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E. coli and Salmonella Contamination of Tomato Marketed and Consumed in Nairobi Metropolis

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Abstract
Tomato, a worldwide consumed commodity for its nutritive values can harbour *Salmonella* and *E.coli*. Tomato can contribute to diarrheal illnesses; and associated burden in households. Seasonal bacterial analyses to detect enterobacteria were conducted from January to June 2017 in Nairobi. The study shows that, the vegetable during the study period is 94% contaminated with *E. coli* and 28% with *Salmonella*. February had the highest contamination during the dry season (2.37 $\log_{10}$cfu.ml^{-1} >2; p≤ 0.05) and May (2.8 $\log_{10}$cfu.ml^{-1} >2; p≤ 0.05) the highest in wet season. Thus, seasons have influence on microbial contamination in tomato. Bacteria multiplication slows in dry period and increases in wet season. Increase of bacteria from March (end of dry season or beginning of rains) to high presence in May (end of rains) might come with more health concerns if attention is not paid to ready-to-eat vegetables. Consumers purchasing from open air markets seem more at risk of bacterial infection (Kangemi 1.84±0.159; Githurai 2.02±0.1815; Wakulima 1.97±0.24 of *E. coli* contamination) compared to those who use supermarkets (Nakumatt W. 1.54±0.134; Uchumi Sarit C. 1.27±0.105). Although most tomatoes were washed and cleaned, bacteria levels were still a threat to health. Surfactants from pesticides might contribute to tomatoes infection as they are able to wound skins of crops and open ways to bacterial contamination. With sudden bacterial increase in wet seasons (Kangemi 2.98±0.225^{kl}; Githurai 2.75±0.157^{efghi}; Wakulima 2.69±0.067^{ghijk}; Nakumatt 1.78±0.092^{bcd}; Uchumi 1.54±0.215^{cde}), consumers might experience more symptoms of enteric bacteria. Special attention should be paid in wet times as best quality of tomato at sight is not necessarily safe for direct consumption without further processing. These findings might help in understanding why consumers of salad might be exposed to symptoms of enteric bacteria in wet times. Food handlers, health workers, consumers and policy designers should be informed of this risk.
Keywords: *E. coli*, *Salmonella* spp, bacteria, season, contamination

Introduction

Tomato is grown and consumed throughout the world and its consumption improves health (Nelly et al., 2016). A quantity of 100 to 162 million metric tons of the raw produce is annually traded in global markets (de Vos et al., 2011; Tao Lin et al., 2014). The plant has gained lots of interest due to its vitamins A and C, potassium, phosphorous, magnesium, calcium, molybdenum and manganese (Srinavasan, 2010; Romero-González and Verpoorte, 2011) and antioxidants such as polyphenol (Manach et al, 2004). The fruit is good for consumption when ripe and 25 mg are recommended for a daily intake. Consumption of adequate quantities of tomato improves vision, gastrointestinal health and reduces risk of heart diseases, diabetes, stroke, anemia and rheumatoid arthritis (Esra et al., 2010; Dias, 2012).

Tomato can harbor *Salmonella* and *E. coli* (Orozco et al., 2008; Ganyu et al. 2011; Razzak et al., 2014) which are gram negative, non-spore forming, facultative anaerobic and rod-shaped bacilli of the Enterobacteriaceae family (Nanne, 1998; Forsythe and Hayes, 1998; Razzaq et al., 2014; Thilini et al., 2016). *E. coli* presence in food is an indicator of fecal contamination and a sign of potential presence of *Salmonella* (Mensah et al., 2012; Nelly et al., 2016). *E. coli* had been recovered after 12 months in chocolate and *Salmonella* after 19 months (Baylis et al., 2004). *Salmonella* can subsist for 24 months at a temperature of 4^{o}C (Finn et al., 2013). These infective agents have been of high interest in the world and outbreaks were recorded in advanced world (Finn et al., 2013; Thilini et al., 2016). More attention should be given to these contaminants in tomato as they have been implicated in outbreaks related to vegetables in the USA (Pierangeli et al., 2014).

E. coli is a bacillus of 0.5µm in diameter and, 1.0µm to 3.0µm in length (Rodney, 2006). Some genera of *E. coli* can cause food poisoning (Forsythe and Hayes, 1998). The bacterium is a saprophyte able to survive in environment and conquer new hosts (Blattner et al., 1997; Rodney, 2006). Some diarrheal strains grow in acidic milieu enduring a pH of 2.0 (Rodney, 2006; Deering at el., 2015).

Depending on the species, *E. coli* can cause a number of diseases in human including enteric, nosocomial septicemia, pulmonary, neonatal meningitis, surgical site infections, urinary infection, pulmonary, long-term infirmity and death (Blattner et al., 1997; Kimutai, 2010; WHO, 2015). Most infants' diarrheal infections in developing countries are linked to pathogenic *E. coli* (Motarjemi et al., 1993). This variety has been responsible for several epidemics ending with 53 deaths in North America and Europe (WHO, 2015).

Salmonella is a flagellated bacillus of 2-3µm in length contributing to zoonosis worldwide. The strain enterica was first isolated from the intestine of pigs (Zubay et al., 2004). *Salmonella* can live in fruits, seeds and leaves of various plants (Berger et al., 2009, Ganyu et al., 2011). Several outbreaks of *Salmonella* were linked to fresh fruits and vegetables (Chang et al., 2013). The source of the pathogen in developed countries is often mammal transporters and animal feed (Finn et al., 2013; Thilini et al., 2016). In the developing world, frequent transmissions are through vegetables, water and human to human path (Wegener, 2003), and the type habitually found is *Typhi* (Yadav et al., 2014). Infective dose of the genus is 15 to 20 cells depending on the age of the host and the type of strain (Wesonga, 2010).

Salmonella spp can live at a low pH through self-endocytosis forming vesicle (Zubay et al., 2004) or establishes a pH homeostasis when its intracellular pH corresponds to the surroundings. The genera can manage and repair damages from acids at post-shock and even build a cross-tolerance-induce response for self-protection against acid stress (Thilini et al., 2016).

The estimated yearly cases of global *Salmonella* food infection is 93.8 million people with 155,000 deaths. In the USA, on 1.4 million sicknesses, 600 expiries were annually attributed to Salmonellosis (Hendriken et al., 2011; Thilini et al., 2016). In Denmark, the burden from *Salmonella* infection was estimated at US$15.5 million (Wegener, 2003). Efforts to decrease incidences of *Salmonella* food infection showed improvement. For US$ 26.5 million spent in control US$ 25.5 million were saved (Wegener, 2003) and associated economic losses at the national and international levels were reduced.

For a total of 2274 intestinal infections illnesses recorded in England and Wales from 1992 to 2006, 4% were related to consumption of raw vegetables (Little and Gillespie, 2008). In Mexico and USA, records of fatal cases were related to fresh produce as tomato. The USA singularly recorded 15 confirmed deaths from contaminated fresh produce caused by *Salmonella*, hepatitis A and *E. coli* O157:H7 (FAO and WHO, 2008). Diarrhea, a common symptom of foodborne disease (WHO, 2010) able to be caused by unsafe tomato is responsible of 6% of deaths in Kenya (Kenya Vision 2030, 2013).

This study sought to determine the presence of *Salmonella* and *E. coli* pathogen on tomato sold in some open air markets and supermarkets.

MATERIALS AND METHODS

Study site and period of collection

The study was done in five sites in Nairobi Metropolis, the Capital City of Kenya. Three open air markets - Githurai, Wakulima, Kangemi and two supermarkets- Nakumatt Westgate and Uchumi Sarit Centre were chosen. The Nairobi City is situated between latitudes 1°16'59"S and 36°49'00"E.

Samples of tomato were collected for a period of six months during the dry and wet seasons. Wet seasons in Nairobi correspond to the period with abundant tomato of good quality sold at a low price by retailers in supermarkets and open air markets. Dry season corresponds to the period with less tomato of good quality at sight sold at a high price in markets. The sites of sample collection represented all the social classes (high, middle and low). The supermarkets selected targeted the retailing points for high and middle social classes whereas the open markets targeted the middle and the lower classes. Additionally, Wakulima and Githurai markets were selected because they are both for wholesales and retails.

Study design

A cross sectional study for analysis of raw tomato sold and consumed in Nairobi Metropolis was done for a period of six months during dry and wet seasons. Samples were collected twice a month from selected sites. The first batch was collected between the first and the tenth day of the month while the second set was collected between the twenty third and the twenty eighth day of the month. Four samples per open market were randomly picked from four different retailers at four different nooks of the markets to cover the whole retailing areas. Samples from supermarkets were collected according to the layout and following the design effect of boxes containing tomato. These open markets and supermarkets were selected for their locations and representativeness of the City.

Sample collection and preparation for analyses

A total of 240 samples, 120 during dry and wet seasons each were collected from tomato retailers in selected points from January to June 2017. Criteria for samples collected were: Red and hard tomato, without disease, no wound and no soft part. Samples were prepared for analyses and detection of *Escherichia coli* (*E. coli)* and

Salmonella spp in the laboratory of bacteriology. Aseptic procedures were keenly followed during sample analysis of whole tomato. Samples from different retailers and from each market were pooled and screened as one sample. The samples were analyzed to determine the monthly and seasonal levels of contamination and also to establish whether bacterial exposure differs between social classes.

Biochemical tests

Indole, Methyl red, Voges-Proskauer and Citrate (IMViC) biochemical tests were used for characterization of *E. coli*. Deamination of phenolalanine, Sulfite and Indole production in SIM Agar, motility test, decarboxylation of lysine, growth in potassium cyanide, fermentation of glucose and sucrose in triple sugar, urease activity were used for *Salmonella* characterization.

Isolation, culture and Identification

E.C Broth Agar medium for *E. coli* and Xylose Lysine Deoxycholate (XLD) Agar for *Salmonella*; Urea Broth and Triple Sugar Ion (TSI) Agar, Plate Count Agar, (from HiMedia Laboratories Pvt. Ltd., India); Violet Red Bile Agar (from Oxoid Ltd., England) were used and prepared according to the manufacturer's instructions.

Raw samples pooled were vigorously rubbed separately for one minute by hand with glove in a mortar previously cleaned with 70% ethanol and flamed. Twenty five grams (25g) of raw tomato were weighed using a balance (Mentor Ohaus Corp. Pine Brook, NJ USA - China). They were then placed in different sterile stomacher bags containing 225 ml diluent for homogenization using Stomacher (Stomacher 400 Lab Blender, England) for 2 minutes. Serial dilutions to 10^{-5} corresponding to infective dose of typhoidal salmonellosis causing enteric fever (Zubay et al., 2004) were made in sterile dilution bottles. Content of dilution bottles were vortexed for homogenization. A quantity of 0.1ml of each preparation of 10 ml was pipetted and transferred into sterile petri dishes.

For *Salmonella spp*, spread plate method (Wesonga, 2010) was employed to examine the presence and incubation was done at 37^{o}C for 24 hours. Examination of plates with XLD was done by observation of black colonies resulting from non-fermented lactose. Pour plate technique (Waitaka et al., 2014) was employed for enumerating *E. coli* and incubation was done at 45^{o}C for 24 hours.

Enumeration of colonies forming units (CFU) was done using the colony counter (Schneider & Co.AG Vorm. J.E. Gerber & Co, Zurich- Suisse) and the results recorded as Log CFU/g (Razzaq et al, 2014). A minimum limit of 10^{-2} CFU for reporting the results obtained was adopted. The chosen limit corresponds to the highest acceptable concentration of generic *E. coli* in the whole commodity of vegetables analyzed as done by Nelly et al. (2016).

The levels of bacterial counts in this study were compared with the EU Commission for Regulation of Food No 2073/2005 for *Salmonella* and *E. coli*.

Biochemical characterization

All the bacterial isolates were confirmed using biochemical tests. *Salmonella* was confirmed using Urea broth and Triple Sugar Iron Agar (TSI), while IMVIC biochemical tests were used for *E. Coli* (Thi Thu Hao, 2007; Kipkurui, 2011) (Table 1)

Table 1: Biochemical test for *E. coli*

Samples	Lactose fermentation	Indole	Methyl Red	Vogues-Proskauer	Citrate	Type of *E. coli*
1	(+)	(+)	(+)	(-)	(-)	Typical
2	(+)	(+)	(+)	(-)	(-)	Typical

Presence of Salmonella was confirmed with a negative (-) urea test for biochemical characterization.

Statistical analysis

Raw data from monthly analyses were first entered into Excel sheet with all parameters including sites of samples collection in Nairobi and the results from samples analyzed. Data analysis focused specifically on *E. coli* as *Salmonella* was rarely detected in samples analyzed. Counts from bacterial contamination were converted into $\log_{10}$CFU/ml as done by Penteado et al. (2016) on analysis of fresh tomatoes marketed in Rio de Janeiro-Brazil, using excel sheet. Data translated were imported into GenStat (General Statistics) to determine the presence or prevalence of *E. coli*. Means of $\log_{10}$CFU.ml^{-1}, standard deviation (±SD), p-values were determined. The grand mean was used to determine overall infection of samples for the study period. Linear regression was used to establish predictive models using the statistical package for social sciences (SPSS) IBM version 20. Significance of level of contamination per site of collection and per month was considered at 95% level of confidence.

RESULTS

Overall analysis: Total Viable Count, Total Coliform, *E. coli* and *Salmonella* detections

A total of 60 analyses per pathogen equivalent to 120 for both *Salmonella* and *E. coli* were conducted. Analysis of samples collected in January including Total Coliform (to assess faecal pollution indicator), Total Viable Count (to ascertain the presence of bacteria in samples); detection of *Salmonella* and *E. coli* showed positive results. Other suspects as Shigella and Klebsiella were also detected but were not of interest in this study. This first analysis showed colonies forming units varying from 10^2 cfu.ml^{-1} (equivalent to 3 in $\log_{10}$cfu.ml^{-1}) to 10^5cfu.ml^{-1} (6 $\log_{10}$cfu.ml^{-1}) for Total Coliform and Total Viable Count. *Salmonella* and *E. coli* was detected from 10^1 cfu/ml (equivalent to 2 in $\log_{10}$cfu.ml^{-1}) to 10^3 cfu.ml^{-1} (equivalent to 4 in $\log_{10}$cfu.ml^{-1}). Colonies of *Salmonella* were found in tomato from Uchumi Sarit Centre and in samples from Githurai (**Table 2 and 3**).

Table 2. Total Coliform, Total Viable Count, *E. coli*, *Salmonella* on tomato sampled from five sites in Nairobi (January, 2017). Results are given in (Means of $\log_{10}$cfu ± SD).

	T.C.	TVC	*E. coli*	*Salmonella*
Nakumatt	4.05±0.77	5.14±1.26	2.60±0.17	0.00±0.00
Kangemi	5.16±0.37	6.37±0.78	4.33±0.15	0.00±0.00
Uchumi	2.90±0.61	4.63±0.07	1.85±0.22	0.50±0.70
Wakulima	4.98±0.70	5.89±0.87	2.65±1.46	0.00±0.00
Githurai	3.64±1.34	6.28±0.92	3.57±1.37	1.07±1.86

1. Values are mean of two determinations ± standard deviation

Confirmation of *E. coli*

Presence of *E. coli* was confirmed by the morphological characterization using a microscope and a biochemical test. Morphological characterization for *E. coli* reveals that, the organism was short, gram negative, non-motile and non-spore forming. Its biochemical characterization using the Indole Methyl red Vogues-Proskauer Citrate showed that, the bacterium fermented lactose as described in Table 1.

Results from these analyses disclosed significant differences ($p< 0.05$) in the counts between open and supermarkets (**Fig 1**). The most contaminated site per month for *E. coli* was Githurai with the highest number (4/5) of most hazardous samples ($\log_{10}$cfu.ml^{-1} > 2) followed by Kangemi (3/5), Wakulima (2/5), Nakumatt Westgate (1/5) and Uchumi Sarit Centre (0/5) (**Table 4**). Seasonal differentiation into highest contamination period (with $\log_{10}$cfu.ml^{-1} >2), bordering limit (for $\log_{10}$cfu.ml^{-1} = 1.301) and lowest ($\log_{10}$cfu.ml^{-1} < 1.301) contaminated term was denoted (**Fig 2**). The month of May had the highest contamination (3 sites with highest colonies), followed by February (3 sites but slightly minor compared to contamination of May), March (2 sites recorded with high contamination $\log_{10}$cfu.ml^{-1} >2), April (1 site recorded contamination of $\log_{10}$cfu.ml^{-1} >2) and June (1 site recorded high contamination slightly lower than April). Lowest contamination of the analytes in June clearly shows the influence of seasonal variation on tomato infection (**Table 4**).

Table 4: Prevalence of Escherichia coli (Means of $\log_{10}$ cfu.ml^{-1} ± SD and p-value)

	Kangemi	**Githurai**	**Wakulima**	**Nakumatt W.**	**Uchumi Sarit**
February	2.44±0.227defghi	2.29±0.365fgh	2.40±0.175fghij	1.62±0.135cde	1.29±0.11bc
March	0.0±0.0a	2.47±0.1defghi	1.70±0.015bcd	2.21±0.197efgh	0.68±0.042b
April	2.17±0.127g	0.52±0.035a	1.94±0.06bcde	0.93±0.107b	1.40±0.065bcd
May	2.98±0.225kl	2.75±0.157efghi	2.69±0.067ghijk	1.78±0.092bcd	1.54±0.215cde
June	1.62±0.225bc	2.06±0.25fgh	1.11±0.88bc	1.16±0.137b	1.44±0.093bcd
Grand Mean	1.84±0.159	2.02±0.1815	1.97±0.24	1.54±0.134	1.27±0.105

1. Values are mean of two determinations ± standard deviation
2. Values with the same letter in the same column are not significantly different.
3. Values with different letter in the same column are significantly different at $p < 0.05$

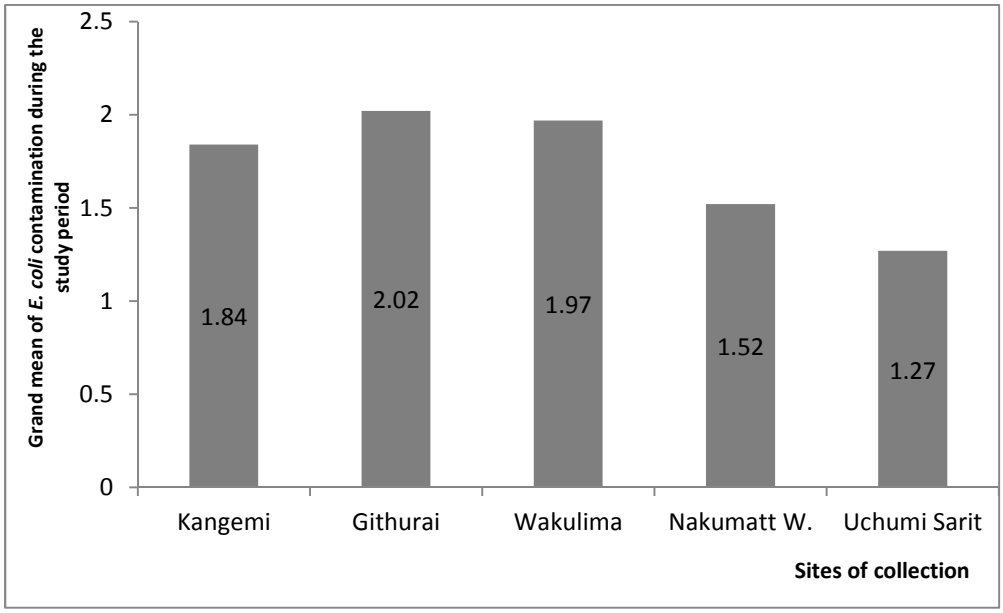

Figure 1: Grand mean of log_{10} cfu.ml^{-1} vs sites of collection for *E. coli*

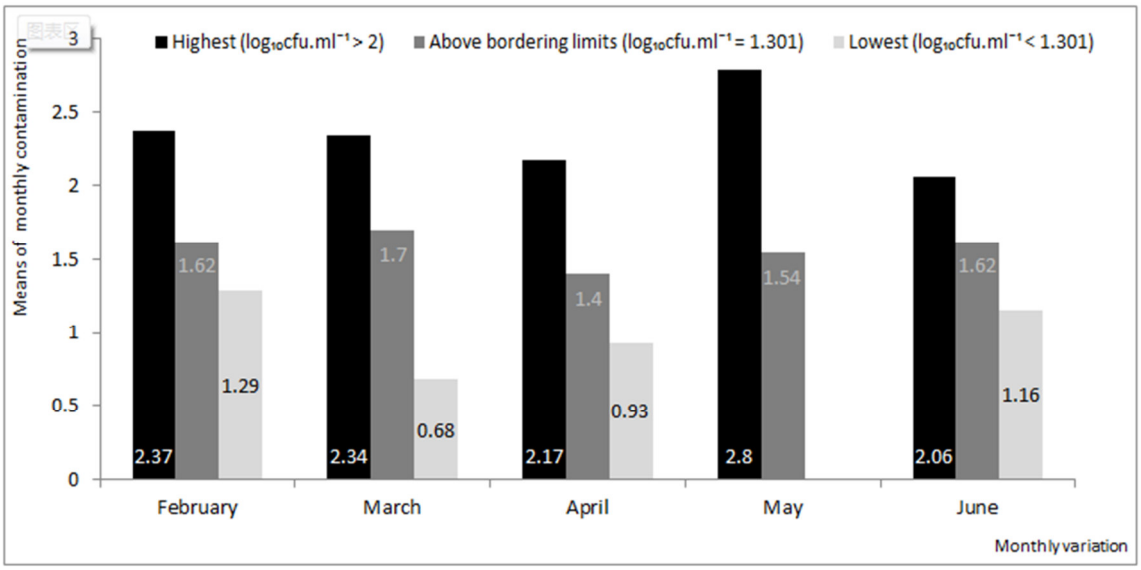

Figure 2: Monthly variation of contamination during the study period

Salmonella presence

Table 5: Overall months with infection of *Salmonella* during the study period

	January	**February**	**March**	**May**
Kangemi	No	No	No	No
Githurai	Yes	No	Yes	Yes
Wakulima	No	No	No	No
Nakumatt W.	No	No	Yes	No
Uchumi	Yes	Yes	No	No

Table 6: Comparison of contamination between *E. coli* and *Salmonella* during study period

	January	**February**	**March**	**May**
E. coli (log_{10}cfu.ml^{-1})	3.145	3.230	7.477	3.636
Salmonella (log_{10}cfu.ml^{-1})	2.115	2. 91	4.477	2.178

It was noticed that, concentrations of *E. coli* was always higher than of *Salmonella* in most samples where they were found together (**Table 6**). The pathogen was mostly found in dry season and only Githurai was

positive in May. Finally, *Salmonella* analysis pointed Githurai as the most contaminated site (3 months showed contamination) followed by Uchumi (2 months) and Nakumatt (1 month)

Discussion

On the basis of ISO 16649- 1 or 2 for pre-cut fruit and vegetables ready to eat, *E. coli* should not be beyond 100 CFU/g. This indication is supported by the FSAI stressing that, the colony forming unit of *E. coli* should be lower than 20 (CFU < 20) which is equivalent to 1.30103 in Log_{10} (< 1.30103). The borderline or marginal limit should be less or equal to 100 CFU (≤ 100, equivalent in Log_{10} to ≤ 2). With colony forming unit greater than 100 (Log_{10} > 2), the pathogen is most likely to be consumed in food. Thus, the produce is harmful for health and, is a potential source of infection for consumers (Commission regulation, 2005; FSAI, 2016).

1- *E. coli* prevalence on tomato

Data analysis of *E. coli* showed the comparison per sites and per month. Ninety-four per cent of the tomatoes analyzed during the study period were contaminated with the pathogen. This level of contamination seems normal since it is known that, tomato farming in open field is subject to enterobacteria contamination through fauna, irrigation water, soil, runoff, manure and workers (Orozco et al., 2008). Accordingly, finding high contamination of fresh tomatoes with *E. coli* has been linked to presence of the pathogen in contaminated water for irrigation (Lopez-Galvez et al., 2014). This result supports the finding of Orozco et al. (2007) in Mexico who found colonies of *E. coli* in hydroponic tomato greenhouses. They align with the work of Penteado et al. (2016) in Brazil who analyzed tomatoes from open air markets and supermarkets with strict hygiene from production to handling and found 1.1% *E. coli* contamination. By analyzing tomato as whole, results could not indicate whether, contamination was external or internal. As such, these results tend to support somewhat the work of Heaton and Jones (2007) disclosing presence of enteropathogens like *E. coli* in tomatoes as a way of subsistence. This work agrees with the findings of Ogundipe et al. (2012) in Nigeria and Nelly et al. (2016) in Canada. The first stipulated that, tomato from Lagos in Nigeria is highly infected with different pathogens and can constitute public health concerns if consumed fresh. The latter said- raw vegetables infected with number of pathogenic agents are a considerable concern for food safety.

Substantial differences were found at 95% level of confidence within same months and from one market to another. In February for instance, infection from Kangemi (2.44±0.227 $log_{10}cfu.ml^{-1}$) compared to the one from Nakumatt, (1.62±0.135 $log_{10}cfu.ml^{-1}$) and Uchumi Sarit Centre (1.29±0.11 $log_{10}cfu.ml^{-1}$) were significantly different ($p<0.05$). This reveals that, there is no similarity of markets within the same period. As such, consumers may be infected differently due to consumption of tomatoes from different sites. Contamination found is not bound to sites of collection. It has been linked to different sources of contamination as handling (Kutto et al., 2011), infection in farms by pests (Orozco et al., 2008), from water for irrigation (Deering et al., 2015) and soil (Heaton and Jones, 2007). This variation between sites of collection contradicts the work by Pierangeli et al. (2014) in Philipines who found no significant differences between open air markets and supermarkets in analyses of raw produce including tomato. In the present study, open air markets were highly contaminated ($log_{10}cfu.ml^{-1}$ >2) compared to supermarkets which had moderate ($log_{10}cfu.ml^{-1}$ >2) and lowest ($log_{10}cfu.ml^{-1}$ < 1.301) contamination. These findings are in agreement with the work done in Calabar- Nigeria by Obieze et al. (2010) who observed microbial pollution (including *E. coli*) of vegetables from different markets. Poor hygienic handling by retailers may not be the only source of contamination as some studies have reported possibilities of bacteria living inside the crop (Wright et al., 2017). Accordingly, this finding suggests careful processing when the vegetable is consumed fresh or, thorough cooking as stipulated in food safety standard (FSAI, 2016).

1.1. Influence of seasons on tomato infection with *E. coli*

The seasonal analysis showed a significant difference between dry period (February, March) and wet season (April, May). In fact, the month of May showed highest *E. coli* contamination (**Fig 2**) with a peak at 2.8 or $logcfu.ml^{-1}$ >2. Even, no lowest ($log_{10}cfu.ml^{-1}$ < 1.301) contamination was recorded in May. This month normally could have been among the least contaminated ones due to rains which might be washing the skins of the crop. Moreover, retailers had clean and attracting tomatoes during wet season. Some were even pouring water on the vegetable displayed on tables to show the cleanness and beauty of good fruits to attract customers. With this mean of contamination above the ones in dry season, the increase of bacterial load on fruits might be linked to seasons. Rainfall period might be pointed as contributing to boost the level of contamination of tomatoes. Maybe, the level of contamination detected might be including internal contamination and probably, availability of water might favor *E. coli* presence and multiplication on fruits. This observation allows saying that, high bacterial infections recorded might correspond to wet season in farming areas. Their increase might be linked to sufficient water availability favoring biological activity within the plant and fruit. The results seem to indicate that, multiplication of *E. coli* increases during wet period when plants absorb enough water from the surrounding to nourish the trees and fruits. On the contrary, low level of bacteria in dry season might correspond to low levels of water in farming areas. Perhaps, hot weather reduces bacterial presence on tomatoes. This may

be due to evaporation shortening the level of water needed for the multiplication of pathogens. Thus, dry period might create competition for water between the environment (soil and atmosphere) and tomato plants while reducing tomato contamination.

Contamination during dry season was below 2.5 in $\log_{10}$cfu.ml^{-1} and had its peak at 2.47±0.1 in February. It can be said that, season plays a role on levels of microbial infection of raw tomatoes. Relatedly, the dry season can be seen as period with reservoir of bacteria ready to multiply from the inception of rains. This finding corroborates the work done by Ogundipe et al. (2012) who asserted that, an elevated amount of water in tomato favors bacterial growth.

1.2. Monthly ranking of tomatoes' infection with *E. coli*

Around 40% of overall tomatoes investigated showed highest contamination ($\log_{10}$cfu.ml^{-1} > 2); 32% bordered limits or had moderate levels of contamination (1.30103 < $\log_{10}$cfu.ml^{-1} < 2); 24% reflected lowest contamination ($\log_{10}$cfu.ml^{-1} < 1.301) and 4% were hazards free. Month of May had the highest contamination with a mean of (2.80±0.151 in $\log_{10}$cfu.ml^{-1}); February second (2.37±0.202 in $\log_{10}$cfu.ml^{-1}); March third (2.34±0.070 in $\log_{10}$cfu.ml^{-1}); April fourth (2.17±0.078 in $\log_{10}$cfu.ml^{-1}) and June (2.06±0.317 in $\log_{10}$cfu.ml^{-1}). Decrease of high levels of contamination noticed in March and April seems to reveal water scarcity in farming areas. This probably has a negative effect on microbial growth in environment, plants and fruits. It can explain why March (the third month) in dry season appears with two sites poorly contaminated (Kangemi: 0.0±0.0; Uchumi: 0.68±0.042) while April following the end of dry or beginning of wet season (Kutto et al., 2011) also had weak contamination (Githurai: 0.52±0.035; Nakumatt: 0.93±0.107) (**Table 4**). On the contrary, sudden increment in May reveals the latency and potential of bacteria to multiply and get the highest peak once water is permanently enough confirming the finding of Ogundipe et al. (2012). Such information is useful to consumers of raw vegetables as well as providers of ready-to-eat vegetables. This result is useful to understand periods of high infection and those of low contamination of vegetables. It increases the level of management of pathogens on fresh vegetables being given out for immediate consumption. It is worth emphasizing that, safety measures should be observed all along preparation of ready-to-eat vegetables but specific care is to be given during wet seasons. Similar fluctuation of *E. coli* infection according to climate (before rains, during wet period and after rains with a drop on level of hazard) was recorded by Orozco et al. (2008) in tomato farms in Mexico. They recorded a high incidence of *E. coli* on tomato during rains and low fruits contamination by the pathogen after rains.

1.4. Sites ranking of tomatoes' infection with *E. coli*

Githurai appears as the most contaminated site (with 40% of high contamination $\log_{10}$cfu.ml^{-1} > 2). The site is followed by Kangemi 30%; Wakulima 20%; Nkumatt Westgate 10% and Uchumi Sarit Centre 0% (**Table 4**). Consumers from Githurai might be more exposed to *E. coli* an indicator of fecal contamination (Pierangeli et al., 2014), while those from Uchumi Sarit Centre might be less at risk of the pathogen. Maybe, the supermarkets are less infected because tomato displayed in boxes is of high quality. Supermarkets care for the quality of tomato purchased for retailing compared to vendors in open air markets. They keep standards to meet the level imposed by clientele. As their tomato was always washed and cleaned, surely water used for washing the crop was of good quality and may justify the acceptable level ($\log_{10}$cfu.ml^{-1} < 1.301) recorded (**Fig 1**). As well, the supermarkets might manage crops received in a special way before displaying in boxes to keep the image. It worth mentioning that only the best fruits, and big in seize, were always collected from supermarkets. Maybe the best and biggest tomato at sight (with no disease, no wound and no damage or soft point) might be less contaminated with the bacillus. This assertion agrees with the finding of Franz et al. (2007) in the Netherlands revealing that, crops contaminated with pathogens weigh less than non-infected ones. This might also corroborate the conclusion of Gu et al. (2011) in the USA disclosing that, plant growth is slightly affected when infested by enteric bacteria.

Retailers from open markets rarely displayed best quality tomato on their tables. Probably, they choose the quality mostly affordable by majority of their customers daily in order to minimize losses. This discrepancy between open air markets and supermarkets expresses the difference of social classes and their level of exposure to foodborne diseases.

2. Prevalence of *Salmonella* on tomato and routes of contamination

Salmonella was rarely found during analysis on contrary to *E. coli***.** Maybe, its presence was still embryonic and needed more time for maturity and detection (Sheppard, 1998).

Referring to EN/ISO 6579 for pre-cut fruit and vegetables ready to eat in the market, *Salmonella* should be absent in all units of 25 g of samples analyzed in the laboratory during the shelf-life of the produce. This recommendation is supported by the Food Safety Authority of Ireland (FSAI) stating that, results of *Salmonella* analysis in 25 g should not show any colony forming unit. Each presence should be considered unsatisfactory and thus, hazardous for human consumption (Commission Regulation, 2005; Finn et al., 2013; FSAI, 2016).

A total of 28% or (7/25) of contamination with *Salmonella* was found during the analysis period. Infected samples included those from Githurai (4/7) or 57.14%; Uchumi (2/7) or 28.57% and Nakumatt (1/7) or 14.28%.

Contaminations were recorded in samples for January, February, March and May (**Table 5**). Specifically, one open air market and both supermarkets had Salmonella contaminated tomatoes. This weak contamination of *Salmonella* can be attributed to handling since samples were analyzed as whole. Maybe, period of collection also influences as most contaminations occurred in dry season (January to March). Tomatoes from supermarkets were always of high and best quality and permanently cleaned; same as some samples from some open markets. Previous studies on *Salmonella* detection on vegetables in Nairobi pointed the level of infection at 18.8% in water used for washing the vegetable and at 4.5% on the vegetable analyzed (Kutto et al., 2011). Similar results on water used for washing were reported in Spain by Lopez-Galvez et al. (2014). Contamination occurring on tomatoes collected may be a random process that cannot be attributed to specific conditions. Related to period of contamination, dry season may be the period in which the vegetable is contaminated by the pathogen. Although this does not reveal high exposure of consumers, these findings serve as a warning to food handlers. Studies on the pathogen in Kenya as those conducted in the Peri-Urban farms by Kutto et al. (2011) decried poor agricultural practices and handling as sources of vegetables' contamination.

Worldwide, studies revealed different avenues of *Salmonella* contamination including seedborne, soilborne and plant infection during growth. Others painted running polluted waters from rains, adulterated water from showers, wrongly converted manure, wounds from pests and adjuvants, human, water of irrigation with unprocessed sewage and airborne contamination (Sheppard, 1998; Heaton and Jones, 2007; Orozco et al., 2008; Gu et al., 2013; Farakos and Frank, 2014; Pierangeli et al., 2014; Deering et al., 2015; Kumar et al., 2017). The present study supports the work done by Pierangeli et al. (2014) in Phillipines. They collected 50 samples of tomato from open air markets and supermarkets and found contamination at 20% equivalent to 10/50 samples contaminated with *Salmonella*. This result supports the work by Finn et al. (2013) disclosing that, the pathogen can live long on surfaces and in matrices of food. It is recommended that, further analyses show interest on the inner side of the crop.

A comparison of *E. coli* and *Salmonella* presence on tomato

Salmonella was rarely found in tomato analyzed during the study period unlike to *E. coli*. As such, no correlation between both pathogens could be established. Exceptions were separately found in March where high presence of *E. coli* (7.477 $\log_{10}$cfu/ml^{-1}) corresponded to strong colonies of *Salmonella* (4.477 $\log_{10}$cfu/ml^{-1}) in samples from Nakumatt (**Table 6**) and also, high detection of *E. coli* (4.100$\log_{10}$cfu/ml^{-1}) corresponded to high presence of *Salmonella* (3.579 $\log_{10}$cfu/ml^{-1}) on samples from Githurai. This supports the work of Lopez-Calvez et al. (2014) in Spain disclosing that, higher presence of *E. coli* on tomatoes analysis corresponded to higher presence of *Salmonella*. However, irregular detection of the pathogen on samples has not allowed more processing and prediction related to sites and seasons. This finding supports the work of Little and Gillespie (2008) in the UK. They analyzed salad ready-to-eat and found inconsistency on human infection with the vegetable during the period of surveillance.

The results of the present study reveal that *Salmonella* presence on tomatoes is random and attention should be paid when preparing raw tomato for salad. Finding both pathogens seem to suggest that, washing by retailers before seems not providing a safer crop due to potential presence in tissues (Holden et al., 2017).

Probable infection by enteric bacteria generated from the use of pesticides in tomato farms

Another source of fresh tomatoes poisoning might be the use of synthetic chemicals in farms together with adulterated water for irrigation. This combination can end up with internal pollution due to damage of skins by surfactants (a surface active constituent of chemicals allowing the solution sprayed to cover entirely the surface targeted in a large zone) present in pesticides. Such reaction creating wounds on skins of tomato in farms exposes them to environmental contamination (Ganyu et al., 2011; Castro et al., 2016). Thus, depending on the use of pesticides in tomato farms (good or bad), cultivators might have been contributing unknowingly to the infection of the produce. Such information should be shared with farmers in order to improve the use of pesticides in tomato farms.

Finding bacteria on whole tomato supports the results from Ganyu et al. (2011) in the USA. They assert for instance that, *Salmonella* can reside in the inner and outer part of vegetables. A potential source of bacteria on tomatoes in Kenya can hold on cattle raring or pastoral activities largely adopted in the country. Animals dropping are easily seen in the environment. Accordingly, Orozco et al. (2008) showed that, animal faeces end up contaminating vegetables with pathogens through pests and humans feet among others. It can be said that, these fecal droppings might be used either by farmers as manure or might even be carried by rains to rivers. The polluted water if not decontaminated can be used for irrigation or for pesticide spray and spoil the crop (Sheppard, 1998). As such, it sounds normal to have detected some bacterial colonies on the whole crop although they seemed always cleaned. Cleaning before processing seems to reduce the level of contamination but might not eliminate completely pathogens present on the skins. That is probably why Kutto et al. (2011) in Kenya and Lopez-Calvez et al. (2014) in Spain were still getting pathogens in water used for washing and on

cleaned vegetables. A potential cycle of pollution between land and crops using rivers for irrigation can also contribute to bacterial contamination of vegetables. This probable contamination of environment agrees with the work of Orozco et al. (2008) in Mexico who explained contribution of rains and floods on pathogens presence on tomatoes.

Generalization of the outcomes

This work shows that although tomatoes might be washed and appear clean, they remain a potential source of health infection. High contamination of *E. coli* and *Salmonella* in March and May reveals that, consumers might experience health concerns related to fresh vegetables consumption from the beginning to the middle or end of wet seasons. It appears to be the favorable moment for bacteria multiplication in crops. As such, special attention should be paid when preparing dishes as salad during this period. It can be revealed that during wet season, consumers are exposed to more bacteria in fresh produce and might experience symptoms of enterobacteria including headache, fever, abdominal pain, nausea, vomiting and diarrhea (WHO, 2015). Bacterial increase between March and May might contribute to explaining health infection in wet periods. This information can be considered by health workers when diagnosing patients in wet times. Manifestation related to symptoms above can be seen as enteric bacterial infection. This assertion might contribute to explaining the occurrence of fever for instance usually considered as symptoms of climate change (when moving from dry to wet period). This study confirms that such a symptom might also be linked to vegetables contamination with enteric bacteria.

It can finally be said that, the best quality tomato at sight (hard fruit, without wound or sign of spoilage, without disease and without physical harm) is not necessarily safe for direct consumption by human without further processing (Falomir et al., 2010; FSAI, 2016). This agrees with Eni et al. (2010) in Nigeria who reported that, vegetables with positive nutritional values useful for human body and health are also involved in disease transmission. Pierangeli et al. [43] (2014) under similar outcomes also concluded that, vegetables are high carriers of environmental bugs such as *Salmonella* and *E. coli*.

Conclusion

Seasonal analysis of tomato has revealed a difference in contamination per sites of collection. Tomato is mostly contaminated with *E. coli*. However, *Salmonella* can also be present but to a much lower extent.. With the criteria established on samples collection, the present study could not clarify where exactly (inside or on the surface or both) tomato was contaminated. Limitation is from the fact that, recent studies are depicting presence of bacteria within the crop. Here, we acknowledge surface contamination and cannot refute internalization of human pathogens. Studies with interest on the inner side of the vegetable are encouraged because they might provide more information. This work is relevant to food handlers, consumers and policy makers.

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