penetrate Arabidopsis and lettuce plants roots, *Klebsiella pneumoniae* has been detected also in numerous plant roots (Alegbeleye et al., 2018). Other studies reported that bacterial pathogens can be found in plant rhizosphere because there are nutrients available where pathogens can survive (Pachepsky et al., 2011).

The guidelines for domesticated and wild animals in the produce safety rule are in Subpart I. Standards are provided for farms that have grazing animals, working animals and for intrusion by wild animals. If there is significant evidence of potential contamination corrective actions should be taken, such as placing flags outlining the affected area and that farmers take measures to identify and not harvest produce that is likely to be contaminated. It is required to visually examine the growing area of the produce. The FDA does not recommend excluding animals from outdoor growing areas, destroying animal habitat, or clearing borders around growing or drainage areas (FDA 2018)

2.6.3. Worker Training and Health and Hygiene

Employees play an important role in the safety of the produce because they are the ones who are in direct contact with food contact surfaces and produce. Several studies have demonstrated that foodborne illness can be traced back to employees improperly handling food, poor food worker hygiene and/or food preparation practices and ineffective employee training (Egan et al., 2007; Gould et al., 2013). In addition, food handlers can be asymptomatic carriers of foodborne pathogens and transmit those to food (Egan et al., 2007). A report from the FDA in 1999 stated that 93% of outbreaks that were related to food handlers involved sick workers (Simonne et al., 2005). A study evaluated the relationship between indicator organisms on worker hands, soil and water and microbial contamination. The results indicated that transfer from worker

hands was the main contributor of contaminants, with no significant relationship between bacteria or phage from soil or irrigation water (Bartz et al., 2017).

Training food handlers is crucial in the food industry and should involve all stakeholders. It is important that growers get guidance from qualified personnel in food safety areas to provide awareness and knowledge to create a food safety culture among farmers and employees (Julien-Javaux et al., 2019). To be effective, employees must be trained according to their level of education and cultural context (Shinbaum et al., 2016). All the rules under FSMA require that all employees including company employees, or any outside temporary employees, have documented training in food safety. Workers should have access to adequate handwashing stations, toilet facilities in the field, employee accommodations and other alternative hygiene products (Simonne et al., 2010). The guidelines for Health and Hygiene are in Subpart D.

2.6.4. Equipment, Tools and Buildings

Reducing or preventing food safety issues should be an integrated approach and a responsibility from all food handlers in each step of the food chain. Infected manure, water, soil, crop debris and farm workers themselves can be a source of contamination for tools and machinery (Hoagland et al., 2018).

Equipment and tools should be maintained in good condition and technical specifications by manufacturers should be followed for the use and maintenance (Gil et al., 2015). The PSR does not require a farm food safety plan; however, it is highly recommended for farms to establish one that includes cleaning and sanitizing procedures to prevent or reduce the incidence of foodborne pathogens (Gil et al., 2015). Throughout, growing and harvesting, equipment and tools should be properly sanitized (Olaimat and Holley, 2012). During harvesting the equipment can be a source

of microbial contamination that can impact to further processes if there is inappropriate cleaning or sanitizing procedures. The monitoring and management on-farm should focus on harvest equipment as important control points to reduce microbial contamination (Izumi et al., 2008).

The produce safety rule provides guidelines regarding equipment, tools and buildings in Subpart L. Standards related to this Subpart addresses processes that are intended or likely to contact covered produce and those instruments or controls used to measure, regulate, or record conditions to mitigate the growth of microorganisms of public health significance. The requirements related to buildings are referred to as fully or partially enclosed buildings used for covered activities, storage sheds or other structures used to store food (FDA, 2018).

2.6.5. Sprouts

Sprouts are grown in controlled environments avoiding potential field sources of contamination. Environmental parameters required for sprouts production include a warm and moist growing condition and are generally hand harvested (Baker et al., 2019). The sprouting of seeds is critical because it provides favorable conditions for microbial growth (Machado- Moreira et al., 2019). Sprouts are harvested while immature (harvested when the cotyledons are still underdeveloped), which make them more vulnerable to human pathogen colonization and internalization (Turner et al., 2020). Sprouts are mostly consumed raw with minimal heat treatment, hence, considered high-risk foods (Baker et al., 2019) making them susceptible to food-borne pathogens. In fact, sprouts have been involved in several foodborne outbreaks (Baker et al., 2019; Benincasa et al., 2019). It is reported that since 1996, 48 outbreaks have been related to sprouts leading to 2,499 cases, 179 hospitalizations and 3 deaths. The most common sources are alfalfa sprouts, clover, mung bean and sprouted chia powder. *Salmonella* spp., *E. coli* and *Listeria* spp. were the most common pathogens implicated (Gensheimer and Gubernot, 2016). A study

evaluating the prevalence of pathogens in sprouts marketed in the United States detected the presence of non- O157 STEC and *L. monocytogenes* from mung bean, alfalfa, and broccoli sprouts (Zhang et al., 2018). An additional challenge with sprouts safety is the demand for organically grown produce, chemical additives are not permitted, and alternative antimicrobials and methods are currently undergoing research, like photosensitization which can be an effective antimicrobial (Žudytė and Lukšienė, 2019). Most of the outbreaks related to sprouts have been related to contaminated seeds, poor sanitation, and lack of hygienic practices (Baker et al., 2019). The guidelines for growing, harvesting, packaging, and holding of all sprouts can be found in Subpart M in the Produce Safety Rule.

2.6.6. Agricultural Water

Water in agriculture is essential and is used for primary production and for postharvest handling. There are a wide variety of uses including irrigation of crops, hand washing, fertilizer preparation, washing tools and equipment for harvesting and many others, and can be a source of contamination. Water is one of the most common routes of contamination of enteric pathogens in horticultural production with correlations known since the past 70 years. The researchers Norman and Kabler in 1953 reported that poor microbiological quality of water for irrigation was associated with human pathogens in leafy vegetables (Pachepsky et al. 2011).

There are several factors that can be involved in water contamination. One can be meteorological conditions, like heavy rains, which have been identified as a potential source of contamination during primary production (Hoagland et al., 2018). The presence of animals near the water source can represent potential run-off that contaminate the water and then the produce (Gil et al., 2015). Pesticides and fungicides if diluted with contaminated water, can enhance the growth of pathogens

like *Salmonella* spp., providing nutrients for microbial growth (Izumi et al., 2008; Uyttendaele et al., 2015).

The method of application for irrigation water can impact microbial spread. Drip irrigation is less likely to contaminate fresh produce in comparison to overhead spray irrigation (Riggio et al., 2019). Drip or surface irrigation reduces the contact with the edible parts of the plant (Uyttendaele et al., 2015). In addition, there is a higher risk of microbial contamination when there is contact between the water and the harvestable part of the crop (Julien-Javaux et al., 2019). Norovirus is a foodborne pathogen of concern in produce, especially in microgreens and sprouts. This virus can attach to fruits and vegetables when contaminated water is used for irrigation (Riggio et al., 2019). In addition, contaminated water can be transferred from roots to edible parts of the plant when using drip irrigation. When using aerial irrigation systems, these can contaminate leaf surfaces and pathogens are able to attach to scar tissues or wounds (Pachepsky et al., 2011).

Water with high microbial counts can contribute to contamination of fruits and vegetables (Hanning et al., 2009). The source of irrigation water is an important factor to consider. Surface water sources are exposed to environmental contaminations and resulting in a poor microbiological quality. In terms of microbial quality, municipal water is considered the best water followed by ground water, gray water and collected rainwater (Riggio et al., 2019).

The neighboring states of Florida, North Carolina, and Virginia regulate alternative water sources for irrigation like recycled water or reclaimed water that can also be a source of microbial contamination (Rock et al., 2019). Several studies associated to irrigation/ agricultural water have been conducted over the US. Research conducted in reclaimed and return flows used for the irrigation of crops from Arizona indicated that those sources of water can be a source of bacterial contamination. Returned flow includes surface and ground water that is unconsumed from

irrigation applications and reclaimed water consist of wastewater that has been treated and then reused. A study conducted by Jokinen et al., (2019) surveying downstream canals and reservoir outlet in Canada of water, indicated that reservoir outlet as water source had lower population of *E. coli* in comparison to canals.

Post-harvest water can be a source of microbial contamination, especially in facilities where water is recycled. To maintain water quality and reduce the build-up of microorganisms, interventions should be taken, such as using chlorine or other disinfection techniques, like UV-C radiation, ozone, hydrogen, and others (Gil et al., 2015). Most RTE produce receive a washing treatment after cutting. This is a critical step because these products do not undergo any thermal treatment before consumption and therefore the water used must be treated to eliminate any remaining adulterant (Gil et al., 2015).

2.6.7. Agricultural Water Criteria by the Produce Safety Rule

The guidelines for agricultural water are included in the Subpart E in the Produce Safety Rule. The final produce safety rule established two sets of criteria for microbiological water quality, both based on the presence of generic *E. coli* which can be an indicator of fecal contamination. The criteria consist of; 1. No detectable generic *E. coli* is allowed for post-harvest water, for example, water used for washing hands during and after harvest, water used on food contact surfaces, water used to directly contact produce (including ice) during or after harvest and irrigation water for sprouts. The rule establishes that if generic *E. coli* is detected then it is necessary to immediately discontinue the water and corrective actions must be taken. In addition, PSR prohibits the use of untreated surface water for any of purposes mentioned previously.

2. The second criteria is for water that is directly applied to growing produce (other than sprouts). For these criteria there are two important parameters: the geometric mean (GM) and the statistical threshold (STV). The GM of samples is 126 or less colony forming units (CFU) of generic *E. coli* per 100 mL of water and the STV of samples is 410 CFU or less of generic *E. coli* in 100 mL of water. The GM is an average and essentially represents the average of generic *E. coli* in a water source. The STV, represents the amount of variability in the water quality and can be described as the level at which 90% of the samples are below the value.

It is important to mention that if the water does not meet the criteria mentioned then corrective actions are needed no later than the following year. In addition, farmers that do not meet the initial microbial agricultural water criteria have the following options; 1. Allowing time for potentially dangerous microbes to die off by using a set time interval between the last application of irrigation and harvest but no more than four consecutive days; 2. Allowing time for potentially dangerous microbes to die off between harvest and end of storage, or to be removed during commercial activities such as washing within appropriate limits, and 3. Treating the water.

Frequency and type of water

The frequency for testing the water depends on the type of water source (surface or ground water). Surface water is considered the most vulnerable source; therefore, for untreated surface water that is applied to growing produce (other than sprouts) the FDA requires farmers to do an initial survey using a minimum of 20 samples. The water needs to be collected as close as is practicable to harvest over the course of two to four years. The data from those samples is used to calculate the GM and the STV to provide a microbial water quality profile for that source. After that it is necessary to update the GM and STV with at least five samples per year. For recalculating the GM, the previous 15 samples plus the new 5 samples are used as data.

For untreated ground water that is directly applied to growing produce (other than sprouts), the FDA requires farmers to perform an initial survey with a minimum of 4 samples, collected as close as is practicable to harvest, during the growing season or over a period of one year. The initial data is used to calculate the GM and STV and determine if the water meets the requirements for the produce safety rule. Determination of the GM and STV is required through the collection of a minimum of one sample per year. The new sample plus the most recent three samples are used to recalculate the GM and STV.

In addition, for untreated ground water that is used for purposes for which no detectable generic *E. coli* is allowed, the Food and Drug Administration (FDA) requires an initial testing with a minimum of four samples per growing season or over a one-year period. If the initial testing meets the requirements, then a minimum of one sample per year is required for the future water testing.

For agricultural water from public water systems or supplies that meet requirements established in the rule there is no requirement to test the water.

Approved methods

The FDA allows methods that are valid and at least equivalent to methods from U.S Environmental Protection Agency (EPA). The approved methods are the following:

1. Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC) (September 2014). U.S. Environmental Protection Agency. EPA-821-R-14-010.
2. Method 1103.1: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC) (March 2010). U.S. Environmental Protection Agency. EPA-821-R-10-002.

3. Method 1604: Total coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium) (September 2002). U.S. Environmental Protection Agency. EPA-821-R-02-024.

4. 9213 D – Natural Bathing Beaches (2007). In: Standard Methods for the Examination of Water and Wastewater, 22nd Edition (Rice E.W., et al., Ed.), 9-46 – 9-48. Washington, DC: American Public Health Association. (2012).

5. 9222 B – Standard Total Coliform Membrane Filter Procedure (1997), followed by 9222 G – MF Partition Procedures (1997) using NA-MUG media. In: Standard Methods for the Examination of Water and Wastewater, 21st Edition (Eaton A.D., et al., Ed.), 9-60 – 9-65, and 9-70 – 9-71, respectively. Washington, DC: American Public Health Association. (2005).

6. D 5392-93 – Standard Test Method for Isolation and Enumeration of *Escherichia coli* in Water by the Two-Step Membrane Filter Procedure. In: Annual Book of ASTM Standards, Volume 11.02. ASTM International. (1996, 1999, 2000).

7. Hach Method 10029 for Coliforms – Total coliforms and *E. coli*, using m-ColiBlue24 Broth PourRite Ampules.

8. IDEXX Colilert Test Kit, but only if using IDEXX Quanti-Tray/2000 for quantification.

9. IDEXX Colilert-18 Test Kit, but only if using IDEXX Quanti-Tray/2000 for quantification.

In addition to the previous methods, the FDA has determined that the following presence/absence methods are scientifically valid and at least equivalent to the method of analysis in § 112.151(a):

1. TECTA™ EC/TC medium and the TECTA™ Instrument: A Presence/Absence Method for the Simultaneous Detection of Total Coliforms and *Escherichia coli* (*E. coli*) in Drinking Water. (2014).
2. Modified Colitag™ Test Method for the Simultaneous Detection of *E. coli* and other Total Coliforms in Water. ATP D05-0035. (2009).
3. IDEXX Colilert Test Kit
4. IDEXX Colilert-18 Test Kit
5. IDEXX Colisure Test Kit
6. E*Colite Bag or Vial Test for Total Coliforms and *E. coli* in Potable Water. Charm Sciences, Inc.
7. 101298 Readycult Coliforms 100. EMD Millipore (division of Merck KGaA, Darmstadt, Germany).

2.7. Compliance dates

Compliance dates for sampling are determined by the size of the farms. FSMA has categorized farms based on their revenue from produce sales. Very small businesses are the ones that, on a rolling basis, average an annual momentary value of produce sold during the previous 2-year period that is more than $25,000 but not more than $250,000. Small businesses are farms that if, on a rolling basis, the average annual monetary value of produce the farm sold during the 3-year period is more than $250,000, but no more than $500,000.

However, there are several ways the farm or grower can be exempt or excluded from the produce safety rule. Some growers may be excluded depending on commodities and average annual produce sales. In addition, some growers can be exempt based on processing activities or average annual food sales to qualify end users. It is important to mention that farms with an average annual value of produce sold during the previous 3-year period that is less than $25,000 are excluded.

Produce items include fruits and vegetables as well mixes of intact fruits and vegetables. Produce that is rarely consumed raw is not covered in the produce safety rule, for example: asparagus, black beans, great northern beans, kidney beans, lima beans, navy beans, pinto beans, garden beets, cashews, sugar beets, sour cherries, chickpeas, cocoa beans, coffee beans, collards, sweet corn, cranberries, dates, eggplants, figs, ginger, hazelnuts, horseradish, lentils, okra, peanuts, pecans, peppermint, potatoes, pumpkins, winter squash, sweet potatoes, and water chestnuts.

Produce not categorized as Raw Agricultural Commodities (RAC) are not covered by the produce safety rule. RAC include any food in its raw or natural state, including all fruits that are washed, colored, or otherwise treated in their unpeeled natural form prior to marketing.

2.8. Water Quality Indicator Organisms

The detection for foodborne pathogens in the environment, including water, is complex. For that reason, to assess the microbiological quality of environmental and drinking water, fecal indicators organisms are used such as enterococci and generic *E. coli* (Masters et al., 2011). Literature suggests that *E. coli* is the best fecal indicator in comparison to other indicators.

Pathogenic microorganisms are rarely found in surface water and tend to be time-consuming and expensive to enumerate. Generic *E. coli*, total coliforms and *Enterococcus spp*. are considered fecal indicator bacteria (FIB) (Jokinen et al., 2019) and are important indicators for human

pathogens in water bodies (Holman et al., 2014). However, it has been demonstrated that *E. coli* is the best indicator for bacteriological quality of water because of the availability of affordable, fast, sensitive, specific and easy to perform methods (Odonkor and Ampofo, 2013).

Escherichia coli is a Gram- negative, facultative anaerobic, non- spore forming and rod-shape bacterium with an optimum growth temperature at 37 ℃, commonly found in warm blooded animals. In anaerobic conditions it will undergo fermentation producing lactate, succinate, ethanol, acetate, and carbon dioxide. Most of *E. coli* strains do not cause illness, but there are some serotypes that can have a significant impact on human health (Odonkor and Ampofo, 2013).

E. coli is commonly found in the large intestine of warm-blooded animals. Its presence in the environment usually indicates fecal contamination, and in food processing facilities it is an indicator of poor sanitations (Odonkor and Ampofo, 2013).

This bacterium can survive in drinking water for up to 12 weeks depending on environmental conditions (temperature, pH, etc.) (Edberg et al., 2000). The use of *E. coli* as an indicator organism in the industry is widely accepted; however, the presence of this bacterium does not necessarily indicate the presence of pathogens. It does indicate unacceptable levels of fecal contamination which can be associated with an increased risk of other fecal microbes like *Salmonella* spp., and hepatitis A (Odonkor and Ampofo 2013). According to Odonkor and Ampofo (2013), some of the challenges of using *E. coli* as an indicator include i) it is outnumbered by other types of fecal bacteria making it more difficult to find, ii) it does do not survive long time outside of the gut and iii) it can be found in tropical environments. The EPA recommends the use of generic *E. coli* and enterococci as indicators with an acceptable level of *E. coli* as 126 CFU/100 ml..

2.9. Sampling Methodologies

For this review three equivalent methods to EPA Method 1603 are described.

EPA Method 1604 (Oshiro, 2002)

The EPA Method 1604 is used for total coliforms and *E. coli* in water by membrane filtration using MI agar or broth, with a simultaneous detection from both total coliforms (TC) *and E. coli.*

Two enzyme substrates, the fluorogenic 4-Methylumbelliferyl-β-D galactopyranoside (MUGal) and a chromogen Indoxyl- β -D-glucuronide (IBDG), are included in the medium to detect the enzymes β -galactosidase and β -glucuronidase, respectively, produced by TC and *E. coli*, respectively.

The volume of water to use is 100 mL, filtered through a 47- mm, 0.45-µm pore size cellulose ester membrane filter that retains the bacteria present in the sample. The filter is placed on a 5-mL plate of MI agar or on an absorbent pad saturated with 2-3 mL of MI broth, and the plate is incubated at 35 °C for up to 24 hours. The bacterial colonies that grow on the plate are inspected for the presence of blue color from the breakdown of IBDG by the *E. coli* enzyme β -glucuronidase and fluorescence under longwave ultraviolet light (366 nm) from the breakdown of MUGal by the TC enzyme β -galactosidase (Oshiro,2002).

E. coli is represented by blue colonies on each MI plate under normal/ambient light. Positive results that occur in less than 24 hours are valid, but the results cannot be recorded as negative until the 24-hour incubation period is complete. When exposing each MI plate to length wave ultraviolet light (366 nm), fluorescent colonies are counted blue/green, fluorescent *E. coli*, blue/white, fluorescent other than *E. coli*, and blue/green with fluorescent edges. (Oshiro,2002).

Hach Method 10029 (mColiBlue 24)

The mColiBlue24 broth causes oxidation, in coliforms form red colonies and blue colonies represent *E. coli* colonies. This broth is made so that acidification of the medium does not occur. As a result, the method can analyze many colonies at the same time for their oxidation reaction. Colonies that are blue after initial 24 hours of incubation are *E. coli*, and enzymatic indicator in the medium causes non- fecal coliform colonies. The selectivity of the enzymatic indicator eliminates the need for confirmation. The low false positive and the false negative rates allow for the detection of at least 95% of all *E. coli*.

EPA 1103.1 (mTEC)

The EPA Method 1103.1 is used for detection and enumeration of *E. coli* in water by membrane filtration using a differential and selective medium (mTEC). The plates are incubated at 35 ± 0.5 °C for 2 ± 0.5 hours to resuscitate injured or stressed bacteria, and then incubated at 44.5 ± 0.2 °C for 22 ± 2 hours. Following incubation, the filter is transferred to a filter pad saturated with urea substrate. After 15 minutes, yellow, yellow-green, or yellow-brown colonies are counted with the aid of a fluorescent lamp and a magnifying lens.

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3. Assessing Microbial Quality of Agricultural Water Used for Irrigation on Small Alabama Farms Exempt from PSR

3.1. Abstract

Produce can become contaminated with foodborne pathogens from water applied in the field. Approximately 46% of foodborne illnesses and 22% of deaths in the US are attributed to produce contamination according to the CDC. To reduce the food safety issues, The Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) set regulations to protect the safety of produce during growing, harvesting, packing, and holding produce for human consumption. However, not all the farms are expected to meet the PSR and those exempted farms can still get people sick. The purposes of this study were: (1) evaluate the microbial quality of agricultural water used for irrigation in PSR exempt Alabama Farms and identify any significant difference between growing seasons. (2) Identify significant differences between three approved generic *Escherichia. coli* enumeration methods. (3) Recognize potential environmental factors that can contribute to the presence of generic *Escherichia. coli* in water source. For that, water samples (1 L) from 5 locations (A, B, C, D and E) was collected during 2019-2020 (n=30). Each location was sampled 3 times per growing season (2 seasons). Generic *Escherichia coli* were enumerated for each sample. Population of the bacteria were calculated per 100 mL and log transformed and enumerated using EPA 1103.1, EPA 1604 and Hach method 10029. The Wilcoxon test was used to compare medians between growing seasons. A Kruskal Wallis test was performed to determine statistical differences between the enumeration methods. And regression analysis was performed to identify relationships between variables (water pH, environmental temperature, and coliform population) and *E. coli* populations. Overall, the results from this research indicated that there was no statistical difference between the 3 enumeration methods evaluated ($P > 0.05$), with all of them producing equivalent results. There was no statistical difference ($P > 0.05$) between the two

growing seasons evaluated. The variable temperature had the strongest correlation (P = 0.02, r = 0.10) causing an impact in *E. coli* population. The information from this research will represent a better understanding from the risk that PSR exempt farms represent as potential source of produce contamination in the state of Alabama.

3.2. Introduction

The food system is very complex and even with modern technologies and resources, there are still challenges. The CDC estimates that 48 million people are sickened each year from foodborne diseases in the US resulting in 120,000 hospitalizations and 3,000 deaths (CDC, 2020). In relation to food categories, it was found that produce represented 46% of illnesses and 23% of deaths from 1998 - 2008 just in the United States (Painter at al., 2013). Produce can become contaminated through various routes at any point in the food chain, water can be one of them. Pathogenic bacteria such as *Escherichia coli* O157:H7 and *Salmonella* spp. are frequently implicated in foodborne diseases related to fresh vegetables due to unsafe agricultural practices and food safety gaps. Therefore, monitoring and establishing microbiological standards is important to guarantee the safety of fruits and vegetables and to reduce the effects on human health, economics, and social impacts.

The Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) was released in 2016 and provides the science-based minimum standards for the safe growing, harvesting, packaging, and holding of fruits and vegetables for human consumption. One of the standards includes regulations regarding agricultural water. The regulation requires produce farmers to monitor the biological quality of pre- and post-harvest water used on the farms, with two criteria of generic *E. coli*, the Geometric Mean (GM) <126 CFU/100 mL and the Statistical Threshold Value (STV) <410 CFU/100 mL. To determine these the FDA

allows methods that are valid and equivalent to methods from U.S Environmental Protection Agency (EPA) (FDA 2018).

The purpose of PSR is to prevent the introduction of known or foreseeable hazards into produce and to provide a reasonable assurance that the produce is not adulterated with biological hazards. The objectives for this research were 1) to evaluate the microbial quality of agricultural water used for irrigation in PSR exempt Alabama Farms for two growing seasons and identify any significant differences between them and 2) to identify significant differences between thee approved generic *E. coli* enumeration methods. This work aims to identify the microbial quality of water used in produce production and get a better understanding of the risk that PSR exempt farms represent for potential produce contamination in the state of Alabama.

3.3. Materials and Methods

Agricultural water from five different geographical areas in Alabama (Figure 3.6.2) was sampled and enumerated for generic *E. coli* during two growing seasons. The FSMA Produce Safety Rule (Subpart E) requires that produce farmers monitor their agricultural water for generic *E. coli* (FDA, 2016). The EPA has a list of approved alternative methods for testing water that are equivalent to the EPA 1603, the method recommended by the Produce Safety Rule. For this research, the methods used were the following 1) EPA Method 1604 (MI agar), 2) Hach Method 10029 (mColiBlue 24 broth), and 3) EPA 1103.1 (mTec).

3.2.1. Study Design

The experimental design was a repeated measures arrangement conducted over summer 2019 and summer 2020 in triplicate. Water samples from sources used to irrigate produce were collected from five different locations across the state of Alabama. Farms were enrolled based on willingness to participate and agreement that farms would be kept confidential. The five locations (A, B, C, D and E) were chosen for this study and all of them are PSR- exempt since most of the produce grown from these locations is sold directly. The evaluated locations had different types of water: surface (Locations A: spring, B: pond, D: pond and E: lake) and ground water (Locations C: well and D: well). Distance from the laboratory was a factor on farm selecting due to specific times allowed for testing. The produce safety rule mentions that samples need to be analyzed within 6 hours. Locations A and E were approximately 0.5-hour from the lab and locations B, C and D were between 1.5- and 2-hours' driving.

3.2.2. Water sampling

Water from the ponds and spring were sampled with a sterile bottle (NALGENE) near the irrigation water intake source at approximately one-foot depth. All other water samples were collected at the source from a tap. Water samples were collected 3 times at each location over the course of two growing seasons (n=30), from May to August, 2019 - 2020. In each case, a 1 L water sample was collected in triplicate in sterile plastic bottles. Environmental temperature was recorded during each visit and water pH was taken. Samples were transported to the laboratory in a cooler with ice. Microbial analysis (Figure 3.6.2) began less than 3 hours after collection in accordance with the Standard Methods for the Examination of Water and Wastewater (Federation et al., 2005).

3.2.3. *Escherichia coli* Detection

The methods used were EPA 1103.1 (mTEC), EPA 1604(MI), and Hach 10029 (mColiBlue24), all which are approved by the FDA for FSMA PSR water testing. Results from the three replicates were averaged for each enumeration method and sampling time (1 to 6). mTEC and MI agar were used to measure generic *E. coli.* MI agar and mColiBlue24 were able to measure coliforms as well. Media were prepared in advance, along with sterile phosphate- buffered saline solution (PBS) in dilution bottles and 10 mL rinse tubes.

The sample was shaken vigorously, and serial dilutions were performed in phosphate-buffered saline (PBS). A volume of 90 - 100 mL was filtered via the membrane filtration method through a 47 mm diameter, 0.45 µm pore size filter (Pall Laboratory, Port Washington, NY, USA). The apparatus used for the filtration was rinsed twice with 30 mL PBS to ensure the entire sample was filtered. After that, the membrane filter was removed and plated onto three different media types to enumerate generic *E. coli* and coliforms in two of the media types.

For method EPA1604, the filter was placed on MI agar and incubated for 24 ± 2 hours at 35 ℃. Blue colonies were counted as *E. coli.* Fluorescent colonies under a UV lamp were recorded as coliforms. In the Hach method 10029, 3 mL of mColiBlue24 broth was poured into a petri plate containing an absorbent pad and then a filter was placed on the absorbent pad. The plate was incubated for 24 ± 2 hours at 35 ℃. Blue colonies were counted as *E .coli*. Red colonies represented coliform bacteria.

For the method EPA 1103.1 the membrane filter was placed on mTEC Agar and placed for 2 hours at 35 ± 0.5 ℃ in an incubator to resuscitate injured or stressed cells and then incubated in a water bath for 24 ± 2 hours at 42 ℃. After the incubation, the membrane filter was transferred to

a urea substrate- saturated absorbent pad that was incubated for 15 min at room temperature. Yellow, yellow-green- and green colonies were counted as *E. coli*.

Generic *E. coli* and coliforms were enumerated and expressed as CFU/100 mL. The averages of the results are reported in Table 3.6.1. The limit of detection (LOD) was 1 CFU/100 mL, ND (none detected). The positive controls used were pure *E. coli* and for the negative controls and *Klebsiella* and *Pseudomonas aeruginosa* were used on each trial sampling day with all three types of media.

3.2.4. Statistical Analysis

Statistical analysis was only performed for surface water because ground water did not show any presence of generic *E. coli* in any of the growing seasons. All statistical analysis was performed in the program R, Version 4.0.2. The CFU of generic *E. coli* and coliforms were transformed to logarithmic scale and the Shapiro-Wilk test was performed to assess the normal distribution ($P < 0.05$). To assess the enumeration methods, Kruskal Wallis Test was performed to determine significant differences between the medians of each method used ($P < 0.05$). To determine if there was any statistical difference between growing seasons a Wilcoxon test ($P < 0.05$) was performed for log transformed *E. coli* median from each location and growing season. Regression analysis was performed to identify correlations between environmental temperature and generic *E. coli* population. For all measures of association, P values of ≤ 0.05 were considered significant.

3.4. Results and Discussion

The factors pH, temperature and coliform population were analyzed as potential predictors for the presence of generic *E. coli* (Table 3.6.4). The results from the simple linear regression indicated that environmental temperature had a positive linear correlation ($r = 0.10$, $P < 0.05$). However, pH from water and coliform population did not show any significant correlation with *E. coli* population. Results reported at various sources of surface water from San Joaquin Valley, California, differ from our research , where the correlation from *E. coli* and coliforms were explained by an $r = 0.53$ denoting significance of the Pearson's correlation value (Sbodio et., 2013). In addition, Sbodio et al, (2013) reported that for *E. coli* populations there was a significant correlation with water temperature and pH ($P < 0.05$). A higher correlation denotates the presence of generic *E. coli* was affected by that external factor. In our study the Pearson's correlation value was lower in comparison to the study conducted by Sbodio. A study conducted on surface and well water among sixty Iowa produce farms reported no significant correlation ($r = 0.20$, $P > 0.005$) between water temperature and generic *E. coli* populations. The average of temperature from water samples collected in those samples was 18.6 ℃ (Bhullar, 2019). Another study, conducted on surface water from canal waters over 3 regions from the Southwest US reported significant correlation between *E .coli* and water temperature ($r = 0.26$, $P < 0.001$) and pH ($r= 0.15$, $P < 0.001$) (Lothrop et al., 2018). Yin and Patel (2018) reported that in surface water enterococci concentration, pH and electric conductivity showed to be statistically significant as predictors of the presence of *E. coli* in a creek water source, but temperature did not show any correlation with the presence of *E. coli* from the creek water (Yin and Patel, 2018). In that same study, they evaluated the correlation between *E. coli* and total coliforms and found significant differences ($r= 0.79$, $P < 0.05$). From four studies two report correlation between *E. coli* and

water temperature. Variability in *E. coli* concentrations can be due to several factors including weather conditions (sunny vs cloudy days) or time and day (AM vs PM). A study conducted in Lake Michigan found that there were higher population of *E. coli* in sunny days on comparison to cloudy days. In the same study, it was observed that there were higher counts of *E. coli* in the morning than in the afternoon. Studies conducted in Massachusetts streams reported similar findings (Pachepsky et al., 2011). For this research most of the water samples were collected in the morning; however, for future research it might be beneficial to consider other variables to predict *E. coli* populations. Our study just evaluated environmental temperature and indicated that had a positive correlation with generic *E. coli* counts. Three of the studies report positive correlation with water pH, different from our results. Our sample size was smaller in comparison to previous studies in could affect the results, although our results did not show any correlation with pH, pH is an important factor that influences microbial growth. However, more information is needed to get a better understanding from the effect of environmental factors on microbial quality from water sources.

The Shapiro test ($P < 0.05$) was performed to identify the distribution of the data. To compare the methods, the non-parametric Kruskal Wallis test was used because there was not a normal distribution from the data. The Kruskal Wallis test indicated no significant differences between the 3 methods used for this research.

Table 3.6.1 shows a descriptive statistics for generic *E. coli* populations in surface water determined by three equivalent methods to EPA 1603.1, in which mTEC resulted in a higher variability. The highest count from generic *E. coli* in the first month was 60 CFU/100 mL in location D (lake) and the number can be attributed to the temperature which was 20 ℃, the pH from that water source was 7.5. Temperature created favorable conditions for the growth of generic

E. coli. The lowest count for the same month was recorded in location A, the pH from that location is 4.6 and the temperature was 30 ℃, although the temperature was high, the pH was very low.

In the second sampling period, the lowest count (2 CFU/100 mL) of *E. coli* was found in water using mColiBlue, in location B (pond) at optimal conditions (29℃ , pH 7). The other 2 media (MI and mTEC) evaluated in the same farm showed 8 and 13 CFU/100 mL water, respectively. The statistical analysis showed that there was no statistical difference between any of the methods but there was always more variability in mTEC, reflected in the SD. The highest number (43 CFU/100 mL) of *E. coli* for that month was in location E (pond), with a correspondent pH of 8 and a temperature of 29 ℃ when the water was sampled.

The third sampling period had the lowest values recorded for the bacteria. All the locations had between 1 and 4 CFU/100 mL except location A which reported 11 CFU/100 mL. The temperatures for that month for all sites were between 25-29 ℃.

The fourth sampling period was May 2020 for most of the locations. The lowest counts of *E. coli* reported were 1 CFU/100 mL in location E (pond) with a temperature of 19 ℃ and a pH of 8. The highest count of *E. coli* for that month was the lake with 40 CFU/100 mL which can be associated with the higher temperature (31 ℃) and pH (7.5)

In the fifth sampling period, the highest count was in location E (lake) with 46 CFU/100 mL and temperature reported for that month was 28 ℃. The locations with lowest counts of *E. coli* were A and D with counts between 1 and 3 CFU/100 mL and temperatures from 27-28 ℃ and pH of 4.6 and 7.5, respectively.

In the last sampling period locations A, B and D had the lowest count of generic *E. coli* between 2 and 7 CFU/100 mL. Location E reported the highest count with 15 CFU/100 mL and a temperature of 31 ℃.

Overall, the locations evaluated were in range from 1- 60 CFU/100 mL of generic *E. coli.* When comparing the water sources, farm E (pond) showed to have the highest counts of the bacteria in 3 of the 6 sampling periods, followed by the lake. Location A, which had a spring, reported the lowest presence of generic *E. coli* during the sampling. Location C did not show any presence of generic *E. coli.* The averages of generic *E. coli* ± SD in surface water are presented in Table 3.6.2. From that table it can be observed that the method EPA 1103.1 presented a higher variability with a SD of ±20.

The information given by the Kruskal Wallis test leads us to conclude that there are no differences between the three methods evaluated. The use of low technology and low-cost methods can be challenging to characterize the acceptability of water sources (Sbodio et al., 2013). A study conducted by Gradl in 2019 identified the cost/plate for the methods EPA 1103.1 (mTEC $0.09), EPA 1604 (MI $0.35) and Hach 10029 (mColiBlue $1.16) (Gradl 2019). The results in our study indicated that there was no statistical differences between the methods used. EPA 1103.1 (mTEC) showed more variability in comparison to the others; therefore, the MI method would be recommended to use in small farms because is more affordable than the Hach 10029 (mColiBlue) and the results are reliable.

Studies evaluating microbial quality of irrigation water in various US regions have found different levels of generic *E. coli*. Gradl (2019) evaluated microbial quality of agricultural water from Alabama Agricultural Experimental Stations (AAES) and did not report the presence of generic *E. coli* in ground or municipal water. Like the results obtained in this research, all the evaluated

sources met with the PSR criteria. Gradl (2019) concluded that there were not significant differences between MI and mTEC but reported differences in the performance from mColiBlue24 which recovered more colonies from generic *E. coli.* A study on surface water conducted by Nowell (2019) evaluated the microbial quality of water from different sites located in Alabama comparing different approved methods by the EPA(EPA Method 1603, Coliscan ® Easygel, 3M^{TM} Petrifilm and Coliscan ® Membrane Filter). The water sources evaluated were used primarly for urban land uses but some of them were used for agricultural and forested areas. Nowell (2019) reports that alternative methods are practical, consistent, and efficient in conducting *E. coli* enumeration for water sampling (Nowell 2019).

Previous Studies conducted in Georgia and Florida (neighbor states of Alabama) evaluated the presence of generic *E. coli* and foodborne pathogens like *Salmonella* and *Campylobacter jejuni*. Populations of *E. coli* were within the same range in both states in comparison to those obtained from Alabama in this study (Rodrigues et al., 2020).

Surface water has been associated with several outbreaks linked to produce like fresh salad, lettuce, spinach, cantaloupe, and others (Uyttendaele et al., 2015). This type of water is exposed to environmental conditions, and can become contaminated with runoff, raw sewage, and animal feces. Untreated surface water used for irrigation poses a higher risk to cause produce contamination with pathogenic bacteria like *Salmonella* spp.(Truitt et al., 2018). In fact, surface water is considered a major reservoir for *Salmonella* spp.

Weather can cause an impact in bacterial population, especially when there are storms and/or strong winds which can result in high bacteria levels in the water column. Rainfall can increase the levels of microorganisms on the surface water, illustrating the vulnerability and variability that impact water exposed to the environment (Uyttendaele et al., 2015). Surface water is the main

source of irrigation water in the US (Pachepsky et al., 2011). The data obtained from this research and the PSR illustrates the need for a higher frequency of testing for surface water; here is higher prevalence of *E. coli* in surface water than in ground water. Marine et al. (2015) conducted research in the Mid-Atlantic region of the US and found that source of water was a significant factor for indicator bacteria, with groundwater samples having lower populations of APC bacteria, generic *E. coli* and TC in comparison to surface water samples.

Ground water is less likely to get contaminated, but should still be tested as some sources of contamination can include failing of septic systems, leaking sewer lines, land discharges and others (Uyttendaele et al., 2015). This type of water can get become contaminated by anthropogenic activities, with these pollutants lasting longer periods of time in the water (Araujo et al., 2017). From the locations evaluated, none of the ground water sources presented *E. coli*. Irrigation system is an additional variable to consider. Drip irrigation has shown to be a lower risk delivery method because there is reduced contact with the edible part of the plant (Allard et al., 2019). Overhead sprinkler systems represent higher risks because there is direct contact of the edible portion of the plant with the irrigation water, especially in leafy greens production (Marine et al., 2015).

Evaluating microbial quality of water for irrigation is still challenging even with current regulations. Sampling different water sources is extremely important, as they have been understudied and are poorly understood (Strawn et al., 2013; Pagadala et al., 2015; Partyka et al., 2018). In addition, sampling points across the water sources can have different bacterial loads, a study conducted by Allard et al., (2019) showed significant differences between sampling locations from a creek when they compared water directly from the creek with the end-of-hose creek collected from the field. For our study we tried to be consistent and collected water samples from the same sample points for the duration of the sampling times.

It is crucial to identify and collect information related to irrigation water and its potential to harbor foodborne pathogens to get a better understanding from the risk factors associated that can potentially be a source of contamination for produce. The presence of *E. coli* clearly performs an important component as an indicator microorganism, but even though the water sources met with the PSR, pathogenic organisms can be present; therefore meeting the PSR may not be enough to guarantee the safety of agricultural water. Data collection is very useful because it helps to create models or other tools that identify the behaviors of foodborne pathogens, creating more efficient food safety systems for each farm as each farm has different characteristics (Strawn et al., 2013).

Alabama is one of the Southeastern states where most fruit and vegetable production occurs, emphasizing the impacts on safe produce production practices is essential. Good Agricultural Practices (GAP) or good manufacturing practices (cGMP), both provide guidelines to reduce food safety issues (Ongeng et al., 2011), but in the state of Alabama, very few farms hold a food safety certification.

In relation to growing season, a Wilcoxon test was conducted, (Table 3.6.3), showing there was no difference between the two growing seasons. Those results are similar to those obtained by Marine et al. (2015) as they did not find any statistical difference between bacterial counts from fall-spring growing seasons. In addition to water samples, they collected leafy greens samples and they did find statistical difference from bacterial counts from the growing two seasons they evaluated (Marine et al., 2015).

3.5. Conclusion

It can be concluded that the samples of PSR-exempt farms from Alabama represent minimal risk to produce safety. All the evaluated locations had averages of generic *E. coli* below the limits required by the PSR. The highest value reported was 59 CFU/100 mL from generic *E. coli*. There was no presence of generic *E. coli* in the ground water just in the surface water which support the lowest frequency for water sampling recommended by the PSR. The averages of generic *E. coli* in surface water for farm A was in a range between 3 and 8 CFU/100 mL. For farm B, it was between 5 and 8 CFU/100 mL. Farm C did not have any presence of generic *E. coli* in the water source which was expected because the source is ground water. Farm D had a range between 13 and 20 CFU/100 mL and farm E had a range between 7 and 19 CFU/100 mL. The medians from the two growing seasons were compared using the Wilcoxon test, but no significant differences were found in any of the evaluated farms ($P > 0.05$).

Overall, no significant difference was found among the three evaluated methods ($P > 0.05$); all methods provided equivalent results. From the external factors evaluated, only environmental temperature showed a positive correlation ($r= 0.10$, $P = 0.005$) with generic *E. coli.*

3.6. Tables and Figures

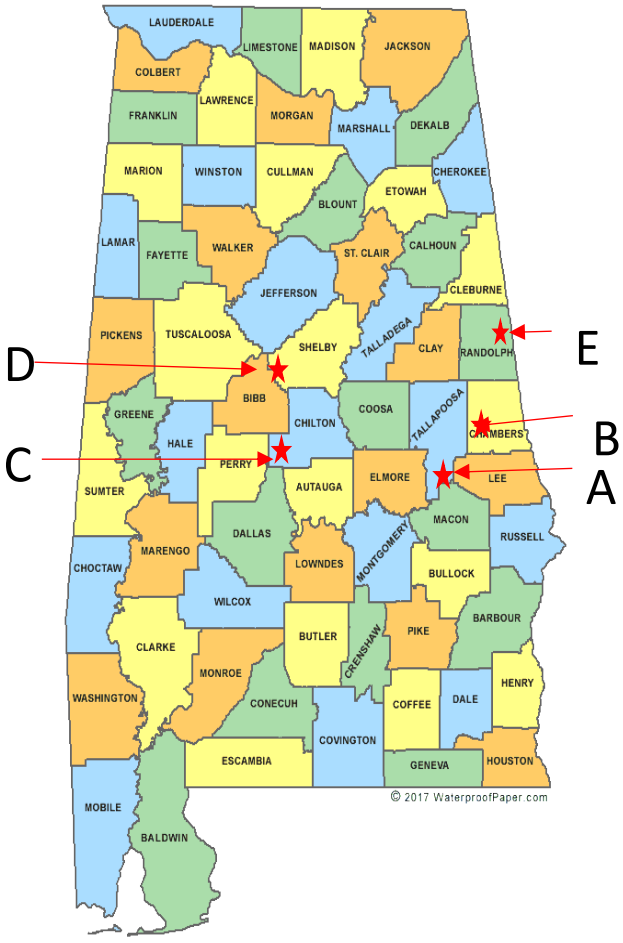

Figure 3.6.1. Map of farm locations evaluated

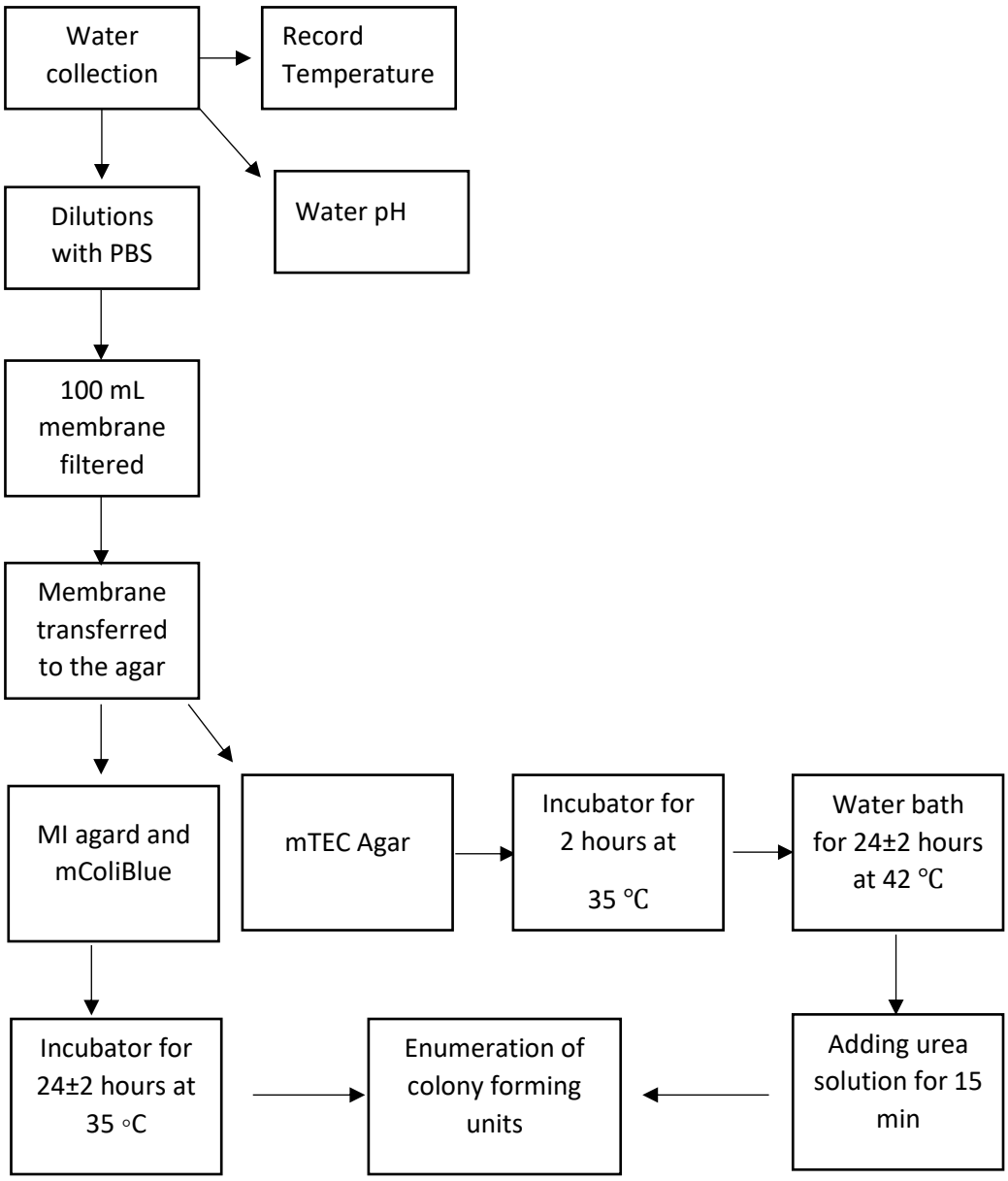

Figure 3.6.2 Microbial analysis for water samples

Table 3.6.1. Descriptive statistics for generic *E. coli* populations in surface water determined by three equivalent methods to EPA 1603.1

Location	Sampling time	Microbial Count (CFU/100 mL) mean[2] ± SD		
		EPA 1103.1	EPA 1604	Hach 10029
		mTec	MI	mColiBlue24
A	1	2±1	2±0	2±0
	2	10±54	14±4	3±1
	3	12±12	11±10	4±5
	4	8±2	17±5	8±4
	5	3±1	2±1	1±0
	6	12±12	7±10	2±1
B	1	4±35	18±3	13±1
	2	13±8	8±3	2±1
	3	1±1	1±1	2±0
	4	10±4	4±1	7±5
	5	11±3	11±3	5±5
	6	3±1	3±1	2±2
D	1	59±58	39±24	20±14
	2	3±4	6±4	4±4
	3	<LOD	<LOD	<LOD
	4	34±7	40±27	35±25
	5	2±2	2±2	5±4
	6	3±3	4±2	2±3
E	1	6±4	12±5	16±17
	2	43±45	20±25	11±7
	3	2±0	2±0	2±0
	4	1±0	1±0	1±0
	5	46±40	21±8	6±3
	6	15±12	13±12	8±5

[1]PSR requirements are GM <126 CFU/100 mL

[2]Average of three replicates

Table 3.6.2. Averages of generic *E. coli* ± SD CFU/100 mL in surface water determined by three equivalent methods to EPA 1603.[1]

Location	EPA 1103.1 mTec	EPA 1604 MI	Hach 10029 mColiBlue 24
A	8±5	9±6	3±3
B	7±5	8±6	5±4
D	20±20	12±8	13±14
E	19±20	12±8	7±6

[1] n = ?

Table 3.6.3 Comparison of growing seasons using the Wilcoxon Test

Location	media	G1	G2	P-value
A	MI	1.04	0.85	0.41
	Mtec	1	0.69	0.65
	McoliBlue	0.47	0.3	0.41
B	MI	0.9	0.6	0.5
	Mtec	0.6	1	0.65
	McoliBlue	0.3	0.69	0.68
D	MI	0.77	0.6	0.65
	Mtec	0.47	0.6	0.65
	McoliBlue	0.69	0.69	0.74
E	MI	1.07	1.11	0.65
	Mtec	0.77	1.17	0.65
	McoliBlue	1.07	0.84	0.2

Table 3.6.4 Descriptive statistics for generic *E. coli* and coliform populations, and environmental factors in surface water determined by two equivalent methods to EPA 1603

Farm	Sampling Time	MI *E. coli* CFU/100 mL	Coliforms CFU/100 mL	Temperature ℃	mColiBlue *E. coli* CFU/100 mL	Coliforms CFU/100 mL	Temperature ℃	pH
A	1	2	1124	30	2	480	30	4.6
	2	14	1274	29	3	161	29	4.6
	3	11	605	29	4	426	29	4.6
	4	12	133	28	8	113	28	4.6
	5	2	313	28	1	494	28	4.6
	6	7	309	28	2	103	28	4.6
B	1	18	338	27	13	1442	27	7
	2	8	998	29	2	182	29	7
	3	1	3517	26	2	3883	26	7
	4	4	8700	18	5	3883	18	7
	5	11	6050	29	5	1827	29	7
	6	3	478	33	2	505	33	7
D	1	39	243	28	20	1391	28	7.5
	2	6	271	29	5	501	29	7.5
	3	1	32	25	1	26	25	7.5
	4	40	999	31	35	114	31	7.5
	5	2	187	27	5	35	27	7.5
	6	4	106	23	2	65	23	7.5
E	1	12	480	25	16	56	25	8
	2	20	110	29	12	49	29	8
	3	2	360	27	2	42	27	8
	4	1	32	19	1	54	19	8
	5	22	216	28	7	121	28	8
	6	13	294	31	8	205	31	8

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4. Assessing Microbiological Quality of Produce and Food Safety Practices on Small AL Farms exempt from PSR

4.1. Abstract

Fresh produce is often associated with foodborne outbreaks as these products easily become contaminated with foodborne pathogens from the environment and poor handling practices. To reduce food safety issues the Food Safety Modernization Act (FMSA) Produce Safety Rule (PSR) was established. However, some farms are PSR exempt due to their small size and may be at risk of produce contamination. The purposes of this study were: 1) identify potential relationships between food safety environment and handling practices on microbial quality of fresh produce and water used for irrigation on PSR exempt Alabama farms; and 2) identify food safety/handling practices implemented in each location. A total of 5 locations were evaluated throughout Alabama, with 63 samples of produce (onions, tomatoes, squash, and others) and 15 samples of irrigation water. Produce samples were analyzed for aerobic plate counts and coliforms. Water samples were enumerated for generic *E. coli* and coliforms using the 1604 EPA method (MI). Farmers were asked to complete a paper-based survey consisting of yes-no questions covering PSR topics. Bacterial loads from produce were compared using ANOVA and Tukey HSD. Simple linear regression was used to determine potential relationship between coliforms from produce and water samples. Coliform loads from produce were between 1.45-5.55 Log CFU/g, aerobic plate counts between 1.66–5.72 Log CFU/g. Coliforms in water were between 1.38-3.51 Log CFU/ 100 mL and 0.30-1.60 Log CFU/100 mL for generic *E. coli.* There was no relationship between water and produce microbial quality ($P = 0.46$). The survey responses indicated that most of the locations implement proper food safety practices. Identifying microbial

indicator loads from fresh produce and gaps in handling practices can aid in determining if further actions are needed to reduce produce contamination on PSR exempt farms in the state of Alabama.

4.2. Introduction

In the United States, several food safety practice guidelines have been implemented in the past but the most recent act is the Food Safety Modernization Act (FSMA), which provides the first regulation considering produce safety to prevent food safety issues. FSMA represented a significant change in food safety laws because the focus is on preventing hazards in a proactive manner, instead of reactive (Astill et al., 2018). Implementing the PSR among small farmers remains challenging. Farmers have larger investment in their water systems (Astill et al., 2019) and, it is difficult to "change farmers' minds". Some farmers have dedicated their entire life in growing produce following their own practices and when there is a new regulation, they tend to resist adapting and applying new practices. The implementation of new practices can represent financial investment for famers, and it can be time consuming because they need to learn new skills or practices. However, farmers are more likely to adopt new farming practices when they have knowledge of the societal importance and feasibility of the new practices, therefore, conducting training among farmers is essential (Baur, P. 2020).

Previous research has shown that farmers are more likely to comply with rules and standards when there is flexibility and those rules and regulations do not impose upon their sense of control over their farm's operations (Baur, P. 2020). The produce safety rule is flexible and addresses each farm as individuals by identifying specific hazards and developing different ways to confront those. By holding certifications in safety handling farms are more appealing to consumers. Research conducted in Thailand showed that customers are willing to pay more for fresh produce with food

safety and brand labels (Wongprawmas and Canavari, 2017). Previous research conducted by Neill and Holcomb (2019) found that consumers pay less for PSR exempt vs produce coming from PSR compliant farms (Holcomb and Garber, 2019).

Foodborne outbreaks are challenging to trace back to the source because of the short shelf life of produce and the possibility of several routes of contamination (Allard et al.,2019). The objectives for this research were: 1) to identify potential relationships between food safety environment and handling practices on microbial quality of fresh produce and water used for irrigation from PSR exempt Alabama farms and 2) to identify food safety/handling practices implemented in each location.

4.3. Materials and Methods

Agricultural water from five different geographical areas of the state was sampled and enumerated for generic *E. coli* and total coliform bacteria during a growing season. Samples of produce were collected from each location and enumerated for coliform bacteria and aerobic plate count. A paper based survey on produce safety practices was delivered to the farm managers.

4.3.1 Water Sampling Process

Water samples were collected with a sterile bottle (NALGENE) near the irrigation water intake source. Samples were collected monthly over a growing season (n=45), where 1L of water was collected in triplicate in sterile Nalgene plastic bottles. Produce samples from each location were collected and placed in sterile bags (75 grams). All the samples were transported to the

laboratory in a cooler with ice. The microbial analysis began less than 6 hours after collection in accordance with the Standard Methods for the Examination of Water and Wastewater.

From each 1 L bottle of water, serial dilutions were performed in phosphate buffered saline (PBS). A volume of 90-100 mL was filtered via the membrane filtration method through a 47 mm diameter, 0.45 µm pore size filter (Pall Laboratory, Port Washington, NY, USA) and placed into MI Agar. Plates were incubated at 35 ℃ for 24 h. After incubation, blue colonies were counted as *E. coli* . The blue coloration in the colonies was due to the production of the enzyme β-D-glucuronidase, that breaks down the indoxyl- β-D-glucuronidase (IBDG) and forms a blue colored compound. This method was it used because was more consistent in comparison to the EPA Method 1103.1 and Hach Method 10029. The results from the three replicates were averaged. The results are shown on table 4.2 The limit of detection (LOD) was 1 CFU/100 mL. *E. coli* was used as a positive control and *Pseudomonas aeruginosa* was used as a negative control.

4.3.2 Fresh Produce Sampling Process

From May 2020 - October 2020 a total of 60 samples of squash, onion, bok choy, kale, blueberry, and fig were collected from five produce safety rule exempt farms in Alabama. Samples were collected fresh from the field and were processed without washing. All samples were placed in Ziploc bags and shipped to the lab in coolers with ice.

Produce samples were analyzed for aerobic plate counts (APC) and coliforms. Each produce sample was weighed (25 grams) and diluted with 0.1% buffered peptone water and then homogenized for 1 min at 230 rpm in a stomacher. A 10- fold serial dilutions was plated on Violet

Red Bile Agar (VRBA; Hardy Diagnostics, Santa Monica, CA) and tryptic soy agar (TSA; Hardy Diagnostics, Santa Monica, CA) in triplicate for each medium. The plates were placed in a 35 ℃ incubator for 24 hours. After 24 hours the colonies were counted and reported in CFU/gram. The results are shown on Table 4.6.2.

4.3.3 Survey

The survey consisted of 10 yes-no questions covering Produce Safety Rule topics focusing on identifying food safety practices that growers implement on each farm. Farm managers from the five locations evaluated were asked to complete a paper-based survey. Results are reported in Table 4.6.1.

4.3.4 Statistical analysis

Data were analyzed using R software version 4.04 for all bacterial counts. All microbial counts were log transformed. Differences in microbiological counts and prevalence among treatment means were determined using one-way ANOVA and Tukey HSD. P -values less than 0.05 were considered statistically significant. Simple linear regression was used to determine potential relationship between coliforms from produce and water samples. Survey data results were compiled and mean response for each question were computed, percentages of completion are shown on table 4.6.1.

4.4. Results and discussion

A total of 105 samples of produce and water were collected from 5 farms. All farms belong to central Alabama and are PSR exempt because of their size. Small farms do not need to meet with PSR requirements, but exempt farms can potentially represent a food safety risk because they sell their products commonly at farmers markets, therefore, it is important to have an overview of their food safety practices and microbial quality of their produce. Previous research indicates that growers managing small farms have minimal training in food safety. In addition, growers from small farms usually identify food safety guidelines as too complex and there is a lack oversight from their food systems (Kilonzo et al., 2018).

The evaluated locations used drip irrigation (Farms A and C) and two farms utilized overhead or sprinkler irrigation (Farms D and E). Microbial quality from the water samples and produce samples were evaluated. The range of generic *E. coli* found in the surface water (All farms except C) was between 0.70 and 0.95 Log CFU/100 mL. The ranges found in surface water for coliforms were between 1.89 and 3.32 Log CFU/100 mL. Farm C supplemented the irrigation system by using ground water, however, the microbial analysis indicated a microbial load below the limit of detection (LOD).

For the produce samples, microbial analysis included aerobic plate count and coliforms. Both analyses are known to be microbial indicators and can reflect actual sanitary conditions from fresh produce. There was no statistical difference between the coliform loads from each produce, with the microbial load range between 1.45 and 5.55 log CFU/gram. For total aerobic plate counts, microbial loads were higher with a range of 1.66 to 5.72 Log CFU/gram. It was expected to find higher microbial concentration in squash and onions as those vegetables are grown directly on the

ground but there were no statistical differences when comparing coliform counts with other produce samples.

The use of plastic coverage for soil is considered a good agricultural practice in produce that is grown near the ground. One of the farms (B) did not cover the soil and the squash was in direct contact with the soil. From table 4.2 it can be observed that squash from farm C had the higher coliform loads (5.33 log CFU/g) in comparison to farms A and C that covered their soil. From the study conducted in Minnesota coliform counts on summer squash were 3.9 log MPN/g for organic products versus 3.3 log MPN/g for conventional produce (Mukherjee et al., 2004). The ranges in coliforms found in this research were between 2.44- 5.33 log CFU/g.

Microbial loads reported from the produce indicated that figs had the higher concentration which can be attributed to the type of irrigation water used for this produce (surface and overhead). Leafy greens (kale and bok choy) because their shapes are more prone to microbial contamination; but our study did not show any statistical difference between microbial load indicators. A study conducted on fresh produce indicated that leafy greens and herbs had higher aerobic plate counts with a range between 5.2 and 6.1 log CFU/g). In the same study it was also reported that produce grown at the surface level like lettuce, spinach, parsley, and cilantro had higher counts of aerobic bacteria and coliforms when compared to produce that grew above the surface like tomatoes and apples (Kilonzo et al., 2019). Contrary to the findings of this research, another study conducted on microbial quality of fresh produce from farmers markets in West Virginia and Kentucky found that aerobic plate count loads were higher in leafy greens (spinach) than other produce commodities (Li et al., 2017). A study conducted on leafy greens from Minnesota farmers' markets found mean coliform counts within a range of 0.1-3.3 log CFU/g (Tong et al., 2017). The leafy greens included in this research were kale and bok choy and the microbial loads for total coliforms

were between 2.04- 4.32 Log CFU/g and 2.76-4.25 Log CFU/g for aerobic plate counts. Mukherjee et al. (2004) reported coliform counts of 3.0 log MPN/g in organic bok choy and 5.4 log MPN/g in conventional bok choy. Coliform counts in bok choy for this research were 2.71 log CFU/g and 2.92 log CFU/g for total aerobic plate counts.

Blueberries from farm D had drip irrigation, and the microbial load for coliforms was below the limit of detection and for total aerobic plate counts there was no difference between the other produce. Blueberries are an important commodity and are considered ready to eat; therefore, it is critical to guarantee their safety. Because of this, blueberries are listed in the top ten riskiest foods by the US FDA. Research conducted in Washington State did not find any presence of generic *E. coli* or pathogenic microorganisms on blueberry samples, and coliform loads were below the detectable limit similar to the findings in this research (Shen et al., 2020). A study conducted of packing lines in blueberry facilities suggest that blueberry microbial loads increase significantly in packing lines and hence it is required to pay special attention to maintenance and sanitation treatments (Quansah et al., 2019).

Overhead irrigation was used at farm E with irrigation from surface water and can be reflected in the higher microbial loads in comparison to the other produce. There were no significant differences on coliform counts from figs when compared with other produce. For aerobic plate counts, figs had the higher microbial load. Villalobos and researchers evaluated microbial quality on figs and report finding bacteria species like *Pseudomonas gessardii*, *Pantoea agglomerans* and *Enterobacter asburiae.* They also found fungal species like *Aureobasidium pulullans*, *Cladosporium cladosporioides* and *Alternaria alternata.* Figs are perishable due to their high sensitivity to physical damage and susceptibility to postharvest contamination (Liu et al., 2020).

Most of the postharvest losses in figs are mainly caused by the growth and propagation of microorganisms (Del Carmen Villalobos et al., 2017).

Research conducted at small farms from Tennessee on fresh tomatoes found microbial loads for aerobic plate count from 3.2 log CFU/g and total coliforms from 2.3 log CFU/g (Kilonzo et al., 2018); results from farm C in our study indicated that tomatoes had total aerobic plate count between 1.45 and 2.07 log CFU/g, and between 1.66 – 2.25 log CFU/g for coliform counts. Fresh tomatoes sold in farmers markets from Virginia and Kentucky had an average of 3.7 log CFU/g for aerobic plate counts and 3.8 Log CFU/g total coliforms (Li et al., 2017). Microbial loads found in tomatoes from this research were lower in comparison to fresh tomatoes sold in farmers markets.

For the purposes of this research microbial quality on fresh produce was completed to identify potential correlations with irrigation water and the food safety practices from each location. The statistical analysis showed no correlation between water quality and produce quality ($P = 0.46$). A lack of correlation between microbial quality of water and microbial quality of fresh produce indicates that other parameters need to be included when conducting microbial analysis for fresh produce.

All locations evaluated met the criteria for water quality standards. This information will benefit the farmers when the agricultural water section from the PSR inspection takes place; their water sources will not require investments or corrective actions. The microbial load on water used for irrigation was under the regulation limit and as it can be observed from microbial quality on produce, water did not represent microbial risk on produce. When a water source contains higher microbial loads from indicator microorganism, water can represent pathogen contamination, therefore; maintaining the quality of production and post-harvest produce, water biological quality is crucial (Yousuf et al., 2020).

Currently there are no regulations for fresh produce, just for ready to eat produce. Appearance, freshness, and color are the main attributes to evaluate quality on fresh produce, but there are no microbial standards (Singla et al., 2020). Quality and safety of fresh produce can be affected by microbial load and handling practices. If produce is contaminated or has a bad quality can represent a vehicle for the transmission of food-borne diseases (Yousuf et al., 2020).

Some factors that can alter microbial load in produce include storage temperature, produce pH, water content, and damage during harvesting, transport, and environmental factors. The efficacy of decontamination procedures can be affected by microbial loads (Ziuzina et al., 2020). The United Fresh Produce Association Food Safety & Technology council developed a paper to identify potential points where microbiological testing can be implemented (United Fresh 2020) but no other baseline information is available for sampling fresh produce. Meeting food safety standards is challenging because of the dynamic and complex produce industry due to the highly seasonality, shelf life, and others (Astill et al., 2019).

Minimally processed produce is the closest category to fresh produce and has very few steps before consumption. Minimally processed fruits and vegetables are prone to biological contamination due to the presence of imperfections in the surface like cuts, moisture content, the lack of microbial stability due to the minimal processing, the metabolism from the tissues and the type of packaging (Perez- Rodriguez et al., 2018). After harvesting, produce is usually washed to reduce contamination, but more recent information suggests that post-harvest washes can be a high-risk contamination point, indicating that alternative post- harvest decontamination technologies need to be implemented like irradiation treatments, ultraviolet light, cold plasma irradiation, pulsed light, high hydrostatic pressure, gas phase (ozone and chlorine dioxide), and hydroxyl radicals generated through advanced oxidative process or gas plasma (Murray et al., 2017).

Assessing the food safety practices at the farm level can provide useful data to determine food safety risks from produce and suggest proper food safety measures to minimize those risks (Neill et al., 2019). From the five farms evaluated, the surveys indicated that most of the farmers follow food safety practices which can be reflected in the microbial loads in both water and produce. All of the farmers (n= 5) have access to food safety trainings and have completed previous trainings. Having training in food safety practices provides science-based knowledge to growers that they can apply at their farms. Today, food safety trainings are necessary for growers due to an increase in public awareness on produce safety. Because of this, food safety education has become an important research and extension topic that can be reflected in the increased number of papers and research published on produce safety in the past years (Chen et al., 2021). Assessment of food safety trainings are necessary and is important to validate the farmers' knowledge of how to manage produce safety at their farms. Resources should be accessible for farmers for consultation, as a lack of resources may impede the transfer of knowledge into practice (Jayawardhana et al., 2020).

All farm managers reported the use of disinfectants and appropriate cleaning of tools used during production and harvesting. In addition, they wash their produce before it leaves the farms. Washing is considered an essential step where microbial contamination can be reduced (Kilonzo et al., 2018); however, deficiencies in packing house sanitation and equipment conditions can represent sources of microbial contamination (Gutierrez and Adhikari, 2019; Lepper et al., 2019). Some of the most common disinfectants among the produce industry include chlorine, calcium hypochlorite and sodium hypochlorite. Furthermore, the produce industry is looking for different alternatives for disinfectants such as irradiation, ozone, cold plasma, and other technologies (Yousuf et al., 2020).

The PSR emphasizes the importance of record keeping, including employee training, records from water analysis and cleaning and sanitizing. Most of the farms evaluated during this study were farms owned by small families and with few having additional employees. Eighty percent of the farm managers reported that they kept training records from their employees. The PSR does not require a food safety plan, but it is highly recommended, providing documentation of the on- farm processes. Just 60% of the farms had a written food safety plan. Only 60% of farms have had third party audits in the past. All growers reported the use of biological soil amendments. Composting manure is a technique used commonly in organic farming where parameters like time and temperature perform a crucial role in killing foodborne pathogens (Mukherjee et al., 2004). The PSR has identified biological soil amendments as a potential risk and untreated biological soil amendments of animal origin such as manure, must be applied without having contact with the produce. The regulation provides two valid examples of scientifically valid composting methods that meet the PSR standards (US FDA, 2018). From the survey it was identified that 80% of the farms use supplemental irrigation. And from the previous chapter we can conclude that all of them met with the produce safety rule criteria. It has been demonstrated that water sources can represent a route of microbial contamination, especially surface water (Gutierrez and Adhikari, 2018). Domestic and wild animals represent a source of contamination because they can shed microorganisms and transmit them to the fruits and vegetables, soil or even water sources (Gutierrez and Adhikari, 2018); 60% of the farms evaluated have domestic animals near the growing area. Overall, the data recovered from the survey indicated that produce growers follow good agricultural practices but there is a deficiency in documentation and farm record keeping.

4.5. Conclusion

Coliforms and total aerobic plate counts are indicator bacteria that can be used to determine poor hygiene and can be used to assess microbiological quality of fresh produce and water sources. A lack of correlation between microbial quality of water and microbial quality of fresh produce indicates that other parameters need to be evaluated when performing microbial analysis for fresh produce. Overall, the data recovered from the survey indicated that there is a deficiency in documentation and farm record keeping but most of the growers follow good agricultural practices.

4.6.Tables and Figures

Table 4.6.1 Survey related responses

Questions	Response	
Do you use biological soil amendments derived from animal origin (like raw manure, bone mean or other)?	y[1]=100%	n[2]=5
Do you have a written food safety plan for your farm?	y=60%	n=3
Have you ever had a third-party audit of your farm to verify food safety practices?	y=60%	n=3
Do you have domestic animals (cows, dogs, cats, pigs, chickens, turkeys, etc.) between 10-400 fts close to the land used for growing crops?	y=60%	n=3
Do you have access to training related to good agricultural/ Food safety practices?	y=100%	n=5
Are you trained in farm food safety practices such as those listed in the Food Safety Modernization Act Produce Safety Rule or those included in Good Agricultural Practices (GAPs)?	y=100%	n=5
Do you keep training records from your employees?	y=80%	n=4
Do you use supplemental irrigation?	y=80%	n=4
Are you currently testing your irrigation or wash water source(s)?	y=80%	n=4
Do you wash your produce before it leaves the farm?	y=80%	n=4
Do you disinfect or clean any tools like knives, scissors?	y=100%	n=4

[1]y = yes

[2]n = number of responses

Table 4.6.2. Microbial loads from water and produce samples from small Alabama farms over a three-month period.

Location	Date Sampled	*E. coli* in water	Coliform in water (MI)	Produce	TCC[1]	APC[2]
A	Month 1	1.07±0.69	2.11±0.79	Squash	4.89±1.49	5.46±1.77 [ab, 3]
				Onion	3.42±0.11	4.92±0.57 [ab]
	Month 2	0.30±0.10	2.48±0.09	Squash	3.21±0.03	3.02±0.51 [ab]
				Onion	4.34±0.94	4.64±0.73 [ab]
	Month 3	0.84±1.0	2.39±0.30	Squash	2.65±0.90	3.85±0.18 [ab]
	Average	**0.74±0.40**	**2.33±0.19**			
B	Month 1	0.60±0.01	3.76±0.19	Onion	3.71±1.02	3.60±1.30 [ab]
				Bok Choi	2.71±0.38	2.91±0.15 [ab]
				Kale	2.04±0.80	2.76±0.26 [ab]
	Month 2	1.04±0.47	3.51±0.29	Kale	4.32±0.76	4.25±0.47 [ab]
				Squash	5.33±0.81	5.40±1.20 [ab]
	Month 3	0.47±0.01	2.68±0.07	Squash	2.44±0.97	4.31±1.10 [ab]
	Average	**0.70±0.30**	**3.32±0.57**			
C	Month 1	<LOD	<LOD	Tomato	1.45±0.39	1.66±0.37[b]
				Squash	2.84±1.27	2.57±0.33 [ab]
	Month 2	<LOD	<LOD	Tomato	2.07±0.22	2.25±0.53[b]
				Squash	3.06±0.69	2.45±0.52 [ab]
	Month 3	<LOD	<LOD	Squash	2.84±0.14	2.47±0.21 [ab]
	Average	**<LOD**	**<LOD**			
D	Month 1	1.60±1.43	3.08±0.27	Blueberry	<LOD	2.51±0.38 [ab]
	Month 2	0.30±0.30	1.95±0.61	Blueberry	<LOD	2.38±0.29 [ab]
	Average	**0.95±0.92**	**2.52±0.80**			
E	Month 1	0.30±0.10	1.38±0.32	Fig	4.65±0.95	4.64±0.95 [a]
	Month 2	1.34±0.90	2.15±0.42	Fig	5.55±0.79	4.97±0.37 [a]
	Month 3	1.11±1.07	2.13±0.67	Fig	3.92±0.10	5.72±0.26 [a]
	Average	**0.92±0.55**	**1.89±0.44**			

[1] TTC = total coliform count.

[2] APC = aerobic plate count (mesophilic aerobic bacteria) :

[3] Treatments with the same letter are not significantly different.

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5. Evaluation of Current Usage of Food Safety Practices among Produce Growers from Alabama

5.1. Abstract

Produce can become contaminated at any point in the food chain and accounts for 46% of illnesses and 22% of deaths from foodborne illness in the US. To reduce the food safety issues, The Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) set regulations related to the growing, harvesting, packing, and holding of produce for human consumption. On-farm implementation for the PSR can be challenging due to the investment required by farmers. With limited information on food safety practices implemented among farmers in Alabama, the objective of this study was to collect data to estimate the current usage of food safety practices specified by the PSR among growers from different regions of Alabama. A survey with 11 yes-no questions was developed and administered both paper based and electronically using Qualtrics Software. With the assistance of extension agents, farmers from different locations in Alabama were contacted via personal emails, farmer's markets and Facebook groups and were asked to complete the survey. From 66 completed surveys, the most relevant data indicated that most of the farmers (66.61%) have access to food safety trainings of those only 46.97% of them have completed trainings on food safety practices, PSR or Good Agricultural Practices (GAPs). Results also showed that 75.75% of the farmers use irrigation but just 37.88% have analyzed their water sources for microbial quality and only 36.36% currently test their water sources on regular basis. It was identified that 62.12% of the farms surveyed had access to toilet facilities near the work area and 28.79% have a written food safety plan. Identifying current usage and gaps on food safety practices among produce growers can help actions to develop tools for food safety training among growers and reduce produce contamination and foodborne outbreaks in Alabama.

5.2. Introduction

The US Department of Agriculture is currently conducting on farm inspections in AL based on the Produce Safety Rule excluding the water provision. Establishing a background or baseline information for farm food safety practices implemented in each state is relevant to get a better understanding of the potential impacts on growers before the PSR inspections are conducted. The Produce Safety Rule has some exemptions and not all farms are required to meet the PSR. Most of the exempt farms sell fresh fruits and vegetables in small quantities, for example, farmers markers or for personal consumption. If farms do not follow appropriate food safety practices, people can become sick. There is a lack of monitoring of the microbial quality in these types of farms, and previous research has demonstrated that microbial levels in produce from farmer markets were higher when compared with produce from retail establishments (Mohammad et al., 2020). It is therefore essential to train growers and produce handlers in food safety practices to mitigate produce contamination.

There has been a recent increase in the consumption of locally produced food and produce, which has led to an increase in on- farm pathogenic contamination resulting in foodborne outbreaks (Sinkel et al., 2018). Lack of testing and proper record keeping, and traceability are just a few contributing factors that contribute to microbial contamination. Consumers are more aware of food safety through recalls and media (Chen et al., 2021). This knowledge has had an influence in the produce market, when there is an outbreak or recall they react instantly by reducing consumption and purchases (Pivarnik et al., 2018). In addition, consumers fuel trends like organic produce and clean labels which have an impact in the market. When comparing traditional produce vs organic produce consumers, it is thought that organic produce is safer, which is not necessarily accurate.

Literature has demonstrated with various produce types that organic farming can compromise food safety due to the use of biological soil amendments, especially those from animal origins (Pivarnik et al., 2018).

Currently in Alabama there are a few farms that hold GAP certification. Research conducted with growers from Kentucky suggested that some challenges for obtaining and implementing GAP certification includes the lack of time, the audit process, and the cost of certifications (Sinkel et al., 2018). In addition, farmers incur costs due to the training needed for workers, investment in supplies, inputs, infrastructure, equipment, and others (Schmit et al., 2020). The implementation of the produce safety rule can be burdensome especially for small farms or sustainable farm operation. A study conducted in Minnesota showed that small farmers, the implementation of PSR require and investment of 10% of gross revenue versus an average- sized farm that would incur costs from 2% and up of their gross revenue (Adalja and Lichtenberg, 2018). A study conducted in the New England (NE) region indicated that the primary motivation to become GAP certified was buyer requirements and market channels (Pivarnik et al., 2018). Growers from New York indicated that most had a personal commitment to food safety practices on the produce that they grow and sell (Schmit et al., 2020). Farmers from Oregon signaled that their main motivation to adopt GAP or food safety certifications was to keep their current customers (71%), followed by improving food safety, preparing for FSMA regulations and extend their customer base (Prenguber and Gilroy, 2013).

Some benefits of implementing food safety practices include expanding market channels, adding new markets and buyers, and strengthening the farm brand to prospective buyers' due confidence in the food safety improvements (Schmit et al., 2020). Consumers are more attracted to produce that is safe; therefore, by decreasing microbial contamination and improving food safety practices

consumer will increase their consumption of produce. The implementation of the PSR will be positive according to the FDA which estimates that there will be a reduction of $1.04 billion annually on foodborne illnesses (Prenguber and Gilroy, 2013). Also, previous research reported that consumers are willing to pay more for produce coming from produce safety rule compliance farms (Neill et al., 2019).

It has been identified that there is a need to reinforce and create awareness among farmers in the Southeastern region of the United States which have previously been engaged in a several unsafe practices including the use of non- composted soil amendments and little or no sanitizing of food handling surfaces (Sinkel et al., 2018). For instance, research finds numerous farms in Georgia use litter from chickens as fertilizers, but mostly without composting which can compromise food safety (Rodrigues et al., 2020).

Currently there is limited research on the knowledge and perceptions/implementation of food safety practices by Alabama Farmers. Through this research, we wanted to determine the level of knowledge and implementation of food safety practices on farms from Alabama with the purpose to assess current farm management practices utilized by growers and evaluated their knowledge in food safety.

5.3. Materials and methods

The survey, both a paper based and online survey using Qualtrics Survey Software, were administered to vendors at farmers' markets. Farm managers were recruited through Alabama extension agents, personal invitations, by email, Facebook groups and farmers markets and asked to complete the survey. Farmers did not receive any compensation for participating in this study and the participation in this study was anonymous and voluntary. The survey consisted of 11 yes-

no questions produce safety related. The questionnaire was approved by Auburn University's Institutional Review Board (IRB).

5.4. Results and discussion

A total of 66 growers completed the survey. All growers were from different regions in Alabama. For the purpose of this research, question responses were categorized as; training, animal, record keeping, cleaning and hygiene, or water related (Table 5.6.1) .

5.4.1 Training related responses

Growers were asked if they have access to food safety practices/good agricultural practices (GAP) training. It was reported that 60.61% of them have access to these types of trainings and just 49.67% reported the completion of on farm food safety practices such as those listed in the Food Safety Modernization Act produce safety rule or those included in GAP. A survey conducted among 226 farmers and 45 market managers from Georgia, South Carolina and Virginia reported that only 41.2% of them had previously offered trainings on sanitation (Harrison et al., 2013). According to Rodrigues et al., (2020), a survey conducted between Georgia farmers indicated that from 120 growers just 29.7% of them previously received the Produce Safety Alliance Grower Training Course and just 28.2% of them hold GAP training (Rodrigues et al., 2020).

A survey conducted with Kentucky farmers reported that 90% (144) of the produce growers surveyed were familiar with GAP (Sinkel et al., 2018). Similar results are reported by Lichtenberg and Page (2016) from the Mid-Atlantic region where 87% of the growers provide food safety education and training to their employees. Pivarnik et al., (2018) reported that a survey conducted among growers (n = 301) in the New England region indicated that 87% of the farmers have

attended GAP or equivalent food safety training, 67% have participated in GAP auditing and all of them are currently implementing food safety practices on their farms. Usually, farmers markets venders undergo formal inspection only when required by a buyer and rarely by choice (Mohammad et al., 2020). A survey conducted among farmers from Arkansas and Texas reported that 36.7% (n=45) have received formal food safety training previously. In addition, the same study reported that farmers market managers provided guidelines or outreach material to farm managers and vendors regarding handwashing training, standard operating procedures (SOPs) and others (Mohammad et al., 2020). Harrison et al., (2013) reports that in Virginia, Georgia, and South Carolina over 75% of the market managers reported a lack of sanitation training of their workers or vendors. Perry and others (2019) conducted a study in Iowa, Illinois, Indiana, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota, Ohio, and Wisconsin and found that most of farmers had a lack of understanding on topics like biological soil amendments of animal origin (BSAAO) and agricultural water (Perry et al., 2019).

The Produce Safety Alliance (PSA) has developed trainings to educate growers and extension agents and other interested in the produce industry (Woods et al., 2020) but there is a need for more available resources. Training and education tools among growers are crucial because through access to information, farmers can learn to identify potential hazards or contamination risks points. A study conducted in Kentucky reported no statistical relationship between the respondents' knowledge of sources of microbial contamination and level of education; however, they reported that growers with college degrees were able to identify more sources of contamination versus farmers with high school degrees or less (Sinkel et al., 2018). Pivarnik and researchers (2018) reported that there were no significant differences in knowledge based on farm size (medium vs

small) but trainings on food safety practices had significant impacts on farmers knowledge ($P<0.005$).

More awareness and education are needed among farmers, and previous research has identified that growers prefer hands-on/experiential events as well as text-based materials as techniques for learning new information (Perry et al., 2019). It has been demonstrated that enhancing food safety among consumers is relevant as it may lead to a decrease in foodborne illnesses (Mohammad et al., 2020). More awareness is also needed from the grower's side as previous research reports that some growers consider that food safety standards are unnecessary and are unlikely to undergo third party audits (Preguber and Gilroy). Food safety is a shared responsibility among food handlers and food safety can be achieved when the knowledge is translated into a food safety culture among all the interested parties (Yiannas, 2015; Mohammad et al., 2020). Farmers Market managers and farmers should be more aware of increasing training programs among small and medium sized farms, especially those that do not have a food safety certification. In addition, when foodborne illness is traced back to local markets, it can compromise the future of locally grown food and reduce economic viability (Harrison et al., 2013).

Additionally, there is a need for the development of visual-based educational materials for diverse audiences (Strohbehn et al., 2018). When visiting farmers markets, most of the participants mentioned having poor knowledge regarding the Produce Safety Rule. In addition, there is a need for educational tools for Hispanic and other non-English speaking communities, where some of the farmers from Latino origin mentioned the lack of understanding of the PSR due to the language barrier. Research conducted in Iowa described challenges in the PSR implementation including the lack of understanding of food safety requirements and best practices. In addition, challenges in worker training include workers with different backgrounds and primary languages, lack of

record keeping, trainers with previous on-farm experience. In terms of delivery for the trainings, some farmers reported that they prefer to work at their own pace by using technologies like virtual platforms, but others reported that they prefer human contact (Strohbehn et al., 2018).

Research conducted on consumer behavior reported that consumers have very few understandings of food safety practices for handling produce, for instance 50% reported that they do not wash their hands before handling produce (Scott et al., 2009). The same research reported that educational programs are effective among consumers, showing statistical differences among consumer's attitudes towards produce safety before and after the training. Some of the statements evaluated included topics related to foodborne illnesses caused by bacteria on produce, washing and sanitizing produce, storage, and others (Scott et al., 2009).

5.4.2. Animal related responses

Microbial contamination from soil is generally associated with the presence of manure, in the form of treated or untreated biological soil amendments (Gutierrez & Adhikari, 2018). Farmers (57.58%) indicated the use of biological soil amendments derived from animal origin like raw manure, bone meat or others. Sinkel et al., (2018) reported in their study that in Kentucky, 54% of the surveyed farmers use composted manure. A study conducted by Chen et al., (2016) suggested that growers have limited knowledge about soil amendments criteria in the PSR even though most of them reported the use of manure on their farms. Harrison and others (2013) reported that in Georgia, South Carolina and Virginia, most of the farmers surveyed the use manure 56% (n = 128). Most of them reported the use of poultry litter or manure including cattle, horses, and other

BSAAO. The use of untreated or poorly treated BSAAO can compromise food safety, emphasizing the necessity to educate growers in this field.

In addition, the presence of animals themselves close to the produce fields can compromise the microbial quality of produce. From our survey, 43.94% of farmers reported having domestic animals like cows, dogs, cats, pigs, chicken, turkey, or other animals in a proximity of 10-400 ft to the land used for growing crops. The study conducted in Georgia, South Carolina and Virginia reported that 51.8% of the farmers had farm or domesticated animals (Harrison et al., 2013)

5.4.3 Agricultural water

Microbial parameters for water are established by the PSR. The USDA is not currently conducting inspections in agricultural water. It is important for farmers to determine a baseline before full inspections take place. Agricultural water can be a source of bacterial, viral, and parasitic human pathogens (Draper et al., 2016), indicating how essential is to meet with the PSR parameters. Corrective actions for agricultural water are expensive and challenging because of several factors that can affect the prevalence of microbial contamination in water sources (Rodrigues et al., 2020). The most common foodborne pathogens associated with produce in the past decades include Norovirus, *Salmonella enterica* serovar Typhimurium and *Escherichia coli* O157:H7. Evidence suggests that crops can internalize these pathogens. Internalization of foodborne pathogens depends on production systems, initial inoculum, pathogen type, plant type, route of entry and microbial ecology factors (Garcia et al., 2020). Many growers are not fully aware of the PSR and research conducted by Chen and others (2021) indicated that there is limited

knowledge and understanding on the water parameters established by the PSR, hence, more education and awareness is needed (Chen et al., 2021)

From our survey it was identified that 75.76% of the growers supplement their water needs with irrigation but only 37.88% have previously tested their water sources for microbiological purposes and just 36.36% are currently testing the microbial quality from the water sources. Post- harvest water used for washing produce can also be a vector of microbial contamination, therefore microbiological quality of water is crucial. Growers (78.79%) reported washing their produce before it leaves the farm, similar to the findings from Lichtenberg, and Page (2016) that reported that 60% growers in the Mid-Atlantic region of the US wash produce after harvesting. In the past there was a lack of research in agricultural water quality conducted in the southeast region. States like Georgia and Florida are now conducting research in microbial quality of water sources (Rodrigues et al., 2020). Research conducted among growers from Georgia, South Carolina and Virginia reported that most farmers used surface water to supplement their irrigation needs. In the same research, it was reported that 39.8% of the respondents used tested well water, 9.7% used municipal water followed by 15.6% who used untested well water sources. The remaining respondents mentioned the use of other water sources. For washing the produce, less than half (30.4%) of the growers used tested well water (Harrison et al., 2013). Results obtained by Sinkel et al., (2018) indicated that in Kentucky only 47% of the surveyed growers reported managing water quality and just 29% of the participants tested their water sources. A study conducted in the Mid- Atlantic region of the US (Pennsylvania, New Jersey, Maryland, Massachusetts, New York, Ohio, Vermont, and West Virginia) indicated that 36% of the growers tested their water sources. The same study reported that water testing was more frequent among larger farms (Lichtenberg and Page, 2016). In previous research it is mentioned that both education and personalized

attention to on-farm food safety practices have improved the knowledge among farmers as well attitudes and motivation to implement on-farm food safety practices (Pivarnik et al., 2018)

5.4.4 Cleaning and hygiene

Equipment and tools used during production and post harvesting practices can be a source of microbial contamination. Our results reported that 84.85% of the growers disinfect or clean the tools used. Similar results are reported by Lichtenberg and Page (2016) where 72% of growers in the Mid- Atlantic region confirmed the sanitation and cleaning of the facilities.

Toilet facilities are important because farmers and their employees can be vectors of microbial contamination if they do not wash their hands properly. From the data collected it was indicated that 62.12% of the growers have access to toilet facilities near the work area. Mohammad and researchers (2020) reported that most farmers markets in Arkansas and Texas do not have appropriate facilities for handwashing, refrigeration and restrooms which can represent food safety issues and challenges in the proper implementation of food safety practices. Harrison et al., (2013) reported that 66.8% (n = 151) and 66.4% (n = 150) of the farmers that completed the survey indicated the access of hand washing and bathroom facilities near the field. Research conducted in Europe reported that most farmers markets have access to electricity, hand washing facilities, toilers, rubbish collection and cleaning (Worsfold et al., 2004).

5.5. Conclusion

The information recovered from this survey can be used as a baseline for Alabama food safety educators in developing tools and education materials for growers. Identifying current usage and gaps in food safety practices among produce growers can help develop tools for food safety

training among growers to reduce produce contamination and foodborne outbreaks in Alabama. It can be concluded from this research that more awareness about produce safety is needed, as well more accessible educational materials and tools.

5.6. Tables and Figures

Table 5.6.1 Survey responses of Alabama farms on food safety practices

Question	YES (%)	NO(%)
Do you have access to training related to good agricultural/ food safety practices?	60.61	39.39
Are you trained in farm food safety practices such as those listed in the Food Safety Modernization Act Produce Rule or those included in Good Agricultural Practices (GAPs)?	46.97	53.03
Do you supplement your water needs with irrigation?	75.76	24.24
Have you ever tested your irrigation or wash water source(s)?	37.88	62.12
Are you currently testing your irrigation or wash water source(s)?	36.36	63.64
Do you use biological soil amendments?	57.58	42.42
Do you have a written food safety plan for your farm?	28.79	71.21
Do you have domestic animals?	43.94	56.06
Do you have toilet facilities near the work area?	62.12	37.88
Do you wash your produce before it leaves the farm?	78.79	21.21
Do you disinfect or clean any tools like knives, scissors?	84.85	15.15

*n=66 farmers completed the survey

5.7. References

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