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Indicator microorganisms in fresh vegetables from "farm to fork" in Rwanda

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ABSTRACT

Microbial safety of ready-to-eat vegetables is currently a global concern. We studied indicator microorganisms in fresh vegetables from "farm to fork" in Rwanda, to identify possible trends in microbial counts along the supply chain in a developing country. A total of 453 samples were taken across the vegetable supply chain (farm, market and food service establishment level) and analyzed for indicator microorganisms; *Enterobacteriaceae*, *Listeria* spp., aerobic plate count and coagulase - positive staphylococci. The sampling at farm and market covered 11 types of vegetables commonly eaten raw in salads. Results show that the mean count of *Enterobacteriaceae* and *Listeria* spp. in vegetables were respectively 5.8 and 4.6 log cfu/g at farm, 6.3 and 4.9 log cfu/g at market, 6.0 and 5.1 log cfu/g upon arrival at food service establishments, and finally 3.3 and 2.9 log cfu/g in ready-to-eat salads. Aerobic plate count and coagulase-positive Staphylococci were on average 6.8 and 4.6 respectively at start of salad preparation and 4.9 and 3.0 in the final product. Unit operations like washing with or without sanitizers, trimming and peeling significantly reduced indicator counts by on average 2.1 log cfu/g from start to end of salad preparation. Results also show that 91% (51/56) and 22% (12/56) of ready-to-eat salads prepared by food service establishments met the guidelines for coagulase - positive staphylococci (10^4 cfu/g) and presumptive *Listeria* spp. (10^2 cfu/g). The high counts of these indicator microorganisms along the vegetable supply chain, raises concern about the potential presence of foodborne pathogens. This study calls for improved adherence to GAPs and GHPs in the fresh vegetable supply chain so as to minimize the potential risk from foodborne pathogens.

1. Introduction

Global production and consumption of fresh vegetables has been increasing for the last three decades (FAO STAT, 2015), concurrently, the reported foodborne outbreaks linked to fresh vegetables have surged (Callejon et al., 2015; Herman, Hall, & Gould, 2015; Painter et al., 2013; Lynch, Tauxe, & Hedberg, 2009). Pathogens most implicated in these vegetable related outbreaks include *Norovirus*, *Salmonella* spp., *Escherichia coli* and *Shigella* spp. (Callejon et al., 2015; Kozak, Macdonald, Landry, & Farber, 2013). To minimize these vegetable associated outbreaks internationally, guidelines such as those from the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) (CAC/RCP 53-, 2003) have been developed to prevent or control the conditions or factors leading to microbial contamination, survival or growth along the "farm to fork" continuum. To investigate the effectiveness of the control measures in these guidelines, researchers from mainly developed countries have continued to study foodborne pathogens and indicator microorganism (IMOs) at different stages of the vegetable supply chain (Holvoet, Sampers, Seynnaeve, & Uyttendaele, 2014; Schwaiger, Helmke, Hölzel, & Bauer, 2011; Ward et al., 2015). Because pathogens are usually prevalent in low numbers, appear sporadically or absent at times, IMOs like aerobic plate count (APC), faecal coliforms, *Enterobacteriaceae*, *Listeria* spp. can provide more information to the detect the changes in control or preventive measures (Buchanan & Oni,

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2012). Indicator microorganisms have been defined as a species of microorganisms or a group of microorganisms that indicate if food has been exposed to conditions that pose an increased risk to be contaminated with a pathogen or has been held under conditions that would allow pathogen proliferation (Buchanan & Oni, 2012). Although researchers (Holvoet, Sampers, et al., 2014; Schwaiger et al., 2011; Ward et al., 2015) have used IMOs to investigate the extent of contamination of vegetables, most studies do not cover the whole vegetable supply chain (VSC) *i.e.* the "farm to fork" continuum. A full overview of microbial levels across the entire supply chain may be of more practical use in preventing foodborne outbreaks at food service level.

In this study, we examined IMOs in the VSC in Rwanda from "farm to fork" to identify possible trends in microbial counts (growth or contamination, inactivation, survival) along the VSC. By investigating the microbial counts of IMOs across the entire VSC, we aim to contribute to practical approaches and information for risk managers in implementing microbial safety guidelines. Three major stages of the VSC were selected for investigation, farm, market and food service establishments (FSEs). To represent the final stage of the VSC, we chose FSEs over households, because in Rwanda, preparation and consumption of raw vegetables salads is more common in FSEs than in households (most people in homes consume cooked vegetables). Four specific objectives were set, (i) determining the difference between counts of IMOs in vegetables at farm and market, (ii) investigating the ability of the different FSEs to eliminate or reduce IMOs counts from start to the end of salad preparation, (iii) benchmarking of the microbial counts in FSE-RTE salads with existing guidelines or regulatory requirements and (iv) comparing the relation between the counts of IMOs at start of salad preparation at FSEs with the counts in the ready-to eat (RTE) salads. *Enterobacteriaceae* and *Listeria* spp. were selected as IMOs along the supply chain based on the expected vast abundance in farm vegetables (Little, Roberts, Youngs, & De Louvois, 1999; Zhu & Hussain, 2015) and hence the ability to provide observable trends (increase or decrease) across the VSC. At FSEs, we included other IMOs *viz.* aerobic plate count (APC) and coagulase - positive staphylococci (CP-staphylococci) the former, to indicate the exposure of the vegetables to contamination and proliferation of microorganisms in general (Aycicek, Oguz, & Karci, 2006) and the latter to indicate personnel hygiene behaviors (Balzaretti & Marzano, 2013; Jacxsens et al., 2009) during salad preparation.

2. Materials and methods

2.1. Study design, sampling points and area

Selected IMOs were analyzed from 453 samples taken along the vegetable supply chain (three major stages: farm, market and FSE) in Rwanda from February to October 2015. The samples at farm and market covered 11 types of vegetables commonly eaten raw, *viz*; beet root, cabbage, carrot, celery, cucumber, garlic, green pepper, lettuce, onion, parsley and tomato (each vegetable type sampled nine times). At farm, the study concentrated on the vegetable growing regions of Rwanda. Based on availability and the "one farm one sample" approach, we took 30, 26, 21, 16 and 6 samples from the Western, Southern, Northern and Eastern provinces and the peripherals of the City of Kigali respectively. Markets were selected based on the availability of the 11 chosen vegetables sold in built-open markets and supermarkets in the City of Kigali (15), the Southern (3), Western (2), Northern (1) and Eastern (1) provinces of Rwanda. Sampling in FSEs (hotels, restaurants and bars) was done in two cities of Rwanda (Kigali (51) and Musanze (5)). Food service establishments buy whole vegetables from either from markets or from the farms directly and during salad preparation, different vegetables are mixed, washed and cut. One FSE can buy different vegetables from different markets or growing regions depending on the price or availability and no fresh cut vegetables are available before the food service level. The selection of each FSE was based on the maximum transit time of two hours between the FSEs and the laboratory to minimize holding time of prepared salad before analysis. The samples were stored in cooling boxes during transportation. To prepare the FSEs for the study, we organized a consent meeting in which managers of FSEs were briefed about the study and its importance in improving food safety. Out of 280 FSEs invited, 168 FSE managers showed interest to participate in the study and were provided with consent forms to register. To investigate the ability of FSEs to decrease microbial load during salad preparation, a sample was taken at the start and at the end of salad preparation. The samples were provided for free and after the laboratory analysis, we shared the test report and feedback with each individual FSE.

2.2. Sample collection

2.2.1. Farms

Each of the 11 types of vegetables was sampled 9 times leading to total of 99 vegetable samples which were purchased randomly from 99 farms. The sampling procedure slightly differed for the three categories of vegetables (fruit, subterranean, leafy). Fruit vegetables (*i.e.* cucumber, green pepper, tomato) were picked at maturity from the plant. Subterranean vegetables like carrot, beet root, garlic and onion, the vegetable roots, tubers or bulbs were uprooted, hand shaken to remove the attached soil and the aerial part cut off and discarded. Leafy vegetables such as lettuce, cabbage, celery and parsley, the samples consisted of only aerial parts which were cut from the root base. For cabbage, ten heads were collected from each farm. For other farm vegetables, a pooled farm sample (~2 kg) was collected as far apart as possible depending on the farm size. Farm size ranged from around 6 m^2 to over 4000 m^2 and several of these farm units conglomerate to form a vegetable farming area and in each farm one type of vegetable is grown. Sterile materials such as gloves and knives were used throughout the sampling process and changed between each farm sample.

2.2.2. Markets

Twenty two markets were visited and in each market 11 types of vegetables were purchased leading to a total of 242 samples. To obtain a representative sample for a given market, we randomly purchased small units of vegetables from 6 to 10 vendors to get a pooled sample of about 2 kg for each type of vegetable in retail markets. In supermarkets (single vendors), packaged units were sampled from the shelves of each vegetable type. For cabbage, ten heads were purchased from each market.

2.2.3. Food service establishments (FSEs)

A total of 56 FSEs (43 hotels and 13 restaurant/bars) were randomly selected and sampled. Each FSE provided 2 samples, one of whole mixed vegetables (FSE-WMV) at start of salad preparation (about 1–2 kg) and another of ready-to- eat (FSE-RTE) vegetables (about 0.5–1 kg). For the 56 FSEs, a total of 112 (56 × 2) vegetables samples were collected. About 70% (39/56) of the visited FSEs washed vegetables with sanitizers, while others did not use any sanitizer but rinsed vegetables with either boiled water or containerized drinking water. Different sanitizers were used; 2% of FSEs used sodium troclosene (25–75 ppm), 12% used sodium