MICROBIOLOGICAL AND CHEMICAL QUALITY OF LEAFY GREEN VEGETABLES IN ACCRA, GHANA

by

JOYCELYN KWANSIMA QUANSAH

(Under the Direction of Jinru Chen)

ABSTRACT

The study was undertaken to determine vegetable farming and selling practices in Accra, Ghana; microbial quality of leafy green vegetables grown or sold in the area; antibiotic resistance profiles of *Salmonella* isolated from the vegetables; and the effectiveness of sanitation methods commonly used by Ghanaian households in inactivating *Salmonella*. Questionnaires were administered to vegetable farmers (n = 102) and sellers (n =37), and a total of 328 exotic (lettuce and cabbage) and indigenous (*Amaranthus*, *Solanum macrocarpon, Hibiscus sabdariffa*, and *Corchorus olitorius*) leafy green vegetables were subsequently collected. Microbial quality of collected samples was determined. Lettuce and cabbage inoculated with *Salmonella* were treated with sterile water or sanitizers commonly used by Ghanaian households. The effectiveness of the treatments was compared and consumer preference on treated vegetables evaluated. Survey results revealed that water from waste drains and poultry manure were commonly used in vegetable farming. Vegetables were transported in sacks (87%) and stored under non-refrigerated conditions. Results of microbial analysis revealed mean aerobic bacteria, yeast and mold, fecal coliform, and enterococcus counts on collected vegetables were 8.80, 4.95, 4.90, and 3.67 log CFU/g, respectively. *Salmonella* was isolated from 10% of the vegetables. The *Salmonella* isolates (n = 33) were resistant to at least one antibiotic and approximately 30% of the isolates were multidrug resistant. One (3%) *Salmonella* isolate tested positive for integrase gene and class 1 integron gene cassette (800 kb in size) with a single gene, *dfrA7*. Results of the sanitation study suggest that treatments of cabbage with chlorine, citric acid, peracetic acid, and vinegar and lettuce with citric acid were significantly ($p \leq 0.05$) effective in reducing *Salmonella* counts compared to the other evaluated sanitizers. A 97-member consumer panel preferred ($p \leq 0.05$) cabbage treated with citric acid, vinegar, and water and lettuce treated with citric acid and water. In conclusion, vegetable farmers and sellers in the area need additional trainings on food safety. The sampled leafy green vegetables had poor microbial quality, therefore consumption without sanitizing or heat treatment should be discouraged. Sanitizing vegetables with vinegar or citric acid at homes is an effective approach to prevent vegetable transmitted infectious diarrheal diseases.

INDEX WORDS: Leafy green vegetables, vegetable farming practice, vegetable selling practice, *Salmonella*, antibiotics, integrons, sanitation

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by

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DEDICATION

I dedicate this work to the Lord Almighty for seeing me through my studies successfully. I also dedicate it to my husband, Elvis Richard Ganyo for encouraging me to pursue my dreams abroad, and my family especially my parents, Rev. and Mrs. Quansah, and siblings, Jonathan Quansah (MB ChB) and Jennifer Quansah, for their support.

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CHAPTER 1

INTRODUCTION

Increasing agricultural activities in urban and peri-urban areas have been observed in many industrialized and developing countries in the world. Urban and peri-urban agriculture are major sources of fresh foods, income, and dietary variety for the urban poor. These activities also help in waste management in urban areas by recycling most organic waste into compost, which are used in cultivation (FAO, 1996; RUAF, 2009). Vegetable farming is the most practiced urban agriculture in most cities due to the highly perishable nature of produce and closeness to market centers (Oberholtzer et al., 2014; Obuobie et al., 2006).

Vegetables are important components of human diets and are good sources of vitamins, minerals, and dietary fiber. Consumption of fruits and vegetables are encouraged to promote overall human health (USDA and HHS, 2010). Various exotic and indigenous Ghanaian vegetables are cultivated in major cities in Ghana, for either commercial purposes or household sustenance (Danso et al., 2014). Vegetable cultivation in urban areas in most countries including Ghana are challenged with access to land and potable water, as well as environmental and industrial pollution which makes use of waste water very common (Adedeji and Ademiluyi, 2009; Danso et al., 2014). There are numerous public health concerns associated with the use of untreated or improperly treated waste water, surface water, animal manure, and other organic waste in vegetable cultivation. Multidrug resistant human pathogens have been isolated from animal fecal matters or irrigation waters used in vegetable farming in Ghana, Nigeria, and Portugal (Aibinu et al., 2007; Andoh et al., 2016; Araújo et al., 2017; Sekyere and Adu, 2015). Previous studies have linked vegetable farming practices such as the use of polluted irrigation water and manure with high incidence of human pathogens, enteric bacteria, helminths, viruses, and chemical contaminants in leafy green vegetables (Castro-Rosas et al., 2012; Ensink et al., 2007; Keraita et al., 2014; Woldetsadik et al., 2017; Yu et al., 2016).

Contaminated leafy vegetables have been involved in several foodborne illness outbreaks in U.S., Canada, as well as some European and African countries (CDC, 2004; Der et al., 2013; EFSA and ECDC, 2015; Herman et al., 2015). Most of these outbreaks were caused by bacteria (*Salmonella* and *E. coli*) or viruses (hepatitis A and norovirus virus) which can be transmitted through the fecal-oral route (Callejón et al., 2015; De Roever, 1999; Kozak et al., 2013). Leafy green vegetables may be consumed raw with minimal processing or no kill steps, thus have been identified as potential vehicles for transmitting bacterial pathogens and other microbiological hazards (CDC, 2014; FAO/WHO, 2008). Although no sanitizing method can completely remove contaminants from leafy vegetables, washing of vegetables before consumption is recommended to minimize foodborne illness (FDA, 2011; Zander and Bunning, 2014). Previous studies have shown that washing practices have varying efficacy in reducing the microbial counts on vegetables (Amoah et al., 2007b; Fishburn et al., 2012; Pezzuto et al., 2016).

The objectives of this study were:

1. To identify agricultural and selling practices of leafy green vegetable farmers and sellers in Accra, Ghana

2. To determine the microbial quality of exotic and indigenous leafy green vegetables grown or sold in Accra, Ghana
3. To determine the antibiotic resistance profile of *Salmonella* isolated from leafy green vegetables
4. To compare effectiveness of some sanitation methods commonly used by Ghanaian households to a few sanitation approaches used by the fresh produce industry in the U.S. in reducing the population of *Salmonella* artificially inoculated on leafy green vegetables

CHAPTER 2

LITERATURE REVIEW

1.0 Urban and peri-urban agriculture

Urban and peri-urban agriculture (UPA) include agricultural activities such as production, processing, and distribution, which occur within and around cities for home consumption and or for the urban market. UPA includes production of crops, poultry, livestock, fisheries, and forestry (FAO, 2010; RUAF, 2009). Marked differences may exist between urban and peri-urbans areas, but there are no universally accepted differences or descriptions for urban agriculture and peri-urban agriculture. These are due to varying population sizes considered as urban area between countries, use of administrative boundaries of cities for urban areas, distinguishing between boundaries of urban and peri-urban areas and between peri-urban and rural areas (Drechsel et al., 2014, 2006).

Urban and peri urban agricultural activities utilize resources such as lands and water that could also serve other purposes to satisfy the requirements of the urban population. UPA occurs on backyards, community gardens, rooftops, as well as used and unused public and private spaces such as school grounds and prisons. UPA is a major source of fresh and perishable foods, dietary variety, and food security, especially to inhabitants of urbans areas (FAO, 1996; RUAF, 2009). According to Lee et al. (2010), UPA supplies about 50% of urban fresh vegetables in some cities. About 70% of the lettuce sold in major cities in Ghana originate from urban farms (Henseler and Amoah, 2014). UPA is also a source of income for the under or unemployed in urban centers and recycling of organic urban waste as compost in farming (FAO, 1996; RUAF, 2009). UPA is the main source of income for about 80%, 77%, or 96% of people engaged in its activities in some cities in Sierra Leone (Lynch et al., 2013), Nigeria (Lawal and Aliu, 2012), and Zambia (Simatele and Binns, 2008), respectively.

UPA is practiced in both developed and industrialized countries in the world including Ghana, Nigeria, Burkina Faso, Australia, Hong Kong, Singapore, China, Argentina, Bolivia, Brazil, Mexico, and U.S.A. About 800 million people were estimated to be engaged in UPA in the 1990's and this number has been projected to increase over the years. Increase in UPA activities has been attributed to the increasing rate of urbanization, especially in most developing countries that has affected food supplies from the rural areas and increasing population of the urban poor (FAO, 2014, 2010; Oberholtzer et al., 2014; Smit et al., 2001).

Vegetable farming is the most practiced form of UPA in most West African countries, Asia, and U.S.A. due to their highly perishable nature, close proximity to the market centers, minimal transportation cost, and lack of appropriate cold transport and storage facilities, especially in developing nations (Lee et al., 2010; Oberholtzer et al., 2014; Obuobie et al., 2006).

1.1 Characteristics of urban vegetable farming

UPA can be categorized according to their location, crops cultivated, tenure modality, scale of production, seasonality, and produce target market (Drechsel et al., 2006). The major forms of UPA practiced in some west African countries are open-space farming where high-value products are grown all year round or seasonally mainly for commercial purposes, home or backyard farming where produce are cultivated usually for home consumption and livestock rearing in or around one's compound. Indigenous and exotic vegetables are cultivated in urban farms in most West African cities (Drechsel et al., 2006).

UPA usually takes place on smaller land sizes (less than 0.2 ha) in the urban areas and larger land sizes (about 2 ha) in the peri-urban areas. Most urban farmers cultivate on the same piece of land for several years. According to Drechsel and Dongus (2010) about 70% of urban farmers in Ghana have been cultivating on the same piece of land for 10 to 20 years. Family members or laborers are hired to assist in the farming activities. The farming activities are usually laborious with lot of manual activities like land preparation, weeding and irrigation. People engaged in urban vegetable farming in most West African cities are migrants from the rural areas with agrarian background, especially those who cannot secure alternative jobs (Adedeji and Ademiluyi, 2009). Increasing urbanization and human activities coupled with the poor state of sanitation infrastructure and waste management systems in some developing countries have led to contamination of most surface water bodies. Access to potable water is usually limited and expensive for the farmers, therefore they rely on polluted water bodies for irrigation (Obuobie and Hope, 2014).

Men are generally involved in urban agriculture more than women in most Latin American and West African countries. Men are associated with commercially oriented UPA, while women are involved with small scale UPA for home consumption. Women also have less access to lands and capital needed for urban agriculture (Drechsel et al., 2006; RUAF, 2009). However, women are usually engaged in urban farming in most South and East African countries (Kenya, Uganda, Zambia and Zimbabwe), mainly due to their primary responsibility of home management, and because women have less access to education thus limited job opportunities in the formal sector (Obuobie and Hope, 2014).

1.2 Farming and post-harvest practices in urban agriculture

Rain-fed agriculture and seasonal cropping predominate in most cities in West Africa where access to other sources of water is limited (Bellwood-Howard et al., 2015). To meet year-round vegetable production demands, various sources of water are employed for irrigation of urban farms. Most farms are situated around major water bodies like rivers and lakes. Other water sources used include domestic pipe water, waste drains, shallow wells, roof catchment tanks, and streams as observed in Kenya, Ghana, India, Togo, and other African and Asian countries (Ensink et al., 2008; Mukundi et al., 2014). The farmers prefer wastewater for irrigation because of it is available in sufficient quantities almost all the year, costless, and perceived to be rich in nutrients. Irrigation is usually done in cool times of the day (morning or late afternoon). Irrigation appliances or methods employed include drip, furrow, bucket, and watering cans. The farmers often use 15-liter watering cans to repeatedly convey water from the water body to the farm, with an estimated distance of 50 to 100 m. Small motorized pumps are increasingly being employed in spray irrigation to ease labor (Obuobie and Hope, 2014; RUAF-CFF, 2007).

Fertilizers are used to maintain soil fertility in urban agriculture. This is due to the intensive use of most lands and short fertility recovering fallow periods. Manures are often used in West African countries with or without inorganic fertilizers in vegetable cultivation. Improperly composted manures are commonly used by some farmers. Depending on the quality of the soil, as much as 20 to 100 tons of manure are applied per hectare of land in a year. Some farmers prefer to use poultry manure due to its low cost, high nutrient content, and fast nutrients release for vegetables (Drechsel et al., 2006).

Vegetables attract a wide range of pests and diseases, thus making intensive use of pest management in farming activities very necessary. The continuous use of the same land in most urban farms promotes build-up of pests and diseases. Different types of pesticides (herbicides, insecticides, fungicides and other agrochemicals) are used in vegetable farming to control pest and disease, reduce crop losses, and improve yield (Dinham, 2003).

Women are predominantly involved in harvesting, processing, and marketing of vegetables in UPA (RUAF-CFF, 2007). Some of the women vegetable sellers enter into contracts with some farmers to pay in part in advance for some crops. This enables the farmers to purchase farm inputs to ensure good harvest. These sellers do harvest the best vegetables for themselves first (Obuobie and Hope, 2014). Hope et al. (2008) observed that some vegetable sellers in Kumasi, Ghana wash their vegetables with irrigation water on the farms to remove soil particles and earthworms to make the vegetables look good. The vegetables are transported to various markets and other retail points under non-refrigeration conditions. According to Amoah et al. (2007a), some market sellers wash their vegetables with water before display, although such waters may not be frequently changed.

1.3 Challenges of urban and peri-urban agriculture

Despite the numerous benefits of urban agriculture, UPA is associated with numerous challenges in both developed and developing countries. According to Oberholtzer et al. (2014), access to land, zoning, city plans, access to water and water management, livestock within cities, and environmental contamination are major challenges faced by UPA farmers in the U.S.A. Similar challenges are encountered by farmers in Nigeria, Ghana, and Zambia (Drechsel et al., 2014; Lawal and Aliu, 2012; Simatele and Binns, 2008). Adedeji and Ademiluyi (2009) observed that access to land and the fear of eviction from various farm lands prevent the farmers in Nigeria from investing in their activities and tend to grow only fast growing seasonal crops. Other challenges associated with UPA are poor access to capital, transport and storage infrastructure, and market facilities (Lawal and Aliu, 2012).

1.4 Urban vegetable farming in Ghana

Ghana is a country in the West African sub-region with a population of about 25 million people in 2010 (Ghana Statistical Service, 2012). Accra is the capital and the largest city in Ghana. It is located in the Greater Accra region of Ghana which is the smallest region by land size, but the second most populous region in Ghana, with over 4 million people in 2010 (Ghana Statistical Service, 2012).

UPA is practiced in major cities of Ghana including Accra, Kumasi, Tamale, Takoradi, and Cape Coast. About 47 ha of land within Accra is under year-round vegetable cultivation and seasonal vegetable farming mixed with cereals occurs on 251 ha for commercial purposes (Danso et al., 2014). About 70 ha of land are used for individual homes or backyard farming. Plot sizes used by the farmers ranged from 0.01 to 0.02 ha in the urban areas and about 2.0 ha in the peri-urban areas. UPA serves as job for over 2,000 urban exotic vegetable farmers and 5,300 street food sellers in these cities. UPA is also source of food for about 800,000 daily consumers (Drechsel et al., 2014).

Vegetable farming is the most practiced urban agriculture. Over eight towns within Accra are noted for urban vegetable farming for decades. Three main forms of UPA vegetable farming are practiced in Ghana. They are urban farming where vegetables are grown all year round mainly for commercial purposes, peri-urban farming where vegetables are grown on seasonal basis, and home or backyard farming where vegetables are cultivated usually for home consumption (Drechsel et al., 2014).

Exotic Ghanaian vegetables such as lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea* var. *capitata*), green pepper (*Capsicum annuum*), spring onion (*Allium fistulosum*), cucumber (*Cucumis sativus*), and cauliflower (*Brassica oleracea* var. *botrytis)* are cultivated by most vegetable farmers. Indigenous Ghanaian vegetables such as tomatoes (*Solanum lycopersicum*), okro (*Abelmoschus esculentus*), *Corchorus* sp., *Amaranthus* sp., garden egg (*Solanum aethiopicum* L. Gilo), and hot pepper (*Capsicum frutescens)* are cultivated in addition to some exotic vegetables (Danso et al., 2014).

The vegetable farms are usually situated near to a source of water including pipe, wells, streams, and drains to meet year-round irrigation supply for vegetable cultivation (Drechsel et al., 2014). Organic fertilizers (poultry and cow manure) are mostly used in vegetable cultivation in addition to inorganic fertilizers such as nitrogen-phosphorus-potassium. Various types of pesticides are used for disease and pest control on the vegetables (Danso et al., 2014).

The market sellers usually harvest the vegetables with or without support from the farm laborers. Harvested vegetables are transported to the markets under non-refrigeration conditions, usually in sacks or baskets. The vegetables do not undergo major processing.

They are washed to remove soil and other unwanted materials, and old leaves trimmed to make them look attractive for sale (Danso et al., 2014).

2.0 Microbial quality of urban leafy green vegetables

Previous studies have linked some vegetable farming practices such as the use of improperly composted manure and polluted irrigation water in urban and peri-urban areas with high incidence of indicator microorganisms, human pathogens, helminths, viruses, and chemical contaminants in leafy green vegetables (Castro-Rosas et al., 2012; Ensink et al., 2007; Keraita et al., 2014; Woldetsadik et al., 2017; Yu et al., 2016).

The use of soils amended with improperly composted manures in vegetable cultivation have been observed to contribute to vegetable contamination by pathogens (Amoah et al., 2005). Bartz et al. (2017) found a significantly positive correlation between levels of microorganisms (coliforms, enterococcus, and *Escherichia coli*) on vegetables and those found in soil, irrigation water, and on hands of farm workers. Castro-Rosas et al. (2012) reported that about 99% (129/130) or 85% (110/130) of ready to eat salads from urbans farms irrigated with untreated sewage water tested positive for fecal coliforms and *E. coli*, respectively in Pachuca-City, Mexico. Cabbage, lettuce, and spinach irrigated with waste water in Ethiopia had aerobic mesophilic, total coliform, and fecal coliform counts of 7.9 - 8.3, 6.5 - 6.8 and 5.4 - 5.8 log CFU/g, respectively (Benti et al., 2014). Cobbina et al. (2013) observed that lettuce irrigated with wastewater in Tamale, Ghana had total coliform, fecal coliform, and *E. coli* counts of 4.1, 3.7 and 3.3 log CFU/g, respectively. Total coliform, fecal coliform, and *E. coli* counts on vegetables correlated positively with those in wastewater used for irrigation. Abakpa et al., (2013) reported fecal coliforms counts of 11.61 and 12.29 log CFU/g on lettuce irrigated with waste water during the dry and wet seasons, respectively in Nigeria. Lettuce cultivated with poultry manure and irrigated with water from well, stream, and waste drain had fecal coliform counts of 3.0 - 8.0 MPN/100 g in Ghana (Amoah et al., 2007a). Yu et al. (2016) observed 92.1% of 214 fresh vegetables cultivated in an urban area in China contained organophosphorus pesticide residue while 23.4% of the vegetables contained organophosphorus pesticide residue above maximum residue limits.

Harvested vegetables can be contaminated due to improper handling during processing, transportation, and storage. Amoah et al. (2007a) reported that vegetables followed from the farm gate to the retail point were high in microbial counts, although the difference was not significantly different from counts of the vegetables collected at the farm gate. Ensink et al. (2007) followed harvested vegetables from the farms to the market centers and observed the market samples had higher *E. coli* counts compared to the same batch of vegetables collected from the farm.

2.1 Indicator and pathogenic microorganisms

Although some fresh produce may be contaminated with pathogenic microorganisms, detection or quantifying of these pathogens on fresh produce can be challenging. This is due to their low population levels and non-uniform distribution in the farm environment as well as relatively long time required for their detection (Heredia et al., 2016).

Pathogen indicators are microorganisms or group of microorganisms whose presence in number/quantity above specific limits indicate the presence of a pathogen (Cordier and ICMSF, 2013). Detection of representative indicator microorganisms is easier and could be used to indicate the presence of pathogenic microorganisms. Some microorganisms are, therefore used to indicate product safety or quality to some degree. However, use of a single indicator is not ideal, and therefore, the use of multiple indicators is recommended in assessing food quality. Commonly used indicator organisms include total coliforms, fecal coliforms, fecal streptococci, *E. coli*, and enterococci (Tyagi et al., 2006). *E. coli* habits in intestines of vertebrate animals, thus its presence in food can indicate the possibility of fecal contamination and presence of pathogens of fecal origin (Tyagi et al., 2006). Enterococci habit intestines of warm blooded animals and humans. Their presence in foods indicate possible fecal contamination (Boehm and Sassoubre, 2014).

Holvoet et al. (2014) observed no correlation between indicator microorganisms and the presence of pathogens in leafy green vegetables, however, high counts of indicator microorganisms have been observed by Tyagi et al. (2006) to significantly correlate with presence of pathogenic microorganisms. Furthermore, Pan et al. (2015) observed a significant association between the populations of fecal coliforms and *E. coli* with *Salmonella* presence on leafy and non-leafy vegetables. They reported that with additional fecal coliforms and *E. coli* increase in 100 CFU per g of vegetable, the probability of *Salmonella* contamination increased by 15%. Ijabadeniyi et al. (2011) observed that aerobic bacteria counts and anaerobic spore former counts could predict the presence of *Listeria monocytogenes* and *Salmonella*, respectively on broccoli and cauliflower. High *E. coli* and coliform counts were found to be meaningful indicators of *Salmonella* in lettuce and strawberries (Moneim et al., 2014).

2.1.1 *Salmonella*

Salmonella are rod-shaped, gram-negative, flagellate, and facultative anaerobe in the *Enterobacteriaceae* family. The genus *Salmonella* is divided into two species, *Salmonella enterica* and *Salmonella bongori*. *S. enterica* is further recognized as six subspecies; I, II, IIIa, IIIb, IV, and VI based on their biochemical reaction and genomic relatedness (Li et al., 2013). Biochemical identification of *Salmonella* is based on serological methods involving agglutination of bacterial surface antigens with *Salmonella* specific antibodies such as somatic (O) lipopolysaccharides on bacterial outer membrane, flagella (H) antigens, and the virulence capsular (Vi) antigen which occurs only in *S*. Typhi, *S*. Paratyphi C, and *S*. Dublin (Li et al., 2013; Pui et al., 2011).

Salmonella are ubiquitous microorganisms that can survive in different environments including food production environments. They are prevalent in most food animals such as poultry and cattle and their products (Li et al., 2013). *Salmonella* can also contaminate fresh produce due to inappropriate pre- and post-harvest practices like the use of inadequately composted manure and contaminated irrigation water (Berger et al., 2010; Islam et al., 2004).

Consumption of *Salmonella*-contaminated foods leads to the onset of salmonellosis with symptoms of gastroenteritis, fever, and abdominal pains (Li et al., 2013). *Salmonella* infection leads to about 16 million cases of typhoid fever, 1.3 billion cases of gastroenteritis, and 3 million deaths globally each year (Pui et al., 2011). According to the World Health Organization report (2015), non-typhoidal *Salmonella* (NTS) is responsible for about 32,000 deaths per year in Africa. Typhoid fever was among the 20 leading causes of outpatient illness in Ghana from 2002 to 2016, with about 384,704 cases recorded in

2016 (Ghana Health Service, 2017). Abdullahi et al. (2014) observed that 43.7% of patients diagnosed with pyrexia and gastroenteritis in three hospitals in Katsina state Nigeria tested positive for NTS. Among children <15 years admitted to a rural hospital in Mozambique, 26% of the 1,550 cases were caused by NTS (Sigauque et al., 2009). In Kenya, 10.8% of 3,296 children on admission with bacteremia were infected with NTS (Muthumbi et al., 2015).

2.2 Foodborne illness associated with leafy green vegetables

Leafy green vegetables are rich sources of vitamins, minerals, and other nutrients to human. However, consumption of contaminated leafy vegetables have been implicated in several outbreaks in Australia (16%), Brazil (18%), Canada (12%), Finland (13%), and Sweden (5%) from 1996 to 2006 (FAO/WHO, 2008).

Contaminated leafy vegetables were involved in three outbreaks of infections from 2007 to 2012 in some European countries (EFSA and ECDC, 2015). Leafy green vegetables were implicated in 78% of 501 outbreaks in the U.S. that occurred between 1998 to 2012 (Herman et al., 2015). Salad vegetables were identified as the source of an outbreak of infection in Koforidua, Ghana (Der et al., 2013). In Zambia, consumption of raw vegetables were linked to cholera epidemic in 2003 to 2004 (CDC, 2004). Most of these outbreaks are caused by bacteria (*Salmonella* and *E. coli*) or viruses (hepatitis A and norovirus) which can be transmitted through the fecal-oral route (Callejón et al., 2015; De Roever, 1999; Kozak et al., 2013).

3.0 Susceptibility of vegetable-borne pathogens to antibiotics

Pathogenic microorganisms on vegetables may gain resistance to antibiotics through farming practices such as the use of incompletely composted manure. Fecal materials from food animals commonly used as manure in vegetable production have been reported to contain pathogens (*Salmonella, E. coli, L. monocytogenes*), with some pathogens being multidrug resistant (MDR) (Bako et al., 2017; Nightingale et al., 2004; Sekyere and Adu, 2015). MDR *Salmonella* have been isolated from fecal matter of poultry, cattle, or pigs in Ghana (Adzitey et al., 2015; Andoh et al., 2016; Sekyere and Adu, 2015), Kenya (Nyabundi et al., 2017) and Egypt (Abdel-Maksoud et al., 2015). Antibiotic resistant bacteria or resistance genes can also be transmitted to fresh produce like fruits and vegetables through the use of contaminated irrigation water and/or contaminated processing environments or surfaces and human processors (CDC, 2017; Verraes et al., 2013).

3.1 Antibiotics

Antibiotics are low molecular weight substances used to kill or inhibit microorganisms. They are used for the treatment and prevention of illness in humans and food animals (Giguère, 2013a). Antibiotics can be grouped into various classes based on site of action, mechanism of activity, class of microorganism, bacteriostatic or bactericidal activity, and time or concentration dependent activity (Giguère, 2013a).

3.2 Classes of antibiotics based on site of action

The main sites of antibiotic action in bacteria are on the cell wall (beta lactams), ribosome (aminoglycoside, macrolides, tetracyclines and chloramphenicol), nucleic acid (quinolones and novobiocin), cell membrane (polymyxins and ionophores) and folate synthesis (sulphonamides and diaminopyrimidines) (Greenwood and Whitley, 2003).

Beta-lactam antibiotics are antibiotics possessing a four-membered lactam ring in addition to varying side chains. Their mechanism of action is by inhibiting the formation of bacteria cell walls during the final stages of peptidoglycan biosynthesis by destruction of the amide bond of the beta-lactam ring by beta-lactamases (Prescott, 2013a). Beta-lactams block the cross link of glycopeptide polymeric units of the cell walls *via* selective inhibition of penicillin-binding proteins (PBPs) (Greenwood and Whitley, 2003; Prescott, 2013a). Examples of beta lactams are penicillin (amoxicillin and ampicillin), cephalosporins (cefotaxime, cefoxitin and ceftriaxone), monobactams, carbapenems, and vancomycin (Greenwood, 2003a; Bush, 2003).

Aminoglycoside antibiotics consist of amino sugars linked to aminocyclitols by glycosidic bonds. They have specific affinity to the 30S subunit of bacterial ribosome and cause misreading of the genetic code, leading to interruption and inhibition of protein synthesis (Dowlings, 2013; Dzidic et al., 2008; Greenwood and Whitley, 2003). Examples of aminoglycosides are streptomycin, gentamicin, kanamycin and neomycin (Boehr et al., 2003).

Sulphonamides are synthetic analogs of para-aminobenzoic acid, which competitively inhibit the activity of dihydropteroate synthetase (DHPS) from catalyzing condensation of para-aminobenzoic acid (PABA) and dihydro-6-hydroxymethylpterin-pyrophospahte (DHPPP) to dihydropteroic acid in the early stage of folic acid synthesis in bacteria. This leads to inhibition in dihydrofolic acid formation (Prescott, 2013b; Sköld, 2000). Common sulphonamides include sulfamethoxazole, sulfisoxazole, sulfadiazine, and sulfamethizole (Greenwood, 2003b). Sulphonamides are normally used in combination with diaminopyrimidines to inhibit sequential stages in folic acid synthesis and thus purines needed for DNA synthesis. Diaminopyrimidines inhibit the activity of dihydrofolate reductase which interferes with synthesis of tetrahydrofolic acid from dihydrofolate in the later stages of folic acid synthesis (Greenwood and Whitley, 2003; Prescott, 2013b). Example of diaminopyrimidines is trimethoprim (Greenwood and Whitley, 2003).

Macrolides are antibiotics that inhibit protein synthesis by reversibly binding to the 50S subunit of ribosome. They inhibit transpeptidation and translocation processes, leading to detachment of incomplete polypeptide chains (Giguère, 2013b). Examples of macrolides are erythromycin and clarithromycin (Bryskier and Butzler, 2003).

3.3 Mechanisms of antibiotic resistance

Changes in the susceptibility of antibiotics do occur with time and that reduces the therapeutic value of antibiotics (Li et al., 2013). Bacterial resistance to antibiotics can occur naturally over time due to random mutation in chromosomal genes and horizontal gene transfer. Horizontal gene transfer is a common mode for bacterial cells to acquire antibiotic resistance genes (Blair et al., 2014; Munita et al., 2016).

Misuse and overuse of antibiotics in food animal production especially in cattle and poultry production have been suggested to exert a selective pressure for the development of antibiotic resistant bacteria (Gillings, 2014; WHO, 2017). The main mechanisms of bacterial resistance to antibiotics are by i) inactivation of antibiotic before or after entry into bacterial cell by production of specific enzymes, ii) modification of bacterial cell wall to become less permeable and accessible by antibiotics, iii) alteration in antibiotic target site thus decreasing its affinity for the antibiotic, iv) activation of efflux systems to expel antibiotic from the cells, and v) modulation of metabolic pathways to bypass antibiotic target (Blair et al., 2014; Munita et al., 2016; Struelens, 2003).

Antibiotic resistant genes are located on mobile DNA elements such as integrons, plasmids, bacteriophage, and transposons. Integrons are site-specific recombination systems that allow a bacterium to capture and express exogenous genes (Gillings, 2014; Munita et al., 2016). They are often involved in the dissemination of antibiotic resistance, especially among gram-negative bacterial pathogens (Boucher et al., 2007; Gillings, 2014). Integrons consist of *intI*, a gene which encodes for integron integrase required for site-specific recombination, a contiguous recombination site (*attI*) that is recognized by the integrase, and a promoter (Pc) for transcription and expression of gene cassettes present (Gillings, 2014). Plasmids are self-replicating genetic elements that carry genes that impart selective advantage to their host bacterium (Boelin and White, 2013). Transposons are discrete segments of DNA capable of changing their position within a chromosome by transposition. They consist of repetitive DNA sequences at ends to facilitate their excision from the genome and transposase genes required to catalyses the excision. Transposons re-enter the genome at random positions (Boelin and White, 2013).

3.4 Antibiotic use in animal production and its impact on vegetable safety

Use of antibiotics in food animal production is a major transmission route of antibiotic resistant bacteria into animal meats and fecal droppings (CDC, 2017; Verraes et al., 2013). Antibiotics are used in food producing animals to treat or prevent disease and to promote growth. They are commonly used in poultry, cattle, and swine production. There has been an increase in the use of antibiotics in food animal production due to growth in consumer demands for animal products attributed to rising incomes, especially in developing countries (Van Boeckel et al., 2015). About 24% increase in antibiotic use in food animal production was observed in the U.S.A from 2009 to 2015 (FDA, 2016). Farmers have shifted to cost-efficient and vertically integrated intensive production system or large-scale farms which depend on antibiotic use to maintain healthy animals and increase productivity (Van Boeckel et al., 2015). In 2010, the countries that used antibiotics the most in food animal production globally were China (23%), U.S.A (13%), Brazil (9%), India (3%), and Germany (3%) (Van Boeckel et al., 2015).

Abuse of antibiotics in food animal production as observed in some developing countries such as Ghana (Boamah et al., 2016; Sekyere, 2014), Nigeria (Adesokan et al., 2015), Rwanda (Manishimwe et al., 2017), Vietnam, Indonesia, and Thailand (Usui et al., 2014) is among the factors contributing to development of bacterial resistance to antibiotics in farm animals (WHO, 2017). The antibiotic resistant bacteria and genes are passed into animal fecal matter, therefore, use of such contaminated manure to improve soil fertility in vegetable production can lead to transfer of antibiotic resistant bacteria into vegetables (CDC, 2017; Verraes et al., 2013).

There are several public health concerns associated with increasing use of antibiotics in food animal production and the emergence of MDR pathogens which can affect the safety of fresh produce. Consumption of such contaminated vegetables will be associated with increased burden of illness, increased virulence and limitations of treatment choices. Diseases caused by MDR pathogens are also associated with treatment failure and complications leading to increased mortality (Molbak, 2005; Wegener, 2012).

4.0 Vegetable washing or sanitizing practices

Leafy green vegetables are often consumed raw with minimal processing or no kill steps, thus have been identified as potential vehicles for transmitting bacterial pathogens and other microbiological hazards (CDC, 2014; FAO/WHO, 2008). Although no sanitizing method can completely remove contaminants from leafy vegetables, washing of vegetables before consumption is recommended to minimize food borne illness (FDA, 2011; Zander and Bunning, 2014).

Various agents are employed for cleaning or sanitizing of vegetables; and these agents have been observed to differ between countries. According to Amoah et al. (2007b), bleach, potassium permanganate, salt, lemon juice, and water were commonly used for cleaning vegetables in some Francophone West African cities. These authors also observed that the use of bleach and potassium permanganate was not familiar to Ghanaian consumers, but rather salt, vinegar, lemon juice, and water were used mostly for cleaning vegetables in Ghana. Woldetsadik et al. (2017) identified tap water and solutions of salt, vinegar, detergents, and commercial vegetable sanitizers (not specified) to be the common vegetable washing methods used by some consumers in Ethiopia.

In Italy, some ready to eat vegetable producers used tap water, solutions of sodium hypochlorite, peracetic acid, and percitric acid for washing vegetables while some consumers used tap water, sodium bicarbonate, vinegar, common salt, and some commercial products for washing vegetables (Pezzuto et al., 2016). Some vegetable washing practices in the U.S.A. include the use of running tap water, ozonated water, bleach, veggie wash, and electrolyzed water (Fishburn et al., 2012).

4.1 Efficacy of some vegetable cleaning or sanitizing agents

4.1.1 Chlorine

Chlorine bleach is the most commonly used disinfectant in the food industry due to its efficacy in killing microorganism and cost effectiveness (Joshi et al., 2013; McGlynn, 2004). Available chlorine in the form of hypochlorite and hypochlorous acid and within the pH 6.5 to 7.5 is the effective form of chlorine. Chlorine solutions with pH lower than 6.0 are corrosive and those above pH 8.0 lose their effectiveness as a sanitizer. Chlorine is used in the range of 50 - 200 ppm on fresh produce. The produce must be washed with potable water after chlorine treatment (Goodburn and Wallace, 2013; Joshi et al., 2013; McGlynn, 2004). Chlorine losses its effectiveness in the presence of organic material, dirt, and oil, thus washing of vegetables to remove dirt before disinfection is recommended (Joshi et al., 2013). Treatment with chlorine (200 ppm) solution reduced *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* counts on cabbage by 0.88, 1.61, and 1.29 log CFU/g, respectively (Lee et al., 2014). Lee et al. (2014) also reported 100 ppm chlorine solution reduced *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* population on cabbage by 0.6, 1.45, and 1.08 log CFU/g, respectively. Chlorine (70 ppm free chlorine) treatment led to 2.05, 2.34, and 2.16 log unit reduction in *Salmonella, E. coli* O157:H7, and *L. monocytogenes* counts, respectively, on lettuce (Fishburn et al., 2012).

4.1.2 Ozone

Ozone is a tri-atomic oxygen molecule formed by addition of singlet oxygen to oxygen molecule. Ozone is unstable in both aqueous and gaseous form, and degrades to form hydroxyl, hydroperoxyl, and superoxide radicals (Joshi et al., 2013). These radicals can oxidize cellular membrane glycoproteins and/or glycolipids and subsequently oxidize cellular components like DNA and RNA (Joshi et al., 2013; Ölmez, 2012). Efficacy of ozone against microorganisms is affected by ozone concentration, pH, and temperature (Joshi et al., 2013). Ozone has to be generated on site for use because it is unstable and decomposes in water. It can be used in the aqueous or gaseous form. Use of ozone does not pose residue problems on fresh produce because it rapidly decomposes to oxygen (Joshi et al., 2013; Ölmez and Kretzschmar, 2009).

Some previous studies found ozone to be effective against various microorganisms. Ölmez (2010) observed that bubbling ozone and ozonated water reduced *E. coli* on lettuce by 1.97 and 1.19 log CFU/g, respectively. Treatment with ozonated water reduced *S. enterica, E. coli* O157:H7, and *L. monocytogenes* counts on lettuce by 1.4 to 1.8 log CFU/g, respectively (Fishburn et al., 2012). Karaca and Velioglu (2014) reported of 2.07, 1.70, and 2.20 log CFU/g reductions in *E. coli* populations on lettuce, spinach, and parsley, respectively after ozonated water (12 ppm) treatment.

4.1.3 Peracetic acid

Peracetic or peroxyacetic acid is a strong oxidizing agent and equilibrium mixture of hydrogen peroxide and acetic acid. Peracetic acid oxidizes by electron transfer and produces reactive oxygen species, oxidize sulfhydryl and disulphide bonds, disrupts cellular membranes, and denatures proteins and enzymes (Joshi et al., 2013; Kitis, 2004). It is effective in inactivation of bacteria, viruses, and microbial spores. Efficacy of peracetic acid is affected by temperature and pH. It is more effective at pH 7 and loses its efficacy at higher pH values. Efficacy of peracetic acid is not affected by organic compounds (Joshi et al., 2013). Peracetic acid for use on non-raw agricultural commodities must not exceed 80 ppm in wash water (CFR, 2007). It is used as wash water for fresh produce and sanitizing of food contact surfaces. Lee et al. (2014) observed treatment of cabbage with 100 ppm peractic acid reduced the population of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* on cabbage by 0.84, 1.38, and 0.98 log CFU/g, respectively. Samadi et al., (2009) reported treatment with 100 ppm peracetic acid reduced the counts of mesophilic microbial counts, total coliform, fecal coliforms, and fecal streptococci by 2.8, 1.3, 1.8, and 1.5 log CFU/g, respectively on leafy green vegetables.

4.1.4 Organic acids

Acetic, citric, and lactic acids are common organic acids used in sanitizing fresh produce. Reduction in pH is the main mechanism of antimicrobial action of organic acids. At low pH, undissociated organic acids can penetrate bacterial cell membranes, ionize and lead to lower internal pH and subsequently affects cellular enzymes function, nutrient transport, and alteration of cell membrane permeability (Brul and Coote, 1999; Theron and Lues, 2010). Akbas and Ölmez (2007) observed that treatments with 1% lactic acid, 1% citric acid, 1% acetic acid, and 1% ascorbic acid caused 3.0, 3.1, 2.4, and 2.1 log CFU/g reduction, respectively on *E. coli* populations on lettuce. They also reported that treatment with 1% lactic acid, 1% citric acid, 1% acetic acid, and 1% ascorbic acid caused 2.2, 1.8, 1.4, and 1.3 log CFU/g reduction, respectively, of *L. monocytogenes* counts on lettuce. Sengun and Karapinar (2005) reported lemon juice with 4.16% citric acid and vinegar with 3.95% acetic acid reduced *S.* Typhimurium counts on rocket leafy (*Eruca sativa*) vegetables by 2.95 and 2.20 log CFU/g, respectively after 15 min treatment. Use of organic acids may have a negative effect especially at high concentration on the sensory quality of fresh produce (Ölmez and Kretzschmar, 2009).

4.1.5 Electrolyzed oxidizing water

Electrolyzed water or electrolyzed oxidizing water is ionized water produced by passing diluted salt solution through an electrolytic cell across a bipolar membrane (Huang et al., 2008; Joshi et al., 2013). During electrolysis, positively charged ions (Na^+, H^+) are drawn to the cathode, resulting in sodium hydroxide and negatively charged ions (Cl^-, OH^-) drawn to the anode which results in hypochlorous acid and hydrochloric acid. Two types of water are produced simultaneously; an acidic solution (acid electrolyzed water) with low pH (2-3) and high oxidation reduction potential (>1000 mV) and basic solution (basic electrolyzed water) with high pH (10 – 11.5) and low oxidation reduction potential (<800 mV). Neutralized electrolyzed water is produced by mixing the positively charged ion solution with OH^- or using a single-cell chamber. It has pH of 7-8 and oxidation reduction potential of 750 mV (Hricova et al., 2008; Joshi et al., 2013).

The main antimicrobial action of electrolyzed water is through the ability of hypochlorous acid to penetrate cell membranes, dissociate into ions, oxidize cellular components and disrupt metabolism activities. Other proposed antimicrobial mechanisms of electrolyzed water include destruction of microbial membranes by chlorine compounds or oxidizing strength due to the high oxidation reduction potential (Hricova et al., 2008; Huang et al., 2008). Antimicrobial action of electrolyzed water is affected by temperature, agitation, and presence of organic compounds. High temperature makes bacterial membranes fluid-like and enables easy penetration of the electrolyzed water across the membrane of bacteria. Agitation also promotes entry of electrolyzed water into inner cellular components (Hricova et al., 2008). Stopforth et al. (2008) observed acidified electrolyzed water reduced counts of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* on leafy green vegetables by 2 – 2.5 log CFU/g. Neutralized, acidified, and alkaline electrolyzed water reduced counts of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* on cabbage by 0.85-1.68, 0.75-1.78, and 0.91-1.92 log CFU/g, respectively (Lee et al., 2014).

4.1.6 Cooking salt

Cooking salt is composed primarily of sodium chloride. They serve other purposes such as drying fishes or sanitizing vegetables aside in cooking to enhance the taste of foods. The efficacy of salt as a sanitizing agent is due to its ability to cause alternation of trans-membrane osmotic pressure. High salt concentration puts osmotic pressure on bacterial cytoplasmic membrane, pulls it away from the cell wall, causing lysis and eventually death of bacterial cells (Hogg, 2005; Nester et al., 2003). According to Amoah et al., (2007b), 7 ppm salt solution caused 1.4 log MPN reduction in fecal coliform counts on lettuce. They also observed that increasing the salt concentration to 35 ppm resulted in 2.1 log MPN reduction. However, increasing salt concentration had deteriorating effect on the lettuce (Amoah et al., 2007b).

4.1.7 Water

Water is the most common cleaning agent used at homes or in the fresh produce industry for washing vegetables. Water is used to remove soil and other unwanted materials attached to vegetables. It may be used alone or in addition to other sanitizing agents (O’Connor-Shaw, 2004). Although water has no antimicrobial properties, previous researchers have observed reduction in microbial counts when used to wash vegetables. Water reduced the level of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* counts on lettuce by 0.21 - 0.61, 0.4 - 0.89 and 0.16 - 0.87 log units, respectively (Lang et al., 2004). Fishburn et al. (2012) reported that rinsing lettuce with running tap water reduced *Salmonella, E. coli* O157:H7, and *L. monocytogenes* counts on lettuce by 1.50 to 1.60 log units. Amoah et al. (2007b) observed washing lettuce with running tap water reduce fecal coliforms counts by 2.2 log MPN.

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CHAPTER 3

LEAFY GREEN VEGETABLE FARMING AND SELLING PRACTICES IN ACCRA, GHANA

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Abstract

Vegetable farming is the most practiced urban agriculture in cities in Ghana. Exotic and indigenous Ghanaian vegetables are cultivated usually for commercial purposes. This study determined the agricultural practices of urban vegetable farmers as well as post-harvest and selling practices of vegetable sellers in Accra, Ghana. Exotic (lettuce and cabbage) and indigenous (*Amaranthus* sp., *Solanum macrocarpon, Hibiscus sabdariffa*, and *Corchorus olitorius*) leafy green vegetables farmers, and sellers were identified before survey questionnaires were administered to 102 farmers from 12 different farming areas and 37 vegetable sellers from 4 market centers. Survey results revealed that vegetable farming was dominated by men while women dominated vegetable selling in the same region. Most farmers cultivated exotic leafy green vegetables more than the indigenous ones. Water from waste drains pumped into shallow wells were the common source of irrigation water. Availability of water and distance to water source influenced the choice of water used for irrigation. Improperly composted manure was commonly used in farming. Vegetables were usually harvested using bare hands and knives and vegetables were transported in sacks to market centers under non-refrigerated conditions. Vegetable sellers mostly considered availability of vegetables when buying from farmers or middlemen. Majority of leafy vegetables sold in Accra markets originated from farming areas outside of Accra, Ghana. Most farmers and sellers who participated in the survey disagreed that the use of polluted irrigation water can contaminate vegetables or make consumers sick. Results suggest that vegetable farmers and sellers in the region need essential trainings on microbial safety of fresh vegetables. Production of vegetables with clean irrigation water and properly composted manure should be encouraged. Harvested produce should be stored under cool temperatures.

Key words: leafy green vegetable**,** vegetable farming practice, vegetable selling practice, manure, irrigation

1.0 Introduction

Urban and peri-urban agriculture (UPA) are agricultural practices taking place within and around cities which compete for resources that could also serve other purposes to satisfy the requirements of the urban population (FAO, 2010). Globally, over 800 million people are estimated to be engaged in UPA and this is attributed to increasing urbanization, which has affected food supply from rural areas. UPA is practiced in some countries in Africa (Ghana, Nigeria, Mali, Cameroon, Burkina Faso, Togo), Asia (Hong Kong, Singapore, China), and Latin America (Argentina, Bolivia, Brazil, Mexico). It is a significant source of fresh and perishable food, income, and food security (FAO, 2014, 2010, 1996).

UPA is practiced in major cities of Ghana including Accra, Kumasi, Tamale, Takoradi, and Cape Coast. Vegetable farming is the most practiced urban agriculture in Ghana (Danso et al., 2014). Most Ghanaian vegetable farmers plant exotic vegetables such as lettuce, cabbage, green pepper, spring onion, cucumber, and cauliflower, while other farmers plant indigenous vegetables including tomatoes, okro, *Corchorus* sp., *Amaranthus* sp., garden egg, and hot pepper in addition to some exotic vegetables (Danso et al., 2014). The main types of vegetable farming include urban farmers that grow vegetables all year round for commercial purposes, peri-urban farmers that grow vegetables on seasonal basis, and home or backyard gardeners that grow vegetables usually for home consumption. Most vegetable farms are situated close to a water source for irrigation (Drechsel et al., 2014). Fertilizers and pesticides are used in vegetable cultivation. The vegetables are usually bought by wholesalers and retailers who transport them to various markets (Amoah et al., 2007).

The objective of this study was to determine agricultural practices of urban vegetable farmers as well as postharvest and selling practices of vegetable sellers in Accra, Ghana.

2.0 Materials and Methods

2.1 Survey and administration of questionnaire to vegetable farmers and sellers

Vegetable farms in and around the Accra Metropolis district of Ghana where exotic (lettuce and cabbage) and indigenous (*Amaranthus* sp., *Solanum macrocarpon, Hibiscus sabdariffa*, and *Corchorus olitorius*) leafy green vegetables are cultivated were identified. Vegetable sellers in four major market centers within the same region were also identified. Vegetable farmers and sellers who were willing to participate were included in the study during the period from March 2016 to March 2017. Questionnaires were administered to 102 farmers from 12 different farming areas to obtain information on some farming and postharvest practices associated with leafy green vegetable cultivation. Questionnaires were also administered to 37 vegetable sellers from four market centers to obtain information on some postharvest handling and selling practices.

2.2 Statistical analysis

Fecal coliform and *Enterococcus* counts of leafy green vegetables subsequently collected from the farmers and sellers that participated in the study and the results of microbial surveys have been reported in Chapter 4 of this dissertation (Quansah et al., 2018). In the present chapter, the Seemingly Unrelated Regression (SUR) model of Statistical Data Analysis (STATA) Software (2010) was used to correlate the fecal coliform or enterococci counts recovered from the collected vegetables reported in Chapter

4 with vegetable farming and selling practices reported by the farmers in their responses to circulated questionnaires. The basic SUR system assumes that for each individual observation *i* there are M cross-sectional units, each with its own linear regression model (Greene, 2003):

$$(1) \qquad y_{ij} = X_{ij}\ \beta_j + \varepsilon_{ij}, \qquad i=1, \ldots, N, j=1, \ldots, M.$$

The distinct property of the SUR model is that it allows nonzero covariance between error terms ε_{ij} and ε_{ik} for a given individual *i* across equations *j* and *k*:

$$(2) \qquad Cov(\varepsilon_{ij}, \varepsilon_{ik}) = \sigma_{ij}$$

$$(3) \qquad Cov(\varepsilon_{ij}, \varepsilon_{i'k}) = 0 \qquad if\ i \neq i'.$$

In this study, we used the SUREG procedure available in STATA which uses the asymptotically efficient, feasible generalized least-squares algorithm developed in Greene (2003). The resulting GLS estimator, which was designed to address heteroscedastic and autocorrelated disturbances, is given by the following:

$$(4) \qquad \beta = [X'\Omega^{1}X]^{-1} X'\ \Omega^{1}\, y = [X'\ (\Sigma^{1} \otimes I)\ X]^{-1} X'\ (\Sigma^{1} \otimes I)\ y.$$

Our model includes the following two equations, one for each of the two measures of possible contaminants as the dependent variable. The explanatory variables include the demographic attributes of farmers, enterprise dummy variables capturing types of leafy green vegetables planted, a set of farming, irrigation, and post-harvest practices employed by the farmers, extension or training services received by the farmer respondents, and food safety knowledge. These two estimating equations are defined as follows:

$$(5) \qquad FEC_t = \beta_{01} + \beta_{11}\, DEM_t + \beta_{21}\, LGV + \beta_{31}\, FPP + \beta_{41}\, EXT + \beta_{51}\, FSK + \varepsilon_1$$

$$ENT_t = \beta_{02} + \beta_{12}\, DEM_t + \beta_{22}\, LGV + \beta_{32}\, FPP + \beta_{42}\, EXT + \beta_{52}\, FSK + \varepsilon_2$$

where FEC is the fecal coliform counts and ENT is the *Enterococcus* counts of sampled leafy green vegetables. LGV is the leafy green vegetable enterprise dummy variable (taking on a value of 1 for each type of leafy vegetable and 0 if a different vegetable is planted, with lettuce as the excluded category), FPP is a set of variables capturing varied farming, irrigation, and post-harvest practices, EXT is a set of dummy variables for the types of extension services or training assistance received by the farmer respondents, and FSK are several ordinal variables that collect the farmers' opinions on and perceptions of certain food safety issues. This system of equations is estimated for a general model based on all farm observations in the sample. The SUR approach to this empirical issue is justified by the results of the Breusch and Pagan (BP) test of independence conducted on the different models. The BP test yielded a chi-square test statistic with significant *p*-value that refutes the BP test's null hypothesis of independence. In other words, this result indicates the presence of contemporaneous correlation between residuals of the equations in the system, thus justifying the use of the SUR estimation technique. Microsoft Excel 2016 was used to sum the various variables.

3.0 Results

3.1 Demography of the vegetable farmers

Most people engaged in leafy green vegetable farming in Accra, Ghana were males (98%; Table 3.1). Approximately, 70% of the farmers were aged between 18 and 45 years, 20.6% were between the ages of 46 and 60 years while 9.8% were older than 60 years. About 50% of the farmers had no formal education while none of them had tertiary education. However, some of the farmers had education up to the primary (16.7%), junior high school (20.6%), and senior high school (13.7%) levels. Most of the farmers were Muslims (90.2%) and were of the Mole-Dagbon and other ethnic groups (92.2%) whose homeland is in the three Northern regions of Ghana.

3.2 Farming characteristics

Vegetable farming in urban areas of Accra, Ghana were observed to be on small-scale, with 70.6% of the farmers having farms with sizes less than 0.4 ha, 25.5% had farm sizes between 0.4 to 1.2 ha and 3.9% had farm sizes greater than 1.2 ha. Approximately, 57% of the farmers worked alone on their farms while 41.2% of farms had 2 to 4 farm workers (Table 3.2). Some of the farmers had been cultivating vegetables for less than 10 years (29.4%), 11 to 20 years (34.3%), 21 to 30 years (25.5%), and 31 to 50 years (9.8%). It was observed that most of the farmers (57%) had been planting on their current farm lands for 10 years or less. Few farmers (4%) had been planting on the same farm land for 31 years or above, and one of the farmers had been planting vegetables on the same farm land for over 50 years (Table 3.2).

The farmers were observed to cultivate exotic leafy green vegetables more than the indigenous ones. All the farmers involved in this study cultivated lettuce, while 84.3% of the farmers cultivated cabbage (Table 3.2). Among the indigenous Ghanaian leafy green vegetables, *Amaranthus* sp., *Corchorus olitorius, Hibiscus sabdariffa*, and *Solanum macrocarpon* were cultivated by 46.0%, 35.3%, 25.5%, and 28.4% of the farmers, respectively. Market demands (73.5%) and weather conditions (50%) were found to be the major factors that influenced the type of vegetables cultivated. Soil conditions (20.6%) and expertise (13.7%) were other factors that the farmers considered on the type of vegetables to plant. The farmers rotated the types of vegetables they cultivated due to low soil fertility (59%), to minimize the spread of pests and diseases (44%), and due to changes in weather conditions (6%). Other vegetables cultivated by the farmers included cauliflower (*Brassica oleracea* var. *botrytis)*, onions (*Allium cepa*), carrot (*Daucus carota* var *sativus*), beet root (*Beta vulgaris*), Chinese cabbage (*Brassica rapa* var *chinensis*), and okro (*Abelmoschus esculentus*) (Table 3.2). More farmers planted cabbage once or twice a year and lettuce 5 to 6 times in a year. Indigenous leafy vegetables were mostly cultivated once or twice a year, but some were planted as often as 7 to 8 times a year (Table 3.3).

3.3 Irrigation

Although all the farmers depended on rainfall for their vegetable farming, they also depended on various forms of surface water to irrigate the vegetables. Water from waste drains pumped into shallow wells (70.6%) were the most common source of irrigation water. Water from rivers (19.6%), pipe (8%), pond (3%), stream (2%), and bore hole (2%) were other sources of water used for irrigation (Table 3.4). Availability of water (98%) and distance to water source (65.7%) were the major factors influencing the choice of water used for irrigating leafy green vegetables. Cleanliness of the water (3.9%) and cost of water (6.9%) were the factors least considered in choosing irrigation water. Watering can (70.6%), small motorized pumps connected to sprinkler (60.8%), and water hose (4.9%) were the appliances used for irrigating vegetables. Most of the farmers irrigated their vegetables 3 to 4 times (49%) or 7 to 8 times (34%) in a week.

3.4 Fertilizers and pesticides use

It was observed that both organic and inorganic fertilizers were used in vegetable cultivation in Accra, Ghana. Nitrogen-phosphorus-potassium (NPK) was the most used inorganic fertilizer in addition to ammonia and urea (Table 3.4). All the farmers that participated in this study (99%) except one used poultry manure and 8.8% of farmers used cow manure in cultivation. About 70% of the farmers used manure after composting for 30 days or less, 17.6% of the farmers used manure after 60 to 120 days of composting, while 4% and 1% used manure after 150 to 180 days and 360 days of composting, respectively. The farmers usually waited for at least 1 to 4 weeks or 5 to 8 weeks after fertilizer application before harvesting their vegetables. Fertilizers were usually applied once during the growing cycle of both exotic and indigenous vegetables. Some farmers also used manure two or three times during a cycle while a few applied manure four times in cabbage growing cycle (Table 3.3).

Various pesticides were used in vegetable production to control pests and diseases. Attack (Emamectin benzoate, 63.7%) and Bypel (*Pieris rapae* Granulosis virus and *Bacillus thuringiensis,* 41.2%) were the pesticides used by most farmers for cultivation. Akape (Imidacloprid, 28.4%), Mectin (Abamectin, 23.5%), Golan (Acetamiprid, 20.6%), Sunhalothrin (Lambda cyhalothrin, 14.7%), and fungicide (copper hydroxide, 6.9%) were the other pesticides used in vegetable farming (Table 3.4). About 62% of the farmers harvested their vegetables 1 to 7 days after pesticide application. Other farmers (27.5%) also harvested their produce 8 to 14 days after pesticide application. Most farmers applied pesticides about five (20.6%) or eight (36.3%) times during the growing cycle of cabbage (Table 3.3). Some farmers (10.8%) applied pesticides about 10 times during the growing cycle of cabbage. Majority of the farmers applied pesticides four (30.4%) or seven (20.6%) times during lettuce growing cycle. Pesticides were applied less often on the indigenous leafy green vegetables during a growing cycle compared to exotic leafy green vegetables.

Pesticides were applied once or twice during the growing cycle of indigenous vegetables (Table 3.3).

3.5 Harvesting and post-harvest practices

The farmers usually harvested the vegetables with bare hands (96%) and knives (71.6%). After harvest, the vegetables were placed into sacks (79%), baskets (51%), rubber bags (12.7%), buckets (7%), or unto bare grounds (45%; Table 3.5). The farmers did not store or carried out any form of processing on the harvested leafy vegetables. The vegetables were harvested usually when the buyer was ready to pick them up. The buyers came to the farms to buy the vegetables, but a few of the farmers (4%) transported the harvested vegetables to busy buyers at the buyer's cost. Vegetable sellers (99%) were the main customers that bought vegetables from the farmers. Household (54%), street side and small food vendors (44%), and operators of hotels and restaurants (33%) also bought vegetables from the farmers. The harvested vegetables were transported to various market centers, retail outlets or homes in sacks (85%), baskets (29%), and rubber bags (27%; Table 3.5).

3.6 General food safety knowledge

Most of the farmers that participated in the survey disagreed that the use of polluted irrigation water can contaminate the vegetables (53%) or make consumers sick (52%) while other farmers (36% and 38%, respectively) had opposite opinions (Table 3.6). About 46% of the farmers agreed while 41% of the farmers disagreed, that chemicals or contaminants in pesticides and fertilizers can get into vegetables. Approximately 42% of the farmers agreed and 46% of the farmers disagreed that chemicals or contaminants in pesticides and fertilizers can get into soil and surrounding waters.

3.7 Demography of market vegetable sellers

Vegetable sellers from the four markets centers were all females. About 62% of them were between the ages of 18 to 45 years, 32% were between 46 to 60 years while 5% were more than 60 years of age (Table 3.7). Out of the 37 sellers, 10 had no formal education and 14, 11, and 2 had primary, junior high school, and senior high school education level, respectively. About 49% of the vegetable sellers belonged to the Akan ethnic group, others were Ewe (13.5%), Ga-Dangme (16.2%), and Mole-Dagbon and other Northern Ghana ethnic groups (21.6%). The sellers were either Christians (75.7%) or Muslims (24.3%).

3.8 Vegetable selling practices

It was observed that vegetable sellers who sold exotic leafy green vegetables did not sell indigenous leafy green vegetables and likewise. Exotic vegetable sellers sold carrot, cauliflower, broccoli, and other salad vegetables in addition to cabbage and lettuce. In addition to the four indigenous leafy green vegetables used in this study, indigenous vegetable sellers sold okro or other indigenous leafy green vegetables. Availability of the vegetables (75.6%), market demands (54%), and expertise (32.4%) were the main factors that influenced the types of vegetables a seller sold.

Majority of the sellers had been selling vegetables for 10 years or less (59.5%). About 32% and 8% of sellers had been selling for 11 to 25 years or more than 25 years, respectively (Table 3.8). Availability (75.7%) of a vegetable was the major factor considered in buying vegetables from a farmer or middleman. Appearance (18.9%) of the vegetable, farming practices (18.9%) of the farmers, vegetable price (8.1%), and customer relations with the farmers (8.1%) were other factors considered in buying vegetables (Table 3.8).

Most of the leafy green vegetables sold in the markets in Accra were found to be cultivated in areas outside of Accra or Ghana. According to the sellers, most of the vegetables originated from the Ashanti (35%), Eastern (27%), and Volta regions (16%) of Ghana with few coming from the Greater Accra region (21.6%). Some vegetables sold in Accra markets came from neighboring countries like Togo and Burkina Faso (Table 3.8).

Leafy green vegetables were transported to the various market centers usually in sacks by trucks, vans, and taxicabs. Rubber bags and baskets were sometimes used for transporting the vegetables (Table 3.8). At the end of a day, unsold vegetables were packed into sacks, rubber bags, or wooden boxes, and kept on bigger wooden shelves in the open space or storage rooms at ambient temperature. Only one seller reported of storing her vegetables in cold storage at a fee. About 92% of the vegetable sellers washed their produce with water to remove soil before display.

3.9 General food safety knowledge

Most of the vegetable sellers disagreed (49%) that the use of contaminated irrigation water can contaminate the vegetables or make consumers sick (Table 3.9). Some of the sellers agreed that chemicals or contaminants in pesticides and fertilizers can get into vegetables (22%) or into soil and surrounding waters (32%) while others disagreed (38%, Table 3.9).

3.10 SUR models

From the SUR model (Table 3.10), leafy green vegetables grown in the farming areas 10, 11, and 12 were associated with lower fecal coliform counts compared to other farming areas. Farms operated by older farmers, by farmers with no formal education, or by farmers planting on current farm lands for a longer period of time were associated with producing vegetables with higher fecal coliform counts compared to all other farmers. Additionally, farmers with smaller farm size produced vegetables with higher fecal coliform counts compared to those with a large farm size. Lettuce was associated with higher fecal coliform counts than cabbage and *S. macrocarpon*, while *C. olitorius* was associated with higher fecal coliform counts compared to lettuce.

Farmers who used higher quantities of nitrogen-phosphorus-potassium fertilizer or poultry manure produced vegetables with high fecal coliform counts. The use of higher quantities of Golan pesticide was also associated with high fecal coliform counts while the use of lower quantities of Mectin pesticide was associated with high fecal coliform counts. The shorter the waiting period after pesticide application before harvest of vegetables, the higher the fecal coliform counts.

According to the SUR model, vegetables from the farmers who disagreed that use of contaminated water can make consumers sick or agreed that chemical in pesticides and fertilizers can get into vegetables had high fecal coliform counts. Farmers who received less training in pesticide use or farmers with more training in fertilizer use produced vegetables with high fecal coliform counts.

Leafy green vegetables grown in farming areas 4, 5, and 6 had higher enterococcus counts compared to the other farming areas. Farmers with no formal education or farmers who had been planting for more years on their current farm lands produced vegetables with higher enterococcus counts (Table 3.10).

According to the model, use of lower quantities of NPK fertilizer or Golan pesticide are associated with production of vegetables with high enterococcus counts or use of higher quantities of Bypel pesticide are associated with production of vegetables with high enterococcus counts.

Farmers that agreed the use of contaminated water can contaminate vegetables or that chemical or contaminants in pesticides and fertilizers can get into surrounding water were associated with production of vegetables with high enterococcus counts. Farmers, who disagreed that the use of contaminated water can make consumers sick, were associated with production of vegetables with high enterococcus counts. Farmers who received less training in pesticide use or farmers with more training in fertilizer use were associated with production of vegetables with high enterococcus counts.

4.0 Discussion

It was observed in this study that vegetable farming was undertaken in various part of Accra, Ghana. Most of the vegetable farmers were male, similar to observations previously made by Drechsel et al. (2006) in Accra and other West Africa cities (Table 3.1). This observation may be due to societal definition of gender roles that farming is a man's job because most of the farming activities such as land preparation and irrigation are done manually (Hope et al., 2009; Obuobie and Hope, 2014). Majority of the farmers were between the ages of 18 and 45 years, which is representative of the working class that have migrated to the cities to search for jobs and ended up with urban farming when they were unable to secure what they had hoped for (Obuobie and Hope, 2014). Islam is the predominant religion in the Northern, Upper East and Upper West regions of Ghana;

therefore, it is not surprising that most of the urban vegetable farmers were Muslims. These farmers are usually migrants from such regions and were engaged in vegetables farming in the study area. The approximately 49% illiteracy rate of the vegetable farmers observed in this study were comparable to reported 48% illiteracy rate of vegetable farmers in Accra area reported by Danso et al. (2014).

As the capital city of Ghana, most lands in Accra are used for infrastructural development, therefore, urban vegetable farmers have difficulty in accessing land for farming. Farming usually occurs on lands belonging to governmental institutions and private developers who have not yet started construction (Danso et al., 2014). It is, therefore, not surprising that most farm sizes were less than 0.4 ha (1 acre) as observed by Ojo et al. (2011) in Nigeria.

Most of the vegetable farmers that participated in the current study cultivated exotic vegetables more than indigenous vegetables (Table 3.2), as reported by previous studies in Ghana and other West African countries (Danso et al., 2014). This observation can be due to the high economic returns associated with the exotic vegetables compared to the indigenous vegetables. Market demands, soil conditions, and expertise are some of the factors that influence the type of vegetables the farmers cultivated as observed in other previous studies (Danso et al., 2014). Crop rotation was practiced by the vegetable farmers in this study due to low soil fertility or as mechanism to control pest and disease infestation. These findings are different compared to those by Danso et al. (2014) who reported that the farmers practiced crop rotation mainly due to seasonal demands.

Wastewater from drains, rivers, and streams were used by most of the farmers to irrigate their vegetables. This may be due to declining availability of fresh water associated with rapid urbanization and lack of waste treatment facilities. The use of wastewater for irrigation in urban vegetable farming has been observed in several countries including Ethiopia (Woldetsadik et al., 2017), South Africa (Gumbo et al., 2010), India (Yadav et al., 2016), and Pakistan (Ensink et al., 2007). Availability of water for irrigation and distance to the water source were more important to the farmers in this study than the cleanliness or cost of the water. This observation may be attributed to the importance of water to meet year-round vegetable cultivation. Watering cans were the common appliance used for irrigating the vegetables as observed in some cities in Ghana, Togo (Keraita et al., 2007, 2003), and Nigeria (Ojo et al., 2011). Small motorized pumps connected with sprinklers were also used by some farmers who could afford them.

Almost all the farmers that participated in this study used poultry manure with or without other inorganic fertilizers for soil fertility maintenance required for year-round vegetable farming. This may be because poultry manure is relatively inexpensive and easily available compared to inorganic fertilizers (Amoah et al., 2007). Some farmers in this study applied poultry manure, with or without proper composting, directly to the soil usually during land preparation before transplant of vegetables as observed by Mensah et al. (2001) in Accra and Kumasi, Ghana.

The farmers applied pesticides to control diseases and pests on the leafy vegetables. It was observed that pesticides were applied more often on exotic vegetables (four or more times per growing cycle) than on indigenous vegetables (three or less times per growing cycle) (Table 3.3). This may be due to the high economic returns and market demands associated with the exotic vegetables compared to the indigenous ones and the comparatively high pest infestation associated especially with cabbage production as claimed by the farmers.

At maturity, the leafy green vegetables were harvested mostly with bare hands or knives. The vegetables were harvested when the buyers were ready to pick up the produce because there were no storage facilities on the vegetable farms visited in the study. Most of the vegetables were sold to vegetable sellers at the farm gate because the sellers wanted to avoid middlemen costs and obtain high quality vegetables for sale (Henseler and Amoah, 2014).

All the vegetable sellers that participated in this study were females as opposed to the male dominance in urban vegetable farming, similar to the report of Hope et al. (2009). According to Obuobie and Hope (2014), this observation may be attributed to the Ghanaian tradition that retailing is a woman's job.

It was observed that availability of vegetable and market demands influenced the type of vegetable the sellers sold. Most of the vegetable sellers claimed their vegetables originated from farms outside of Accra and even outside Ghana while few vegetable sellers (21.6%) obtained their vegetable from farms in Accra. This is similar to report of Henseler and Amoah (2014) that only 35% of lettuce sold in Accra came from farms within Accra.

Vegetables were transported to the market centers usually in sacks without cold transport. The vegetables were also stored at the market centers in the open space due to lack of storage facilities with or without cold temperature and cost associated with using such facilities (Weinberger and Pichop, 2009). Some of the vegetables were washed before display using water in buckets or bowls, although the water was not regularly changed. This practice can lead to the contamination of the vegetables.

Most of the vegetable farmers and sellers in this study disagreed that there is any potential link between irrigation water quality and vegetable quality. Majority of the farmers and sellers also disagreed that there was any potential link between irrigation water quality and consumers sickness, similar to reports of previous studies (Mayilla et al., 2017; Ouedraogo, 2002; Owusu et al., 2012). Similar to our observation, Ouedraogo (2002) reported that farmers in Ouagadougou, Burkina Faso saw no direct link between irrigation water quality and the health of consumers. Owusu et al. (2012) observed that some farmers (192/202) in Kumasi, Ghana using wastewater for irrigation think their produce were wholesome for human consumption. Mayilla et al. (2017) found farmers in Tanzania had very positive perceptions about the use of polluted water for irrigating vegetables. However, according to Keraita et al. (2008), farmers knew that the use of contaminated irrigation water was not accepted but put up defensive strategies by underestimating the risks associated with its use when talking with local authorities, media, health personnel or general public and overestimating the risks associated with its use when they see probability of receiving donations such as farm inputs or equipment.

Vegetables from farming areas 10, 11, and 12 were associated with lower fecal coliform counts compared to those from the other farming areas involved in the study according to the SUR model. This may be attributed to the farming practices like the use of improperly composted manure and contaminated irrigation water for vegetable cultivation on farms associated with produce of higher coliform counts. It was observed in the study that older farmers were more likely to be reluctant in changing their farming practices passed to them by their fathers even after being educated of the detrimental effects from use of these practices. Previous studies in Northern Ghana (Udimal et al., 2017) and

Nepal (Ghimire and Huang, 2016) observed older farmers were less likely to adopt new practices to improve their farming activities compared to younger ones because of lack of interest in long term investment, especially if no children are expected to take over the farms.

A significant relationship existed between farmers with no education and the microbial quality (fecal coliform and enterococci counts) of their produce. Willingness to implement new farming methods that can improve vegetable qualities such as the use of properly composted manure and clean irrigation water can be affected by education. Education increases the likelihood of farmers to adopt new practices because farmers can better understand requirements and implications of these practices (Ghimire and Huang, 2016; Waller et al., 1998). The perception of farmers influence their farming practices (Waller et al., 1998). This supports our observation that farmers who disagreed that the use of contaminated water can make consumers sick were more likely to produce vegetables with high microbial counts because they do not see anything harmful about it.

Livestock manure may contain various microorganisms including *E. coli, Salmonella, Campylobacter*, and*, Enterococcus.* Application of such manure on farm land increases microbial populations in the soil. Continual application of manure on the same piece of land for years increases microbial populations in the soil and subsequently the contaminates vegetables (Atidégla et al., 2016; de Freitas et al., 2003; Venglovsky et al., 2009). It is therefore not surprising that there was likelihood of farmers that cultivated on the same land for more years to produce vegetables with high fecal and enterococcus counts.

Agricultural extension services provide support, scientific research-based information, and skills to solve problems encountered by farmers to improve their productivity and income (Anderson, 2007). Although extension services were provided to most of the vegetable farmers that participated in this study, there was significant correlation between farmers receiving training on fertilizer use and high fecal coliform and enterococcus counts on their vegetables. This observation can be due to the farmers not adopting what they learn from the extension agents, or inefficiency in knowledge transfer process (Asiedu-Darko, 2013). Adoption of practices from extension agents in Ghana have been reported to be affected by the posture of extension personnel, for example treating the farmers as illiterate and can lead to the farmers refusing to adopt the measures because they perceive to be disrespected (Asiedu-Darko, 2013).

5.0 Conclusions

Contaminated irrigation water and improperly composted manure were used in vegetable cultivation in Accra, Ghana. Production of vegetables with clean irrigation water and properly composted manure should be encouraged. Harvested produce should be stored under refrigerated conditions. Vegetable producers and sellers should be educated on how to produce safer food and maintain their quality at all times.

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Table 3.1 Demography of surveyed vegetable farmers in Accra, Ghana

Survey attributes	Frequency
Farming areas (FA) were participating farmers were from	
FA 1	20
FA 2	3
FA 3	6
FA 4	11
FA 5	32
FA 6	8
FA 7	6
FA 8	2
FA 9	3
FA 10	6
FA 11	3
FA 12	2
Age range (years) of participating farmers	
18-30	25
31-45	46
46-60	21
>60	10
Sex of participating farmers	
Male	100
Female	2
Ethnic group of participating farmers	
Akan	1
Ewe	5
Ga-Dangme	2
Mole-Dagbon and other Northern Ghana ethnic groups	94
Religion of participating farmers	
Christian	10
Muslim	92
Level of education of participating farmers	
None	50
Primary school	17
Junior high school	21
Senior high school	14
Tertiary	0

Table 3.2 Characteristics of participating vegetables farms in Accra, Ghana

Survey attributes	Frequency
Farm size (ha)	
0 to 0.40	72
0.41 to 0.80	14
0.81 to 1.20	12
1.21 to 1.60	3
>1.60	1
Number of farm workers	
1	58
2	15
3	17
4	10
5	1
6	0
7	1
Main vegetables cultivated	
Amaranthus sp.	47
Cabbage	86
C. *olitorius*	36
H. sabdariffa	26
Lettuce	102
S. macrocarpon	29
Reason influencing type of vegetable cultivated	
Expertise	14
Market demands	75
Soil conditions	21
Weather conditions	51
Reasons for practicing crop rotation	
Changes in weather conditions	6
Low soil fertility	60
Minimize spread of pest and disease	45
Other vegetables cultivated by farmers	
Beet root	9
Carrot	10
Cauliflower	31
Chinese cabbage	7

Okro	6
Onion	17
Vegetable cultivation experience (years)	
1 to 5	13
6 to 10	17
11 to 15	19
16 to 20	16
21 to 25	12
26 to 30	14
31 to 40	8
41 to 50	2
>50	1
Planting on current farm land (years)	
1 to 5	35
6 to 10	23
11 to 15	15
16 to 20	10
21 to 25	5
26 to 30	10
31 to 40	3
41 to 50	1
>50	0

Table 3.3 Planting practices of participating vegetable farms in Accra, Ghana

Survey attributes	*Amaranthus* sp.	Cabbage	*C. olitorius*	*H. sabdariffa*	Lettuce	*S. macrocarpon*
How often vegetables were planted in a year						
1 – 2	57	47	64	75	2	71
3 – 4	6	31	7	7	11	3
5 – 6	10	3	6	1	45	7
7 – 8	23	0	21	14	20	17
9 – 10	0	0	0	0	7	0
11 – 12	2	0	2	1	12	1
13 – 14	0	0	0	0	1	0
How often pesticides were applied in a growing cycle						
1	16	2	14	8	7	12
2	10	3	7	5	12	6
3	13	6	8	6	15	7
4	0	4	1	1	31	0
5	0	21	0	0	4	0
6	0	3	0	0	6	0
7	0	0	0	0	21	0
8	0	37	0	0	2	0
9	0	0	0	0	0	0
10	0	11	0	0	0	0

How often fertilizers were used in a growing cycle						
1	12	47	8	9	76	8
2	4	2	4	6	24	1
3	0	9	0	0	2	0
4	0	3	0	0	0	0
5	0	0	0	0	0	0

Table 3.4 Irrigation and pesticide use practices of participating vegetable farms in Accra, Ghana

Survey attributes	Frequency
Source of irrigation water	
Bore hole	2
Pipe	8
Pond	3
River	20
Stream	2
Waste drains	71
Factors influencing choice of irrigation water	
Availability	100
Cleanliness of water	4
Cost	7
Distance to water source	67
Appliance used in irrigation	
Small motorized pumps with sprinkler	62
Watering can	72
Water hose	5
How often vegetables were irrigated per week (times)	
1 to 2	4
3 to 4	50
5 to 6	3
7 to 8	35
9 to 10	0
> 10	9
Inorganic fertilizers used	
Ammonia	37
NPK (Nitrogen-phosphorus-potassium)	74
Urea	12
None	1
Organic fertilizers used	
Cow manure	9
Poultry manure	101
None	1
Duration of composting manure before use (days)	

30	71
60	6
90	7
120	5
150	1
180	3
210	0
240	0
270	0
300	0
330	0
360	1
Waiting period after fertilizer use before harvest (weeks)	
1 to 4	40
5 to 8	49
9 to 12	0
13 to 16	1
17 to 20	0
>20	0
Pesticides used	
Akape (Imidacloprid)	29
Attack (Emamectin benzoate)	65
Bypel 1 (PrGv.Bt)	42
Fungicide (copper hydroxide)	7
Golan (Acetamiprid)	21
Mectin (Abamectin)	24
Sunhalothrin 2.5 EC (Lambda cyhalothrin)	15
Waiting period after pesticide use before harvest (days)	
1 to 7	63
8 to 14	28
15 to 21	5
22 to 28	1
>28	1

Table 3.5 Harvest and post-harvest practices of participating vegetable farmers and sellers in Accra, Ghana

Survey attributes	Frequency
Vegetable harvesting tools	
Bare hands	98
Knives	73
garden fork	2
Case for vegetable harvesting	
Bare ground	46
Basket	52
Sack	81
Buckets	7
Rubber bags	13
Did you store harvested vegetables	
Yes	1
No	101
Storage of harvested vegetables	
Airy / open place	1
Cold room	0
Selling of harvested vegetables	
Sellers come to farm and buy	102
Take vegetables sell at the market	4
Main Vegetable buyers	
Street side food vendors	45
Hotels and restaurants	34
Households	55
Vegetable vendors	101
Case for vegetable transport to the market center	
Baskets	30
Rubber/poly bags	28
Sacks	87
Did you carry out any form of processing	
Yes	0
No	102
Extension services	
Receive training	93

Training on disease and pest control	65
Training on fertilizer use	69
Training on pesticide use	85

Table 3.6 General food safety knowledge of participating vegetable farmers in Accra, Ghana

Survey attributes	Agree	Neither agree or disagree	Disagree
Use of contaminated irrigation water can contaminate vegetables	37	11	54
Use of contaminated water can make consumers sick	39	10	53
Chemicals or contaminants in pesticides and fertilizers can get into vegetables	47	13	42
Chemicals or contaminants in pesticides and fertilizers can get into soil and surrounding waters	43	12	47

Table 3.7 Demography of participating market sellers in Accra, Ghana

Survey attributes	Frequency
Sellers interviewed from individual market center (MC)	
MC 1	12
MC 2	11
MC 3	9
MC 4	5
Age range (years) of participating vegetable sellers	
18-30	8
31-45	15
46-60	12
>60	2
Sex of participating vegetable sellers	
Female	37
Male	0
Level of education of participating vegetable sellers	
None	10
Primary school	14
Junior high school	11
Senior high school	2
Tertiary	0
Ethnic group of participating vegetable sellers	
Akan	18
Ewe	5
Ga-Dangme	6
Mole-Dagbon and other Northern Ghana ethnic groups	8
Religion of participating vegetable sellers	
Christian	28
Muslim	9

Table 3.8 Vegetable selling practices of participating market sellers in Accra, Ghana

Survey attributes	Frequency
Vegetable selling experience (years)	
1 to 5	11
6 to 10	11
11 to 15	4
16 to 20	2
21 to 25	6
26 to 30	1
31 to 40	1
41 to 50	0
>50	1
Main types of vegetables sold	
Amaranthus sp.	11
Cabbage	19
C. *olitorius*	18
H. sabdariffa	10
Lettuce	19
S. macrocarpon	17
Other vegetables sold	
Carrot	15
Cucumber	13
Green pepper	19
Okro	5
Factors that influence types of vegetables sold	
Expertise	12
Market demands	20
Availability	28
Area/Regions the vegetables come from	
Greater Accra	8
Kumasi, Begoro; Ashanti	13
Aburi - Akuapem mountain; Eastern	10
Aflao; Volta	6
Togo	13
Burkina Faso	1
Vegetables transported to markets in	
Rubber bags	4

Sacks	35
Baskets	6
Washing of vegetables at market center before display	
Yes	34
No	3
Source of vegetable washing water	
Pipe	34
Others	0
Vegetables stored at markets in	
Baskets	20
Rubber bags	2
Wooden boxes	15
Temperature of store room	
Airy / open space	36
Cold room	1
Factors considered when buying vegetables from farmers	
Appearance	7
Availability	28
Customer relation to farmer	3
Farming practice	7
Price	3

Table 3.9 General food safety knowledge of participating vegetable sellers in Accra, Ghana

Survey attributes	Agree	Neither agree or disagree	Disagree
Use of contaminated irrigation water can contaminate vegetables	10	9	18
Use of contaminated irrigation water can make consumers sick	10	10	17
Chemicals or contaminants in pesticides and fertilizers can get into vegetables	12	11	14
Chemicals or contaminants in pesticides and fertilizers can get into soil and surrounding waters	8	14	15

Table 3.10 Seemingly unrelated regression model of leafy green farming practices on fecal coliform and *Enterococcus* counts on the vegetables in Accra, Ghana

Variables	Coefficient	Standard error
Fecal coliform counts		
Farming area 10 -12	-2.3027	0.6267
Age of farmer	0.0293	0.0152
Farmers with no formal education	1.5220	0.5008
Years of planting on current farm land	0.0727	0.0325
Size of farm	-0.2387	0.1136
Cabbage	-1.3903	0.5037
Corchorus olitorius	1.3576	0.5048
Solanum macrocarpon	-1.0303	0.5143
Quantity of NPK fertilizer applied	0.0236	0.0125
Quantity of poultry manure applied	0.0032	0.0009
Quantity of golan pesticide applied	1.3631	0.8273
Quantity of mektin pesticide applied	-2.1544	0.8320
Waiting period after pesticide application before harvest	-0.1192	0.0358
Use of contaminated irrigation water can make consumers sick	-0.6240	03505
Chemical or contaminants in pesticides and fertilizers can get into vegetables	0.6611	0.3368
Receive training in pesticide use	-1.7245	0.6249
Receive training in fertilizer use	1.5482	0.4879
Enterococcus counts		
Farming areas 4 - 6	0.9932	0.4080
Farmers with no formal education	0.6456	0.3275
Years of planting on current farm land	0.0375	0.0211
Quantity of NPK fertilizer applied	-0.0129	0.0075
Quantity of bypel pesticide applied	0.8730	0.2925
Quantity of golan pesticide applied	-1.1821	0.5719
Use of contaminated irrigation water can contaminate vegetables	0.4072	0.2236
Chemicals or contaminants in pesticides and fertilizers can get into surrounding water	-0.4814	0.2154
Receive training in pesticide use	-1.4100	0.4065
Receive training in fertilizer use	1.2633	0.3199
Model statistics		
Breusch-Pagan text of independence, χ^2	13.132	

R^2	0.0003

CHAPTER 4

MICROBIAL QUALITY OF LEAFY GREEN VEGETABLES GROWN OR SOLD IN ACCRA METROPOLIS, GHANA

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Abstract

Samples of two exotic (lettuce and cabbage), and four indigenous (*Amaranthus* sp., *Solanum macrocarpon, Hibiscus sabdariffa*, and *Corchorus olitorius*), leafy green vegetables were collected from 50 vegetable farms in 12 farming areas (n=175) and 37 sellers in 4 major market centers (n=153) in Accra metropolis, Ghana. Microbial quality of collected samples was assessed by isolation of *Salmonella* and enumeration of total aerobic bacteria, yeasts and molds, fecal coliforms, and enterococci. Mean total aerobic bacteria, yeast and mold, fecal coliform, and enterococcus counts on collected vegetables were 8.80, 4.95, 4.90, and 3.67 log CFU/g, respectively. Approximately 75.4% of the vegetables from the vegetable farms 96.0% and 84.3% of the vegetables from 97.3% of the vegetable sellers tested positive for enterococci, and 81.1% of the vegetables from 96.0% of the farms and 83.7% of the vegetables from 94.6% of the vegetable sellers tested positive for fecal coliforms. *Salmonella* were isolated from 5.1% of the vegetables from 16.0% of the vegetable farms and 15.7% of the vegetables from 24.3% of the vegetable sellers. Vegetable source and type had significant influence on the microbial counts. Results revealed that the sampled leafy green vegetables had poor microbial quality. Consumption of fresh leafy green vegetables without sanitizing or heat treatment should be discouraged.

Keywords: indigenous vegetables, cabbage, lettuce, farming areas, market centers

1.0 Introduction

Leafy green vegetables are important components of Ghanaian diets serving as sources of vitamins, minerals, and other nutrients. The vegetables are used as part of a main course or side dish. Both indigenous (cocoyam leaves, *Amaranthus* sp, *Solanum macrocarpon, Hibiscus sabdariffa*, *Corchorus olitorius*, cowpea leaves), and exotic (lettuce, cabbage, spinach, broccoli, Chinese cabbage), leafy green vegetables are currently cultivated and consumed in Ghana (Drechsel et al., 2014). Although indigenous leafy vegetables are often cheaper and more nutritious, exotic leafy vegetables are patronized more, especially by urban dwellers due to changes in lifestyle and diets and lack of public knowledge about nutritional benefits of indigenous vegetables (Darkwa, and Darkwa, 2013).

Vegetable farming usually takes place in the rural areas of Ghana, and harvested vegetables are transported to markets in urban areas. Over the past decades, vegetable farming activities have increased in the urban and peri-urban areas mainly due to increasing market demands, lack of jobs, and changes in lifestyle and diets. Urbanization and increasing population size has, however, led to scarcity of land and water with most famers having access to smaller land size for farming as observed in other West African countries (Drechsel et al., 2014). The farmers situate their farms close to various water sources such as pipe, wells, streams, and drains for irrigation (Drechsel et al., 2014). Both inorganic and organic fertilizers (poultry and cow manure) are used for vegetable cultivation, with poultry manure being commonly used because it is relatively cheaper and easily available (Amoah et al., 2007). Leafy green vegetables are harvested by hand with or without knives into buckets, baskets, or sacks, and then transported to market centers and other retail points under non-refrigeration conditions by market women or middle men. Vegetables are sometimes washed with water to remove dirt before display for sale.

Increased consumption of fresh produce has been associated with an increasing number of foodborne outbreaks in the US, Canada, and European countries (Callejón et al., 2015; Kozak et al., 2013; Lynch et al., 2009). Majority of such outbreaks are caused by bacteria (*Salmonella* and *E. coli*) or viruses (Hepatitis A and Norwalk virus) which can be transmitted through the fecal-oral route (Callejón et al., 2015; De Roever, 1999; Kozak et al., 2013). About 420,000 cases of foodborne illness are reported in Ghana annually with 65,000 deaths (Ababio and Lovatt, 2015). These incidences are believed to be underestimates because most cases of illnesses are not reported to health facilities in Ghana (Ababio and Lovatt, 2015). The number of foodborne illness is likely to increase in Ghana if the consumption of fresh produce increases, a trend that have been observed in other countries (Callejón et al., 2015; Kozak et al., 2013; Lynch et al., 2009).

Indigenous Ghanaian leafy vegetables are mostly used for making stews or soups, which requires minimum to high heat treatments before consumption. Most exotic vegetables are, however, used as side dishes requiring no or minimal heat treatments such as in salads or coleslaws. These leafy vegetables may receive some degree of washing before use. However, research has shown that washing alone is insufficient to reduce microbiological counts on leafy green vegetables to acceptable levels (Almeida De Oliveira et al., 2012; Fishburn et al., 2012). Consumption of leafy vegetables with no or minimal heat treatments makes them probable vehicles for foodborne infections. The objective of this study was to determine the microbial quality of selected leafy green vegetables that are grown and sold in urban areas in Accra Metropolis, Ghana by isolation of *Salmonella* and enumeration of total aerobic bacteria, yeasts and molds, fecal coliforms, and enterococci.

2.0 Material and Methods

2.1 Sample collection

Samples of two exotic, lettuce (*Lactuca sativa*) and cabbage (*Brassica oleracea*), and four indigenous; African spinach (*Amaranthus* sp.), African eggplant leaves (*Solanum macrocarpon),* roselle leaves *(Hibiscus sabdariffa)*, and jute leaves (*Corchorus olitorius)* leafy green vegetables (Fig. 4.1; n=175) were collected in duplicate from 50 farms in 12 different farming areas in Accra Metropolis district of Ghana from March 2016 to March 2017 (Fig 4.2, Table 4.1). Leafy green vegetable samples (n=153) were also collected from 37 sellers in 4 major market centers in the same region. The vegetable samples were collected from farmers who cultivate them or market venders who sell them and were willing to participate in the study. Collected leafy green vegetables were placed into sterile, plastic Ziploc bags (Nasco, Fort Atkinson, WI), kept in a car cooler (Rubbermaid; Newell Brands Inc, Atlanta, GA USA) with ice packs (VWR, Lutterworth, UK), and transported to a microbiological laboratory in the Department of Nutrition and Food Science at University of Ghana. The samples were analyzed immediately upon arrival at the laboratory.

2. 2 Microbial enumeration

Each leafy vegetable sample (25 g) was placed in a sterile whirl-pak bag, and 225 ml of 0.1 M phosphate buffered saline (pH 7.4) was added to the bag. The vegetable samples were rinsed by shaking on a platform shaker (Lab-Line Instrumental Co., Melrose Park, IL, USA) at100 rpm for 30 min at room temperature. A 0.1 ml of appropriate dilutions of vegetable-rinsing buffer was inoculated on four different microbiological media including tryptic soy agar (TSA), Enterococcus agar (EA), MacConkey agar (MAC), and potato dextrose agar (PDA) acidified with 10% tartaric acid to pH 3.5 (Becton, Dickinson and Company, Sparks, MD, USA). Inoculated plates of TSA were incubated at 37 ºC for 24 h and those of EA were incubated at 37 ºC for 24-48 h. The MAC plates were incubated at 44.5 ºC for 24 h and plates of PDA were incubated at 25 ºC for 48-72 h. Colonies of total aerobic bacteria on TSA, yeasts and molds on PDA, presumptive enterococci on EA, and presumptive fecal coliforms on MAC were enumerated after the incubation. *Enterococcus* colonies were confirmed by culturing selected colonies in tryptic soy broth (Becton, Dickinson and Company) with 6.7% NaCl (Fisher Scientific, Pittsburgh, PA, USA) and fecal colonies were confirmed by growth in EC broth (Oxoid Ltd, Basingstoke, Hampshire, England) with inverted fermentation tubes and on triple sugar iron slants (Becton, Dickinson and Company).

2.3 *Salmonella* isolation

For *Salmonella* isolation, a leafy vegetable sample (25 g) was rinsed in 225 ml 0.1% peptone water by shaking on a platform shaker at100 rpm for 30 min at room temperature. The rinsing buffer was incubated at 37 ºC for 24 h and followed by selective enrichment in Rappaport-Vassiliadis (RV) broth with incubation at 42 ºC for 24 h. Subsequently, 0.1 ml of RV broth was inoculated on XLT4 agar with supplement (Becton, Dickinson and Company) for isolation of presumptive *Salmonella* colonies. The colonies were confirmed by growth, on triple sugar iron agar (Becton, Dickinson and Company) and lysine iron agar slants (Becton, Dickinson and Company), and slide agglutination test using *Salmonella* O antiserum poly A- I and VI (Becton, Dickinson and Company).

2.4 Statistical analysis

One-way analysis of variance test was performed, and Fisher's Least Significant Difference test was used to compare the means ($p \leq 0.05$) using the Statistical Analysis Software (Version 9.4). The effect of sample source (farm or market) and vegetable type on vegetable-borne microbial counts were determined.

3.0 Results

The mean total aerobic counts on all sampled leafy green vegetables ranged from 8.30 to 9.20 log CFU/g. The mean yeast and mold counts were from 4.25 to 5.73 log CFU/g (Table 4.2). Mean fecal coliform counts ranged from 4.28 to 5.81 log CFU/g and enterococcus counts from 2.93 to 4.53 log CFU/g. Cabbage samples had the highest mean total aerobic count. Lettuce samples, nevertheless, had the lowest mean total aerobic count which was significantly ($p \leq 0.05$) different from the mean total aerobic counts from the other five types of leafy green vegetables sampled in the study (Table 4.2). Lettuce samples also had the lowest mean yeast and mold count while *C. olitorius, H. sabdariffa*, and *S. macrocarpon* samples had higher yeast and mold counts. *C. olitorius* and *S. macrocarpon* samples also had higher fecal coliform counts and enterococcus counts compared to other types of vegetables sampled in the study. Cabbage and lettuce samples had the lowest mean fecal coliform and enterococcus counts, respectively. In general, the indigenous leafy vegetables sampled in the study had higher microbial counts than lettuce and cabbage, except the counts on *Amaranthus* sp. which were comparable to those from the two exotic vegetables.

There were significant differences in the microbial counts on vegetables collected from various farming areas or market centers (Table 4.2). Vegetables from farming areas

1, 3, and 11 had lower ($p \leq 0.05$) mean total aerobic counts. Yeast and mold counts were higher ($p \leq 0.05$) on vegetables from all the market centers and farming area 3, 4, 6, 7, 11, and 12. Vegetables from farming areas 2, 3, 4, and 5, and markets centers 2, 3, and 4 had significantly ($p \leq 0.05$) higher fecal coliforms counts. Vegetables from market centers 2, 3, and 4 also had higher ($p \leq 0.05$) enterococcus counts and those from farming area 11 had the lowest ($p \leq 0.05$) enterococcus counts.

Mean total aerobic counts on leafy green vegetables collected from the farms *vs*. markets were not significantly ($p \geq 0.05$) different, although most of the counts from market vegetables were relatively higher (Table 4.3). Farm samples had significantly ($p \leq 0.05$) lower fecal coliform and enterococcus counts compared to the market samples, with the exception of cabbage. Indigenous vegetables collected from the farms had lower yeast and mold counts compared to those from the markets. The yeast and mold counts on cabbage and lettuce collected from farms *vs*. market centers were not significantly different.

Amaranthus sp. and lettuce from the farms had lower ($p \leq 0.05$) mean total aerobic counts compared to the other farm vegetables, and there were no significant ($p \geq 0.05$) differences in the total aerobic counts on different vegetables collected from the market centers (Table 4.3). Lettuce from the farms had the lowest ($p \leq 0.05$) yeast and mold count and *C. olitorius, H. sabdariffa* had the highest ($p \leq 0.05$) counts. Among the vegetables collected from the market, cabbage and lettuce had lower ($p \leq 0.05$) yeast and mold counts while *C. olitorius, H. sabdariffa* had higher ($p \leq 0.05$) counts. No significant ($p \geq 0.05$) difference in enterococcus counts was observed on vegetables collected from the farms. Lettuce and cabbage from the market were lower ($p \leq 0.05$) in fecal coliform and enterococcus counts than the indigenous vegetables.

In total, 75.4% of the vegetables from 96.0% of the vegetable farms and 84.3% of the vegetables from 97.3% of the vegetable sellers tested positive for enterococci, and 81.1% of the vegetables from 96.0% of the farms and 83.7% of the vegetables from 94.6% of the vegetable sellers tested positive for fecal coliforms. Prevalence of enterococci and fecal coliforms was 50% or greater in vegetables collected from all the farming areas or market centers (Fig. 4.3). Over 80% of the *Amaranthus*, *C. olitorius*, and *H. sabdariffa* collected from the farms, and *C. olitorius*, *H. sabdariffa,* lettuce, and *S. macrocarpon* collected from the market centers tested positive for enterococci (Fig. 4.4). With the exception of *Amaranthus*, there were higher incidences of enterococci on vegetables collected from the markets than those from the farms. More samples of *Amaranthus*, *C. olitorius,* lettuce, and *S. macrocarpon* collected from the markets tested positive for fecal coliforms than those from the farms (Fig. 4.5).

Salmonella was isolated from 9 vegetable samples (5.1%) collected from 8 farms (16%) in 4 farming areas (33.3%) and 24 vegetable samples (15.7%) collected from 9 sellers (24.3%) in 3 markets centers (75%). Approximately 7.7, 6.7, 2.7, and 31.2% of the vegetables from farming areas 1, 2, 5, and 7 carried *Salmonella* whereas 8.9, 46.3, and 5.3% of the vegetables from market centers 2, 3, and 4 tested positive for the pathogen (Fig. 4.3). The prevalence of *Salmonella* among farm-collected lettuces, cabbage, *C. olitorius*, and *H. sabdariffa* were 7.6, 5.9, 12.5, and 3.8%, respectively and 20.6% and 41.2% of the *C. olitorius* and *H. sabdariffa* samples collected from the market centers carried *Salmonella* (Fig. 4.6).

4.0 Discussion

The levels of total aerobic bacteria counts recovered from leafy green vegetables in this study are comparable to findings of previous studies from certain geographic areas. A study in Lebanon found 9.41 and 8.78 log CFU of aerobic bacteria per gram of lettuce (Halablab et al., 2011). Abdullahi and Abdulkareem (2010) and Manjunath et al. (2017) reported a total bacterial count of 8.36 log CFU/g of cabbage and 7.83 log CFU /g of *Amaranthus*, respectively. Mngoli and Ng'ong'ola-Manani (2014) reported fecal coliform counts of 5.08 to 5.84 log CFU/g of lettuce sampled in Malawi. Fecal coliform counts observed by Cobbina et al. (2013) were 3.7, 3.5, and 3.1 log CFU/g of lettuce, *Amaranthus*, and cabbage, respectively. Some of these reported fecal coliform counts are lower than the results of the present study. Amoah et al. (2006) found that lettuce sampled from some cities in Ghana had higher fecal coliform counts than those from cabbage samples, a finding which is similar to the observation of this study. The recovery of fecal coliforms and enterococci from leafy green vegetables indicates possible fecal contamination and potential presence of enteric pathogens (Boehm and Sassoubre, 2014; New Hamspire department of environmental services, 2003).

Le Quynh Chau et al. (2014) reported a *Salmonella* incidence of 17.6% on fresh leafy vegetables sold in Vietnam. Nma and Oruese (2013) detected *Salmonella* in 42.7% of the cabbage and lettuce samples purchased from markets in Nigeria. Uyttendaele, Moneim, Ceuppens, and Tahan (2014) found that 38.9% and 43.3% of the lettuce collected from farm or retail outlets in Egypt carried *Salmonella*. Although the *Salmonella* incidence observed in this study was relatively lower compared to the incidences reported in some of the developing countries, the presence of the pathogen on leafy green vegetables, especially on lettuce and cabbage which are usually consumed with minimal or no heat treatment is a major food safety concern.

Low microbial quality of leafy green vegetables is usually the results of poor farming practices such as the use of polluted irrigation water, improperly-composted manure, and inappropriate postharvest handling practice (Adetunde et al., 2015; Amoah et al., 2006; Cobbina et al., 2013). Streams, shallow wells, and waste drains are common sources of irrigation water used in vegetable cultivation in Ghana. Wastewater from both households and industries are mostly released into the environment in the untreated form, and eventually ends up or empties into major streams or water bodies used in vegetable irrigation (Keraita et al., 2014). Random testing of irrigation water in the vegetable-growing areas in the present study revealed the presence of high microbial loads (data not shown), which makes it a possible source of vegetable contamination. Adetunde et al. (2015) and Cobbina et al. (2013) analyzed the microbial quality of irrigation water and irrigated leafy green vegetables and found a strong positive correlation between the microbial counts on vegetables and in irrigation water. Adetunde et al. (2015) observed that wild and domestic animals could be the source of irrigation water contamination.

The use of composted and inadequately-composted manure is very common in vegetable production in Ghana and other African countries (Drechsel et al., 2014). Increase in temperature during the composting process inactivates most of the pathogens present (Jones and Martin, 2003). Properly-managed composting can reduce the number of pathogens like *E. coli* and *Salmonella* to non-detectable levels (Lung et al., 2001). However, fecal coliforms and human pathogens could remain viable in inadequately-composted manures, leading to the contamination of the soil and leafy green vegetables.

Amoah et al. (2006) have isolated *Salmonella* from poultry fecal matter, dust, water, and feed samples in Accra and Kumasi, Ghana. Amoah, Drechsel, and Abaidoo (2005) observed that soils contaminated due to prior exposure to inadequately- or un-composted-manures could lead to vegetable contamination. Amoah et al. (2005) analyzed the fecal coliforms in inadequately-composted poultry manure and lettuce grown on soil enriched with the manure. The level of fecal coliforms in inadequately-composted poultry manure was found comparable to the level of the bacteria on leafy green vegetables.

Most indigenous leafy vegetables sampled in the present study had higher microbial counts than the two exotic vegetables and this observation might be attributed to the use of pesticides, which often include fungicides (manganese ethylene-bis-dithiocarbamate and benzimidazole) and biocides (sodium sulfide and 1,2-benzisothiazolin-3-ones), during exotic vegetable production. Pesticides are more frequently used in the production of exotic vegetables, to control pests and diseases due to their high economic values or returns, than in the indigenous vegetable production. The use of pesticides especially at high concentrations has been shown to kill soil microorganisms (Kalia and Gosal 2011; Ayansina and Oso 2006) or temporally inhibit their growth (Filimon et al., 2015). Ottesen et al. (2015) observed lower counts of *Salmonella* and *Paenibacillus* on tomato leaves and fruits that were regularly sprayed with pesticides as compared to controls which received no spraying, although the difference in the bacterial counts was not statistically significant.

The present study found that leafy green vegetable samples collected from the market centers had higher microbial counts than the farm samples. Market vegetables also had higher prevalence of enterococci, fecal coliforms, and *Salmonella* compared to farm vegetables. Ensink, Mahmood, and Dalsgaard (2007) observed that leafy green vegetables collected from agricultural fields had lower *E. coli* counts compared to the counts of the same batch of vegetables transported to the markets. Unsanitary postharvest practices were reported by Ensink et al. (2007) as the major source of vegetables contamination. Unhygienic postharvest handling practices and improper storage conditions could be attributed to the poor microbial quality of market vegetables in this present study. Vegetables transported to the markets are stored under non-refrigeration temperatures in sacks or boxes kept in rooms or in the open at the markets centers. Some vegetables are washed to remove dirt before being displayed at the market for sale using water which is not changed regularly. This practice promotes cross contamination and microbial growth on vegetables. Different from the results of the present study, Amoah et al. (2007) observed no significant differences in the contamination levels on lettuce from the farm through the value chain to retail outlets in some cities of Ghana. The authors believed that the on-farm contamination levels were so high, which overshadowed additional contamination after harvest.

Other postharvest practices that could contribute to the poor microbial quality of leafy green vegetables include the activities of handlers and processers. It was observed during our study that vegetables were mostly handled with bare hands which were cleaned on the farms using polluted irrigation water. Furthermore, sanitation infrastructures are lacking in most of the market centers and vegetable farms in Ghana. Use of washrooms without running water for hand washing can also contribute to the contamination of leafy green vegetables.

5.0 Conclusion

High fecal coliform and *Enterococcus* counts were found on leafy green vegetables sampled in the present study. On average, market vegetables had higher microbial counts than the vegetables collected from the farms. Indigenous leafy green vegetables with the exception of *Amaranthus* sp. had higher yeast and mold counts, fecal coliform counts, and *Enterococcus* counts than lettuce and cabbage. *Salmonella* was isolated from both exotic and indigenous vegetables collected from the farms and markets. This study suggests that leafy green vegetables grown and sold in some urban areas of Ghana are associated with high microbial counts and some of them are contaminated with *Salmonella*. Consumption of fresh leafy green vegetables without sanitizing or heat treatment should be discouraged.

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Table 4.1 Number of leafy green vegetable samples collected from individual farming areas and market centers in Accra metropolis, Ghana

	Number of leafy green vegetables collected						
Source	*Amaranthus*	Cabbage	*C. olitorius*	*H. sabdariffa*	Lettuce	*S. macrocarpon*	Total
FA 1	2	5	2	0	17	0	26
FA 2	2	1	2	2	6	2	15
FA 3	0	0	0	2	0	2	4
FA 4	0	0	2	2	6	1	11
FA 5	6	0	6	6	9	10	37
FA 6	4	2	0	6	4	4	20
FA 7	4	0	0	2	8	2	16
FA 8	2	4	0	2	4	0	12
FA 9	0	0	0	0	2	0	2
FA 10	5	5	2	2	6	2	22
FA 11	0	0	0	0	2	0	2
FA 12	2	0	2	2	2	0	8
MC 1	4	12	10	4	10	8	48
MC 2	7	10	10	6	2	10	45
MC 3	6	8	10	5	2	10	41
MC 4	1	6	4	2	2	4	19
Total	45	53	50	43	82	55	328

Table 4.2 Mean microbial counts recovered from leafy green vegetables collected from vegetable farming areas and market centers in Accra metropolis, Ghana

	TAC	Y&M	Fecal coliforms	Enterococcus
			(Log CFU/g)	
Leafy green vegetables				
Amaranthus sp.	8.78^{a}	4.79^{c}	4.72^{bc}	3.57^{c}
Cabbage	9.20^{a}	4.63^{c}	4.28^{c}	3.66^{c}
Corchorus olitorius	9.00^{a}	5.73^{a}	5.81^{a}	4.53^{a}
Hibiscus sabdariffa	8.95^{a}	5.55^{a}	4.89^{b}	3.68^{c}
Lettuce	8.30^{b}	4.25^{d}	4.51^{bc}	2.93^{d}
Solanum macrocarpon	8.87^{a}	5.21^{b}	5.48^{a}	4.13^{b}
Sampling area				
FA 1	7.47^{cd}	4.34^{de}	4.66^{cde}	2.35^{def}
FA 2	9.37^{ab}	3.99^{e}	5.17^{abcd}	2.19^{ef}
FA 3	7.36^{d}	5.01^{abc}	5.54^{abc}	3.50^{bc}
FA 4	9.28^{ab}	4.95^{abcd}	5.67^{abc}	3.24^{cd}
FA 5	9.20^{ab}	4.42^{cde}	5.01^{abcd}	3.44^{bc}
FA 6	8.73^{ab}	5.32^{a}	3.49^{ef}	2.52^{cde}
FA 7	8.61^{ab}	4.88^{abcd}	4.01^{def}	2.92^{cde}
FA 8	8.91^{ab}	4.62^{bcde}	3.48^{ef}	3.24^{cd}
FA 9	9.62^{a}	4.30^{de}	2.88^{f}	2.91^{cde}
FA 10	8.50^{bc}	4.63^{bcde}	3.05^{f}	2.98^{cde}
FA 11	6.91^{d}	5.06^{abc}	3.01^{f}	1.49^{f}
FA 12	9.05^{ab}	5.21^{ab}	4.02^{def}	2.89^{cde}
MC 1	8.57^{abc}	5.08^{ab}	4.78^{bcd}	4.48^{ab}
MC 2	9.26^{ab}	5.43^{a}	5.62^{abc}	4.57^{a}
MC 3	8.99^{ab}	5.50^{a}	6.23^{a}	4.89^{a}
MC 4	9.02^{ab}	5.02^{abc}	6.05^{ab}	4.72^{a}
Sampling site				
FA	8.68^{a}	4.65^{b}	4.31^{b}	2.88^{b}
MC	8.94^{a}	5.29^{a}	5.57^{a}	4.58^{a}

Means followed by different small letter within a column are significantly different ($p \leq 0.05$).
FA: Farming area; MC: Market center
TAC: total aerobic counts
Y&M: yeast and mold counts

Table 4.3 Effect of sampling site (farm *vs*. market) on the mean microbial counts on leafy green vegetables

Sampling Area	Microbial counts (Log CFU/g)					
	Amaranthus	Cabbage	*C. olitorius*	*H. sabdariffa*	Lettuce	*S. macrocarpon*
	Total aerobic counts					
Farm	$8.61^{b, A}$	$9.65^{a, A}$	$8.90^{ab, A}$	$8.94^{ab, A}$	$8.20^{b, A}$	$8.86^{ab, A}$
Market	$9.03^{a, A}$	$8.99^{a, A}$	$9.00^{a, A}$	$8.96^{a, A}$	$8.70^{a, A}$	$8.87^{a, A}$
	Yeast and mold counts					
Farm	$4.54^{bc, B}$	$4.71^{abc, A}$	$5.20^{a, B}$	$5.18^{a, B}$	$4.22^{c, A}$	$4.89^{ab, B}$
Market	$5.16^{b, A}$	$4.60^{c, A}$	$5.97^{a, A}$	$6.21^{a, A}$	$4.36^{c, A}$	$5.40^{b, A}$
	Fecal coliform counts					
Farm	$3.96^{b, B}$	$4.11^{ab, A}$	$5.02^{a, B}$	$4.15^{ab, B}$	$4.33^{ab, B}$	$4.63^{ab, B}$
Market	$5.85^{ab, A}$	$4.36^{c, A}$	$6.17^{a, A}$	$6.18^{a, A}$	$5.26^{b, A}$	$5.98^{a, A}$
	Enterococcus counts					
Farm	$2.89^{a, B}$	$3.13^{a, A}$	$3.01^{a, B}$	$3.02^{a, B}$	$2.71^{a, B}$	$2.91^{a, B}$
Market	$4.59^{b, A}$	$3.91^{c, A}$	$5.24^{a, A}$	$4.86^{ab, A}$	$3.87^{c, A}$	$4.84^{ab, A}$

Means followed by different small letter within a row are significantly different ($p \leq 0.05$).
Means followed by different capital letter within a column are significantly different ($p \leq 0.05$).

Figure Legends

Fig. 4.1 Vegetables sampled in the study. A: *Amaranthus* sp.; B: *Corchorus olitorius;* C: *Hibiscus sabdariffa*; D: *Solanum macrocarpon;* E: green leaf lettuce (*Lactuca sativa*); F: cannon ball cabbage (*Brassica oleracea* var *capitata*).

Fig. 4.2 Vegetable farming area and market center sampling sites in Accra metropolis, Ghana (modified from a google map).

Fig. 4.3 Prevalence of *Enterococcus*, fecal coliforms, and *Salmonella* on all vegetable samples collected from individual farming areas and market centers.

Fig. 4.4 Prevalence of *Enterococcus* on individual types of leafy green vegetables collected from all farming areas and market centers.

Fig. 4.5 Prevalence of fecal coliforms on individual types of leafy green vegetables collected from all farming areas and market centers.

Fig. 4.6 Prevalence of *Salmonella* on individual types of leafy green vegetables collected from all farming areas and market centers.

Fig. 4.1


Kwabenya
Haatso
Madina
Ogbojo
Taifa
N4
N1
N6
EAST LEGON
Sakumono
Lapaz
Accra
N1
Awoshie
Gbawe
Mallam
OSU
Densu Delta Protected Area
○ = sampling site
Google
Map data ©2017 Google
United States
Terms

Fig. 4.2

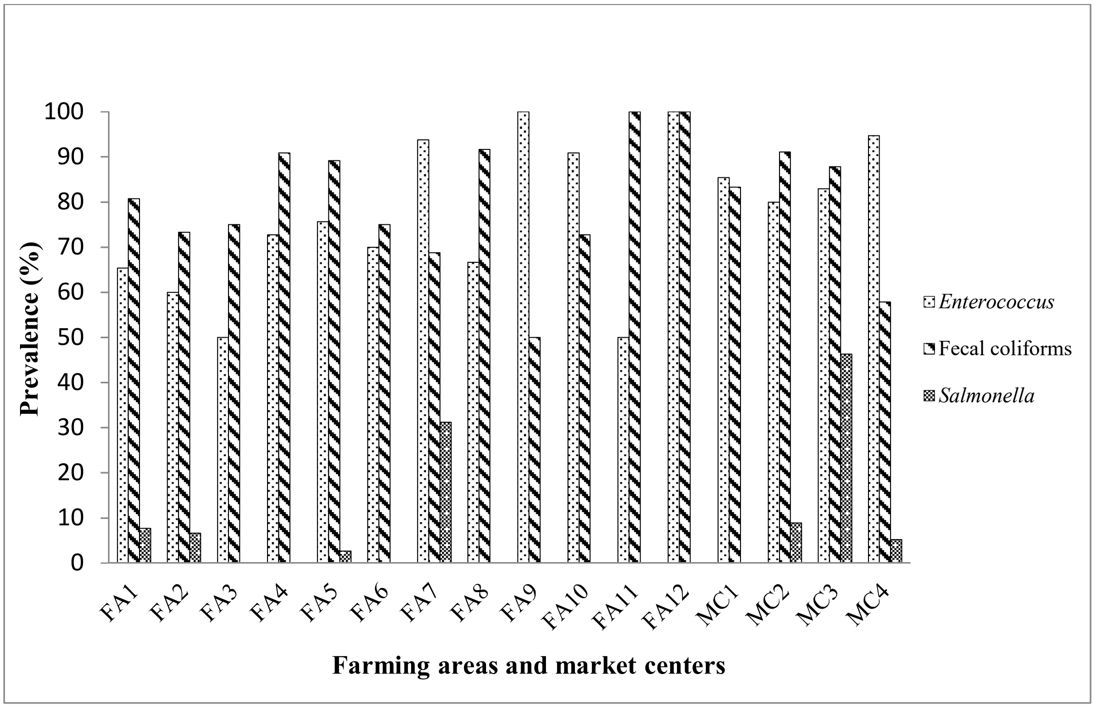
100
90
80
70
60
50
40
30
20
10
0
Prevalence (%)
FA1
FA2
FA3
FA4
FA5
FA6
FA7
FA8
FA9
FA10
FA11
FA12
MC1
MC2
MC3
MC4
Farming areas and market centers
Enterococcus
Fecal coliforms
Salmonella

Fig. 4.3

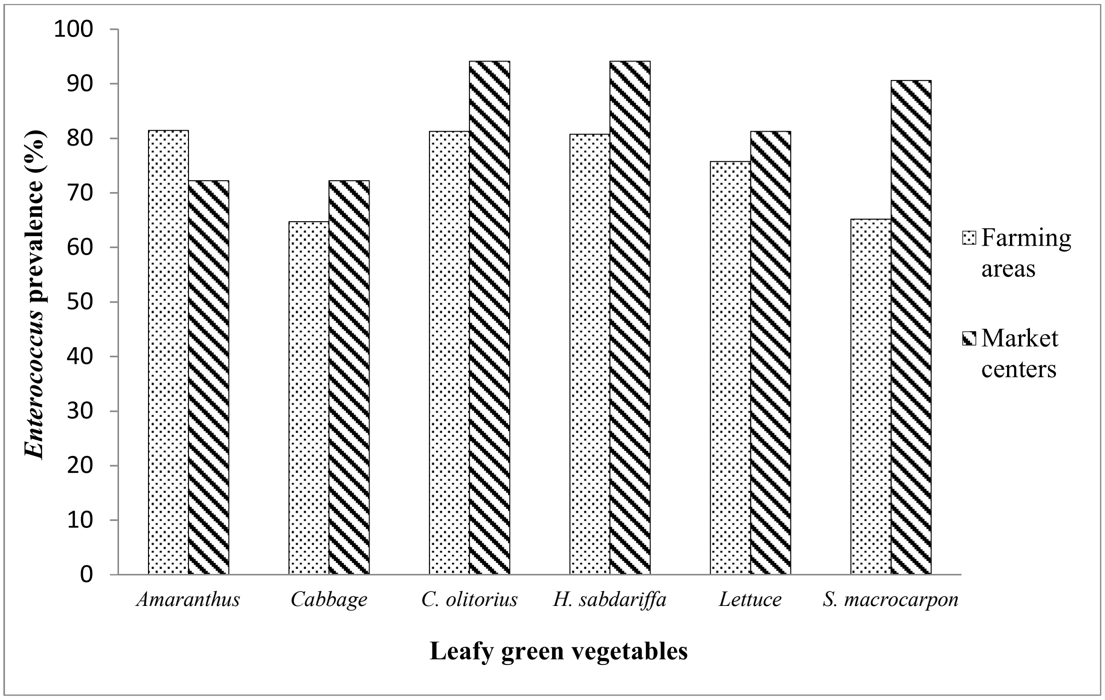
100
90
80
70
60
50
40
30
20
10
0
Enterococcus prevalence (%)
Amaranthus
Cabbage
C. olitorius
H. sabdariffa
Lettuce
S. macrocarpon
Leafy green vegetables
Farming areas
Market centers

Fig. 4.4

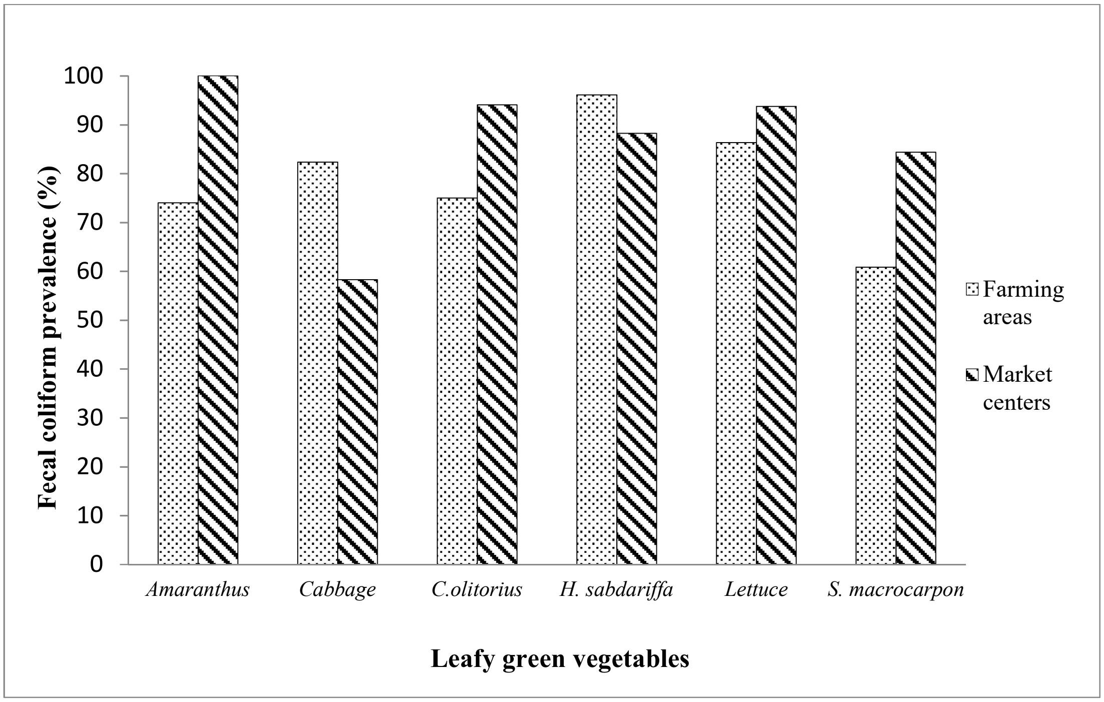
Fecal coliform prevalence (%)
100
90
80
70
60
50
40
30
20
10
0
Amaranthus
Cabbage
C.olitorius
H. sabdariffa
Lettuce
S. macrocarpon
Leafy green vegetables
Farming areas
Market centers

Fig. 4.5

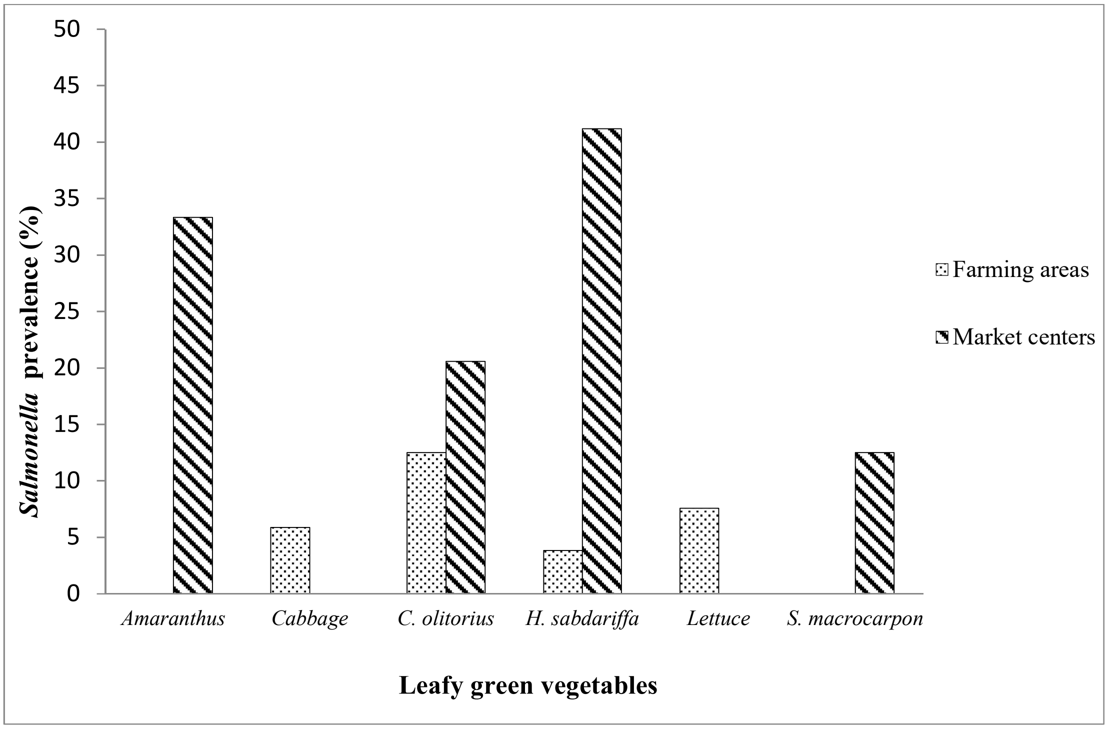
Salmonella prevalence (%)
50
45
40
35
30
25
20
15
10
5
0
Amaranthus
Cabbage
C. olitorius
H. sabdariffa
Lettuce
S. macrocarpon
Leafy green vegetables
Farming areas
Market centers

Fig. 4.6

CHAPTER 5

ANTIBIOTIC RESISTANCE PROFILE OF THE *SALMONELLA* STRAINS ISOLATED FROM LEAFY GREEN VEGETABLES IN GHANA

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[1] Quansah, J. K. and J. Chen. To be submitted to *Food Control.*

Abstract

Antibiotic resistant pathogens including *Salmonella* have been isolated from leafy green vegetables in the U.S. and elsewhere. However, similar work has not been done in Ghana and surrounding countries. The aim of this study was to determine the antibiotic resistance profile of *Salmonella* strains isolated from leafy green vegetables (n=328) in Accra, Ghana and the distribution of integrons and structure of integron gene cassette in antibiotic resistant *Salmonella*. Antibiotic resistance profiles of isolated *Salmonella* to 12 antibiotics were determined using the disc diffusion assay. The minimum inhibitory concentrations (MICs) of five antibiotics most commonly resisted by the *Salmonella* isolates were determined using the two-fold macrodilution method. PCR was used to detect the presence of integrons and integron gene cassette in *Salmonella* cells. Amplified integron gene cassette was purified and sequenced using the Sanger sequencing techniques. The *Salmonella* isolates (n = 33) were resistant to at least one antibiotic and approximately 30% (10/33) of the isolates were multidrug resistant (MDR). Most of the isolates were resistant to sulfisoxazole (27/33) and cefoxitin (13/33). The MICs of ampicillin, cefoxitin, streptomycin, tetracycline, and sulfisoxazole was ≥ 64, 32, 64, 16, and > 1,024 µg/ml, respectively. Eight different patterns in antibiotic resistance were observed among the *Salmonella* isolates and the common MDR pattern was AAuFox. One (3%) *Salmonella* isolate tested positive for integrase gene and class 1 integron gene cassette (800 kb in size). Nucleotide sequencing revealed the class 1 integron carried a single gene, *dfrA7*. This study shows that leafy green vegetables in Ghana are contaminated with *Salmonella* with mobile antibiotic resistant genes. Consumption of contaminated vegetables can be a possible route for acquiring antibiotic resistant *Salmonella*.

Keywords: antibiotics, leafy green vegetable, antibiotic resistance patterns, integrons, *Salmonella*

1.0 Introduction

Salmonella are ubiquitous microorganisms that can survive in different environments including food production environments. They are prevalent in most food animals like poultry and cattle and their products (Li et al., 2013). *Salmonella* can also contaminate fresh produce due to inappropriate pre- and post-harvest practices such as the use of inadequately composted manure and contaminated irrigation water (Berger et al., 2010; Islam et al., 2004).

Consumption of *Salmonella*-contaminated foods leads to the onset of salmonellosis with symptoms of gastroenteritis, fever, and abdominal pains (Li et al., 2013). A report from the World Health Organization (2015) indicates that non-typhoidal *Salmonella* (NTS) is responsible for about 32,000 deaths per year in Africa. Typhoid fever is among the 20 leading causes of outpatient illness in Ghana, with about 384,704 cases recorded in 2016 (Ghana Health Service, 2017). Abdullahi et al. (2014) found 43.7% of patients diagnosed with pyrexia and gastroenteritis in three hospitals in Katsina state, Nigeria tested positive for NTS. Among children <15 years admitted to a rural hospital in Mozambique, 26% of the 1,550 cases were caused by NTS (Sigauque et al., 2009). In Kenya, 10.8% of 3,296 children on admission with bacteremia were infected by NTS (Muthumbi et al., 2015).

Antibiotics are commonly used for treatment of salmonellosis. However, changes in the susceptibility of bacteria to antibiotics can occur, which reduces the therapeutic value of antibiotics (Li et al., 2013). Bacterial resistance to antibiotics can occur naturally over time due to random mutation during DNA replication or exchange of genetic materials among bacterial cells. Horizontal DNA transfer is a common mode for bacterial cells to acquire antibiotic resistant genes (Blair et al., 2014). Misuse and overuse of antibiotics in food animal production especially in cattle and poultry production have been suggested to exert a selective pressure for the development of antibiotic resistant bacteria. Some antibiotic resistant genes are located on mobile DNA elements such as integrons, plasmids, and transposons. Integrons are site-specific recombination systems that allow a bacterium to capture and express exogenous genes (Gillings, 2014). They are often involved in the dissemination of antibiotic resistance, especially among gram-negative bacterial pathogens (Boucher et al., 2007; Gillings, 2014).

MDR *Salmonella* have been isolated from meat, animal feeds, as well as fecal matter of poultry, cattle, and swine in Ghana (Adzitey et al., 2015; Andoh et al., 2016; Sekyere and Adu, 2015). Fecal matter of poultry and cattle are commonly used as fertilizer, either untreated or improperly-composted, in vegetable production. *Salmonella* was isolated from some exotic and indigenous leafy green vegetable samples collected from vegetable farms and market centers in Ghana (chapter 4). The objectives of this study were to determine the antibiotic resistance profile of the isolated *Salmonella* and the distribution of integrons and structure of integron gene cassettes in the *Salmonella* isolates that were resistant to antibiotics.

2.0 Methods

2.1 *Salmonella* strains

Salmonella (n=33) isolated from two exotic (cabbage and lettuce), and four indigenous (*Amaranthus* sp., *Corchorus olitorius, Hibiscus sabdariffa* and *Solanum macrocarpon*) leafy green vegetables (n=328) collected from 50 vegetable farms in 12 farming areas and 37 vegetable sellers in 4 major market centers in Accra, Ghana (Quansah et al., 2018) were used in this study (Table 5.1). The *Salmonella* isolates were retrieved from frozen storage and inoculated on tryptic soy agar (Becton Dickinson, Sparks, MD) and grown at 37°C for 24 h before being used in the following experiments.

2.2 Antibiotic susceptibility of the *Salmonella* isolates

Antibiotic resistance profiles of the *Salmonella* isolates were determined using the standard disc diffusion assay according to the guidelines provided by the Clinical and Laboratory Standard Institute (CLSI, 2012a, 2007). The isolates were inoculated in tryptic soy broth (TSB) (Becton Dickinson, Sparks, MD) and incubated at 37 °C for 2 – 6 h. The turbidity of the inocula was adjusted with TSB to that equivalent to a 0.5 McFarland standard. A sterile cotton swab was immersed into each inoculum, pressed against side of the test tube to remove excess fluid, and then used to inoculate the entire surface of a Mueller-Hinton agar (Becton Dickinson) plate in at least two directions. Antibiotic discs were applied within 15 min of the inoculation, and the inoculated agar plates were incubated at 37 °C for 24 h. Twelve antibiotics were used including amoxycillin/clavulanic acid (Au; 30 µg), ampicillin (A; 10 µg), cefoxitin (Fox; 30 µg), ceftriaxone (Cx; 30 µg), chloramphenicol (C; 30 µg), ciproflaxin (Cip; 5 µg), gentamycin (G; 10 µg), nalidixic acid (N; 30 µg), streptomycin (S; 10 µg), tetracycline (T; 30 µg), trimethoprim (W; 5 µg) (Oxoid Ltd, Basingstoke, Hants, UK), and sulfisoxazole (Su; 0.25 mg) (Becton Dickinson). The diameter of the zone of inhibition around each disc was measured to the nearest whole millimeter, and each *Salmonella* isolate was classified as resistant, intermediate, or sensitive to the antibiotics tested based on CLSI guidelines (CLSI, 2007).

2.3 Minimum inhibitory concentration (MIC)

The MICs of five antibiotics most commonly found to be resistant by the *Salmonella* isolates were determined using the twofold macrodilution method (CLSI, 2012b). These antibiotics were ampicillin, streptomycin (Fisher, Fair Lawn, NJ), cefoxitin (Chen-Impex, Wood Dale, IL), sulfisoxazole, and tetracycline (Sigma, St Louis, MO). All antibiotics were weighed, dissolved, diluted in the appropriate diluents and added to Mueller-Hinton broth to make dilution series of 1 to 256 µg/ml for ampicillin, 2 to 256 µg/ml for cefoxitin, 4 to 256 µg/ml for streptomycin, 64 to 1,024 µg/ml for sulfisoxazole, and 1 to 128 µg/ml for tetracycline. The MIC was determined as the lowest concentration of each antibiotic that completely inhibits the growth of *Salmonella* cells.

2.4 Detection of integrase gene

Salmonella isolates that demonstrated resistance to selected antibiotics were inoculated on TSA and incubated at 37 ºC for 24 h. After 24 h of incubation*,* the *Salmonella* cultures were transferred into TSB and incubated at 37 ºC for 24 h. An aliquot of each *Salmonella* culture was boiled in 100 µl of sterile water for 10 min and centrifuged using Eppendorf centrifuge 5415 C (Brinkman Instruments, INC., Westbury, N.Y.) at 14,000 *g* for 2 min. The supernatant fluid was used as DNA template. Presence of integrons in *Salmonella* cells was determined using PCR by amplifying the integrase gene *intI* using degenerate primers hep 35 and hep 36 (Invitrogen life technologies) as described by White et al. (2001). DNA template (5 µl) was added to 20 µl of PCR mix containing 0.5 U of *Taq* DNA polymerase (Thermo scientific, Luthonia), 0.1 mM of each deoxynucleoside triphosphate (Thermo scientific, Waltham, MA), 2.5 µl of 1.5 mM $MgCl_2$ (Thermo scientific), 2.0 µl of 10x *Taq* buffer with KCl, and 9.5 µl of water. A PE 480

DNA thermal cycler (Perkins Elmer, Waltham, MA) was used for amplification. The reaction parameters were 30 cycles at 94ºC for 2 min, 59ºC for 1 min, and 72ºC for 1 min, followed by a final extension at 72ºC for 1 min. The samples were maintained at 4ºC after amplification.

2.5 Detection of integron gene cassettes

The structure of identified integron was determined by amplifying class 1 and class 2 integron cassette regions using primers hep 58 and hep 59, and primers hep 51 and hep 74 respectively, as described by White et al. (2001). DNA template (5 µl) was added to 20 µl of PCR mix containing 0.5 U of *Taq* DNA polymerase (Thermo scientific), 0.1 mM of each deoxynucleoside triphosphate (Thermo scientific), 1.5 µl of 1.5 mM $MgCl_2$ (Thermo scientific), 2.0 µl of 10x *Taq* buffer with KCl, and 9.5 µl of water. A PE 480 DNA thermal cycler (Perkins Elmer, Waltham, MA) was used for the amplification. The reaction parameters were 30 cycles at 94 ºC for 2 min, 59 ºC for 1 min, and 72 ºC for 1 min, followed by a final extension at 72 ºC for 1 min. The samples were maintained at 4 ºC after amplification.

2.6 DNA sequencing

The PCR product of amplified class 1 integron gene cassette was submitted to Eurofins Genomics, a Eurofins MWG Operon company (Louisville, KY) for purification using exonuclease I and shrimp alkaline phosphate and sequenced using Sanger sequencing techniques. The sequencing results were compared against those in the NCBI database.

3.0 Results

3.1 Antibiotic susceptibility test

Results of the antibiotic susceptibility test showed that the *Salmonella* isolates were resistant to at least one antibiotic (Table 5.2). It was observed that 51.5% (17/33) of the isolates were resistant to one antibiotic, 8.2% (6/33) to two antibiotics, and 30.3% (10/33) were MDR. None of the *Salmonella* isolates were resistant to ceftriaxone, ciproflaxin, and gentamycin. Twenty-seven out of the 33 isolates were resistant to sulfisoxazole (81.8%), 13 to cefoxitin (39.4%), 10 to amoxycillin/clavulanic acid (30.3%), ampicillin (30.3%), and streptomycin (30.3%). Five isolates were resistant to tetracycline (15.2%), 3 to trimethoprim (9.1%), and 2 to chloramphenicol (6.1%) and nalidixic acid (6.1%) (Table 5.2).

It was also observed that *Salmonella* isolated from certain types of leafy vegetables were resistant to specific antibiotics. All six *Salmonella* strains isolated from *Amaranthus* sp. were resistant to only sulfisoxazole, although the vegetables were from three different vegetables sellers in one market center (MC 3) (Table 5.1 and 5.3). The only *Salmonella* isolate from cabbage was resistant to multiple antibiotics including amoxycillin/clavulanic acid, ampicillin, cefoxitin, streptomycin, and sulfisoxazole. All nine isolates from *C. olitorius* were resistant to sulfisoxazole, while 3 out of the 9 isolates were resistant to amoxycillin/clavulanic acid, ampicillin, cefoxitin, streptomycin, and tetracycline, and 2 out of the 9 isolates were resistant to chloramphenicol, nalidixic acid, and trimethoprim. Among the 8 isolates from *H. sabdariffa*, 5 were resistant to sulfisoxazole, 3 to cefoxitin, 2 to amoxycillin/clavulanic acid and ampicillin, and 1 was resistant to streptomycin and trimethoprim. All the 5 isolates from lettuce were resistant to cefoxitin, 3 to amoxycillin/clavulanic acid and ampicillin, and 2 to streptomycin and sulfisoxazole. All the 4 isolates from *S. macrocarpon* were resistant to sulfisoxazole, 3 to streptomycin, and 1 was resistant to amoxycillin/clavulanic acid, ampicillin, cefoxitin, tetracycline, and trimethoprim (Table 5.3).

At least one vegetable sample collected from each of the 4 farming areas (FA) and 3 market centers (MC), whose vegetables tested positive for *Salmonella*, was resistant to sulfisoxazole (Table 5.4). Apart from vegetables purchased from MC 4, vegetables from the other FA or MC were resistant to amoxycillin, ampicillin, cefoxitin, and sulfisoxazole. Only vegetables from FA 2 and FA 5 exhibited resistance to chloramphenicol and nalidixic acid.

3.2 Antibiotic resistance patterns and MIC

Eight different patterns in antibiotic resistance were observed among the *Salmonella* isolates examined in the study (Table 5.5). About 51.5% of the isolates had resistance to a single antibiotic (sulfisoxazole or cefoxitin) while 18.2% of the isolates had resistance to two antibiotics (streptomycin and sulfisoxazole, SSu). The remaining isolates (n=10) had resistance to 3, 5, 7, 8, and 9 antibiotics. MDR patterns of AAuFox, AAuFoxSSu, ASSuWAuFoxT, ACTAuFoxNSuW, and ACSSuTAuFoxNW were exhibited by 3, 4, 1, 1, and 1 *Salmonella* isolates, respectively (Table 5.5).

The MIC of ampicillin against the *Salmonella* strains ranged from 64 to >256 µg/ml (Table 5.6). The MICs of cefoxitin, streptomycin, and tetracycline were 32 to >256 µg/ml, 64 to >256 µg/ml, and 16 to > 128 µg/ml, respectively. The MIC of sulfisoxazole was > 1,024 µg/ml.

3.3 Integrease genes and integron gene cassette

Only one out of the 33 (3%) *Salmonella* isolates tested positive for *intl*. The isolate has a class 1 integron gene cassette of approximately 800 kb. Nucleotide sequencing revealed the class 1 integron carried a single gene for dihydrofolate reductase (*dfrA7*) (GenBank accession no. kx807610.1) which encodes resistance to trimethoprim.

4.0 Discussion

The present study found that the *Salmonella* isolated from the leafy green vegetables grown or sold in Ghana were resistant to antibiotics, and 30.3% of the isolates are MDR (Table 5.2). This finding suggests that consumption of contaminated leafy green vegetables could be a potential route of acquiring MDR pathogens(CDC, 2017; Chang et al., 2015). It is well known that foodborne illness caused by MDR pathogens is more likely to be associated with treatment failure or complications, which in turn increases the cost of health care and mortality rate (O'Neill, 2014; Wegener, 2012).

Use of antibiotics in food animal production is a major transmission route of antibiotic resistant bacteria into animal meats and products (CDC, 2017; Verraes et al., 2013). Antibiotic resistant bacteria or resistant genes can be also transmitted to plant produce like fruits and vegetables through the use of contaminated irrigation water and/or improperly composted manure, contaminated processing environments or surfaces, and human processors (CDC, 2017; Verraes et al., 2013),

Although antibiotics are mainly used to treat and prevent diseases during animal production in Ghana, previous studies have documented the misuse and overuse of antibiotics among farmers (Andoh et al., 2016; Boamah et al., 2016; Sekyere, 2014). Some of such practices include the use of higher than recommended dosage of antibiotics, administration of antibiotics meant for disease treatment to animals in the absence of disease, and not completing the recommended antibiotic courses (Andoh et al., 2016; Boamah et al., 2016; Sekyere, 2014). These practices are among the factors contributing to development of bacterial resistance to antibiotics in farm animals (WHO, 2017). Indeed, previous studies have isolated antibiotic resistant bacterial pathogens from fecal matter of food animals. Andoh et al. (2016) isolated 94 *Salmonella* from 200 samples of poultry fecal matter, bedding materials, food, water, and slaughterhouses in Ghana. Eighty nine out of the 94 *Salmonella* were isolated from fecal matter and bedding, with MDR prevalence of 24.5%. Sekyere and Adu (2015) found 72 fecal samples from 108 pig farms in the Ashanti region of Ghana were positive for *S.* Typhimurium and MDR prevalence was 40.3%. Antibiotic resistant bacteria in manure can be transferred to the soil used for vegetable production (Byrne-Bailey et al., 2009; Götz and Smalla, 1997; Heuer et al., 2011), causing the contamination of vegetables by antibiotic resistant bacteria.

Uptake of antibiotics from manure-amended soils by leafy and non-vegetables (corn, potato) have been documented in previous studies (Bassil et al., 2013; Dolliver et al., 2007; Kumar et al., 2005). The concentration of the antibiotics absorbed by the plants increased with increasing antibiotic concentration in the manure. Azanu et al. (2016) observed uptake of antibiotics by lettuce and carrot from irrigation water spiked with antibiotics. The antibiotics absorbed by plant tissues are the likely pressure for selection of antibiotic resistant human and plant pathogens.

Antibiotic resistant *Salmonella* were isolated from both indigenous and exotic leafy green vegetables in Ghana in the present study. *Salmonella* isolated from indigenous leafy green vegetables in Malaysia (Yoke-Kqueen et al., 2008), lettuce in Ethiopia (Guchi and Ashenafi, 2010), ready to eat salad vegetables in Tukey (Taban et al., 2013), and leafy and non-leafy vegetables in Bangladesh (Saha et al., 2016) have been found resistant to one or more antibiotic, as observed in this study. However, the prevalence of MDR *Salmonella* from leafy green vegetables in our study (30.3%) was lower compared to the 100% incidence (8/8) from Ethiopia (Guchi and Ashenafi, 2010), but higher than the 18% (6/33) incidence from Malaysia (Yoke-Kqueen et al., 2008).

The most common resistance exhibited by *Salmonella* used in this study was to sulfisoxazole (42.4%; Table 5.2). Sulfonamides, like sulfisoxazole are synthetic analogs of para-aminobenzoic acid which competitively inhibit the activity of dihydropteroate synthetase (DHPS) in the early stage of folic acid synthesis in bacteria, inhibiting the formation of dihydrofolic acid (Prescott, 2013a; Sköld, 2000). Mutation in chromosomal gene *folP* leads to altered DHPS production that has reduced affinity to sulfonamides, thus conferring bacterial resistance to sulfonamides (Sköld, 2000; Struelens, 2003). Plasmid-borne resistance to sulfonamides are common in enteric bacteria due to the acquisition of *sul1*, *sul2*, and *sul3* genes. These genes encode for variants of DHPS enzymes (Prescott, 2013a; Sköld, 2000). Plasmid and integron-mediated sulphonamide resistance are often linked to other resistant genes including trimethoprim (*dfr*) or streptomycin (*strA*, *strB*) resistant genes (Prescott, 2013a). Co-existence of trimethoprim and sulfisoxazole resistance was found in two *Salmonella* isolates in the present study whereas resistance to streptomycin and sulfisoxazole was observed in 11 *Salmonella* isolates (Table 5.5).

Streptomycin is an antibiotic that belongs to the aminoglycoside family. Approximately 30% of the *Salmonella* isolates included in the present study were resistant to streptomycin (Table 5.2). Aminoglycoside antibiotics have specific affinity to the 30S subunit of the bacterial ribosome, inhibiting protein synthesis (Dzidic et al., 2008; Greenwood and Whitley, 2003). One of the mechanisms of bacterial resistance to aminoglycoside is to reduce the binding efficiency of the antibiotics to the bacterial ribosome. Other mechanisms include reduced uptake of the antibiotics, inactivation of the antibiotics by specific aminoglycoside-modifying enzymes (AMEs) and active efflux of intracellular aminoglycosides by efflux pumps (Boehr et al., 2003; Garneau-tsodikova and Labby, 2016). AMEs are usually plasmid-mediated enzymes which modify the hydroxyl and amino groups of aminoglycosides, prevent ribosomal binding, and thus, the antibiotics are unable to inhibit protein synthesis (Dowlings, 2013; Munita et al., 2016).

Approximately 30% of the *Salmonella* isolates included in the present study were resistant to ampicillin (Table 5.2). Guchi and Ashenafi (2010) found that 50% (4/8) of the *Salmonella* examined in their study resisted ampicillin. In the study of Saha et al. (2016), 100% (17/17) of the *Salmonella* isolates were resistant to ampicillin. Beta-lactam antibiotics function through inhibiting the formation of bacteria cell walls during the final stages of peptidoglycan biosynthesis. Beta-lactams block the cross link of glycopeptide polymeric units of the cell walls *via* selective inhibition of penicillin-binding proteins (PBPs) (Greenwood and Whitley, 2003; Prescott, 2013b). Mechanism of beta-lactam resistance is mainly due to destruction of the amide bond of the beta-lactam ring by beta-lactamases (Prescott, 2013b). In gram-negative bacteria, resistance can also be due to low permeability of cell wall, lack of PBPs and production of numerous beta-lactamase enzymes (Greenwood and Whitley, 2003; Prescott, 2013b). It is therefore not surprising that all 10 isolates in this study that were resistant to ampicillin were also resistant to other beta-lactams like amoxicillin-clavulanic acid and cefoxitin (AAuFox; Table 5.2).

The incidence of resistance to chloramphenicol observed in the present study was 6.2% (Table 5.2). *Salmonella* isolated by Saha et al. (2016) and Yoke-Kqueen et al. (2008) had 0% (0/17) and 2% (3/134) resistance to chloramphenicol, respectively which are lower compared to the incidence observed in this study, however Guchi and Ashenafi (2010) reported a 13% (1/8) incidence for the resistance to chloramphenicol. Chloramphenicol was the first choice antibiotic for treating typhoid fever from 1948 to 1970s (Cooke and Wain, 2004; Groß et al., 2011; Mills-Robertson et al., 2002; Wilcox, 2003). It inhibits protein synthesis by binding irreversibly to the receptor site on the 50S ribosomal unit of bacteria. The binding inhibits the elongation of peptide chains by the peptidyl transferase enzyme, and thus, affects bacterial growth (Dowlings, 2013; Greenwood and Whitley, 2003). The widespread use of chloramphenicol led to the development of resistant *S.* Typhi over time (Wilcox, 2003). The mechanism of resistance to chloramphenicol is usually by acetylation of the antibiotic by chloramphenicol acetyltransferases (CATs) (Murray and Shaw, 1997; Schwarz et al., 2004). Other mechanisms of chloramphenicol resistance include efflux of intracellular antibiotic, inactivation by phosphotransferases, mutations of the target site and permeability barriers (Murray and Shaw, 1997; Schwarz et al., 2004). Acetylation of the hydroxyl groups on chloramphenicol prevents it from binding to the 50S ribosomal unit of bacteria (Dowlings, 2013). The genes for CATs are usually located on plasmids, transposons, or integrons, but may transpose to bacterial chromosomes (Dowlings, 2013; Struelens, 2003).

When the pathogens developed resistance to chloramphenicol, trimethoprim /sulfamethoxazole and ampicillin were used in treating typhoid fever until the late 1980's when *S. enterica* serotype Typhi developed resistance to these antibiotics as well (Groß et al., 2011; Mills-Robertson et al., 2002; Parry et al., 2002). Trimethoprim/sulfamethoxazole was an inexpensive and effective treatment of a broad range of diseases. The widespread use of the antibiotic caused dissemination of *dfrA* genes globally (Frank et al., 2007). The presence of *dfrA* genes, especially d*frA1* have been reported in most studies from parts of the world outside Africa, but a high prevalence of *Enterobacteriaceae* with class 1 integron carrying single *dfrA7* cassette have been reported in studies from Africa (Dahmen et al., 2010; Frank et al., 2007; Gassama et al., 2004; Labar et al., 2012). These reports correlate with findings of this study where one *Salmonella* isolate carried a class 1 integron with a cassette containing a single *dfrA7* gene.

Co-existence of resistance to ampicillin-amoxicillin/clavulanic acid-cefoxitin (AAuFox) was the common MDR pattern in our study, and was exhibited by 30% (10/33) of *Salmonella* isolates (Table 5.5). Xu et al. (2018) found AAuFox to be the common MDR pattern (36/185) in *Salmonella* isolated from flies captured on cattle farms in Georgia, USA. Diarra et al. (2014) observed that more than 43% of 193 *Salmonella* isolated from chicken in British Columbia, Canada were simultaneously resistant to AAuFox in addition to ceftiofur and ceftriaxone. About 17% (10/60) of *S*. Typhimurium isolates from laboratory surveillance for diarrheal disease in Jeollanam-do, Korea had the AAuFox resistance pattern in addition to resistance to ampicillin/sulbactam, cefazolin, cafalothin, and tetracycline (Yoon et al., 2017). AAuFox resistance pattern can be due to the presence of a single *bla* gene with broad spectrum resistance and multiple *bla* genes with narrow spectrum resistance (Xu et al., 2018).

The ACT pattern was found in two *Salmonella* isolates included in the study. Interestingly, the ACT was a common pattern observed as part of some MDR *Salmonella* isolates in previous studies in Ghana (Andoh et al., 2017; Mills-Robertson et al., 2003). Mills-Robertson et al. (2003) and Mills-Robertson et al. (2002) found 90.3% (28/31) or 10.3% (6/58) of the *Salmonella* isolated from human body fluid and fecal matter, respectively had the ACT resistance pattern. Andoh et al. (2017) observed 20.7% (6/29) of the *S*. Enteritidis isolated from human body fluid and fecal matter had the ACT resistance pattern.

Resistance to ampicillin, streptomycin, sulfisoxazole, and trimethoprim (ASSuW) was part of the MDR resistance pattern exhibited by one *Salmonella* isolate in the present study in addition to resistance to amoxicillin/clavulanic acid, cefoxitin and tetracycline (AuFoxT). Zewdu and Cornelius (2009) observed that ASSuW was a component of the most common MDR pattern (10/98) in *Salmonella* isolated from foods (poultry, pork, beef, fish and cheese) and human stools in Ethiopia.

Resistance to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (ACSSuT) was part of the resistance pattern of another MDR isolate in the present study in addition to resistance to amoxicillin/clavulanic acid-cefoxitin-nalidixic acid (AuFoxNW). The ACSSuT was the most common MDR pattern of non-typhi *Salmonella* in the US in 2004 (CDC, 2007). About 41% of 798 *Salmonella* strains isolated from human body fluids and stool from 91 hospitals in Taiwan had the resistance pattern of ACSSuT (Lauderdale et al., 2006). Krauland et al. (2009) found ACSSuT pattern among

Salmonella isolates collected from laboratories in South Africa (30/160), Uganda (10/100), Denmark (8/153), Spain (8/151), and Philippines (6/67). This pattern is often indicative of a 43kb region of *Salmonella* genomic island 1 (SGI1) in *S.* Typhimurium designated DT104 (Boyd et al., 2000; FDA, 2013).

5.0 Conclusion

Salmonella isolated from green leafy vegetables in Ghana were resistant to antibiotics, and 30.3% were MDR. One isolate carried a single gene (*dfrA7*) encoding for trimethoprim resistance. This study shows that green leafy vegetables grown or sold in Ghana are contaminated with antibiotic resistant *Salmonella*. Consumption of contaminated vegetables can be a possible route for acquiring antibiotic resistant pathogens.

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Table 5.1 Sampling source of leafy green vegetables contaminated with *Salmonella* in Accra, Ghana

Source	*Amaranthus*	Cabbage	*C. olitorius*	*H. sabdariffa*	Lettuce	*S. macrocarpon*	Total
FA 1		1			1		2
FA 2			1				1
FA 3							
FA 4							
FA 5			1				1
FA 6							
FA 7				1	4		5
FA 8							
FA 9							
FA 10							
FA 11							
FA 12							
MC 1							
MC 2			1	2		1	4
MC 3	6		6	4		3	19
MC 4				1			1
Total	6	1	9	8	5	4	33

FA: farming area, MC: market center

Table 5.2 Antibiotic profile of the *Salmonella* isolated from leafy green vegetables from farming areas and market centers in Accra, Ghana

Salmonella isolate	Au	A	Fox	Cx	C	Cip	G	N	S	Su	T	W
1	R	R	R	S	S	S	S	I	R	R	S	S
17	R	R	R	S	S	S	S	S	S	S	S	S
32	I	I	R	S	S	S	S	I	S	S	S	S
43	I	I	R	S	S	S	S	I	I	S	S	S
47	I	I	R	S	S	S	S	S	I	S	S	S
61	R	R	R	S	S	S	S	I	R	R	S	S
150	S	S	S	S	S	S	S	S	S	R	S	S
153	S	S	S	S	S	S	S	S	R	R	S	S
157	S	S	S	S	S	S	S	S	R	R	S	S
163	S	S	S	S	S	S	S	S	I	R	S	S
167	S	S	S	S	S	S	S	S	I	R	S	S
173	S	S	S	S	S	S	S	S	I	R	S	S
179	S	S	S	S	S	S	S	S	R	R	S	S
183	R	R	R	S	S	S	S	I	R	R	S	S
185	S	S	S	S	S	S	S	S	I	R	S	S
191	S	S	S	S	S	S	S	S	I	R	S	S
244	S	S	S	S	S	S	S	S	I	R	S	S
300	R	R	R	S	S	S	S	I	S	I	S	S
315	S	S	S	S	S	S	S	I	S	R	R	S
323	S	S	S	S	S	S	S	S	I	R	S	S
325	S	S	S	S	S	S	S	S	I	R	S	S
333	S	S	S	S	S	S	S	S	I	R	S	S
337	S	S	S	S	S	S	S	S	I	R	S	S
343	S	S	S	S	S	S	S	I	S	R	R	S
347	S	S	S	S	S	S	S	S	I	R	S	S
383	S	S	S	S	S	S	S	S	R	R	S	S
415	S	S	S	S	S	S	S	S	I	R	S	S
417	R	R	R	S	S	S	S	I	S	I	S	S
490	R	R	R	S	S	S	S	I	R	R	R	R
536	S	S	S	S	S	S	S	S	I	R	S	S
760	R	R	R	S	S	S	S	S	R	R	S	S
793	R	R	R	I	R	S	S	R	I	R	R	R
935	R	R	R	I	R	S	S	R	R	R	R	R

Au: amoxycillin/clavulanic acid, A: ampicillin, Fox: cefoxitin, Cx: ceftriaxone, C: chloramphenicol, Cip: ciprofloxacin, G: gentamycin, N: nalidixic acid, S: streptomycin, Su: sulfisoxazole, T: tetracycline, W: trimethoprim.

S: susceptible, I: intermediate, R: resistant

Table 5.3 Antibiotic profile of the *Salmonella* isolated from six types of leafy green vegetables from farming areas and market centers in Accra, Ghana

Leafy vegetable	Number of isolates	Au	A	Fox	Cx	C	Cip	G	N	S	Su	T	W
Amaranthus sp	6										6		
Cabbage	1	1	1	1						1	1		
C. olitorius	9	3	3	3		2			2	3	9	3	2
H. sabdariffa	8	2	2	3						1	5	1	
Lettuce	5	3	3	5						2	2		
S.macrocarpon	4	1	1	1						3	4	1	1

Au: amoxycillin/clavulanic acid, A: ampicillin, Fox: cefoxitin, Cx: ceftriaxone, C: chloramphenicol, Cip: ciprofloxacin, G: gentamycin, N: nalidixic acid, S: streptomycin, Su: sulfisoxazole, T: tetracycline, W: trimethoprim

Table 5.4 Antibiotic profile of the *Salmonella* isolated from leafy green vegetables from farming areas and market centers in Accra, Ghana

Source of vegetable	Number of isolates	Au	A	Fox	Cx	C	Cip	G	N	S	Su	T	W
FA 1	2	1	1	2						1	1		
FA 2	1	1	1	1		1			1		1	1	1
FA 5	1	1	1	1		1			1	1	1	1	1
FA 7	5	3	3	4						2	2		
MC 2	4	2	2	2						1	3	2	1
MC 3	19	2	2	2						5	18	1	
MC 4	1										1		

FA: farming area, MC: market center

Au: amoxycillin/clavulanic acid, A: ampicillin, Fox: cefoxitin, Cx: ceftriaxone, C: chloramphenicol, Cip: ciprofloxacin, G: gentamycin, N: nalidixic acid, S: streptomycin, Su: sulfisoxazole, T: tetracycline, W: trimethoprim

Table 5.5 Antibiotic resistance patterns of the *Salmonella* isolated from leafy green vegetables from farming areas and market centers in Accra, Ghana

Resistance patterns	Number of antibiotic resisted	Number of isolates
AAuFox	3	3
AAuFoxSSu	5	4
ACSSuTAuFoxNW	9	1
ACTAuFoxNSuW	8	1
ASSuWAuFoxT	7	1
Fox	1	3
SSu	2	6
Su	1	14

Au: amoxycillin/clavulanic acid, A: ampicillin, Fox: cefoxitin, C: chloramphenicol, N: nalidixic acid, S: streptomycin, Su: sulfisoxazole, T: tetracycline, W: trimethoprim

Table 5.6 Minimum inhibitory concentration of the *Salmonella* isolated from leafy green vegetables from farming areas and market centers in Accra, Ghana

Salmonella isolate	Ampicillin	Cefoxitin	Streptomycin	Sulfisoxazole	Tetracycline
	µg/ml				
1	128	128	64	> 1024	2
17	> 256	> 256	< 4	> 1024	4
32	> 256	128	32	> 1024	2
43	256	64	16	> 1024	4
47	256	256	16	> 1024	2
61	256	128	128	> 1024	2
150	4	4	32	> 1024	4
153	2	8	32	> 1024	< 1
157	4	4	32	> 1024	4
163	4	4	32	> 1024	4
167	2	4	32	> 1024	4
173	4	4	32	> 1024	2
179	4	4	32	> 1024	2
183	128	128	32	> 1024	2
185	8	32	<4	> 1024	< 1
191	16	32	32	> 1024	2
244	2	4	16	> 1024	2
300	> 256	128	8	> 1024	2
315	4	16	16	> 1024	>128
323	2	4	32	> 1024	2
325	2	8	16	> 1024	2
333	2	4	16	> 1024	2
337	2	4	32	> 1024	2
343	4	16	16	> 1024	> 128
347	2	4	32	> 1024	2
383	< 1	8	32	> 1024	2
415	2	4	32	> 1024	2
417	> 256	128	8	> 1024	2
490	> 256	>256	>256	> 1024	128
536	4	4	16	> 1024	2
760	64	64	64	> 1024	2
793	>256	>256	32	> 1024	16
935	>256	>256	64	> 1024	16

CHAPTER 6

COMPARISON OF SANITATION METHODS COMMONLY USED BY THE US FRESH PRODUCE INDUSTRY OR GHANAIAN HOUSEHOLDS IN INACTIVATING *SALMONELLA* ARTIFICIALLY INOCULATED ON LEAFY GREEN VEGETABLES

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Abstract

A previous survey conducted by our laboratory revealed a poor microbial quality of, and the presence of *Salmonella* on leafy green vegetables grown or sold in Accra, Ghana. The aim of this study was to compare the effectiveness of some sanitation methods commonly used by Ghanaian households to several sanitation approaches used by the fresh produce industry in the U.S. in reducing the population of *Salmonella* on vegetables. Cabbage and lettuce artificially inoculated with each of 3 *Salmonella* cocktails were treated with sterile water and 6 different sanitizers. The efficacies of the sanitizers in inactivating *Salmonella* were evaluated using standard plate count assay. The effect of the treatments on overall preference was evaluated by a consumer panel. Treatments with chlorine, citric acid, peracetic acid, and vinegar resulted in a 2.44 to 2.67 log CFU/g reduction in *Salmonella* counts on cabbage and were significantly ($p \leq 0.05$) more effective compared to the other treatments used in the study. Treatment with citric acid was most ($p \leq 0.05$) effective in reducing the level of *Salmonella* on lettuce (3 log CFU/g). Treatments with ozonated water, salt, and water were significantly ($p \leq 0.05$) less effective, compared to other treatments used in the study, in reducing *Salmonella* counts on both types of vegetables (0.02 to 1.32 log CFU/g). The consumer panel concluded that citric acid, vinegar, and water treated cabbages were the more preferred ($p \leq 0.05$) than chlorine treated ones. Lettuce treated with citric acid and water were more preferred ($p \leq 0.05$) whilst salt treated lettuce was the least preferred. Thus, among sanitation method commonly used in Ghanaian households, citric acid and vinegar were effective in reducing microbial counts on vegetables with lesser detrimental effect on their sensory quality.

Key words: cabbage, lettuce, sanitizers, citric acid, water, vinegar

1.0 Introduction

Leafy green vegetables are good sources of vitamins, minerals, and dietary fiber. Consumption of leafy green vegetables in addition to other fruits and vegetables have been encouraged to promote overall health (USDA and HHS, 2010). Leafy green vegetables are usually consumed raw with minimal processing or no kill steps, thus have been identified as potential vehicles for transmitting bacterial pathogens and other microbiological hazards (CDC, 2014; FAO/WHO, 2008a)

Leafy vegetables and herbs contaminated with pathogenic microorganisms have been implicated in outbreaks of infections in Australia (16%), Brazil (18%), Canada (12%), Finland (13%), and Sweden (5%) from 1996 to 2006 (FAO/WHO, 2008a). Contaminated leafy vegetables were also involved in 3 outbreaks of infections from 2007 to 2012 in some European countries (EFSA/ ECDC, 2015). In the U.S., leafy green vegetables were implicated in 78% of 501 outbreaks that occurred between 1998 to 2012 (Herman et al., 2015).

Although leafy green vegetables are presumed to be associated with some disease outbreaks in Ghana, none were reported before 2008 due to poor surveillance system (FAO/WHO, 2008b). In a 2013 report by Der et al. (2013), however, salad vegetables were identified as the source of an outbreak of infection in Koforidua, Ghana. In other African countries such as Zambia, consumption of raw vegetables were linked to cholera epidemic in 2003 to 2004 (CDC, 2004).

Vegetables can be contaminated with human pathogens at various stages from cultivation to the consumer's plate. During production, leafy green vegetables can be contaminated from contaminated irrigation water, soil, inadequately composted manure, grazing animals, and human workers. During post-harvest, handling, packing, transport, storage, and processing of leafy green vegetables can lead to contamination (Beuchat, 1995; Harris et al., 2003).

The U.S. Food and Drug Administration (FDA) (2011) recommends washing of vegetables before slicing or consumption to minimize foodborne outbreaks. Microorganisms can attach to the surfaces of fresh produce or internalize plant tissues. No sanitizing method can completely remove contaminants from leafy vegetables (Zander and Bunning, 2014). However, different cleaning or decontamination practices can be used to reduce microbial loads on vegetables. Some common household leafy vegetables sanitizing methods used in Ghana and other West African countries include the use of water, salt solution, bleach, vinegar, lemon juice, and potassium permanganate (Amoah et al., 2007). Woldetsadik et al. (2017) identified the use of tap water and solutions of salt, vinegar, detergents, and commercial vegetable sanitizers (not specified) to be the common vegetable washing methods in Ethiopia. Running tape water, vinegar solution are used at homes, while ozonated water, electrolyzed oxidizing water, sodium hypochlorite, peracetic acid, and commercial vegetable washes are commonly used by the fresh produce industry in U.S. and European countries (Fishburn et al., 2012; Pezzuto et al., 2016; Zander, A and Bunning, 2014).

Previous studies have shown washing practices have varying efficacy in reducing the microbial counts on vegetables (Amoah et al., 2007; Fishburn et al., 2012; Pezzuto et al., 2016). Some physical and chemical cleaning practices have been observed to affect the visual quality of vegetables (Amoah et al., 2007; Petri et al., 2015; Sanz et al., 2002). The aim of this study was to compare the effectiveness of some sanitation methods commonly used by Ghanaian households to a few sanitation approaches used by the fresh produce industry in the U.S. in reducing the population of *Salmonella* on cabbage and lettuce. The overall preference of the leafy green vegetables as affected by the different sanitizer treatments was compared by a consumer panel.

2.0 Materials and Methods

2.1 *Salmonella* strains

In a previous study of our laboratory (Quansah et al., 2018), a total of 33 *Salmonella* were isolated from two exotic (cabbage and lettuce), and four indigenous (*Amaranthus* sp., *Corchorus olitorius, Hibiscus sabdariffa and Solanum macrocarpon*), leafy green vegetables (n=328) collected from 12 farming areas and 4 market centers in Accra, Ghana. In the current study, 9 of the isolates were selected based on their source of isolation – from different type of vegetables, farms, and market centers. The isolates were retrieved from frozen storage, inoculated on tryptic soy agar (TSA) (Becton Dickinson, Sparks, MD), and grown at 37 ºC for 24 h before being used in the following experiments.

2.2 Inoculum preparation

The isolates were inoculated into tryptic soy broth (Becton Dickinson, Sparks, MD) and incubated at 37 ºC for 24 h. The absorbance of the cultures was read at 600 nm and its optical density adjusted to 0.8 to 0.9 (approximately 1.5 x 10^8 CFU/ml). Each culture (1 ml) was centrifuged at 14,000 *g* for 2 min using Eppendorf centrifuge 5415 C (Brinkman Instruments, INC., Westbury, N.Y.). Each resulting pellet was suspended and washed with 1 ml of sterile water, and re-centrifuged as described above. This step was repeated, and after the final washing, the pellet was re-suspended in 1 ml of sterile water and an equal volume of three *Salmonella* cultures was combined to form a three-strain cocktail. A total of 3 cocktails consisting of the 9 *Salmonella* isolates described above were used in the study.

2.3 Vegetables preparation and inoculation

Lettuce (green leaf) and cabbage (cannonball) were purchased from a local grocery store. The outer leaves of lettuce and cabbage were discarded. Vegetables were cut to 5 cm diameter pieces with a cookie cutter (Harold Import Co., Lakewood, NJ). The vegetables were disinfected with 20,000 ppm sodium hypochlorite by shaking on a platform shaker (Lab-Line Instrumental Co., Melrose Park, IL, USA) at 30 rpm for 15 min. Dey-Engley (DE) neutralizing broth (Becton Dickinson, Sparks, MD) was added to the vegetables to remove residual chlorine by shaking under the conditions described above. Sterile water was then added to the vegetables to wash and remove any residues. This washing step was repeated once.

Forty-five pieces ($\geq$ 135 g of cabbage and $\geq$ 50 g of lettuce) of the vegetables were placed into 3 L of sterile water inoculated with 6 ml (approximately 1.0×10^8 CFU/ml) of a three-strain *Salmonella* cocktail. Cells of *Salmonella* were allowed to attach to the surface of the vegetables for 5 h at room temperature with shaking at 30 rpm on the platform shaker. The vegetables were rinsed twice with sterile water under the conditions described above to remove unattached and loosely attached *Salmonella* cells. The vegetables were subsequently spread out to dry on sterilized paper towels placed on plastic trays (34 x 43 cm, Molded fiber glass tray company, Linesville, PA) for 2 h in a biosafety cabinet.

2.4 Efficacy of sanitizers

Vegetables with attached *Salmonella* cells were treated with 2% citric acid (Ball citric acid, Jarden home brands, Canada), 200 ppm chlorine (household GV bleach$_1$, Bentonville, AR), 3 ppm ozonated water, 80 ppm peracetic acid (Acros organics, Austria), 20% sodium chloride (GV iodized salt, Bentonville, AR), 40% vinegar (5% acetic acid, GV distilled white vinegar, Bentonville, AR), and sterile deionized water. Inoculated but untreated vegetable leaves were included in the study as controls. Ozonated water was generated using a 1 KNT generator (Oxidation technologies, Inwood, IA, USA) by bubbling ozone into deionized water at controlled flow rate of 4 LPM. A small commercial (industrial) oxygen cylinder (D) (Airgas USA LLC, Kennesaw, GA) with 95% oxygen purity and an output pressure of 5-10 PSI was utilized to achieve a final concentration of 3 ppm ozonated water. Prepared ozonated water was used within 12 min of production.

Vegetables (5 pieces, ≥ 15 g of cabbage and ≥ 5.5 g of lettuce) were treated with 300 ml of each sanitizer with shaking on a platform shaker at 30 rpm for 10 min. After the treatments, the sanitizer solutions were removed, and the leaves transferred into sterile sampling bags (Twirl'em, Labplas, Sainte-Julie, QC, Canada) for analysis. A 50 ml aliquot of each of the following solutions was added to the vegetables to neutralize the sanitizers. Peptone (0.1%) water was added to untreated and water treated leaves, phosphate saline buffer (pH 7.0) to vinegar and citric acid treated leaves, DE buffer to chlorine and ozonated water treated leaves, water to salt treated leaves, and 1% sodium thiosulphate solution was added to peracetic acid treated leaves.

2.5 Microbial enumeration

The vegetables were homogenized, using a stomacher (Seward stomacher 400 lab system, England), at normal speed for 1 min. A 0.1 ml of appropriate dilutions of each vegetable homogenate was inoculated on TSA and Xylose lactose tergitol 4 (XLT4) agar with supplements (Becton Dickinson, Sparks, MD). Inoculated plates of TSA and XLT4 agar were incubated at 37 ºC for 24 h and resulting colonies were enumerated after the incubation.

2.6 Preference ranking test

A forced-choice consumer preference ranking test was carried out on uninoculated cabbage and lettuce samples treated with the seven cleaning or sanitizing agents. The test was approved by University of Georgia's Institutional Review Board (STUDY00004592) before being carried out. A panel of 97 consumers (Female – 59 and Male – 38) were recruited based on their usage of the two vegetables. Treated vegetables (5 pieces) were served in 96-ml clear polypropylene portion cups (Prime source, St Louis, MO) to the consumers. Two sets for each of the two vegetables were prepared and served separately. Within a set, the samples were randomly presented to consumer panelists for ranking from 1 (most preferred) to 8 (least preferred) based on the overall perception of the vegetables, mainly appearance and odor, without tasting the samples. The data was collected using Compusense Cloud (Compusense Inc., Guelph, Ontario, Canada).

2.7 Statistical analysis

One-way analysis of variance (ANOVA) test was performed and Fisher's Least Significant Difference test was used to compare the means ($p \leq 0.05$) using the Statistical Analysis Software (Version 9.4). The effects of sanitizer in reducing *Salmonella* counts on vegetables were determined. The effects of sanitizing treatments on the overall preference of treated vegetables were also determined.

For the preference ranking test, Friedman's test (nonparametric one-way ANOVA) was carried out on the data. Nemenyi's procedure (two-tailed) was used for post-hoc mean separation at 5% level of significance.

3.0 Results

Chlorine, citric acid, peracetic acid, and vinegar treatments led to a 2.44 to 2.67 log CFU/g reduction in *Salmonella* counts on cabbage leaves, which were significantly ($p \leq 0.05$) higher compared to the efficacies of the other treatments evaluated in the study (Table 6.1). Sanitizing cabbage leaves with ozonated water and salt solution also significantly reduced *Salmonella* counts with an overall reduction of 0.61 to 0.90 log CFU/g. However, washing cabbage leaves with sterile water did not significantly reduced *Salmonella* counts on cabbage leaves.

Sanitizing of lettuce with citric acid was most effective ($p \leq 0.0$), which resulted in a reduction of *Salmonella* counts by 3.0 log CFU/g. Treatments with peracetic acid and vinegar achieved a 2.48 and 2.62 log CFU/g reduction, respectively on lettuce leaves, and these levels of reduction were similar ($p \leq 0.0$) to those caused by the treatments with citric acid and chlorine (2.15 log CFU/g). Washing lettuce with water caused a 0.71 log CFU/g reduction in *Salmonella* counts, which was not significantly ($p \geq 0.05$) different from the 0.85 and 1.32 log CFU/g reductions caused by the treatments with ozonated water or salt solution. The efficacy of washing lettuce with water to reduce *Salmonella* counts was comparable ($p \leq 0.0$) to that of untreated lettuce.

A total of 97 consumer panel evaluated the overall preference of cabbage and lettuce leaves treated with water and various sanitizers used in this study. The consumer panel consisted of White/Caucasian (75%), African-American (15%), Asian (7%), and Hispanic/Latino (3%). They were aged 21 to 30 (20.6%), 31 to 40 (25.8%), 41 to 50 (15.5%), 51 to 60 (23.7%), 61 to 70 (11.3%) and only three consumers were aged 71 years or more (3.1%).

Citric acid, vinegar, and water treated cabbage leaves were preferred more ($p \leq 0.05$) by the consumer panel, while chlorine treated cabbage leaves was the least preferred ($p \leq 0.05$). However, there were no significant ($p \geq 0.05$) difference in consumer preference for the untreated control, ozonated water, peracetic acid, and salt treated cabbage leaves, from other treated samples (Fig. 6.1).

Lettuce samples treated with citric acid and the control were preferred more ($p \leq 0.05$) while salt treated lettuce was less preferred (Fig. 6.2) by the consumer panel. Consumer preference to chlorine treated lettuce was significantly ($p \leq 0.05$) different from the untreated control, as well as citric acid, and salt treated ones. Preferences for lettuce leaves treated with ozonated water, peracetic acid, vinegar and water were not significantly ($p \geq 0.05$) different from untreated control leaves as well as leaves treated with citric acid and chlorine (Fig 6.2).

4.0 Discussion

The use of citric acid solution in sanitizing cabbage and lettuce leaves was generally most effective ($p \leq 0.0$) in reducing *Salmonella* counts compared to the treatments with other sanitizers included in the study (Table 6.1). In previous research, rocket leafy (*Eruca* *sativa)* vegetable treated with lemon juice with 4.16% citric acid (pH 3.0) and vinegar with 3.95% acetic acid (pH not specified) for 15 min led to a 2.95 and 2.20 log CFU/g reduction, respectively in *S.* Typhimurium counts (Sengun and Karapinar, 2005). These findings are comparable to the 2.67 - 3.03 log CFU/g reduction caused by citric acid treatments but lower than the 2.62 - 2.67 log CFU/g reduction resulted from vinegar treatments in the current study. Citric acid and acetic acid are the main organic acids in lemon juice and vinegar, respectively. Lipid permeability of organic acids in undissociated form is their main mechanism of antimicrobial action (Theron and Lues, 2010). The mean pH of citric acid solution in this study was 2.06 ± 0.08 which is below its pKa_1, pKa_2, and pKa_3 of 3.13, 4.76 and 6.40, respectively (Silva et al., 2009). The mean pH of acetic acid solution in this study was 2.54 ± 0.12, which was also below its pKa of 4.8 (Rodríguez Cordero et al., 2015). At such low external pH, undissociated citric and acetic acid can penetrate bacterial cell membranes. Once the organic acids reach the cytoplasm, the high cytoplasmic pH causes the dissociation of the organic acid into ions, leading to the accumulation of protons. The decreasing intracellular pH affects cellular enzymes function, nutrient transport, and alteration of cell membrane permeability (Brul and Coote, 1999; Theron and Lues, 2010).

Neal et al. (2012) observed that treatment of spinach with ozonated water (1 ppm), peracetic acid (80 ppm), and water caused a 0.9, 0.8 and 0.7 log CFU/g reduction, respectively in *Salmonella* populations. These observations are comparable to the 0.85 - 0.90 and 0.02 - 0.70 log CFU/g reductions caused by treatments with ozonated water, and sterile water but lower than the 2.44 to 2.48 log CFU/g reduction resulting from treatment with peracetic acid in the present study. Lee et al. (2014) observed treatment of cabbage with 100 ppm peracetic acid or 200 ppm sodium hypochlorite for 1 min reduced *S.*

Typhimurium populations by 1.38 and 1.61 log CFU/g respectively, which were lower compared to the 2.44 and 2.63 log CFU/g reduction observed in the present study. This difference in *Salmonella* population reduction can be attributed to different bacterial strains and experimental conditions used in the two studies (ÖLmez, 2010; Tang et al., 2012). Lee et al. (2014) exposed vegetables to the bacterial inocula at 5 ºC for 16 to 18 h compared to 21 ºC for 5 h used in this study. According to Tang et al. (2012), longer inoculation time could led to increased levels of bacterial attachment on fresh produce and this can subsequently affect the efficacy of sanitizers in removing attached bacterial cells. Peracetic acid is a strong oxidizer and a mixture of hydrogen peroxide and acetic acid. Peracetic acid oxidizes by electron transfer and produces reactive oxygen species, oxidize sulfhydryl and disulphide bonds, disrupts cellular membranes, and denatures proteins and enzymes (Joshi et al., 2013; Kitis, 2004). The antimicrobial activity of ozone is mainly by attack of molecular ozone at low pH and sometimes action of free radicals formed because of ozone decomposition at high pH. Ozone oxidizes cellular membrane glycoproteins and/or glycolipids and subsequently oxidizes cellular components, leading to leakage of cellular components, cell lysis, and eventual cell death (Guzel-Seydim et al., 2004; Ölmez, 2012). The 0.85 - 0.90 log CFU/g reduction by treatment with ozonated water observed in the present study was lower compared to findings of Fishburn et al. (2012) where ozone (0.75 ppm) reduced *S. enterica, E. coli* O157:H7, and *L. monocytogenes* on lettuce by 1.4 to 1.8 log CFU/g, although our study used a higher concentration of ozone (3 ppm). This observation can be attributed to differences in pH of ozonated water used. The ozonated water used by Fishburn et al. (2012) had pH value of 5 while the pH of ozonated water used in this study was 8.5. At higher pH, ozone decays at a faster rate, which affects the stability and thus efficacy of ozone as a sanitizer (Gardoni et al., 2012).

Decontamination of lettuce using chlorine led to a 2.15 log CFU/g reduction in the present study, which is significantly ($p \leq 0.05$) higher compared to the levels of reduction by salt, ozonated water and water. This observation is similar to the findings of Fishburn et al. (2012) that chlorine (70 ppm free chlorine) treatment achieved a 2.05, 2.34 and 2.16 log unit reduction in *Salmonella, E. coli* O157:H7, and *L. monocytogenes* counts, respectively on lettuce. According to Stewart and Olson (1996), the antimicrobial action of chlorine on bacteria is due to alterations in cellular permeability that leads to leakage of essential cellular components. Other antimicrobial action include interference in cell membrane functions, irreversible binding of sulfhydryl groups, leading to impairment of enzyme and protein functions and denaturation of nucleic acids (Stewart and Olson, 1996).

Treatment with salt was the least effective ($p \leq 0.05$) in reducing *Salmonella* counts on the vegetables with an average reduction of 0.61 and 1.32 log CFU/g on cabbage and lettuce, respectively. The main mechanism of action of salt is by alternation of trans-membrane osmotic pressure. High salt concentration puts osmotic pressure on bacterial cytoplasmic membranes, pulls it away from the cell wall, causing lyses and eventually death of bacterial cells (Hogg, 2005; Nester et al., 2003).

Treatment with water did not significantly reduce *Salmonella* counts on cabbage and lettuce leaves. *Salmonella* counts on water treated leaves were similar ($p \leq 0.0$) to those on untreated control leaves. This is not surprising because water has no antimicrobial properties and will be effective in removing only loosely attached bacterial cells. Lang et al. (2004) reported that water reduced the level of *Salmonella, E. coli* O157:H7, and *L.*

monocytogenes by 0.21 - 0.61, 0.4 - 0.89 and 0.16 - 0.87 log units, respectively on lettuce, similar to the 0.02 - 0.71 log CFU/g reduction observed in this study.

Patel and Sharma (2010) reported that *Salmonella* cells attach more strongly to lettuce than cabbage leaves which may lead to difficulty of removing attached bacterial cells from lettuce compared to cabbage leaves. The authors attributed this observation to the presence of fewer damaged cuticles on surfaces of cabbage than lettuce leaves which provide niches and sites for *Salmonella* colonization. The tougher nature of cabbage leaves minimizes damage to cuticles on leaf surfaces. However, there was relatively higher log reduction of *Salmonella* counts on lettuce after treatment, with three out of the six sanitizers used in this study, in addition to the control (water), compared to cabbage.

The appearance or visual quality of vegetables are more likely to affect consumer preference than other product attributes like taste, aroma, and texture (Barrett et al., 2010). Some sanitizers like salt, chlorine, and vinegar used in this study affected the appearance and odor of the treated vegetables, which may have affected their preference by the consumer panel. Salt treated lettuce was the least preferred, which may be because of the effect of osmotic pressure that caused dehydration of vegetable leaves. Comments by the consumer panel (not shown) indicated that they could perceive the smell of chlorine on the vegetables, which may have also affected their preference (Figs. 6.1 and 6.2). Similar to our observation, Vijayakumar and Wolf-Hall (2002) reported that, among lettuce treated with household sanitizers like water, lemon juice, chlorine and vinegar, lettuce treated with 4% chlorine was among the least preferred by the consumer panel.

5.0 Conclusion

Washing vegetables with only water was not effective ($p \leq 0.0$) in reducing *Salmonella* counts on vegetables. However, some of the sanitizers used in the study were effective in reducing ($p \leq 0.0$) *Salmonella* counts on vegetables. Citric acid and vinegar were the most effective ($p \leq 0.0$) Ghanaian household's sanitizers in reducing *Salmonella* counts on the vegetables and had less effect on their sensory quality.

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Extension. 9–10.

Table 6.1 Efficacy of sanitizers in reducing *Salmonella* counts on leafy vegetables

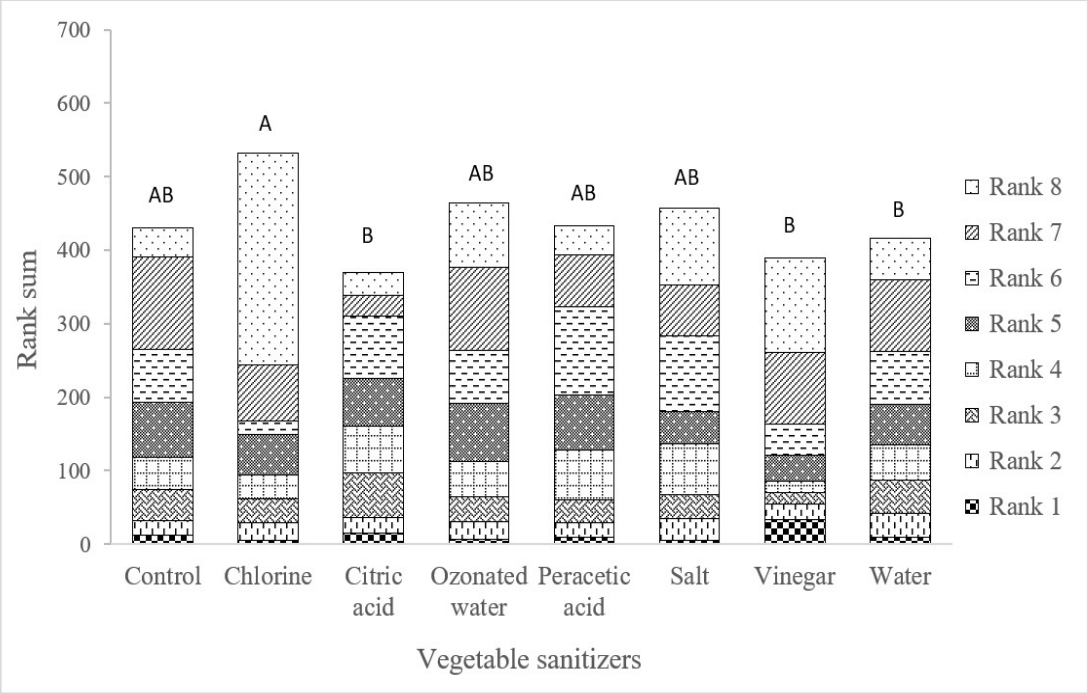
Leafy green vegetable	Treatment	TSA	XLT4
		Log reduction (CFU/g)	
Cabbage	Untreated	0.00^{d}	0.00^{c}
	Water	0.25^{cd}	0.02^{c}
	Salt (20%)	0.57^{bc}	0.60^{bc}
	Ozonated water (3 ppm)	0.87^{b}	0.90^{b}
	Citric acid (2%)	2.35^{a}	2.67^{a}
	Vinegar (40%)	2.44^{a}	2.67^{a}
	Peracetic acid (80 ppm)	2.62^{a}	2.44^{a}
	Chlorine bleach (200 ppm)	2.85^{a}	2.63^{a}
Lettuce	Untreated	0.00^{c}	0.0^{d}
	Water	0.65^{b}	0.72^{cd}
	Salt (20%)	1.00^{b}	1.32^{c}
	Ozonated water (3 ppm)	0.98^{b}	0.85^{c}
	Citric acid (2%)	2.38^{a}	3.03^{a}
	Vinegar (40%)	2.15^{a}	2.62^{ab}
	Peracetic acid (80 ppm)	2.37^{a}	2.48^{ab}
	Chlorine bleach (200 ppm)	2.19^{a}	2.15^{b}

Means of *Salmonella* population, on each type of vegetable leaves, followed by different letter within a column are significantly different ($p \leq 0.05$).

Figure Legends

Fig. 6.1 Preference ranking of cabbage by a consumer panel for the various cleaning and sanitizing treatments.

Fig. 6.2 Preference ranking of lettuce by a consumer panel for the various cleaning and sanitizing treatments.



700
600
500
400
300
200
100
0
Rank sum
AB
A
B
AB
AB
AB
B
B
Control
Chlorine
Citric acid
Ozonated water
Peracetic acid
Salt
Vinegar
Water
Vegetable sanitizers
Rank 8
Rank 7
Rank 6
Rank 5
Rank 4
Rank 3
Rank 2
Rank 1

Fig 6.1

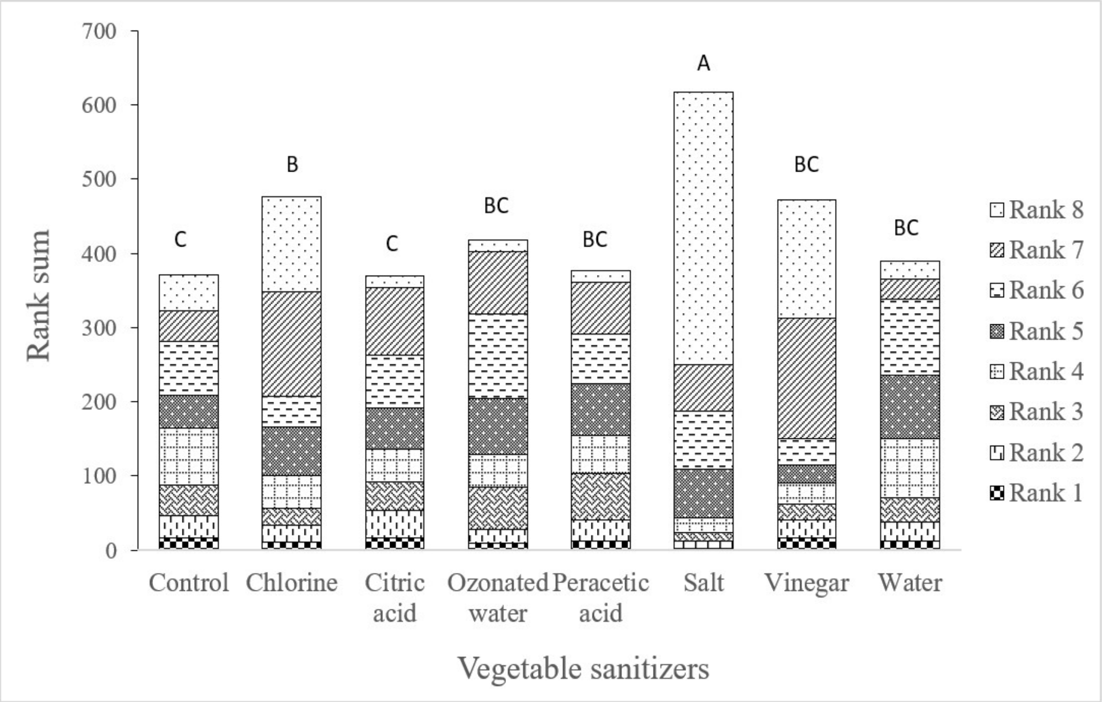

700
600
500
400
300
200
100
0
Rank sum
C
B
C
BC
BC
A
BC
BC
Control
Chlorine
Citric acid
Ozonated water
Peracetic acid
Salt
Vinegar
Water
Vegetable sanitizers
Rank 8
Rank 7
Rank 6
Rank 5
Rank 4
Rank 3
Rank 2
Rank 1

Fig 6.2

CHAPTER 7

CONCLUSIONS

1. The use of polluted irrigation water and improperly composted manure were common in vegetable farms in Accra, Ghana. Vegetables were harvested with bare hands, transported and stored under non-refrigerated conditions. Production of vegetables with clean irrigation water and properly composted manure should be encouraged. Harvested produce should be stored under refrigerated conditions. Vegetable producers and sellers should be educated on how to produce safer vegetables and maintain their quality at all time.

2. Leafy green vegetables sampled in the study had high fecal coliform and enterococcus counts. Market vegetables generally had higher microbial counts than the vegetables collected from the farms. Apart from *Amaranthus* sp., indigenous leafy green vegetables had higher yeast and mold, fecal coliform, and *Enterococcus* counts than lettuce and cabbage. *Salmonella* was isolated from both exotic and indigenous vegetables collected from the farms and markets. This study suggests that leafy green vegetables grown and sold in some urban areas of Ghana are associated with high microbial counts and *Salmonella*. Consumption of fresh leafy green vegetables without sanitizing or heat treatment should be discouraged.

3. *Salmonella* isolated from leafy green vegetables collected from farms and sellers in Accra, Ghana were resistant to antibiotics, of which 30.3% were multidrug resistant. One isolate carried a single gene (*dfrA7*) encoding for trimethoprim resistance. These results show that green leafy vegetables grown or sold in Ghana are contaminated with antibiotic resistant *Salmonella*. Consumption of contaminated vegetables can be a possible route for acquiring antibiotic resistant pathogens.

4. Washing of vegetables with only water was not effective ($p \leq 0.0$) in reducing *Salmonella* counts on vegetables. However, some of the sanitizers (citric acid, vinegar, peracetic acid and chlorine) were effective in reducing ($p \leq 0.0$) *Salmonella* counts. Citric acid and vinegar were the most effective ($p \leq 0.0$) Ghanaian household sanitizers in reducing *Salmonella* counts on the vegetables without jeopardizing their sensory quality.

APPENDIX A

PESTICIDE RESIDUES IN LEAFY GREEN VEGETABLES GROWN IN ACCRA METROPOLIS, GHANA

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[1] Quansah, J. K., A. P. Kunadu, F. K. Saalia, and J. Chen. To be submitted to *African Journal of Food Science*.

Abstract

Different types of pesticides are used in vegetable farming in Ghana to control pests and disease, reduce crop losses and improve yield. The objective of this study was to determine pesticide residues on leafy green vegetables grown in Accra, Ghana. Two exotic and 4 indigenous Ghanaian vegetables (n= 34) were examined for 15 organochlorines, 13 organophosphorus, and 9 synthetic pyrethroids pesticide residues using the modified QuEChERS procedure. Pesticide residues were detected on 50% (17/34) of tested vegetable samples. Although no organochlorine pesticide residue was detected, five organophosphorus (chlorpyrifos, diazinon, dimethoate, malathion, pirimiphos-methyl) and 6 synthetic pyrethroids (bifenthrin, cypermethrin, deltamethrin, fenvalerate, lambda-cyhalothrin, permethrin) pesticide residues were detected on the tested vegetable samples. Chlorpyrifos and deltamethrin were the most detected pesticide residues in 9 and 7 vegetable samples respectively. Among the 17 vegetable samples that tested positive for pesticide residues, 3 (two lettuces and one *H. sabdariffa*) had residues exceeding adopted EU MRLs values. Vegetable farmers should be informed about the health effects associated with pesticide abuse and be encouraged to comply with appropriate measures associated with pesticide use.

Keywords: pesticide residue, leafy green vegetables, maximum residue limit, organochlorines, organophosphates, synthetic parathyroids

1.0 Introduction

Vegetable farming is practiced in major cities in Ghana and is a significant source of income, as well as fresh produce for the table. Various types of exotic and indigenous Ghanaian vegetables are cultivated (Danso et al., 2014). According to Dinham (2003), vegetables attract a wide range of pests and diseases, thus, an intensive pest management program is necessary. Different types of pesticides (organochlorines, organophosphates and synthetic parathyroids) are used in vegetable farming in Ghana to control pests and diseases, reduce crop losses and improve yield. However, previous studies have reported rampant abuse of pesticide in vegetable farming in Ghana (Amoabeng et al., 2017; Asare and Sefa, 2015), Nigeria (Ugwu et al., 2015), and Ethiopia (Mengistie et al., 2017).

Misuse of pesticides in farming can lead to increased concentrations of pesticide residues on vegetables. Residues of pesticides can persist in vegetables and be ingested by consumers. Some pesticides have been associated with several diseases in humans, including cancers, birth defects, and reproductive disorders (Kim et al., 2017; Mostafalou and Abdollahi, 2013). Pesticides can also persist in the soil and pollute surrounding surface and ground water bodies (Aktar et al., 2009).

Washing and processing of fresh produce such as peeling, blanching, and canning may reduce pesticide residue levels, but cannot completely remove the residues from the vegetables (Bonnecherè et al., 2012; Keikotlhaile et al., 2010). The objective of this study was to determine pesticide residues on leafy green vegetables grown in Accra, Ghana

2.0 Materials and Methods

2.1 Sample collection

Samples of two exotic, lettuce (*Lactuca sativa*) and cabbage (*Brassica oleracea*), and four indigenous (*Amaranthus* sp., *Solanum macrocarpon, Hibiscus sabdariffa*, and *Corchorus olitorius*) leafy green vegetables (n= 34) were collected in duplicate from 15 farms in 10 different farming areas in Accra Metropolis of Ghana from March 2016 to September 2016 (Table A1). Approximately 1 kg of each vegetable sample was collected from the farmers who cultivated them and were willing to participate in the study. Collected leafy green vegetables were placed into sterile, plastic Ziploc bags (Nasco, Fort Atkinson, WI), kept in a car cooler (Rubbermaid; Newell Brands Inc, Atlanta, GA USA) with ice packs (VWR, Lutterworth, UK), and transported to a pesticide residue laboratory in the Ghana Standard Authority, Accra, Ghana.

2.2 Extraction and clean up

The extraction procedure described by Biziuk et al, (2015) was used with modification. The vegetable samples were homogenized, and 10 g of comminuted samples were weighed into 50 ml centrifuge tubes. Acetonitrile (10 ml) was added and vortexed for 1 min. A mixture of magnesium sulphate anhydrous (4 g), sodium chloride (1 g), sodium citrate dihydrate (1 g), and sodium hydrogen citrate (0.5 g) was added and vortexed for 1 min. The samples were centrifuged at 2,700 g for 5 min. Six ml of supernatant was transferred into another centrifuge tube containing 150 mg of primary secondary amine and 900 mg of magnesium sulphate, shaken for 30 sec, and centrifuged at 2,000 g for 5 min. Later, 4 ml of the supernatant was transferred into a round bottom flask, 40 ml formic acid solution in acetonitrile was added and concentrated to dryness with a rotary evaporator (below 40 °C). After evaporation, 1 ml ethyl acetate was added for reconstitution and the extract was transferred into a 2 ml vial and placed in the autosampler of a Varian gas chromatograph (GC) CP-3800 (Varian Inc, Walnut Creek, CA). A matrix blank and reagent blank were also analyzed.

2.3 Analysis of organochlorine and synthetic pyrethroids pesticide residues

A Varian GC CP-3800 (Varian Inc, Walnut Creek, CA) equipped with ^{63}Ni electron capture detector (ECD) and pulse flame photometric detector (PFPD) was used to analyse organochlorine (OC) and synthetic pyrethroids (SP) residues. The following GC parameters were used for analysis: capillary column coated with VF-5 ms (30 m+10 EZ Guard, 0.25 mm, 0.25 µm film thickness). Nitrogen gas was used as carrier gas and make-up gas at a flow rate of 1.0 and 29 ml/min, respectively. The temperature of the injector operating in splitless mode was held at 270 °C, and ECD was set at 300 °C. The column oven temperature was programmed at: 70 °C for 2 min, and increased steadily at a rate of 25 °C min^{-1} to 180 °C and increased at 5 °C min^{-1} up to 300 °C. The injection volume of the GC was 1 µl. A Varian Saturn GC (CP-3800 2200 MS/MS) mass spectrometer was used to detect the residues.

The organochlorine pesticide residues tested were lindane, dieldrin, p,p'-DDT, beta-HCH, delta-HCH, p,p'-DDD, p,p'-DDE, aldrin, endrin, heptachlor, gamma-chlordane, alpha-endosulfan, beta-endosulfan, endosulfan sulfate and methoxychlor Synthetic pyrethroids pesticide residues tested include allethrin, lambda-cyhalothrin, cypermethrin, deltamethrin, fenvalerate, bifenthrin, cyfluthrin, fenpropathrin and permethrin (Dr. Ehrenstorfer GmbH, Augsburg, Germany).

2.4 Analysis of organophosphorus pesticide residues

A Varian GC CP-3800 equipped with pulse flame photometric detector (PFPD) was used to analyze organophosphorus pesticide residues. The GC parameters used for analysis was: capillary column coated with VF-1701 ms (30 m, 0.25 mm, 0.25 mm film thickness). Nitrogen gas was used as carrier gas at a flow rate of 2.0 ml/min with Air 1, Air 2 and H_2 flow rate 17, 10 and 14 ml/min respectively. The temperature of injector operating in splitless mode was held at 270 °C, and PFPD was set at 280 °C. The column oven temperature was programmed at: 70 °C for 2 min, and increased steadily at a rate of 25 °C min^{-1} to 200 °C min^{-1} and increased at 20 °C min^{-1} up to 250 °C. The injection volume of the GC was 2 µl. A Varian Saturn GC (CP-3800 2200 MS/MS) was used to detect the residues.

Organophosphorus pesticide residues tested include chlorpyrifos, diazinon, methamidophos, phorate, fonofos, parathion, chlorfenvinphos, malathion, profenofos fenitrothion, ethoprophos, dimethoate and pirimiphos-methyl.

2.5 Pesticide standard solutions

Pesticide standards (obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany)) were dissolved in ethyl acetate to make 1 mg/ml pesticide spike solution. The spike solution was added to the matrix after sample for extraction was weighed. The matrix blank was fortified with pesticide solutions to achieve 0.05 mg/kg and allowed to stand for 10 min to enhance pesticide interaction with matrix.

The calibration standards were made by diluting pesticide solutions with ethyl acetate to make concentration of 0.005, 0.01, 0.02, 0.05, 0.1, 0.50 and 1.0 mg/ml. Level of detection of organochlorines, organophosphorus and synthetic pyrethroids pesticide residues was 0.005, 0.010 and 0.010 mg/kg respectively.

3.0 Results

Pesticide residues were detected in 50% (17/34) of the vegetable samples (Table A2). While no organochlorine pesticide residue was detected, five organophosphorus (chlorpyrifos, diazinon, dimethoate, malathion, pirimiphos-methyl) and 6 synthetic pyrethroids (bifenthrin, cypermethrin, deltamethrin, fenvalerate, lambda-cyhalothrin, permethrin) pesticide residues were detected in the vegetables (Table A3 and A4). Bifenthrin, fenvalerate, malathion, permethrin and pirimiphos-methyl residues were detected in one vegetable sample each. Dimethoate and diazinon were detected in 2 vegetable samples. Cypermethrin and lambda-cyhalothirn were detected in five vegetable samples. Chlorpyrifos and deltamethrin were the most detected pesticide residues, in 9 and 7 vegetable samples, respectively. Three out of the 17 contaminated vegetables (two lettuces and one *H. sabdariffa*) exceed adopted EU maximum residue limits (MRLs) values and the other 14 vegetables had detectable pesticide residues, but the levels of the residues were within the EU MRLs. The residues chlorpyrifos (0.340 mg/kg in lettuce), dimethoate (0.100 mg/kg in lettuce) and lambda-cyhalothrin (0.625 mg/kg in *H. sabdariffa)* were detected above their EU MRLs in 3 vegetable samples. None of the pesticide residues detected in the vegetable samples were above the available U.S. MRLs.

Apart from the 2 cabbage samples from farming area 8, pesticide residues were detected in leafy green vegetables collected from the other 8 farming areas involved in the study (Table 3 and 4). Pesticides residues were detected in vegetables from FA 1(3/8), FA

2(1/3), FA 3(2/2), FA 4 (1/2), FA 5 (4/5), FA 6 (2/4), FA 7 (1/4), FA 10 (2/2) and FA 12 (1/2).

Deltamethrin was the only pesticide residue detected in 38% (3/8) of *Amaranthus* samples. Five organophosphorus pesticide residues (chlorpyrifos, diazinon, dimethoate, malathion, pirimiphos-methyl) were detected in 33.3% (1/3) of the cabbage samples (Table 3). Chlorpyrifos (2), cypermethrin (1), deltamethrin (1), lambda-cyhalothrin (2), and permethrin (1) were detected in 75% (3/4) of *C. olitorius* samples. Chlorpyrifos and lambda-cyhalothrin were detected in both (2/2) *H. sabdariffa* samples. Chlorpyrifos (5), dimethoate (1), cypermethrin (3), deltamethrin (1), and fenvalerate (1) were detected in 42.9% (6/14) lettuce samples. Diazinon (1), cypermethrin (1), deltamethrin (2), and lambda-cyhalothrin (1) were detected in 66.7% (2/3) of *S. macrocarpon* samples (Table 5).

4. Discussion

No organochlorine pesticide residue was detected in the leafy green vegetables. This is not surprising because the use of organochlorine pesticides have been banned for over two decades in Ghana and most countries because they have high persistence and accumulate in the human body and environment, and are associated with carcinogenic and neurotoxic effects in humans (Kleanthi et al., 2008; Singh, 2016). This observation is in contrast with previous studies by Amoah et al. (2006) who found 3 organochlorine pesticide residues (DDT, endosulfan and lindane) in lettuce in Ghana, despite the ban on use of organochlorine pesticides at that time.

Pesticide residues were detected in 50% of the vegetables in this study (Table 3 and 4). This observation is not surprising because misuse of pesticide in vegetable farming has been reported in previous studies in Ghana (Amoabeng et al., 2017; Asare and Sefa, 2015). Our findings are similar to the report of pesticide residues detected in 52% of 155 vegetables in Ghana by Blankson et al. (2016). Our observations are lower compared to pesticide residues detected in 92.1% of 214 leafy and non-leafy vegetables in China (Yu et al., 2016) and 65% of 118 leafy vegetables in Chile (Elgueta et al., 2017). These results indicate the high rate of use of pesticides in vegetable farming in many countries. Detection of pesticide residues above their MRLs was observed in about 9% of the leafy green vegetables used in this study. This is low compared to 21% (Blankson et al., 2016), 23% (Yu et al., 2016), and 27% (Elgueta et al., 2017) observed in previous studies.

Chlorpyrifos was the most detected pesticide residues in 26.4% (9/34) of the vegetables in this study. This is similar to observation of Blankson et al. (2016) that chlorpyrifos was detected in 35 out of 155 of non-leafy vegetable samples collected in Ghana. Blankson et al. (2016) attributed this observation to the fact that chlorpyrifos based pesticides are one of the frequently applied organophosphorus pesticides in vegetable production in the region. Sapbamrer and Hongsibsong (2014) reported chlorpyrifos was the most detected pesticide in 14 out of 84 leafy and non-leafy vegetables sampled in Thailand.

Two pesticide residues with levels above their MRLs were detected in 2 lettuce samples and five organophosphorus pesticide residues (chlorpyrifos, diazinon, dimethoate, malathion, pirimiphos-methyl) with levels within their MRLs were detected in one cabbage sample. This may indicate the intensive use of pesticides to control pests and diseases during the cultivation of these vegetables.

5.0 Conclusion

Pesticide residues were detected on 50% of vegetable samples and pesticide residues above their MRLs were detected in about 9% of the vegetables used in the study. No organochlorine pesticide residue was detected on the leafy green vegetables. Chlorpyrifos and deltamethrin were the most detected pesticide residues in the vegetables. Farmers should be trained to minimize the use of pesticide in vegetable farming and comply with appropriate measures associated with their use.

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Table A1: Number of leafy green vegetable samples collected from individual farming areas in Accra metropolis, Ghana

Sampling Source	*Amaranthus*	Cabbage	*C. olitorius*	*H. sabdariffa*	Lettuce	*S. macrocarpon*	Total
FA 1	2	0	2	0	4	0	8
FA 2	2	1	0	0	0	0	3
FA 3	0	0	0	2	0	0	2
FA 4	0	0	0	0	2	0	2
FA 5	0	0	2	0	2	1	5
FA 6	2	0	0	0	0	2	4
FA 7	2	0	0	0	2	0	4
FA 8	0	2	0	0	0	0	2
FA 9	0	0	0	0	0	0	0
FA 10	0	0	0	0	2	0	2
FA 11	0	0	0	0	0	0	0
FA 12	0	0	0	0	2	0	2
Total	8	3	4	2	14	3	34

FA: Farming area

Table A2 Maximum residue limits of the EU and U.S.A for leafy green vegetables used in the study

	Amaranthus		Cabbage		*C. olitorius*		*H. sabdariffa*		Lettuce		*S. macrocarpon*	
	EU	USA	EU	USA	EU	USA	EU	USA	EU	USA	EU	USA
Pesticide residues	mg/kg											
Bifenthrin	0.05	-	1*	4	2	-	0.05	-	2*		0.3	-
Chlorpyrifos	0.05	-	0.01	1	0.05	-	0.05	-	0.05		0.05	-
Cypermethrin	0.07	-	1	2	2	-	0.07	-	2		0.5	-
Deltamethrin	0.5	-	0.1		0.5	-	0.5	-	0.5		0.3	-
Diazinon	0.01	-	0.01	0.7	0.01	-	0.01	-	0.01	0.7	0.01	-
Dimethoate	0.02	-	0.02*		0.02	-	0.02	-	0.02*	2	0.02	-
Fenvalerate	0.02	8.00	0.08		0.02	-	0.02	-	0.2		0.02	-
Malathion	0.02		0.02	8	0.02	-	0.02	-	0.5	8	0.02	-
Lambda-cyhalothirn	0.5	-	0.2	0.4	0.5	-	0.5	-	0.5	2	0.5	-
Permethrin	0.05	20	0.05	6	0.05	-	0.05	-	0.05	20	0.05	-
Pirimiphos-methyl	0.05	-	0.01		0.05	-	0.05	-	0.01		0.05	-

- MRLs not available

*= changes in MRLS after 2016

Bifenthrin changed to 0.4 for cabbage and 0.01 for lettuce in Aug 2017

Dimethoate will change to 0.01 for cabbage and lettuce from 2019

Table A3 Concentration of organophosphorus pesticide residues detected in the leafy green vegetables collected from farms in Accra, Ghana

Farming area	Leafy green vegetable	Chlorpyrifos	Diazinon	Dimethoate	Pirimiphos-methyl	Malathion
		mg/kg				
FA 1	Amaranthus sp	ND	ND	ND	ND	ND
FA 1	Lettuce	0.018	ND	ND	ND	ND
FA 1	*C. olitorius*	ND	ND	ND	ND	ND
FA 2	Cabbage	0.010	<0.010	<0.010	<0.010	0.010
FA 3	*H. sabdariffa*	ND	ND	ND	ND	ND
FA 3	*H. sabdariffa*	0.022	ND	ND	ND	ND
FA 4	Lettuce	0.041	ND	ND	ND	ND
FA 5	*C. olitorius*	<0.010	ND	ND	ND	ND
FA 5	*C. olitorius*	0.040	ND	ND	ND	ND
FA 5	Lettuce	0.010	ND	0.100*	ND	ND
FA 5	*S. macrocarpon*	ND	<0.010	ND	ND	ND
FA 6	Amaranthus sp	ND	ND	ND	ND	ND
FA 6	*S. macrocarpon*	ND	ND	ND	ND	ND
FA 7	Amaranthus sp	ND	ND	ND	ND	ND
FA 10	Lettuce	0.010	ND	ND	ND	ND
FA 10	Lettuce	0.340*	ND	ND	ND	ND
FA 12	Lettuce	ND	ND	ND	ND	ND

FA: Farming area, ND: non-detectable pesticide residue, *: exceed MRL

Table A4 Concentration of synthetic pyrethroids pesticide residues detected in the leafy green vegetables collected from farms in Accra, Ghana

Farming area	Leafy green vegetable	Bifenthrin	Cypermethrin	Fenvalerate	Deltamethrin	Lambda-cyhalothirn	Permethrin
					mg/kg		
FA 1	Amaranthus	ND	ND	ND	0.022	ND	ND
FA 1	Lettuce	ND	ND	ND	ND	ND	ND
FA 1	*C. olitorius*	ND	ND	ND	ND	ND	0.032
FA 2	Cabbage	ND	ND	ND	ND	ND	ND
FA 3	*H. sabdariffa*	ND	ND	ND	ND	0.365	ND
FA 3	*H. sabdariffa*	ND	ND	ND	ND	0.625*	ND
FA 4	Lettuce	ND	0.025	ND	ND	ND	ND
FA 5	*C. olitorius*	ND	0.020	ND	0.010	0.210	ND
FA 5	*C. olitorius*	ND	ND	ND	ND	0.415	ND
FA 5	Lettuce	ND	0.025	ND	0.010	ND	ND
FA 5	*S. macrocarpon*	ND	0.120	ND	0.020	0.010	ND
FA 6	Amaranthus	ND	ND	ND	0.010	ND	ND
FA 6	*S. macrocarpon*	ND	ND	ND	0.020	ND	ND
FA 7	Amaranthus	ND	ND	ND	0.010	ND	ND
FA 10	Lettuce	ND	ND	ND	ND	ND	ND
FA 10	Lettuce	ND	0.085	ND	ND	ND	ND
FA 12	Lettuce	0.010	ND	0.010	ND	ND	ND

ND: non-detectable pesticide residue, *: exceed MRL

Table A5 Number of pesticide residues detected in the leafy green vegetables collected from farms in Accra, Ghana

Pesticide residues	Amaranthus	Cabbage	*C. olitorius*	*H. sabdariffa*	Lettuce	*S. macrocarpon*	Total
Chlorpyrifos	0	1	2	1	5	0	9
Diazinon	0	1	0	0	0	1	2
Dimethoate	0	1	0	0	1	0	2
Malathion	0	1	0	0	0	0	1
Pirimiphos-methyl	0	1	0	0	0	0	1
Bifenthrin	0	0	0	0	1	0	1
Cypermethrin	0	0	1	0	3	1	5
Deltamethrin	3	0	1	0	1	2	7
Fenvalerate	0	0	0	0	1	0	1
Lambda-cyhalothirn	0	0	2	2	0	1	5
Permethrin	0	0	1	0	0	0	1
Total	3	5	7	3	12	5	35