

MICROBIOLOGICAL CONTAMINATION OF SOME FRESH LEAFY VEGETABLES SOLD IN CAPE COAST, GHANA

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Abstract

This study evaluated the microbiological contamination of cabbage, lettuce, and scallions sold in Abura and Kotokuraba markets in Cape Coast, Ghana. These vegetables were analyzed for the presence and levels of microorganisms using standard microbiological procedures. Results revealed bacterial and fungal contaminations of the vegetables from Abura and Kotokuraba markets. Mean bacterial counts recorded in Nutrient Agar, for example, from Kotokuraba market were 1.93×10^8 , 1.23×10^8 , and 1.17×10^8 cfu/ml for cabbage, lettuce and scallion, respectively, higher than mean bacterial counts recorded from Abura market at 9.9×10^7 , 2.8×10^7 , and 6.60×10^7 cfu/ml for cabbage, lettuce and scallion, respectively. Conversely, the mean fungal counts for cabbage, lettuce and scallion were higher at Abura market than Kotokuraba market. Bacteria isolated from the vegetables include *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Salmonella* spp., *Serratia marcescens*, and *Staphylococcus*, whereas fungi of the genera *Aspergillus*, *Candida*, *Fusarium*, *Penicillium*, and *Rhodotorula* were isolated. These results indicate that the vegetables are significantly contaminated, and have poor microbiological quality that could potentially result in outbreak of foodborne illnesses. Contaminations of the vegetables were due to poor pre- and post-harvest handling practices. The implications of findings of this study on tourism and hospitality industries in Cape Coast are discussed.

Keywords: cabbage, food microbiology, foodborne microorganisms, Ghana, lettuce, scallion

Introduction

Vegetables remain one of the most important portions of a balanced diet, because of the many attendant nutritional benefits that inure to their consumption. Vegetables are rich dietary sources of micronutrients, minerals, vitamins and, most importantly, antioxidants and

fibre, all of which are vital to human health, wellbeing and disease prevention (Eni *et al.*, 2010). Overall public awareness of healthy eating habits has been intensified, prompting an increasing demand and incorporation of fresh vegetables in diets, especially because of their convenience, freshness, taste and health

benefits (Olaimat & Holley, 2012). The Food and Agriculture Organization and the World Health Organization strongly recommend more than 400 g/day intake of fruits and vegetables in diets to promote good health (FAO/WHO, 2014).

Despite the health benefits derived from consuming fresh vegetables, the risk of microbiological contaminations in vegetables is of public concern due to the possibility of vegetable contaminations along the food chain, beginning from the vegetable farm to the dinner table (Kuan *et al.*, 2017); this concern is compounded by the fact that these vegetables are mostly eaten fresh (not cooked) and washing may not guarantee decontamination, so that any resident microorganism easily enter the alimentary canal. Thus, these public concerns are justifiably informed based on reported cases of numerous foodborne disease outbreaks caused by consumption of fresh vegetables contaminated by microorganisms like *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* spp (CDC, 2011 & 2012; Maffei *et al.*, 2013; Beuchat 2017). Various estimates of incidences of foodborne disease outbreaks have resulted in illnesses, hospitalizations, deaths, and even food recalls in some countries, particularly United States of America (Scallan *et al.*, 2011; WHO, 2015; CDC, 2016).

Microbiological contamination of vegetables can occur directly or indirectly through (i) contact with soil, dust, water, and (ii) punctures and open cuts of tissues of vegetables; thus, contaminations of vegetables may occur internally or externally during cultivation, harvest, packaging, storage, transporting and marketing (Solomon *et al.*, 2002; Bernstein, 2007; Eni *et al.*, 2010; de Giusti *et al.*, 2010). Contaminated vegetables rot more quickly and become unwholesome

due to microbiological activities of resident microorganisms. Vegetable spoilage by microorganisms is possible, because vegetables serve as suitable substrates that present the microorganisms with a plethora of valuable nutrients essential for microbial growth. This is a concern for consumers and public health and safety practitioners. Vegetables, whether sold as part of street-vended foods, as part of the menu at eateries, or used domestically for family meals, have understandably come under strict scrutiny by the Ghanaian vegetable consumers. This is particularly so since there is widespread belief and acknowledgement among most consumers that vegetables sold on the Ghanaian markets are largely produced and handled under unhygienic conditions and practices, i.e., application of manure to soils during cultivation of vegetables, irrigation of vegetables farms with heavily contaminated water, use of unclean water to wash vegetables, dressing vegetables on the bare floor and concrete slabs on the farm and at the grocery, and insufficient pre- and post-harvest inspections of vegetables (Wachtel *et al.*, 2002a & 2002b). In Ghana, pipe-borne water is the most commonly used water source to irrigate vegetable farms, but due to the high cost and unreliable water supply, most vegetable farmers have resorted to the use of water from streams, wells and storm drains as alternate water sources to irrigate vegetable farms. Unfortunately, these alternative water sources are heavily polluted, especially with pathogenic and toxigenic microorganisms (Keraita *et al.*, 2008; Donkor *et al.*, 2010; Adentunde *et al.*, 2015).

Some studies in Ghana suggest that vegetables sold at the groceries in the markets are contaminated with microorganisms, mostly bacteria (Mensah *et al.*, 2001; Obeng *et al.*, 2007; Keraita *et al.*, 2008; Donkor *et al.*, 2009 & 2010; Amoah, 2014; Adentunde *et al.*,

2015; Abakari *et al.*, 2018). Findings of these studies motivated the need to evaluate the microbiological quality of fresh, leafy salad vegetables that are usually not cooked, but are consumed raw. The present study, therefore, aimed to investigate the microbiological contaminations of cabbage, lettuce, and scallions sold in two markets in Cape Coast, Ghana. The significance of this study stems from the fact that Cape Coast, capital of the Central Region of Ghana, accounted for over 41% of tourist visits to Ghana for the period 2015 – 2018. This is not surprising given that the Central Region hosts what is called the “big three” of Ghana’s tourist attractions – Kakum National Park, Cape Coast Castle, and Elmina Castle. Thus, the outbreak of any foodborne disease may have grave implications for not only citizens, but also tourists. In addition, and equally important, the University of Cape Coast hosts a significant number of international student population that may be susceptible to such potential outbreaks of foodborne diseases. We present the findings of this study, recommend strategies for improving wholesomeness of vegetables before consumption, and discuss the implications of the findings to the tourism and hospitality industries in Cape Coast, Ghana.

Experimental

Sample collection

A headed cabbage (*Brassica oleracea* var. *capitata* L.), a bunch of loose-leaf lettuce (*Lactuca sativa*) and a bundle of scallions (*Allium fistulosum*) were purchased from three randomly selected groceries (named A, B, and C) each at Abura and Kotokuraba markets in Cape Coast. The vegetables were separately packaged in sterile zip-locks, placed on ice in an ice chest, and immediately transported to the laboratory for microbiological analyses using standard microbiological procedures. We in-

terviewed the vegetable vendors at the selected groceries with regards to pre- and post-harvest handling practices during transportation of vegetables to the groceries. Also, observations such as washing of vegetables before sale, frequent cleaning of benches, weighing scales and knives, use of aprons, wearing of hair coverings, proper handling of money, use of gloves while serving, etc. were made during purchase. We also interacted with the selected vegetable vendors about hygienic conditions and handling of vegetables at their groceries.

Study area

The study was conducted in Cape Coast (5° 7’53.44” North, 1° 16’53.11” West) in Ghana. Cape Coast has a population of 169,894 (Yafetto *et al.*, 2019). Most of the residents engage in economic activities such as farming, fishing and petty trading, and the Metropolis, over the last decade, has demonstrated the market potential for local produce and manufactured goods for agriculture and commerce sectors from other parts of Ghana. In addition to its market potential, Cape Coast is a historic, popular tourism destination for both domestic and foreign tourists, and it continues to enjoy an enviable status as one of the most important tourism destinations in Ghana (Ghana Statistical Service, 2010). There a number of markets located within Cape Coast, popular amongst them are the Abura and Kotokuraba markets, from which samples were obtained for this study. Although both markets are located in the populous communities, Abura is a suburb located at the Cape Coast North Constituency, whereas Kotokuraba is located at the Cape Coast South Constituency. Specifically, Abura market is a relatively small market that serves suburbs like Adisadel, Akotokyir, Kakumdo, Pedu, the Cape Coast Technical University and the University of Cape Coast; Kotokuraba

market, on the other hand, is a larger, ultra-modern central market that serves almost all the suburbs in Cape Coast (Fig. 1).

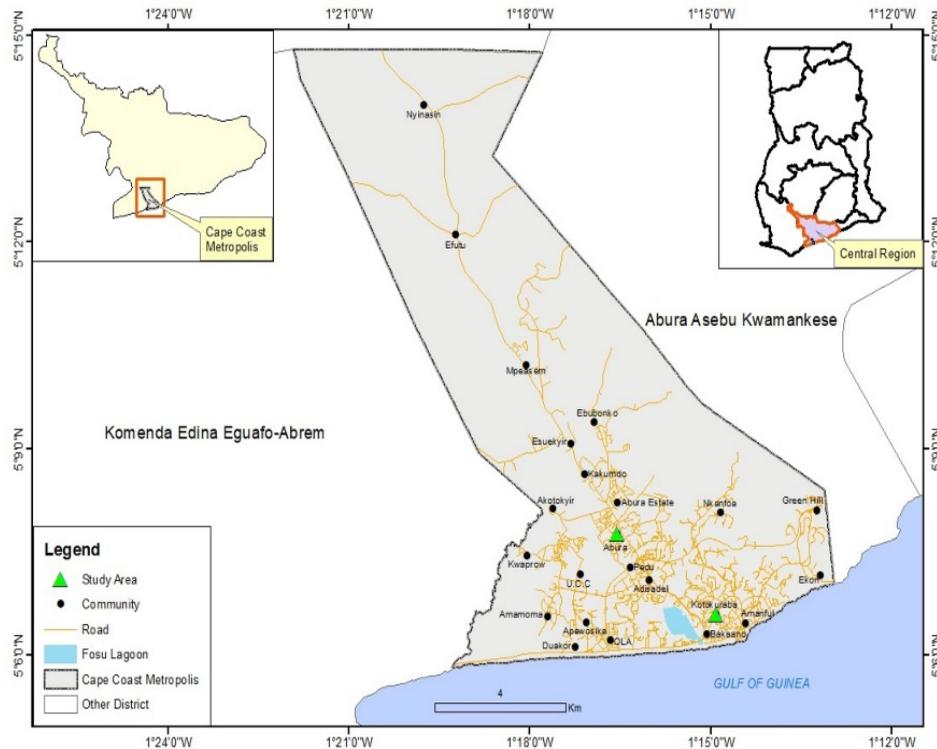


Fig. 1: Cape Coast Metropolis indicating two suburbs, Abura and Kotokuraba, where vegetables were obtained for the study. Source: Richard Adade, Centre for Coastal Management, University of Cape Coast, Cape Coast, Ghana.

Culture media

MacConkey Agar (MAC), Nutrient Agar (NA), and Potato Dextrose Agar (PDA) media (Oxoid Ltd., England) used in this study were prepared according to the manufacturer's instructions. The media were sterilized at a pressure of 1.1 kg/cm² at 121°C for 15 minutes using an autoclave (Shimadzu KY-23D, Omron Corporation, Japan). MAC and NA media were used to isolate bacteria, whereas PDA medium was used to isolate fungi from the vegetables.

Stock Solutions from Vegetables

Stock solutions from vegetables were prepared based on method described by Yafetto *et al.* (2019). Ten grams of leaves from the headed cabbage were weighed and placed in a 200 ml conical flask, after which 90 ml sterile 0.1% peptone water (the diluent) was added. The cabbage leaves and diluent were gently, but thoroughly vortexed on a platform shaker (Scientific Industries, Inc., USA) for 5 minutes to extricate any microbe that may be resident on the surface of the cabbage leaves. The dilu-

ent was decanted and used as a stock solution to prepare serial dilutions for microbiological analyses. Serially diluted stock solution of dilution factor 10^{-3} was used for fungal isolation, while a 10^{-6} dilution was used for bacterial isolation. The same procedure and dilutions were repeated for the preparation of stock solutions from lettuce and scallions.

Isolation of microbes

To isolate fungi, 1 ml aliquot (10^{-3} dilution) of stock solution of cabbage leaves was dispensed into a Petri dish. Twenty milliliters of molten, but cooled PDA medium was added and evenly mixed with the stock solution. The culture plates were prepared in triplicates for cabbage from each of the three randomly selected vendors from a market (i.e., n=9). The culture plates were allowed to solidify and incubated at 25°C for 7 days in an incubator (Plus II, Gallenkamp, England). This isolation procedure was repeated for the microbiological analyses of lettuce and scallions from the two markets.

Bacteria were isolated with the same procedure used to isolate fungi, except that 1 ml aliquot of stock solution with a dilution factor 10^{-6} was dispensed separately into 20 ml molten MacConkey agar and 20 ml molten nutrient agar. The culture plates were prepared in triplicates for cabbage from each of the three randomly selected vendors from a market (i.e., n=9). The plates were then allowed to solidify and incubated at 37°C for 2 days. This isolation procedure was repeated for the microbiological analyses of lettuce and scallions from the two markets.

Identification of isolated fungi

Fungi isolated from cabbage, lettuce and scallions were identified according to the procedure described by Yafetto *et al.* (2019).

A small amount of fungal mycelium was removed from a fungal colony, immersed in a drop of 70% of alcohol on a glass slide, after which it was gently teased out with the inoculation needle. A drop of lactophenol cotton blue dye was added to the mycelium and gently covered with a glass coverslip. Excess dye was removed from the prepared fungal specimen with a Whatman filter paper (Sigma-Aldrich, USA), which was then observed under the microscope. Microscopic identifications of fungi were determined based on morphological and growth features using Leica DM500 Compound Microscope (Leica Microsystems, Switzerland) in consultations with the relevant identification manuals (Davet & Rouxel, 2000; Ellis *et al.*, 2007; Pitt & Hocking, 2009; Watanabe, 2010; Campbell *et al.*, 2013; Kidd *et al.*, 2016).

Identification of isolated bacteria

Sterilized platinum inoculation loops (Thomas Scientific, New Jersey, USA) were used to streak isolated bacteria on MAC and NA to obtain pure bacterial sub-cultures, after which they were incubated at 37°C for 24 hours. Identities of bacteria cultures were confirmed using morphological characteristics, and citrate and catalase standard biochemical tests (Cowan *et al.*, 1993; Cheesbrough, 2006). Images of fungal and bacterial cultures were captured with an in-built, 8 megapixels' digital camera of Infinix NOTE 4 cellphone (Model X572, China).

Colony counts and statistical analyses

Bacterial and fungal colonies were enumerated with digital colony counter (Stuart Scientific, UK), and expressed as mean colony forming units per ml (cfu/ml) and mean \log_{10} cfu/ml (\pm Standard Error), where n=9. Differences in the means were statistically analyzed using ANOVA at confidence level of 95% ($p \leq 0.05$).

Results and Discussion

Bacterial and fungal contaminations of cabbage, lettuce and scallions

This study evaluated the microbiological quality of cabbage, lettuce and scallions sold in Cape Coast, Ghana. Significantly high means of bacterial counts were observed in cabbage, lettuce and scallion from both Kotokuraba and Abura markets than fungi (Tables 1 and 2). Interestingly, mean fungal counts for cabbage, lettuce and scallions were higher at Abura market than at Kotokuraba market (Tables 1 and 2). Most bacteria isolated and the high levels of microbial contaminations reported for cabbage, lettuce and scallions in this study are similar to findings previously reported for other studies both in Ghana and elsewhere (Adebolu & Ifesan, 2001; Mensah *et al.*, 2001; Wachtel *et al.*, 2002a,b; Omemu & Bankole, 2005; Tambekar & Mundhada, 2006; Olayemi 2007; Donkor *et al.*, 2009; Uzeh *et al.*, 2009; Donkor *et al.*, 2010; Eni *et al.* 2010; Yeboah-Manu *et al.*, 2010; Annan-Prah *et al.*, 2011; Goja & Mahmoud, 2013; Maffei *et al.*, 2013; Buyukunal, 2015; Kuan *et al.*, 2017; Yeleliere *et al.*, 2017; Abakari *et al.*, 2018). Results of bacterial and fungal colony counts of cabbage, lettuce and scallions in this study are presented graphically as mean \log_{10} cfu/ml (Figs. 2 and 3). Despite the high microbial contaminations reported in this study, it is important to note that none of the vegetables showed any visible sign of spoilage. However, spoilage of vegetables could occur if not properly washed before stored (Center for Food Safety, 2014). Eni *et al.* (2010) reported that bacterial contamination of vegetables decreased significantly when vegetables were washed with increasing concentrations of vinegar. This suggests that the dire consequence of disease outbreaks that usually occurs as a

result of the consumption of contaminated vegetables can be effectively managed, if vegetables are properly handled with prudent washing regimen.

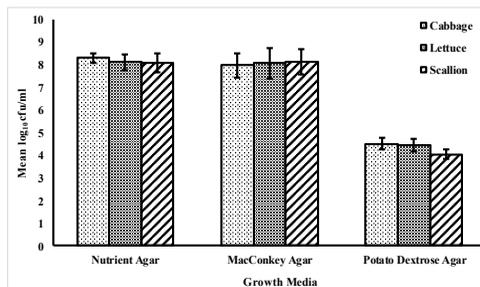


Fig. 2: Mean \log_{10} cfu/ml of bacteria and fungi isolated from cabbage, lettuce and scallion from Kotokuraba market.

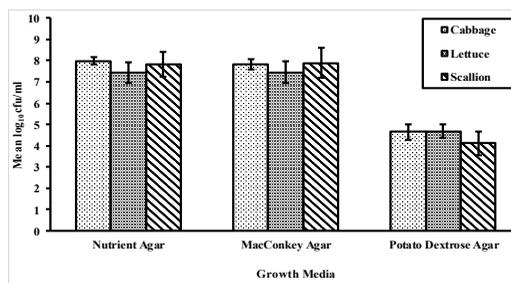


Fig. 3: Mean \log_{10} cfu/ml of bacteria and fungi isolated from cabbage, lettuce and scallion from Abura market.

Bacteria and fungi isolated from vegetables

The selected vegetables evaluated in this study were contaminated with bacteria and fungi (Fig. 4). Bacteria that had contaminated the vegetables were *Enterobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Salmonella* spp., *Serratia marcescens* and *Staphylococcus*. *E. coli*, *Salmonella* spp., and *Staphylococcus* spp. possibly originated from irrigated water contaminated with faecal matter. Eni *et al.* (2010) isolated similar bacteria in their study. *E. coli*, and *Salmonella* spp. have been

particularly implicated in foodborne disease outbreaks associated with consumption of vegetables (Beuchat, 2002; Sivapalasingam *et al.*, 2004; CDC, 2011 & 2012; Maffei *et al.*, 2013). Isolation of these bacteria from vegetables evaluated in this study should raise food safety concerns among consumers, vendors, vegetable farmers, tourists, and health authorities, because of their implications in global foodborne disease outbreaks (Beuchat, 2002; Sivapalasingam, 2004; CDC, 2011, 2012; Scallan *et al.*, 2011; Maffei *et al.*, 2013). Compared to the guidelines of the Health Protection Agency (2009) and Center for Food Safety (2014), based on the mean colony counts reported for this study, it is obvious that the extent of microbiological contamination of the vegetables from the two markets is high (Tables 1 and 2). This suggests that most of the vegetables sold in Cape Coast may be unwholesome for consumption, so that food safety concerns among consumers in the Metropolis is understandable.

Curiously, *Serratia marcescens*, a pink-pigmented Gram-negative bacterium of the family *Enterobacteriaceae* shown in Fig. 4, was isolated from scallion from both markets, and from lettuce from Kotokuraba market only. Surprisingly, *S. marcescens* was not isolated from cabbage from both markets (Table 3). *Serratia marcescens* is an opportunistic pathogen that can be isolated from water, soil, plants, air, insects, and other animals: it has the ability to colonize and survive on materials such as hospital instruments and farm

equipment; it is involved in food spoilage and can cause serious diseases, that can sometimes be fatal, in humans (Rafii, 2014); and it is associated with urinary and respiratory infections, endocarditis, osteomyelitis, *septicemia*, wound infections, eye infections and meningitis (Buckle, 2016). From findings earlier reported in studies in Ghana on microbiological qualities of vegetables, our isolation of *S. marcescens* from lettuce and scallion in this study is likely the first report of its presence on any vegetable sold on the Ghanaian market. This finding is critical, because *Serratia* spp. as opportunistic pathogens, may be widespread on vegetables sold in Cape Coast, and their presence on these vegetables poses serious food safety concern. This situation is worsened considering the detection of the aforementioned pathogenic and toxigenic bacteria in this study (Fig 4). *Serratia marcescens*, has however been reported on some vegetables in other studies elsewhere (Falomir *et al.*, 2010; Sikora *et al.*, 2012; Akoachere *et al.*, 2018).

Fungi isolated and confirmed from the vegetables are of the genera *Aspergillus*, *Candida*, *Fusarium*, *Penicillium*, and *Rhodotorula* (Fig. 4). Annan-Prah *et al.* (2011) reported fungal contaminations of street-vended foods in Cape Coast including ready-to-eat salads that served as accompaniment to mainly rice dishes. Combined fungal and bacterial activities often lead to deterioration and eventual spoilage of vegetables, making them unwholesome for consumption and potential sources of foodborne illnesses.

TABLE 1

Mean microbial counts of leafy vegetables obtained from Kotokuraba market, Cape Coast, Ghana

Culture medium	Mean bacterial and fungal counts (cfu/ml); n=9		
	Cabbage	Lettuce	Scallions
Nutrient Agar	1.93x10 ⁸	1.23x10 ⁸	1.17x10 ⁸
MacConkey Agar	9.40x10 ⁷	1.16x10 ⁸	1.36x10 ⁸
Potato Dextrose Agar	3.10x10 ⁴	2.90x10 ⁴	1.10x10 ⁴

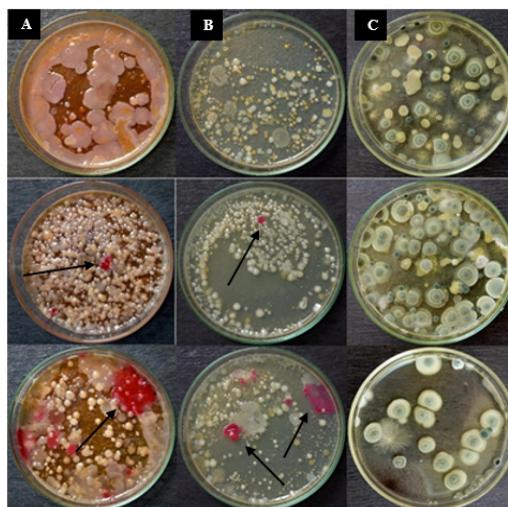


Fig. 4: Bacteria and fungi isolated from cabbage (Top Row), lettuce (Middle Row), and scallion (Bottom Row) on MacConkey Agar medium (column A), Nutrient Agar medium

(column B), and Potato Dextrose Agar medium (column C). Pink-pigmented bacterium, *Serratia marcescens*, isolated from lettuce and scallion (Arrowed).

TABLE 2

Mean microbial counts of leafy vegetables obtained from Abura market, Cape Coast, Ghana.

Culture medium	Mean bacterial and fungal counts (cfu/ml); n=9		
	Cabbage	Lettuce	Scallions
Nutrient Agar	9.90×10^7	2.80×10^7	6.60×10^7
MacConkey Agar	6.80×10^7	2.90×10^7	7.80×10^7
Potato Dextrose Agar	4.50×10^4	4.70×10^4	1.30×10^4

Sources of Microbial Contamination of Vegetables

Bacteria and fungi present on vegetables may be the natural flora of the vegetables. However, the presence of these bacteria and fungi on the vegetables may be attributed to some cultivation practices, including irrigation of vegetable farms with contaminated water, application of animal wastes as manure to enrich the soil, pre- and post-harvest handling practices, transportation, storage and processing (Ray & Bhunia, 2007; Ofori *et al.*, 2009; Eni *et al.*, 2010). It is

well established that most vegetable farmers in Ghana commonly use pipe-borne water, *vis a vis*, water from streams, wells and storm drains to irrigate their vegetable farms. Microorganisms detected in this study are reported also in other studies where water from streams, wells and storm drains were confirmed to have been contaminated with similar microorganisms (Keraita *et al.*, 2008; Donkor *et al.*, 2009; Donkor *et al.*, 2010; Adentunde *et al.*, 2015; Abakari *et al.*, 2018; Akoachere *et al.*, 2018). Our observations and interactions with the

selected vegetable vendors during this study confirmed that vegetables are generally poorly handled when they are offloaded from vehicles that had transported them from the vegetable

farms and at the groceries by the vendors. This poor, unhygienic handling of vegetables is similar to those observed for beef and chevon by Yafetto *et al.* (2019).

TABLE 3

Isolation of Serratia marcescens from vegetables; + (Present); - (Absent)

Presence and absence of <i>Serratia marcescens</i>						
Grocery	Abura market			Kotokuraba market		
	Cabbage	Lettuce	Scallions	Cabbage	Lettuce	Scallions
A	-	-	-	-	+	+
B	-	-	+	-	-	+
C	-	-	+	-	+	+

Implications of Study on Tourism and Hospitality Industries in Cape Coast, Ghana

Food consumed by visitors and tourists is one of the main facilitating factors of the tourism product in a destination. In some destinations, food is the core product and reason for travel. The extent of microbiological contaminations in the food chain, therefore, is a public health concern, especially for a town that has a significant traveler population. In the least, one would expect cases of traveler's diarrhoea, which can disrupt travel plans, in addition to the discomfort caused to the victims. In serious cases, ingestion of contaminated foods can lead to hospitalization or even death. In the present social media era and connected communities, the outbreak of any such cases of foodborne diseases has far-reaching and almost instantaneous implications for destination marketing. Cape Coast and its tourism industry stand to lose a lot if measures are not put in place to improve food handling practices to reduce the level of contaminations in the food chain.

Tourists' perceptions of risks of food safety would influence choice of visiting Cape Coast or not, and, even when they do visit, what and where they eat are greatly influenced by such perceptions. Given that most of these vegeta-

bles are produce of residents in and around the region, outbreaks of foodborne diseases would negatively affect demand for the produce by local farmers, and consequently the distribution of income and benefits from tourism to surrounding local communities.

Recommendations

We recommend that (i) vegetable farmers employ the use of pipe-borne water, instead of water from streams, wells, and storm drains to irrigate their vegetables, and observe good pre- and post-harvest handling practices of vegetables; (ii) vendors should equally observe good personal and environmental hygienic practices at the groceries; (iii) sellers of street-vended foods and consumers must thoroughly wash vegetables before sale and consumption; and (iv) the Food and Drugs Authority, Ghana Standards Authority, Ghana Health Service, Ghana Tourism Authority and National Commission for Civic Education must embark on public education in relation to good agronomic and food handling practices, and strictly enforce compliance to food quality standards at all markets in Cape Coast, Ghana. Future research should be conducted to investigate antimicrobial susceptibility of bacteria isolated from vegetables.

Conclusion

We conclude, based on findings from this study that, (i) microbial contaminations of vegetables evaluated are high, (ii) cabbage, lettuce and scallions sold in markets in Cape Coast, Ghana, are contaminated with bacteria and fungi, (iii) bacterial contaminations of the vegetables are higher than fungal contaminations, and (iv) poor, unhygienic pre- and post-harvest handling of vegetables contribute significantly to contaminations of vegetables. The microorganisms isolated from the vegetables may be pathogenic and/or toxigenic, with the potential of causing foodborne illnesses to consumers. This study provides further impetus to thoroughly investigate the microbiological quality of all kinds of food, whether cooked or raw, to elucidate the extent of food safety risks associated with microbial contaminations of fruits, vegetables, cooked street-vended foods and foods on the menus of restaurants in Cape Coast, Ghana.

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