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ABSTRACT

***Objective*: To assess the microbiological safety of kale (*Brassica oleracea Acephala*) produced from farms and those sold at the markets with special focus on coliforms, *E.coli* and *Salmonella*.**
***Design*: A cross sectional study.**
***Setting*: Peri-Urban farms (in Athi River, Ngong and Wangige), wet markets (in Kawangware, Kangemi and Githurai), supermarkets and high-end specialty store both within Nairobi city.**
***Results*: Mean coliform count on vegetables from farms were $2.6x10^5$ ±$5.0x10^5$ cfu/g while those from the wet markets were $4.6x10^6$ ±$9.1x10^6$ cfu/g, supermarkets, $2.6x10^6$ ±$2.7x10^6$ and high-end specialty store $4.7x10^5$ ±$8.9x10^5$. Coliform numbers obtained on kales from the wet markets and supermarkets were significantly higher ($p<0.05$) compared to those from farms, while kale samples purchased from high- end specialty store had similar levels of coliform loads as those from the farms. *E. coli* prevalence in the wet markets, supermarkets and high-end specialty store were: 40, 20 and 20%, respectively. *Salmonella* was detected on 4.5 and 6.3% of samples collected from the farms in Wangige and wet market in Kawangware, respectively. Fecal coliforms in water used on farms (for irrigation) and in the markets (for washing the vegetables) exceeded levels recommended by World Health Organization (WHO) of 10^3 organisms per 100 milliliter while *Salmonella* was detected in 12.5% of washing water samples collected from Kangemi market.**
***Conclusion*: Poor cultivation practices and poor handling of vegetables along the supply chain could increase the risk of pathogen contamination thus puting the health of the public at risk, therefore good agricultural and handling practices should be observed.**

INTRODUCTION

Vegetables play a role in nutrition; they are a good source of vitamins, minerals and roughage. Thus consumers are encouraged to eat more of these products (1). Vegetable production also contributes to the economy of many countries. There is increased production of these vegetables particularly in and around cities in developing countries and it is estimated that at least 20 million hectares in developing countries are irrigated with low quality water (2). As many as 800 million people in cities and towns world-wide are already raising livestock and cultivating crops in vacant plots, on marginal lands, and in small private plots (3). Studies in nine African cities revealed that, on average, 35% of households engage in some form of agriculture:, this could rise to over 70% depending on their location along the peri-urban to urban transect (4).

In Kenya vegetables are consumed regularly by nearly every household in rural and urban areas (5). There is therefore a commercial vegetable sector that has developed both within Nairobi city and in surrounding areas, taking advantage of the availability of ready and rapid access to urban city markets (6). About 3700 farmers within a 20km radius of Nairobi practice irrigation agriculture and 36% of them use low quality water (7). This low quality water contains the full spectrum of pathogens found in the urban population, many of which can survive for several weeks when discharged onto fields (8). This can be a source of contamination for the vegetables.

Leafy vegetables can also be contaminated by microbial flora along the supply chain. They are exposed to potential microbial contamination at every step including cultivation, harvesting, transportion, packaging, storage and retailing (9). Green Leafy vegetables are thus the commodity group of highest microbiological- safety concern because of their potential to cause large and widespread food borne disease outbreaks; post-harvest processing can "amplify" contamination (10). Kale, a green leafy vegetable is largely produced and consumed by Nairobi city population and therefore chosen for this study. It is a fast growing crop, high yield with high nutritional value. Its local name is *sukumawiki* (to push the week) referring to its importance to many poor people at times of financial hardship (11).

Pathogenic bacteria of major concern on fresh vegetables are: *Salmonella*, *Shigella*, *Escherichia* and *Klebsiella* (12). Of these the most commonly isolated have been *Salmonella* and *Escherichia* (12-13). Total coliform count (including *Escherichia coli, Klebsiella* and *enterobacter*) is normally used to indicate the level of gross bacterial contamination. Contamination by faeces or urine may originate from animals (live or use of animal manure) or human waste disposed into water source used for irrigation. However *E. coli* 0157:H7 is closely associated with contamination from cattle faeces (14). Contamination of fresh produce by *Salmonella* organisms has also been documented (15). Although the presence and sources of pathogenic bacteria found on fresh produce are generally documented (13), this study aimed at determining the bacteriological safety of kale produced from peri-urban areas and sold in various markets of Nairobi. It included bacterial analysis of kale samples at farm and market levels, water used for irrigation at respective farms, and water used for washing of kales at the markets; focusing on coliforms in general, *E. coli* specifically and *Salmonella* organisms. Faecal coliforms served as indicator of faecal contamination and *Salmonella* was chosen because of the potential health hazards it poses to humans. This information is important to health policy makers. It will contribute towards formulation of respective control strategies, for improvement of public health sector.

MATERIALS AND METHODS

Description of sampling sites: Nairobi is at an elevation of 1670m above sea level and covers an area of 700 km^2. The city and its environment receive 1,050 mm of rainfall which is bimodal with the long rains falling between March and May, and short rains between October and December. The mean annual temperature is 17°C, while the mean daily maximum and minimum temperature are 23°C and 12°C, respectively (4). While irrigation activity is expected to occur during the driest months between June and September, for more than 90% of farmers, irrigation is a year round activity (4).

Three peri-urban farming areas were selected based on production practices, proximity to industries and major roads and age of settlement. Athi River represented an industrial area while Ngong and Wangige represented typical peri-urban farming communities whose enterprises are being impacted by rapid urbanisation of Nairobi. It also represented farms of recent settlement and those occupied for over fifty years respectively. Athi River has several manufacturing industries, meat processing plant, wine distillers and hide skin processing industries that discharge their wastes into the Athi river that passes through the town. Most of the farmers in this area utilise the Athi River water to grow the vegetables using furrow irrigation but a small number exploit the untreated effluent water from the meat processing plant. In Ngong site, farmers exploit the streams that flow from the Ngong Hills for the production of the leafy vegetables (kale and spinach). Few farmers use highly contaminated stream water at the Kiserian town. The farmers in Wangige utilise water from streams or boreholes.

Markets were chosen based on perceived cleanliness/sanitation; they also represented market segments frequented by high-income consumers (high-end specialty stores), middle income consumers (supemarkets), and low income consumers (wet markets). Wet markets are normally characterised by poor hygiene and sanitation while supermarkets are perceived to have relatively good hygiene than the wet markets. High-end markets are perceived to be the best in cleanliness, and therefore serve the high income residential estates. All these markets sell a variety of vegetables.

Sampling design for trader and farmers survey: The list of kale traders was developed with the help of respective market management which included local city council for municipal market (Kangemi) and market secretaries for privately managed markets (Kawangware and Githurai). The list included traders who traded during market and non-market days. The kale trader list was verified using the kale trader list done earlier by the project team prior data collection. The sampling list was developed through randomisation, using a table of random numbers. Randomisation was also used to develop the replacement list in case a trader was not available for interview. The number of respondents interviewed in each market was based on the number of traders in a targeted market. That is, probability proportionate to size sampling was used. The survey was conducted early in the morning to late in the evening to enable large coverage of traders who trade at different times of the day. Data were collected both on market and non-market days. Pre-tested questionnaires were administered using personal interview. A total of 80 traders were sampled (Githurai n=30, Kangemi n=24, Kawangware n=26). For farmer survey, list of kale farmers was done with the help of agricultural officers and farmers group leaders in all study sites. The list verification was done prior to the survey. The sampling list was developed through randomisation. Randomisation was also used to develop the replacement list in case a kale farmer was not available for interview. The number of respondents interviewed in each study site was based on size of the kale farmer population. That is, probability proportionate to size sampling was used. Pre-tested questionnaires were administered using personal interview. A total of 120 kale farmers were sampled (Athi River n=24, Ngong=48 and Wangige=48).

Kale and water sampling: From the interviewed traders and farmers list, randomisation was again done to come up with list of traders and farmers from where samples were to be collected. A total of 60 samples (one sample per farmer) were collected (Athi River n=1 6, Ngong=22 and Wangige=22). From the selected farmers, leaves were picked randomly from various locations of the plot by moving in a zigzag manner across the plot while picking leaves from lower, middle and upper part of each selected plant. Samples from each plot were pooled, mixed and sub-sample of 500g taken for analysis. From each selected trader/market, sampling was done by picking bunches from the top, middle and lower part of the display unit/shelves, pooled and sub-sample of 500g weighed and put into sterile paper bags without the sampler touching the vegetables to avoid contamination. In all the sampling sites permission was sought from the owners before the collection of the sample and payment was made for the kale sample(s) collected. Irrigation water samples were taken from the source (where the farmer got water for irrigation); - for the sites where stream water was used, samples were taken at the point where the farmers drew the water. Water used for washing the vegetables at the market was also collected. The respective water samples were collected aseptically into sterile bottles and transported to the laboratory in a cool box, for bacteriological analysis. A total of 150 kale samples and 50 water samples (27 for irrigation and 23 for washing vegetables) were collected for the study.

Sample preparation: Sample preparation and analysis was done at Analabs limited. Each of the kale samples was cut on a sterile chopping board using a sterile knife and blended. The procedure was done in the sterile room. Twenty five (25) grams of the blended sample was put into 225ml of sterile buffered peptone water to make a dilution of 10^{-1} homogenate (16).

Isolation and characterisation of isolates: Coliform count, isolation and characterisation of *E. coli* and *Salmonella* were done according to Health protection Agency (16). Faecal coliforms were counted using the standard most probable number technique according to Bergy (17).

Statistical analysis: SPSS and variance analysis methods (AN OVA) were used in interpretation of results. T-test was used in the evaluation of the significance between the groups. The significance between the values was evaluated at 95% confidence $p<0.05$.

RESULTS

Kale production practices in peri-urban of Nairobi: The results showed that farmers in peri-urban areas produced kale using irrigation. In Athi River 100% of the farmers irrigated kale, while for Ngong and Wangige, it was 96 and 89% respectively giving an average irrigation rate of 95%. Most farmers in Wangige and Ngong used water from shallow wells dug within the farms (67 and 63% respectively), while those in Athi River used river water (50%) and effluent from the Kenya Meat Commission (46%) (Table 1).

The leafy vegetables, mostly kale, were mostly produced using manure particularly in Wangige and Ngong where 97 and 75% of farmers reported that this was the source of the plant nutrient. Only 42% of farmers in Athi River applied manure on kale crop. Thus, on average 72% of the vegetable farmers from peri-urban zone of Nairobi applied manure to their kale. The major source of manure was cattle manure used at rates of 92% in Wangige, 56% in Ngong and 25% in Athi River. About 6 and 2% respectively of farmers from Ngong and Wangige used poultry manure while other 8% of the farmers from Ngong used pig manure. Compost from crop residues/household wastes was also used by farmers. Manure was mixed or incorporated into the soil by 41 % of the farmers. Other farmers spread the sludge from the zero grazing pens onto the plots in the following order Wangige greater than Athi River greater than Ngong (13%, 8% and 6%). Only 3% of peri-urban farmers applied manure in the furrow for planting leafy vegetables.

Table 1
Kale production practices

Farming area	Source of irrigation water					Sources of manure						Method of manure application				
	well	River	Waste water*	Dam	others	Cattle	Poultry	Sheep	Compost	Pig	Others	spread sludge	spread compost*	plowed into soil	placed farrw	other
Athi River	0	11	11	0	1	6	0	1	2	0	15	2	3	5	0	14
n=24	0%	50%	46%	0%	4%	25%	0%	4%	8%	0%	63%	8%	13%	21%	0%	58%
Ngong	30	16	0	0	2	27	3	2	7	4	5	3	11	23	0	11
n=48	63%	33%	0%	0%	4%	56%	6%	4%	15%	8%	11%	6%	22%	47%	0%	25%
Wangige	32	8	0	0	8	44	1	0	3	0	0	6	16	21	4	1
n=48	67%	17%	0%	0%	16%	92%	2%	0%	6%	0%	0%	13%	33%	44%	8%	2%
Total	62	36	11	0	11	77	5	3	11	4	20	11	30	49	4	26
n=120	52%	30%	9%	0%	9%	64%	4%	3%	9%	3%	17%	9%	25%	41%	3%	22%

Compost*- from crop residues/household wastes, Waste water*- Effluent from Kenya Meat Commission

Kale trader retailing practices: Kale traders who operated from the wet markets used different modes of transportation of kale to the market. Most traders/transporters in Githurai and Kangemi transported vegetables using open trucks 50 and 37% respectively. In Kawangware market, most of kale traders used public transport (39%) while some used open trucks (19%). Human transport on back was also used by traders from Githurai: (27%), Kangemi: (16%) and Kawangware: (15%). Generally most traders in wet markets in Nairobi used open trucks to transport their vegetables (average of 36%). The vegetables were carried in sealed bags, Githurai, (79%), Kawangware (73%) and Kangemi (57%) although some traders had open bags, Kangemi: (37%). Githufai: (17%) and Kawangware: (15%). Thus, on average, 70% of traders/transporters in tbe wet markets in Nairobi used sealed bags while 23%used open bags (table 2).

Table 2
Kale traders retailing practices

Market type	Mode of kale transport							Kale packaging method		
	Open trucks	Closed trucks	Public transport*	Human	Wheel-burrow	*Mkokoteni*	Others	Sealed bags	Open bags	Unpacked
Kawangware	5	4	10	4	0	0	3	19	4	3
n=26	19%	15%	39%	15%	0%	0%	12%	73%	15%	12%
Kangemi	9	3	2	4	4	1	1	14	9	1
n=24	37%	13%	8%	16%	16%	5%	5%	57%	37%	3%
Githurai	15	4	2	8	0	0	1	24	5	1
n=30	50%	13%	7%	27%	0%	0%	3%	79%	17%	4%
Total n=80	29	11	14	16	4	1	5	57	19	6
	36%	14%	18%	20%	5%	1%	6%	70%	23%	7%

Human* On back, Public transport "*Matatus*"

Bacteria on kale samples from farms: Mean colifom counts on kale samples from the three farming sites ranged from 1.6x 10^5 to 4.0xl0^{5}cfuJg (table 3) which were not significantly different. Ngong had the highest *E. coli* prevalence of 81.8%. Samples from Wangige had 4.5% prevalence of *Salmonella*. Mean coliform count in irrigation water from the three farming sites were higher than the WHO recommended levels of 10^3 cfull 100ml, (18) (Table 3).

Table 3
Bacterial level on kale purchased from the peri-urban farms of Nairobi

	Coliforms cfu*/g		Prevalence	
Source	Mean	SD*	*E.coli*	*Salmonella*
Athi River n=16	1.9x10^5	±4.0x10^5	6	0
			37.7%	0%
Ngong n=22	4.0x10^5	± 6.2x10^5	18	0
			81.8%	0%
Wangige n=22	1.6x10^5	±4.2x10^5	16	1
			72.70%	4.5%

SD*-Standard deviation, cfu-colony forming units

Bacteria on kale and washing water obtained from the market sites: Mean coliform numbers on kale from the markets ranged from (4.7x10^5 ±8.9xl0^5 tol.2xl0^7 ±1.2xl0^{7}cfu/g of kale leaves (Table 4). There was a significant variation ($P<0.05$) in mean colifom numbers on kale at the three market segments: wet, supermarkets and high-end markets. Coliform numbers in wet and supemarket kale were significantly higher ($P<0.05$) than in farm kales. However, there was no significant difference in coliform count between the farm and specialty store ($P=0.227$). Therefore a general increase in coliform count was observed along the supply chain (figure 1).

The *E. coli* prevalence of 40% at the wet markets was higher than that of supermarkets and specialty store which recorded 20%. Mean faecal colifom count in washing water at the wet markets exceeded the WHO recommended level of 10^3cfu/ 100ml (Table 6). *Salmonella* prevalence on kale in Kawangare market and washing water at Kangemi market was 6.3 and 12.5% respectively. Of all the *Salmonella* isolates in this study, two (66.7%) were *Salmonella* Enteritidis while one (33.3%) was *Salmonella* Typhimurium.

Table 4
Contamination on Kale vegetables purchased from the different market segments in Nairobi

	Colifonns cfu*/g		Bacteria Prevalence	
B	Mean	SD*	*E.coli*	*Salmonella*
Kawangware n=16	1.2x10^7	±1.2x10^7	7	1
			43.8%	6.3%
Kangemi n=16	2.2x10^6	±7.5x10^6	10	0
			62.5%	0%
Githurai n= 18	1.1x10^6	±2.8x10^6	6	0
			33.3%	0%
Supermarkets n=25	2.6x10^6	±2.7x10^6	5	0
			20%	0%
Specialty store n= 15	4.7x10^5	±8.9x10^5	5	0
			20%	0%

SD* Standard deviation, cfu* colony forming units

Table 5
Bacterial contamination in water samples used for irrigation at the peri-urban farms

	Fecal coliforms 100ml		*Salmonella* prevalence
Source	Mean	SD*	
Athi River n=7	2.6x 10^8	±5.9x10^8	0%
Ngong n=10	1.1x10^7	±1.5x10^7	0%
Wangige n=10	3.9x 10^6	±1.1x10^7	0%

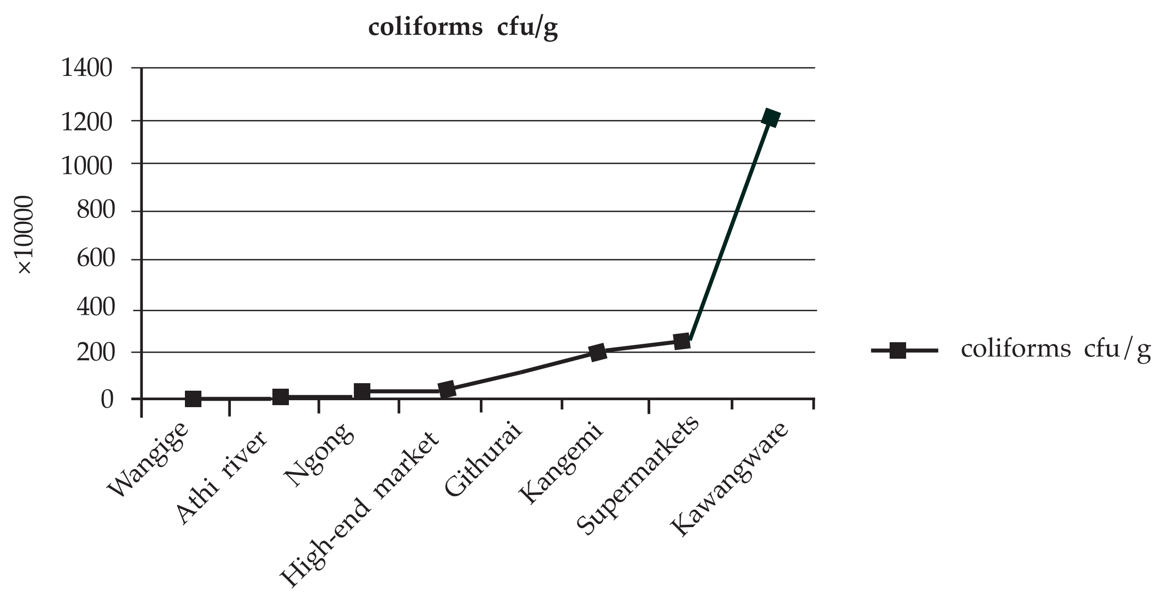
SD*-Standard deviation. cfu-colony farming units

Table 6
Bacterial contamination in water samples used for washing kale at the wet markets

	Faecal coliforms/100ml		*Salmonella* prevalence
Source	Mean	SD*	
Kawangware market n=8	1.0x10^7	±1.6x10^7	0 0%
Kangemi market n=8	8.4x10^6	±4.4x10^6	1 12.5%
Githurai market n=7	2.4x10^6	±6.0x10^6	0 0%

SD* Standard deviation.

Figure 1
Coliforms on kale from farms and markets



DISCUSSION

This study was meant to establish bacterial contamination of kale along the supply chain in Nairobi and its environment. Coliform counts, *E.coli* and *Salmonella* isolations were carried out from kale obtained from farms and from market places, and from water used for irrigation and that used for washing/ refreshing the kale at the markets. There were no significant differences in coliform count on kale from the three farming sites. This can be attributed to the similar farming practices as most farmers irrigated their kale using low quality water and used manure for cultivation of kale. Animal manure is a well known source of food borne pathogenic bacteria and its inappropriate use in vegetable crops contributes a risk to consumer health (19- 20). Cattle manure and that of other farm animals are known to be the predominant reservoir of *E.coli* 0157:H7 which produces vero-toxin (VT) (14, 21). Apart from faecal colifoms, *E.coli* consist strains/serotypes that are pathogenic, including those that produce heat-stable (ST) and heat- labile (LT) toxins, and the vero-toxins (VT). However, this study did not look specifically for these strains/serotypes. *Salmonella* was isolated from 4.5% of kale samples from Wangige farms and this could either be from low quality water used for irrigation or animal manure more so due to spreading as sludge on the plots. *Salmonellae* have been isolated from many types of raw fruits and vegetables (13, 22). As definite example, it has been isolated from the leaf surface of Dodo (*Amaran/hus dubius*) from a contaminated site in Kampala (23).

While sources of kale in the markets might be different from the farms sampled in this study. It is likely that handling increased bacterial loads along the supply chain. Coliform numbers were higher on kale from the wet markets and supermarkets than those from farms, indicating post-harvest contamination, increased bacterial loads along the supply chain. This can be attributed to the use of low quality water by traders in the wet markets for washing the vegetables as well as poor hygiene and sanitation conditions in these markets and this agreed with the observation reported by other studies (24). Other possible sources of post-harvest contamination included the poor handling and packaging as well as transportation systems. Vegetable suppliers to the supermarkets do outsource some vegetables from other farmers to meet the contracted quantities and some of these farmers wash the vegetables with low quality water. Similar study in Nairobi reported a significantly higher mean faecal coliform bacterial numbers in vegetable samples purchased from informal markets (Korogocho and Kibera) in Nairobi compared to those produced by waste water farmers in Kibera (25). It therefore, seems that post-harvest contamination is a major risk, in event of presence of pathogenic bacteria. Guidelines for appropriate agricultural practices and post-harvest handling practices are required so as to reduce bacterial loads on kale hence enhancing consumer safety. The high income grocer such as high-end specialty stores have inspectorate service department that advises farmers on safe production and handling practices such as safe use of water for irrigation, proper use of manure as well as proper packing and transport systems such as closed trucks. Lower coliform count was therefore reported on this market segment. The difference in bacterial loads between vegetable samples from Green grocer and Bazaar has been attributed to cultivation, transportation conditions and personal application (such as washing vegetables and hand contacts) (26). Bacterial loads recorded in this study were above the International commission on microbiological safety of foods (ICMSF) limit of 10^3 to 10^5 coliforms 100 g-1 wet weights of vegetables (27).

In conclusion, the study has shown that bacterial contamination of vegetables occurs both at cultivation and after harvest, along the supply chain. However, post-harvest handling was probably the major contributor to kale contamination. The results of this study will therefore, contribute towards the formulation of focused, local control strategies, for betterment of public health in the country. Application of multiple barrier approach, suggested by WHO (18) involving practices such as: good irrigation practices and vegetable washing using portable water at the market and before food preparation at the household, forms a good basis for the Kenyan approach towards health risk reduction.

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