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Determining the Presence of *Escherichia coli* and *Salmonella sp.* as Indicator Organisms of Contamination from Fresh Produce Sold in Open-Air Markets in Juja

Authors

Ian Mwangi[(1)]; Johnstone Neondo[(2)]; Alfrick Makori[(3)]; Eddy Odari[(4)]

Email: mwangi.njuguna1@students.jkuat.ac.ke

(1.2.3.4) Jomo Kenyatta University of Agriculture and Technology, Kenya

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Abstract

The aim of this study was to determine the presence of *Escherichia coli* and *Salmonella* sp. as indicator organisms of contamination from fresh produce sold in open-air markets in Juja, Kenya. The microbial quality of nine types of fresh produce was obtained from the two selected open markets and was determined by both standard quantitative and Next-Generation Sequencing (NGS) techniques. Purposive sampling technique was used in this cross-sectional study design to collect fresh produce items based on their tendency to be consumed raw or with minimal processing from the two selected open markets. Standard laboratory microbe culturing techniques were used to detect the presence of fecal coliform *E. coli* and foodborne pathogen *Salmonella paratyphi.* DNA was extracted from the surfaces of samples and 16S rDNA sequences were used to analyse the diversity of microbiomes found on the fresh produce using QIIME II software. Members of the Enterobacteriaceae family were in high proportional abundances, and pathogens belonging to this family were detected in the fresh produce. Bla-TEM is one of the most important genes encoding ESBLs, predominantly in the Enterobacteriaceae family, and it is prevalent in the fresh produce resistome. Findings of this study provided the much-needed genomic information about pathogenic bacteria contaminating fresh produce sold in the open market that will guide the development and deployment of reliable control/management strategies against foodborne outbreaks.

Keywords: *Escherichia coli,* contamination, microbiology, *Salmonella sp.*

1.0 INTRODUCTION

Research that has tended to concentrate on pathogen detection and survival in fresh produce vegetables, the composition of this microbiome has largely gone unexplored. However, the richness of this produce-associated bacterial community is starting to become apparent with the use of next-generation 16S rRNA gene sequencing techniques (Jackson et al., 2015). Foods may put customers at an increased risk of contracting a foodborne illness because they are frequently not put through the necessary processes of processing to guarantee the proper removal or inactivation of harmful microorganisms before ingestion (Machado-Moreira et al., 2019). As a result, reports of disease outbreaks linked to RTE (ready-to-eat) fruit and vegetable consumption have significantly increased in recent years, and information on these occurrences is frequently not readily available (Machado-Moreira et al., 2019). For food producers to create effective mitigation strategies, it is essential to identify the kind and source of microbial contamination in these commodities (Machado-Moreira et al., 2019). A wide variety of produce products have been linked to human disease outbreaks all over the world, and some products are more frequently implicated in these outbreaks than others (Al-Kharousi et al., 2016; Amoah et al., 2007; Carstens et al., 2019). For instance, leafy greens like lettuce and spinach, as well as fresh herbs like parsley and basil, are known to be potential sources of bacterial infections (FAO/WHO, 2008b).

Large bacterial populations are known to live in a variety of fresh fruits and vegetables (Rastogi et al., 2012; Oliveira et al., 2010), but our understanding of the diversity of these produce-associated communities is still in its infancy. We are aware that significant human pathogens, such as L. monocytogenes, E. coli, and Salmonella, might be linked to food (Leff et al., 2013). Since fresh produce is frequently consumed raw, these infections can result in large-scale illness outbreaks (Fatica et al., 2011; Critzer et al., 2010). Those microorganisms found in food may have other, less obvious effects on human health in addition to directly causing sickness. Allergy development may be influenced by exposure to non-pathogenic plant microorganisms (Hanski et al., 2012), and consuming raw produce may be a significant way for new commensal bacterial lineages to enter the human body.

The environment has, in recent years, become a significant contributor to the conveyance of antibiotic-resistant bacteria and the emergence of resistant pathogens. Antibiotic resistance in the environment arising both naturally and via anthropogenic induction constitutes reservoirs of antibiotic resistance genes (Manaia, 2017). Studies indicate human pathogens associated with fresh produce in high prevalence, such as *Escherichia coli, Salmonella spp., Listeria monocytogenes* and *Staphylococcus aureus*, herein designated vector bacteria (Birgen et al., 2020; Mbae et al., 2018; Miralles et al., 2019; Paudyal et al., 2017; Rajwar et al., 2016). Most of these bacterial vectors are able to survive in the environment (Carstens et al., 2019; Rajwar et al., 2016) for extended periods and still retain the ability to colonise and invade human body surfaces or internal tissues (Manaia, 2017). Thus, an invasion by these vectors could lead to either dissemination of their antibiotic-resistance genes to commensal microbiota via horizontal gene transfer (HGT) or by constitutively residing in the host system (Manaia, 2017). Furthermore, the broad application of antibiotics creates selective pressures that could select for resistance. Human invasion by these pathogens can cause infections

by attaining certain infectious doses, while debilitated individuals, young children, and the elderly run a greater risk of compounding illnesses (Al-kharousi et al., 2016; Rajwar et al., 2016; Rico, 2015).

Fresh produce has been connected to microbial contamination at various stages in the supply chain (Carstens et al., 2019; Iwu & Okoh, 2019). Plants naturally have epiphytic microorganisms, but various exogenous interactions, from pre-harvest, harvesting and distribution to post-harvest handling, can potentially result in the microbiological contamination of fresh produce (Rico, 2015).

There exists a disparity in the infrastructural development trying to keep up with the rate of urbanisation in developing countries (Amoah et al., 2007; Ndiege et al., 2017). The derelict Juja open market is an example that presents a potential avenue for cross-contamination of fresh produce with pathogenic antibiotic-resistant bacteria via the aforementioned routes, thus presenting a potential risk to public health. Therefore, this study seeks to determine the presence of Escherichia coli and Salmonella sp. as indicator organisms of contamination from fresh produce sold in open-air markets in Juja.

2.0 LITERATURE REVIEW

Microbial Contamination of Fresh Produce

As developing environmental contaminants that pose a rising risk to human health, pathogenic bacteria and antibiotic resistance genes (ARGs) are thought to be present in fresh vegetables (Yin et al., 2022). However, nothing is known about the frequency of pathogens in the phyllosphere of fresh vegetables or how ARGs are related to bacteria that cause disease (Yin et al., 2022). Both as endophytes within plant tissue and as epiphytes on the plant surface, plants are home to a varied bacterial community. Others may be human pathogens, while other plant-associated bacteria behave as plant pathogens or encourage plant growth (Jackson et al., 2013). Numerous sources provide fresh food, which is frequently consumed raw (Li et al., 2017). As a result, it must be handled carefully from the farm to the table to prevent contamination of the product by the farmer, shipper, processor, food service operators, merchants, or customers. As a result, it is sensitive to foodborne pathogen contamination (Li et al., 2017). Production, harvesting, processing, and distribution activities all frequently involve contamination (Murray et al., 2017). Fresh produce, rather than more conventional carriers like poultry, pork, and shellfish, is one of the main food sources of foodborne illness outbreaks (CDC, 2022). ARB in the environment have been detected in fresh produce and consequently attributed to disease outbreaks. Data from the Centers for Disease Control and Prevention (CDC) (Painter et al., 2013) show that fresh produce accounted for 46% of all foodborne illnesses and 25% of deaths in U.S. outbreaks. Additionally, according to CDC data, produce-related outbreaks from 1998 to 2016 totalled between 30 and 60 per year, resulting in 900 to 3000 illnesses (Su et al., 2021). Reports from the United States FDA, CDC and state and local officials estimated that 30 – 60 per cent of outbreaks were linked to fresh produce, especially leafy greens, which accounted for 10 – 40 per cent of the produce-related illnesses. The threat of foodborne illnesses derived from fresh produce has resulted in a substantial burden on public health and, rightly so, has become a concern.

Fresh produce has been connected to microbial contamination at various stages in the supply chain. Fresh produce's microbiological quality is routinely examined for indicator organisms and pathogens, the most frequent of which is the aerobic plate count (APC), total coliforms (TC), and generic E. coli. A good indicator of the microbiological safety of fresh fruit and the environment in which the produce is grown and processed is the absence of coliforms/E. coli (Teplitski et al., 2008). Microbial contamination at the pre-harvest stage is considered the growing field. Johannessen et al. (2002) postulated that plant surface flora mirrors the environmental flora of the field in which it is grown, leading to microbial contamination at the pre-harvest stage. This, therefore, implies the occurrence of pathogenic microorganisms on fresh produce derived from contaminated irrigation water, agricultural soil and untreated or incompletely composted manure. Pathogenic microbes have also compromised the quality of fresh produce in the farm-to-table continuum at the post-harvest stage. Possible sources of microbial contamination can be ascribed to (i) poor construction and maintenance of sanitation facilities and drainage systems; (ii) unchecked source and quality of water being on the fresh produce; (iii) disease-carrying agents such as flies in the proximity of the fresh produce; (iv) uncontrolled conditions such as temperature and humidity during temporal storage of the fresh produce; and inter alia (v) general disregard for hygiene during transaction processes. However, Amoah et al. (2007) concluded that sometimes, the microbial contamination of crops acquired during pre-harvest may be too high to mask any significant contribution of poor handling and transport of the product. Ndiege et al. (2017) also derived no significant association between the observation of hygiene and the microbial burden on food. However, Harris et al. (2018) inferred a significant increase in microbial contamination of fresh produce held in retail markets compared to wholesale markets. This relationship suggests that the transport process or conditions at the retail markets confer contamination on the produce. Tatsika et al. (2019) described the inefficiency of household decontamination methods, but what further aggravates the risk concern is that some fresh produce is consumed raw or lightly processed.

Since there are many stages in the cultivation, harvesting, and processing of open fresh produce, contamination can be introduced at any of these sites and then transferred to the customer (Nuesch-Inderbinen & Stephan, 2016). Prior to now, it was believed that the post-harvest wash procedure was adequate to get rid of contamination picked up in the field. As a result, a lot of studies were done on evaluating or creating efficient sanitisers (Feliziani et al., 2016). As information grew, it became clear that post-harvest washing in commercial settings had a limited ability to eliminate contamination and, at worst, may even result in cross-contamination incidents (Barrera et al., 2012; Gombas et al., 2017). This is the current concept in place to protect fresh fruit from contamination in the field and to reduce cross-contamination during post-harvest handling. Even good agricultural practices (GAP) are insufficient to guarantee that human infections are not introduced into the supply chain for fresh food because it is difficult to prevent contamination in fields or greenhouses (Francis et al., 2012). Applying post-harvest decontamination measures, which can either replace or enhance post-harvest washing, is a more efficient means of control (Meireles et al., 2016).

Instead of directly controlling infections of the decay pathogens within the produce, this is generally accomplished by sanitising wash water, produce surfaces, equipment, and storage areas (Feliziani et

al., 2016). The most used disinfectant is chlorine, which is sprayed on or dipped in water. After sanitisation, the product may be treated with one or more fungicides, which leave behind a residue that prevents pathogens that cause decay from infecting later or evading the sanitisers' effects (Feliziani et al., 2016). Sanitisers are also frequently used to reduce the contamination of produce with viruses that pose a threat to human health (Gómez-López, 2012). Pathogens of fungal degradation are very different from pathogens of humans. Because the plant serves as their principal food source, plant infections can grow quickly inside and digest the host tissue in contrast to Salmonella spp., Listeria spp., Escherichia coli, and other human pathogens and viruses. The control of human diseases manifests as a decrease in colony-forming units since their populations are made up of single cells. On the other hand, fungal post-harvest infections start out as isolated propagules before evolving into a networked fungal mass deep inside the host. The best way to measure their control is a decrease in the proportion of infected specific produce items (Feliziani et al., 2016).

3.0 METHODOLOGY

Study Areas Description and Produce Sample Collection

The study was conducted at markets within the Juja area in Kiambu County, Kenya. It adopted a cross-sectional design that cuts across a variety of fresh produce types and vendors in Juja open markets. The study area was stratified into two sampling sites, and a purposive/ convenient sampling technique was used to collect fresh produce items based on their tendency to be consumed raw or with minimal processing and location of display. The two open-air markets within close proximity to each other were sampled for fresh fruits and vegetables at the point of sale. The two open-air markets differ, by general observation, in terms of the level of infrastructure and the amount of foot traffic they receive. Juja market (latitude -1°6' 3.9168" N and longitude 37°0' 56.368" E) is the main market within Juja while Gachororo market (latitude -1°5' 20.2452" N and longitude 37°1' 11.0748") which is less popular.

Fresh produce collected included carrots, tomatoes, tamarillo fruits, spinach, bell peppers, cabbages, collard greens, zucchinis, mangoes and watermelons. For each produce type, three technical replicates were collected from three different vendors for the same item within a market setting. Samples were inserted into sterile zip bags by the vendor and analysed immediately after reaching the laboratory.

Microbiological Analysis for Fecal Coliform *E. coli* and *Salmonella* sp.

To determine the presence of fecal coliform *Escherichia coli*, surfaces of fresh produce were aseptically sliced to weigh 10 g and inoculated into 90 ml of Lauryl Tryptose Broth (Himedia) for incubation at 44℃ overnight. The overnight broth was then streaked onto sterile plates of MacConkey Agar (Himedia) and incubated overnight at 35 ±2℃ to observe for rose-red colonies. To determine the presence of *Salmonella* sp., surfaces of fresh produce were aseptically sliced to weigh 10 g inoculated into 90 ml of buffered peptone water (BAM media) and incubated for 6 hours at 37℃ (Daquigan et al., 2016). 0.1 ml of the enrichment broth was then inoculated into 10 ml of selective media Rappaport Vassiliadis Medium (BAM Media) and incubated for 24 hours at 41.5 ℃

(Daquigan et al., 2016). The broth was then streaked onto sterile plates of MacConkey Agar (Himedia) and incubated overnight at 35℃ to observe for cloudy, humid colonies.

Pure isolates were then characterised based on their biochemical properties using citrate utilisation test, iodole production test, methyl red test, Voges Proskaeur test, motility test, and catalase test (Akinbankole et al., 2015). The *Salmonella* pure isolates that tested; gram (-), motility (+), indole (-), citrate utilisation (+), methyl red (+), VP (-), urease (+), catalase (+), oxidase (-) were identified as *Salmonella paratyphi* as previously described by Panezai et al. (2018).

Bacterial DNA Extraction, Amplification and Sequencing

Sterile cotton swabs soaked in phosphate buffered saline (PBS) were used to harvest microbiota on the surfaces of fresh produce (Sare et al., 2020). DNA extraction was done using an Isolate II Genomic DNA Kit from Meridian Bioscience Company according to the manufacturer's guidelines. PCR amplification of the 16SrRNA gene V4–V7 variable regions was carried out on the extracted DNA using primers; 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) as previously described by (Ghilamicael et al., 2018). The amplification was done in 30 cycles using 5X MyTaq Bioline® Reaction Buffer under the following conditions: 94°C for 3 min of initial heating, followed by 30 cycles of 94°C for 30 s, 53°C for 40s, and 72°C for 1 min, after which a final extension step at 72°C performed for 5 min and stored at 4°C. The quality of the PCR products was assessed using 2% agarose gel to determine the success of the amplification and the relative intensity of the bands. The PCR products were then pooled together according to the sampling market and the produce type and shipped for sequencing. Sequencing was performed at Molecular Research DNA (www.mrdalab.com, Shallowater, TX, USA) on a MiSeq 2x300bp, following the manufacturer's guidelines.

4.0 RESULTS AND DISCUSSION

Presence of Microbiological Contaminants in Fresh Foods

Microbiological assessment for *E. coli* resulted in rose red colonies on MacConkey agar, while *Salmonella* sp. was detected by cloudy colonies on MacConkey agar plates. Pure isolates of *Salmonella* sp. were identified as *Salmonella paratyphi* using biochemical tests. Fecal coliform *E. coli* was detected in all fresh produce samples except for onion samples, while *Salmonella paratyphi* was present in all samples except apples (Figure 3). Cilantro, spinach, chilli and tamarillo fruit samples had the same levels of contamination with both organisms (Figure 3).

Figure 1. Presumptive *E. coli* colonies on MacConkey agar

Figure 2. Presumptive *Salmonella* sp. colonies on MacConkey agar

Table 1. Results for Biochemical Analysis on Presumptive Pure Isolates

Presumptive Isolate for	*Escherichia coli*	*Salmonella paratyphi*
Gram stain	-ve	-ve
Catalase test	+ve	+ve
Oxidase test	-ve	-ve
Citrate test	-ve	+ve
Motility test	+ve	+ve
Indole test	-ve	-ve
Urease test	-ve	+ve
Methyl red test	+ve	+ve
Triple Sugar Iron	AG/A	AG/NC

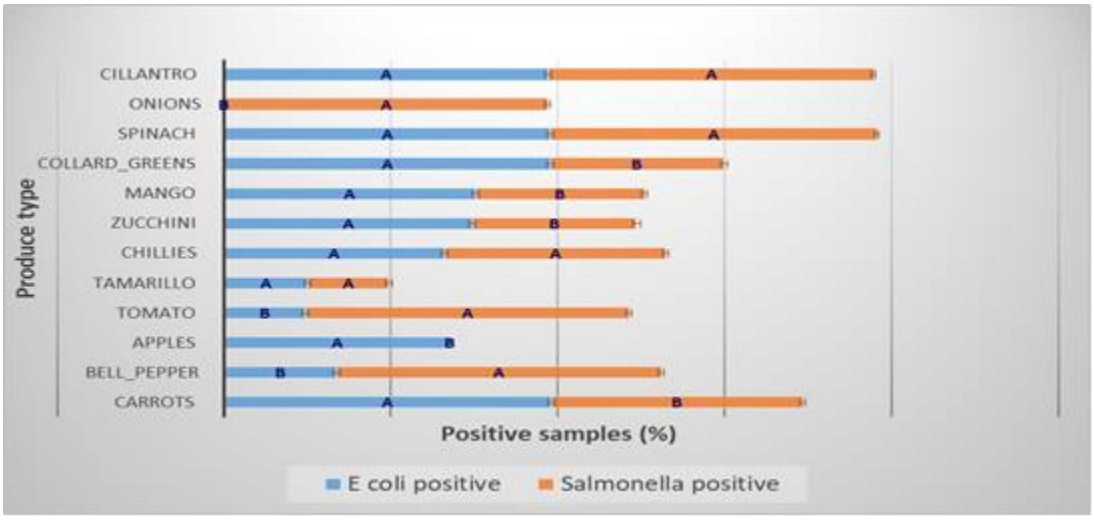

Figure 3. Analysis of Means of Samples Positive for *E. coli* and *Salmonella paratyphi*

Cilantro, spinach, chilli and tamarillo fruits recorded homogeneous means for samples positive for both *E. coli* and *Salmonella paratyphi. E. coli* was absent in all onion samples.

Discussion

According to Berg et al. (2014), vegetables have extremely varied microbiomes that act as reservoirs for opportunistic infections. In Kenya, recent cholera outbreaks that resulted in 76 deaths and 3967 illnesses in the first eleven months of 2017 are most likely caused by tainted food (WHO, 2021). The onset of foodborne outbreaks is significantly influenced by the presence of enteropathogenic bacteria in fresh produce. The fecal indicator organism E. coli was found in abundance in cilantro, spinach, collard greens, mangoes, zucchinis, chillies, tamarillo fruits, tomatoes, apples, bell peppers, and carrots in this investigation. Similar results were observed by Harris et al. (2018), who hypothesised that regional market factors influence produce contamination. Food safety indicator organisms are extensively employed to assess poor sanitation since their presence serves as a marker for the potential appearance of ecologically comparable pathogens (Halkman & Halkman, 2014). Escherichia coli's detection in food has been utilised to signal a higher possibility that pathogens like Salmonella and E. coli O157:H7 were also present in food since it was discovered to be common in feces (Halkman & Halkman, 2014). An ideal food safety indication would be absent from foods that are devoid of the target pathogen and present whenever the pathogen is present. In our investigation, Salmonella paratyphi was found in a variety of foods, including cilantro, onions, spinach, collard greens, mangoes, zucchini, chillies, tamarillo fruits, tomatoes, bell peppers, and carrots. Salmonella is a rod-shaped, motile, gram-negative, non-spore-forming bacterium that belongs to the tribe Salmonellae and the family Enterobacteriaceae. Nature contains a lot of salmonella (Nutrition & Services, 2012). It may thrive in conditions like pond-water sediment and can colonise the intestines of animals (Nutrition & Services, 2012). The fecal-oral route is how it is transmitted (Nutrition & Services, 2012). Only human hosts can harbour S. Typhi and S. Paratyphi, and untreated sewage is typically the source of these organisms' contamination in drinking and/or irrigation water (Nutrition & Services, 2012). Salmonella has been linked to fresh produce, including low-moisture items like raw nuts and spices. Onions, for instance, have been connected to the most current salmonellosis outbreak (CDC, 2021).

5.0 CONCLUSION AND RECOMMENDATIONS

Conclusion: Microbiomes of fresh fruits and vegetables in open-air markets harbour human pathogenic bacteria. Salmonella paratyphi was identified using conventional techniques, and its presence in fresh produce was indicated by the fecal indicator organism Escherichia coli. Human pathogenic bacteria prioritised by the WHO, including Enterobacter sp., Acinetobacter baumannii, Serratia sp., Providencia sp., Morganella mornagnii and Proteus sp., were detected in fresh produce using NGS techniques. These pathogens were discovered in fresh produce at the point of sale. Furthermore, ARGs providing bacterial resistance against antibiotics were also detected. It was also observed that bacterial diversity varied by market.

Recommendations: The complexity of the sample to be investigated and the level of bacterial taxonomic detail required to determine the best sequencing strategy for studying various food matrices. The microbial composition of a food sample could be broadly outlined by an initial 16S rDNA sequencing-based profile. However, this method is unable to give species- or strain-level identification due to its lack of resolution. Additionally, it won't offer an evaluation of these organisms' functional capacity within the sample. Metagenomics and metatranscriptomics would, therefore, be helpful for detailed species-level, strain-level, or functional characterisation of the many members of the microbiome.

6.0 REFERENCES

1. Al-Kharousi, Z. S., Guizani, N., Al-Sadi, A. M., Al-Bulushi, I. M., & Shaharoona, B. (2016). Hiding in fresh fruits and vegetables: Opportunistic pathogens may cross geographical barriers. *International Journal of Microbiology*, *2016*(1), 4292417.
2. Amoah, P., Drechsel, P., Henseler, M., & Abaidoo, R. C. (2007). Irrigated urban vegetable production in Ghana: Microbiological contamination in farms and markets and associated consumer risk groups. *Journal of water and Health*, *5*(3), 455-466.
3. Barrera, M. J., Blenkinsop, R., & Warriner, K. (2012). The effect of different processing parameters on the efficacy of commercial post-harvest washing of minimally processed spinach and shredded lettuce. *Food Control, 25*(2), 745-751.
4. Berg, G., Erlacher, A., Smalla, K., & Krause, R. (2014). Vegetable microbiomes: Is there a connection among opportunistic infections, human health and our "gut feeling"? *Microbial Biotechnology*, *7*(6), 487–495. https://doi.org/10.1111/1751-7915.12159 12(9): 2385-2397.
5. Birgen, B. J., Njue, L. G., & Kaindi, D. M. (2020). *Determinants of Microbial Contamination of Street-Vended Chicken Products Sold in Nairobi County, Kenya.*
6. Carstens, C. K., Salazar, J. K., & Darkoh, C. (2019). Multistate outbreaks of foodborne illness in the United States associated with fresh produce from 2010 to 2017. *Frontiers in Microbiology*, *10*, 2667.
7. CDC. (2022). List of Selected Multistate Foodborne Outbreak Investigations. Retrieved from https://www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbreaks-list.html
8. CDC. (2022). Salmonella Outbreak Linked to Onions. Retrieved on April 6, 2022, from https://www.cdc.gov/salmonella/oranienburg-09-21/index.html
9. Critzer, F. J., & Doyle, M. P. (2010). Microbial ecology of foodborne pathogens associated with produce. *Current Opinion in Biotechnology*, *21*(2), 125-130.
10. Daquigan, N., Grim, C. J., White, J. R., Hanes, D. E., & Jarvis, K. G. (2016). Early recovery of salmonella from food using a 6-hour non-selective pre-enrichment and reformulation of tetrathionate broth. *Frontiers in Microbiology, 7*(DEC), 1–12. https://doi.org/10.3389/fmicb.2016.02103
11. Enya, J., Shinohara, H., Yoshida, S., Tsukiboshi, T., Negishi, H., Suyama, K., & Tsushima, S. (2007). Culturable leaf-associated bacteria on tomato plants and their potential as biological control agents. *Microbial Ecology, 53*, 524–536.
12. Fatica, M. K., & Schneider, K. R. (2011). Salmonella and produce: Survival in the plant

environment and implications in food safety. *Virulence*, *2*(6), 573-579.

13. Feliziani, E., Lichter, A., Smilanick, J. L., & Ippolito, A. (2016). Disinfecting agents for controlling fruit and vegetable diseases after harvest. *Post-harvest Biology and Technology*, 122, 53-69.

14. Francis, G. A., Gallone, A., Nychas, G. J., Sofos, J. N., Colelli, G., Amodio, M. L., & Spano, G. (2012). Factors affecting quality and safety of fresh-cut produce. *Critical reviews in food science and nutrition, 52*(7), 595–610.

15. Ghilamicael, A. M., Boga, H. I., Anami, S. E., Mehari, T., & Budambula, N. L. M. (2018). Potential human pathogenic bacteria in five hot springs in Eritrea revealed by next-generation sequencing. *PLoS ONE, 13*(3), 1–12. https://doi.org/10.1371/journal.pone.0194554

16. Gombas, D., Luo, Y., Brennan, J., Shergill, G., Petran, R., Walsh, R., & Deng, K. (2017). Guidelines to validate control of cross-contamination during washing of fresh-cut leafy vegetables. *Journal of Food Protection, 80*(2), 312–330.

17. Gómez-López, V. M., Gobet, J., Selma, M. V., Gil, M. I., & Allende, A. (2013). Operating conditions for the electrolytic disinfection of process wash water from the fresh-cut industry contaminated with E. coli O157: H7. *Food Control, 29*(1), 42-48.

18. Halkman, H. B. D., & Halkman, A. K. (2014). Indicator Organisms. Encyclopedia of Food Microbiology: Second Edition, 2, 358–363. https://doi.org/10.1016/B978-0-12-384730-0.00396-7

19. Hanski, I., Von Hertzen, L., Fyhrquist, N., Koskinen, K., Torppa, K., Laatikainen, T., & Haahtela, T. (2012). Environmental biodiversity, human microbiota, and allergy are interrelated. *Proceedings of the National Academy of Sciences, 109*(21), 8334-8339.

20. Harris, A. R., Islam, M. A., Unicomb, L., Boehm, A. B., Luby, S., Davis, J., & Pickering, A. J. (2018). Fecal contamination on produce from wholesale and retail food Markets in Dhaka, Bangladesh. *American Journal of Tropical Medicine and Hygiene, 98*(1), 287–294. https://doi.org/10.4269/ajtmh.17-0255

21. Iwu, C. D., & Okoh, A. I. (2019). Preharvest transmission routes of fresh produce associated bacterial pathogens with outbreak potentials: a review. *International journal of environmental research and public health*, *16*(22), 4407.

22. Jackson, C. R., Randolph, K. C., Osborn, S. L., & Tyler, H. L. (2013). Culture dependent and independent analysis of bacterial communities associated with commercial salad leaf vegetables. *BMC Microbiol, 13*, 274.

23. Jackson, C., Stone, B., & Tyler, H. (2015). Emerging perspectives on the natural microbiome of fresh produce vegetables. *Agriculture, 5*(2), 170–187. https://doi.org/10.3390/agriculture5020170

24. Leff, J. W., & Fierer, N. (2013). Bacterial communities associated with the surfaces of fresh fruits and vegetables. *PloS one, 8*(3), e59310.

25. Li, K., Weidhaas, J., Lemonakis, L., Khouryieh, H., Stone, M., Jones, L., & Shen, C. (2017). Microbiological quality and safety of fresh produce in West Virginia and Kentucky farmers' markets and validation of a post-harvest washing practice with antimicrobials to inactivate Salmonella and Listeria monocytogenes. *Food Control*, *79*, 101–108.

26. Machado-Moreira, B., Richards, K., Brennan, F., Abram, F., & Burgess, C. M. (2019). Microbial

contamination of fresh produce: What, where, and how? *Comprehensive Reviews in Food Science and Food Safety*, *18*(6), 1727-1750.

27. Manaia, C. M. (2017). Assessing the risk of antibiotic resistance transmission from the environment to humans: Non-direct proportionality between abundance and risk. *Trends in Microbiology, 25*(3), 173–181. https://doi.org/10.1016/j.tim.2016.11.014

28. Mbae, K. M., Ndwiga, M. K., & Kiruki, F. G. (2018). Microbiological quality of kachumbari, a raw vegetable salad popularly served alongside roast meat in Kenya. *Journal of Food Quality*, *2018*(1), 8539029.

29. Meireles, A., Giaouris, E., & Simões, M. (2016). Alternative disinfection methods to chlorine for use in the fresh-cut industry. *Food Research International, 82*, 71-85.

30. Miralles, M., Maestre-Carballa, L., Lluesma-Gomez, M., & Martinez-Garcia, M. (2019). High-throughput 16S rRNA sequencing to assess potentially active bacteria and foodborne pathogens: A case example in ready-to-eat food. *Foods*, *8*(10), 480.

31. Murray, K., Wu, F., Shi, J., Jun Xue, S., & Warriner, K. (2017). Challenges in the microbiological food safety of fresh produce: Limitations of post-harvest washing and the need for alternative interventions. *Food Quality and Safety, 1*(4), 289–301.

32. Ndiege, M. A., Nyamari, J., & Imungi, J. K. (2017). Food hygiene conditions and microbial contamination of minimally processed fruits in central ward, Nairobi County. *Int J Sci Res*, *7*, 137–140.

33. Nuesch-Inderbinen, M., & Stephan, R. (2016). Epidemiology of extended-spectrum β-lactamase-producing Escherichia coli in the human-livestock environment. *Current Clinical Microbiology Reports*, *3*(1), 1-9

34. Nutrition, A., & Services, H. (2012). Bad Bug Book.

35. Oliveira, M., Usall, J., Vi, I., Anguera, M., Gatius, F., & Abadias, M. (2010). Microbiological quality of fresh lettuce from organic and conventional production. *Food Microbiology, 27*(5), 679-684.

36. Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V., Braden, C. R., Angulo, F. J., & Griffin, P. M. (2013). Attribution of foodborne illnesses, hospitalisations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerging infectious diseases, 19*(3), 407.

37. Panezai, M., Taj, M. K., Nawaz, I., Taj, I., Panezai, M., Panezai, N., Zafar, U., Muhammad, D. G., Essote, S. A., & Muhammade, G. (2018). Research article isolation and identification of salmonella paratyphi from enteric fever patients at different hospitals of Quetta city. *Pakistan Journal of Biological Sciences, 21*(9), 469–474. https://doi.org/10.3923/pjbs.2018.469.474

38. Paudyal, N., Anihouvi, V., Hounhouigan, J., Matsheka, M. I., Sekwati-Monang, B., Amoa-Awua, W., & Fang, W. (2017). Prevalence of foodborne pathogens in food from selected African countries–A meta-analysis. *International journal of food microbiology*, *249*, 35-43.

39. Rajwar, A., Srivastava, P., & Sahgal, M. (2016). Microbiology of fresh produce: Route of contamination, detection methods, and remedy. *Critical Reviews in Food Science and Nutrition*, *56*(14), 2383-2390.

40. Rastogi, G., Sbodio, A., Tech, J. J., Suslow, T. V. & Coaker, G. L. (2012). Leaf microbiota in an agroecosystem: Spatiotemporal variation in bacterial community composition on field-grown

lettuce. *ISME* J, 1–11.

41. Rico, C. M., Barrios, A. C., Tan, W., Rubenecia, R., Lee, S. C., Varela-Ramirez, A., & Gardea-Torresdey, J. L. (2015). Physiological and biochemical response of soil-grown barley (Hordeum vulgare L.) to cerium oxide nanoparticles. *Environmental Science and Pollution Research*, *22*, 10551-10558.

42. Sare, A. R., Stouvenakers, G., Eck, M., Lampens, A., Goormachtig, S., Jijakli, M. H., & Massart, S. (2020). Standardization of plant microbiome studies: Which proportion of the microbiota is really harvested? *Microorganisms, 8*(3), 342. https://doi.org/10.3390/microorganisms8030342

43. Su, Y., Hsu, W. Y., Huang, T. S., & Simonne, A. (2021). Evaluation of the microbiological quality of fresh cilantro, green onions, and hot peppers from different types of markets in three U.S. States. *Horticulturae*, *7*(6), 122.

44. Tatsika, S., Karamanoli, K., Karayanni, H., & Genitsaris, S. (2019). Metagenomic characterisation of bacterial communities on ready-to-eat vegetables and effects of household washing on their diversity and composition. *Pathogens*, *8*(1), 37.

45. Teplitski, M., & Butler, J. D. (2008). Indicator organisms: What every Floridian may want to know about microbiological water quality: SL 259/SS482, 3/2008. *EDIS, 2008*(3). https://doi.org/10.32473/edis-ss482-2008

46. WHO. (2021). Cholera – Kenya. (n.d.). Retrieved September 5, 2022, from https://www.who.int/emergencies/disease-outbreak-news/item/11-december-2017-cholera-kenya-en

47. World Health Organization (WHO). (2008). Microbiological risk assessment series. Microbiological hazards in fresh fruits and vegetables. *World Health Organization*.

48. Yin, Y., Zhu, D., Yang, G., Su, J., & Duan, G. (2022). Diverse antibiotic resistance genes and potential pathogens inhabit in the phyllosphere of fresh vegetables. *Science of the Total Environment*, *815*, 152851.